Design and Evaluation of Oral Controlled Release Tablets of Milnacipran Hydrochloride

THESIS

Submitted in partial fulfilment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

GAUTAM SINGHVI

Under the Supervision of

PROF. RANENDRA N. SAHA

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

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CERTIFICATE

This is to certify that the thesis entitled "**Design and Evaluation of Oral Controlled Release Tablets of Milnacipran Hydrochloride**" and submitted by **Gautam Singhvi**, ID. No. **2008PHXF427P** for award of Ph.D. Degree of the Institute, embodies original work done by him under my supervision.

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Date: **Gauatm Singhvi**

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List of Abbreviations and Symbols

%	Percentage
% CDR	Percentage cumulative drug released
% RSD	Percentage relative standard deviation
% RTD	Percentage remaining to be degraded
λ_{max}	Wavelength of maximum absorbance
<	Less than
=	Equal to
≈	Approximately equal to
^{0}C	Degree centigrade
^{0}C/min	Degree centigrade per minute
5-HT	5- Hydroxy tryptamine
ACN	Acetonitrile
AT	Accelerated temperature
AUC	Area under curve
AUMC	Area under the first moment curve
BCS	Biopharmaceutical classifications system
Cm	Centimeter
C_{max}	Maximum concentration
Conc.	Concentration
cPs	Centipoises
CR	Controlled release
CRT	Controlled room temperature
DCP	Di basic calcium phosphate
DSC	Differential scannimg calorimetry
EC	Ethylcellulose
EDTA	Ethylene di amine tetra acetic acid
et al.	Co-workers
f_2	Similarity factor
FDA	Food and drug administration
FM	Fibromyalgia
F_r	Relative bioavailability
FTIR	Fourier transform infra red
g	Gram

g/l	Gram per litre
h	Hour
HPMC 4K	Hydroxypropyl methylcellulose 4000 cPs
HPMC 15K	Hydroxypropyl methylcellulose 15000 cPs
HPMC 100K	Hydroxypropyl methylcellulose 100000 cPs
HQC	Higher quality control sample
ICH	International conference on harmonization
IP	Indian Pharmacopoeia
IR	Immediate release
J/g	Joule per gram
K	Release rate constant for 'Korsmeyer-Peppas' empirical equation
K_0	Zero order release rate constant
K_1	First order release rate constant
K_{deg}	Degradation rate constant
Kg	Kilogram
kg/cm^2	Kilogram per square centimeter
K_H	Release rate constant representative of square root kinetics
L	Litre
l/h/kg	Litre per hour per kilogram
l/kg	Litre per kilogram
LOD	Limit of detection
LOQ	Limit of quantification
LQC	Lower quality control
MDT	Mean dissolution time
MG	Multi granules
mg	Milligram
mg/ml	Milligram per millilitre
Mg Stearate	Magnesium Stearate
MIL	Milnacipran Hydrochloride
Min	Minute
ml/min	Millilitre per minute
MQC	Medium quality control
MRT	Mean residence time
M_t/M_∞	Fraction of drug released at time t

n	Diffusional exponent indicative of release mechanism in krosmeyer-peppas model
NaCMC	Sodium carboxymethyl cellulose
NE	Norephinephrine
ng/ml	Nanogram per millilitre
$P_{o/w}$	Equilibrium partition coefficient
QC	Quality control
R^2	Regression coefficient
R_f	Retention factor
RH	Relative humidity
RP-HPLC	Reverse phase-High performance liquid chromatography
Rpm	Revolutions per minute
RT	Retention time
S	Slope of the least square regression line
SD	Standard deviation
SNRI	Serotonin and norepinephrine reuptake inhibitors
SSRIs	Selective serotonin reuptake inhibitors
$t_{1/2}$	Half-life
$t_{50\%}$	Time to reach 50% of initial concentration
$t_{90\%}$	Time to reach 90% of initial concentration
T50%	Time taken for 50% of drug release from formulations
T80%	Time taken for 80% of drug release from formulations
TCAs	Tricyclic antidepressants
TDW	Triple distilled water
T_g	Glass transition temperature
TLC	Thin layer chromatography
T_{max}	Time taken to reach maximum concentration
UV	Ultra Violet
Vis	Visible
w/w	Weight by weight
µg/ml	Micro gram per millilitre

Abstract

Milnacipran hydrochloride is a selective serotonin and norepinephrine dual reuptake inhibitor. It is clinically approved drug for the treatment of depression and fibromyalgia. Its short elimination half-life, frequent dosing and associated side effects cause lack of patient compliance and discontinuation of present therapy. To overcome such problems, the objective of present study was to design matrix embeded controlled release formulation using various hydrophilic and hydrophobic polymers. Matrix based CR tablet formulation was decided due to economic and easy process as well as the high reproducibility of matrix formulation.

For characterization of variety of in-process and finished product samples, in-house analytical methods (UV spectroscopic and HPLC) were developed and validated. Various preformulation parameters such as powder characteristics, solubility, partition coefficient, drug-excipient compatibility, solution state and solid state stability at different storage conditions were investigated for stable and bio-available dosage form which can be mass produced.

Controlled release formulations were prepared with hydrophilic polymers (HPMC 15K, HPMC 100K, sodium CMC, carbopol) alone or in combination using wet granulation process. Multi granules based CR tablets were also prepared with different proportion of hydrophilic polymers and hydrophobic polymers. All the formulations were evaluated for their physical characteristics. USP type II apparatus (paddle) at 50 rpm was used for dissolution study for all the formulations. Additionally, the mechanism of drug release from the extended release formulations were evaluated by applying different mathematical kinetic models on drug release profiles. Drug release data were compared with dissolution parameters such as mean dissolution time (MDT), time for 50% and 80% drug release (T50% and T80%) and similarity factor.

The effect of various formulation factors such as polymer type, polymer proportion, polymer viscosity and compression force; and effect of dissolution factors like pH of dissolution medium and agitation speed on the in-vitro drug release were assessed using various dissolution parameters in order to optimize these variables. Based on in-vitro performance, few controlled release formulations were selected for pharmacokinetic study in rabbits.

Results of preformulation investigations indicated that milnacipran hydrochloride was non-hygroscopic and poor flowing powder. Solubility studies in various buffered and unbuffered pH systems showed that milnacipran hydrochloride was highly water soluble (2000 mg/ ml) at all pH. Milnacipran hydrochloride followed first order degradation kinetics in solution state with good stability over the entire pH range. Solid state stability studies showed that milnacipran hydrochloride was stable and compatible with various formulation excipients for sufficient time period. DSC and FTIR studies had further confirmed that there was no interaction between drug and excipients at different storage conditions.

All the designed formulations were found to be within official acceptable quality control limits. In-vitro release profiles and release parameters (T50%, T80% and MDT) indicated that drug release from polymeric matrix was significantly dependent on polymer proportion, hydrophobic content, viscosity of polymer and hardness of tablets. Stability data showed that physical characteristics and release behavior of designed formulations found to be more or less similar with initial results.

In-vivo studies of selected CR formulations in rabbits explicitly indicated that all CR formulations successfully extended the drug release and thus the oral absorption of milnacipran hydrochloride. Level A IVIVC was observed for developed CR formulations.

It can be conclude that stable oral controlled release formulations of milnacipran hydrochloride were successfully designed and evaluated. The proposed method of preparation was simple, economic and reproducible which has scope for commercialization. In-vitro and in-vivo performance of designed formulations proved their potential to maintain plasma drug concentration for longer time and can minimize the frequency of dosing and side effects.

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Milnacipran hydrochloride (MIL) is a serotonin–norepinephrine reuptake inhibitor (SNRI) used in the clinical treatment of major depressive disorder [1] and fibromyalgia [2]. It has been approved since late 1990s in some European and Asian countries for the treatment of depression and has now also been approved for the treatment of fibromyalgia [3-4].

Depression is mean to intense and prolonged sadness. Sadness is an emotion that everyone feels at some time or other, often in response to bereavement, illness or loss. However, depression (also referred to as clinical depression) is different from just feeling sad. Depression is a mood disorder, also called an affective disorder. Depressive signs and symptoms are characterize not only by negative thoughts, moods, and behaviors but also by specific changes in bodily functions such as, crying spells, body aches, low energy or libido, as well as unwillingness for eating, weight loss, or sleeping. The functional changes of clinical depression are often called neurovegetative signs. This means that the nervous system gets changed, cause many physical symptoms that result in diminished participation and a decreased activity level [5-6].

The monoamine hypothesis of depression postulates that the underlying pathophysiologic basis of depression is depletion in the levels of serotonin, norepinephrine, and/or dopamine neurotransmission in the brain. This hypothesized pathophysiology supported by the mechanism of action of current antidepressants that elevated the levels of these neurotransmitters in the brain for patients whose depression was caused by the imbalance of either norepinephrine or serotonin.

Fibromyalgia (FM) is a rheumatologic disorder characterized by widespread musculoskeletal pain and lowered pain threshold. Other prominent symptoms include stiffness, paresthesias, disturbed sleep, fatigue, psychologic distress and tenderness at predefined anatomic sites. Due to these multiple symptoms and high rates of comorbidity with other related disorders, patients with FM often report a reduced quality of life [7].

The etiology and pathophysiology of FM are unclear. Although FM was previously thought to be a muscle disease, it is now considered a disorder of central nervous system perception and regulation of pain [7]. In the central nervous system, both serotonin and norepinephrine have been found to play important roles in pain

perception via their involvement in descending antinociceptive pathways. Dysfunction in these descending pathways is thought to result in the allodynic (painful response to nonpainful stimuli) and hyperalgesic (heightened sensitivity to pain) states experienced by patients with FM [8].

1.1.1 Milnacipran hydrochloride and current available antidepressants

All the existing common antidepressants such as tricyclic antidepressants (TCAs), increase the synaptic concentrations of serotonin (5-hydroxytryptamine, 5-HT) and/or norepinephrine (NE), usually by blocking the reuptake of one or both of the neurotransmitters. However, poor tolerability and toxicity in overdose are most common limitations of TCAs due to their various additional interactions at a variety of neurotransmitter receptors. The idea that "two actions are better than one" has led to the development of compounds that prevent the reuptake of both 5-HT and NE without the nonspecific, side effect-inducing interactions of TCAs. These are introduced as serotonin and norepinephrine reuptake inhibitors (SNRIs) [8-9].

1.1.2 Milnacipran hydrochloride for fibromyalgia

The reduced serotonin and norepinephrine levels observed in patients with fibromyalgia suggest that medications, which increase the levels of these neurotransmitters, may have clinically beneficial effects in fibromyalgia and other chronic pain conditions. A broad array of medications has been used to treat fibromyalgia, including TCAs, selective serotonin reuptake inhibitors (SSRIs), anticonvulsants, non-steroidal anti-inflammatory drugs (NSAIDs), growth hormone, corticosteroids, sedatives and opioids, with varying degrees of success. Before any medication was approved by FDA for treating fibromyalgia, the TCAs were generally used as first-line agents, particularly amitriptyline. However, their use was limited because of adverse effects [10]. From 2007 to 2009, three medications were approved for treating fibromyalgia: pregabalin, duloxetine, and milnacipran hydrochloride. Pregabalin is an alpha-2 delta ligand that was approved for treating fibromyalgia in 2007. Duloxetine and MIL are SNRIs and duloxetine was approved for treating fibromyalgia in 2008 and MIL in 2009. These medications are currently the only approved pharmacologic treatments available [11-12].

1.1.3 Efficacy of milnacipran hydrochloride

It has been proved in clinical studies that MIL has unique pharmacokinetic and pharmacodynamic characteristics such as equipotent serotonin and norepinephrine reuptake inhibition and a linear dose-concentration trend at therapeutic doses that distinguish it from the other SNRIs. In addition, it has negligible effects on any presynaptic or postsynaptic receptors and does not inhibit the cytochrome P 450 system, indicating minimal propensity for drug-drug interactions. The effectiveness of MIL for depression and fibromyalgia has been clearly established in a number of randomized, double blind, placebo-controlled clinical trials [13].

Seven randomized, double-blind clinical trials with similar designs have compared the efficacy and tolerability of MIL and TCAs in patients with major depression. Results indicated that the response rate with MIL (64%) was comparable with that of the TCAs (67%). In contrast with the TCAs, MIL was very well tolerated by patients [5, 13].

A meta-analysis comparative study of MIL at dose 100 mg/day was also compared with SSRIs fluvoxamine (200 mg/day) and fluoxetine (20 mg/day) in moderately to severely depressed patients. Results of this study indicated that MIL was significantly more responded (64%) than the two SSRIs (50%) with a significantly higher remission rate (38.7% versus 27.6%) [14].

Thus, results of clinical studies proved that MIL is undoubtedly effective and better tolerable than TCAs especially in regards to anticholinergic (e.g. dry mouth, constipation) or antihistaminergic side effects (e.g. fatigue, somnolence, weight gain), and even than SSRIs in the treatment of depression, fibromyalgia and may have usefulness for fatigue and anxiety symptoms.

1.2 Problems associated with milnacipran hydrochloride therapy

Unfortunately, MIL has demonstrated numerous adverse effects in depression clinical trials. Some double-blind, randomized, multicenter clinical studies of MIL with 100 mg/day and 200 mg/day reported nausea (12-19%), headache (8-14%), constipation (6-11%), abdominal pain (6-8%) and vomiting (4-8%) as the most frequent spontaneously adverse effects [5].

It is important to note that results of fibromyalgia clinical trials of MIL with 100 mg/day and 200 mg/day indicated that nausea (32-40%), headache (15-18%),

constipation (14-18%), palpitations (6-8%), vomiting (5-8%) and increased heart rate (5-7%) were the most frequent adverse events reported for discontinuation of MIL treatment [14]. Adverse events resulted in approx 27% and 20% premature discontinuation with 200 mg/day and 100 mg/day of MIL dose regimen respectively during fibromyalgia clinical trial. In addition, the recent fibromyalgia clinical trial with the long dose escalation period (four weeks) also reported the abdominal pain and nausea were the most common dose-related side effect [14]. However, controlled study with over 3,300 patients revealed that the incidence of cardiovascular and anticholinergic side effects was significantly lower compared to TCAs [5].

Hence, MIL is effective, safe and better tolerable newer drug for treating depression and fibromyalgia than other available drugs (TCAs, SSRIs, etc) for depression and fibromyalgia but its adverse effects make discontinuation of treatment. MIL needs to titrate over a long period to reach the required dose but its dose related side effects make it limited at high dose.

1.3 Need for the controlled release drug delivery systems of milnacipran hydrochloride

The reports of early antidepressant clinical studies and recent FM clinical trials demonstrated that higher dose (100 to 200 mg/day) of MIL were led to significant improvements in depression, pain and other symptoms of FM. In addition, these trials also indicated high incidence of treatment-emergent side effects that leads to poor patient tolerance at higher dose regimen. Thus, it would be very difficult to reach the upper limits of the dose range with currently available conventional immediate release (IR) formulations of MIL [3, 14].

The conventional IR formulations of MIL may not be suitable for a once-daily dosing regimen for treatment of depression due to its relatively short half-life (8 h). MIL therapy is required for a longer time period to treat depression and fibromyalgia [3]. In this situation frequently administration of IR formulation is not patient compliance and economic.

Thus, there is need to improve the efficacy and safety of this drug through proper control of drug release. Therapeutic efficacy of MIL can be enhanced by delivering as oral controlled drug release formulations (novel drug delivery system)

with better patient compliance. But a thorough study is required to design such products for commercial use.

Therefore, it is planned to design different controlled release formulations of MIL and study different techniques for preparation of oral controlled release delivery systems and effect of various process parameters and excipients on characters of drug release. It is postulated that such drug delivery system will overcome the drawbacks of existing conventional formulations of MIL and provide better outcomes.

1.4 Oral controlled release drug delivery systems

Oral route has been the most popular and successfully used for delivery of various drugs and their dosage forms. There are several reasons for the continued popularity of the oral solid dosage form. The oral route of delivery is non-invasive and patients are able to administer the medicine themselves. For the manufacturer, solid oral dosage forms offer many advantages as they are generally the most stable forms of drugs, they utilize inexpensive technology which provide ease of production at low cost and their appearance can be modified to create brand identification [15-16].

Pharmaceutical products designed for oral delivery as currently available on the prescription and over the counter markets are mostly designed as IR formulations for rapid absorption. Plasma concentration of a drug administered as IR dosage form generally rises quickly to peak and declines. Thus, it is always challenging to formulation scientist to design delivery systems to overcome such problems.

An ideal controlled release drug delivery system is the one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. Thus, unlike conventional immediate release system, the rate of appearance of drug in the system with such formulations is not controlled by absorption process but by release process.

Some of the advantages of a controlled release drug delivery system over a conventional dosage form are such as:

Less frequent drug administration and low plasma concentration

Reduction in fluctuation of steady state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects

Increased safety margin of high potency drugs due to better control of plasma levels
Improvement of bioavailability of some drugs
Maximum utilization of drug enabling reduction in total amount of dose administered
Reduction in health care costs through improved therapy and improved patient convenience and compliance [17].

According to global business intelligence (GBI) research, the oral drug delivery market has seen a significant increase in the number of licensing and partnership deals over the last few years. This has resulted in significant growth in the drug delivery market over the last few years. According to GBI research, the overall oral drug delivery market is forecasted to grow to $199 billion in 2016 from $101 billion in 2009, at a growth rate of 10.3%. Thus, this growth also confirmed the growing interest of pharmaceutical industries towards novel drug delivery systems as a key product for lifecycle management and a strategy to increase patient acceptance and compliance [18].

1.5 Types of oral controlled release drug delivery systems

However, there may different types, but oral controlled release formulation can be divided into three major categories:

1. Membrane systems: In such system, the drug is contained in a core surrounded by a thin rate controlling polymeric membrane.

2. Matrix systems: In this system, the drug dissolved or dispersed in a carrier polymeric matrix. The matrix can be made up of soluble or insoluble polymers.

3. Hybrid system: A combination of membrane and matrix system where drug is dispersed in polymeric matrix and this matrix core is coated with rate controlling polymeric membrane [19].

As this work was mainly on design of controlled release based on matrix system, detailed discussion is made on this system below.

1.5.1 Oral matrix type controlled release formulation

In matrix types of controlled delivery systems, the drug is uniformly distributed in either a hydrophobic or hydrophilic polymer matrix or mixture. In such system, drug is not chemically attached to the polymer. Drug remains as biologically active form, which can exert its effect as it is released from the polymeric matrix. The

major advantage of this type of drug delivery system is that the drug in the polymeric matrix is unaltered. Therefore, its absorption, distribution, metabolism, and excretion after being released from the polymer are the same as that of the native drug. Moreover, its biological or pharmacological effect when released from the polymeric matrix is the same as drug that when used alone [19-20].

Matrix technologies have often proven popular among the oral controlled drug delivery technologies because of their simplicity and ease of manufacturing. The fabrication process for these systems is similar to those for conventional dosage form and is highly reproducible. Matrix based controlled release formulations also provide stability of the raw materials in dosage form and ease of scale-up and process validation. The drug release from polymeric matrix systems remains unaffected by thin spots, pinholes, and other similar defects, which can be a serious problem with reservoir systems. Acceptance and application of matrix systems in pharmaceutical research is reflected by the large number of research publications and patents filed each year and by the commercial success of a number of novel drug delivery systems based on matrix technologies [21].

Two major types of materials are used in the preparation of matrix devices:

(I) Hydrophobic matrix forming agents

As the term suggests, the primary rate-controlling components of hydrophobic matrix are water insoluble in nature. There are two type of hydrophobic matrix forming agents are mostly used in oral matrix formulations:

(i) Digestible base (fatty/wax compounds)- glycerides, glyceryl-tri-stearate, fatty alcohols, fatty acids, carnauba wax, paraffin wax, etc.

(ii) Non-digestible base (insoluble plastics) - poly methylacrylate -methylmethacrylate, polyethylene, ethylcellulose, etc.

Hydrophobic matrix agents are utilized to incorporate drugs into inert water-insoluble matrix materials. Hydrophobic matrix systems are formulated by waxes or insoluble plastic polymer mainly and can be suitable for drugs, which have high water solubility [22]. The use of hydrophobic matrix appears to have several advantages such as chemical inertness, safe application, lower cytotoxicity (due to the absence of solvents), good stability at varying pH conditions and moisture levels, independent on the gastric state, ease of manufacturing with high reproducibility as

well as low production cost. Moreover, as the matrix delivery system passes through the gastrointestinal tract, the active ingredient is slowly release and absorbed [23-24].

Drug release from hydrophobic matrix occurs via a leaching mechanism. Drug particles dispersed in polymer matrix dissolve in the penetrating gastro-intestinal fluids and are released from the tablets by diffusion through the porous network of matrix and pores that are created by dissolution of drug particles. The pore structure of matrix controls the water uptake and drug release. For such matrix, variation of compaction pressure, moisture fraction, particle size, fraction of soluble compound, fraction of channeling agents will result in different porosity of matrix and pore structure which cause change in drug release [25].

(II) Hydrophilic matrix forming agents

Hydrophilic polymer matrix systems are widely used in oral controlled drug delivery because of their most cost effective method of fabrication, flexibility to obtain a desirable drug release profile and broad regulatory acceptance [26-27]. Since last few years, hydrophilic swellable polymers have been widely used to control the release of a drug from matrix tablet formulations. Examples of some of hydrophilic polymers are methylcellulose, sodium carboxy methylcellulose, hydroxypropyl methylcellulose, sodium alginate, xanthum gum, polyethylene oxide and carbopol. Their popularity derives from their non-toxic nature, their ability to accommodate a large percent of drug and negligible influence of the processing variables on drug release rates [27-30].

The mechanism of drug release from hydrophilic matrix is based on swelling of polymer and diffusion or dissolution of drug from swollen polymeric hydrogel [30]. When a hydrophilic matrix is exposed to the aqueous medium, water will be absorbed by the matrix. Diffusion of water into the hydrophilic matrix will differentiate the whole matrix into three distinct regions: “glass” (mostly hydrogel), “tough rubber” (significant proportion of water and hydrogel) and “soft rubber” (mostly water) regions. This basic mechanism affects the drug release. Although drug release is generally affected by type and proportion of polymer, the size of the drug and polymer, drug solubility, drug/ polymer interaction and the glass-rubber transition of the hydrogel particles [31] .

1.5.2 Polymers used in the development of matrix based controlled release formulation

Numerous hydrophilic and hydrophobic polymers have been evaluated for matrix types of drug delivery systems and although it would be impractical to present each of these polymers and its specific application to drug delivery, this chapter will review in general the types of polymers mostly used as matrices for tablet dosage form.

(a) Hydroxypropyl methylcellulose

Hydroxypropyl methylcellulose (HPMC) is a non-ionic, semi-synthetic derivative of cellulose ether. It is a mixture of alkyl hydroxyalkyl cellulose ether containing methoxyl and hydroxypropyl groups [32]. Various grades of HPMC viz. 1000 cps, 4000 cps, 15000 cps, 100000 cps etc. are available based on the viscosity it produces upon hydration. It is highly preferred polymer for the formulation of swellable and hydrophilic matrix systems as it provides a robust mechanism for controlled release of drugs and choice of viscosity grades. Its non ionic nature minimizes interaction problems when used in acidic, basic or electrolytic systems and provides reproducible release profiles [33]. HPMC contains methoxyl and hydroxypropyl substituents on its β-*o*-glucopyranosyl ring backbone, which makes it very resistant to changes in pH or ionic content of the dissolution medium. At pH values from 2 to 13, HPMC is relatively stable and the matrix formulations of any drug prepared using HPMC can show pH independent drug release if the drug has pH independent solubility [34-35]. HPMC has been proved as the most preferred release retardant due to its global regulatory acceptance, excellent stability, safe application, ease of handling, negligible influence of the processing variables on drug release rates, ability to accommodate a large percent of drug and ease of compression with simple tablet manufacturing technology [35-36]. When HPMC hydrated, the polymer chains disentangle from the matrix. HPMC matrix systems are classed as swelling controlled systems and are controlled by the rate of penetration of media and erosion of the matrix [34, 37].

(b) Sodium carboxy methylcellulose

Sodium carboxy methylcellulose (NaCMC) is a polyelectrolyte anionic cellulose derivative, which is sensitive to changes in pH [20]. It is a water-soluble polymer. It is the sodium salt of polycarboxy methyl ether of cellulose, which is produced by reacting alkali cellulose with sodium monochloro acetate under rigidly, controlled conditions. It is an odourless, tasteless and non-toxic polymer, which is highly soluble in hot as well as cold water but stable towards hard water, alkalies, acids and certain electrolytes. It has a long history of use as a suspending agent in liquid pharmaceutical preparations. It is also used as tablet binder. Recent work has confirmed the usefulness of cellulose gum in sustained-release applications [38-39]

Various literatures revealed that drug release from NaCMC matrix was mainly dependent upon the rate and extent of water penetration into the tablet matrix and the relative aqueous solubility of both the matrix material and the drug compound embedded in the matrix. On hydration, polymer chains of NaCMC swell and form a viscous gel layer on the surface. Drug diffusion through swollen gel and erosion of the gel has been found main mechanisms by which this polymer releases the drug [40-41].

(c) Carbopol

Carbopol polymers are synthetic high-molecular-weight polymers of acrylic acid that are chemically crosslinked with either allyl sucrose or allyl ethers of pentaerythritol [42]. These polymers readily hydrate, absorb water and swell quickly up to 1000 times their volume to form a gel when exposed to pH environment above 4 [43]. In addition to their hydrophilic nature and cross-linked structure, their essential insolubility in water make these polymers potential candidates for use in controlled release formulations [44]. Carbopols are efficient matrix forming polymer. When tablets are placed in dissolution medium, the external surface of the tablet hydrated, swells and forms a gel layer (hydrogel) that controls the release of the drug from the tablets.

Carbopol polymers have a pKa of 6 ± 0.5, so at pH 1.2 they are virtually un-ionized; they will start to ionize at pH 4.5. At lower pH values the polymer is not fully swollen, and there are larger regions of low microviscosity; the solvent can penetrate fast and deep into the glassy core and the drug is released faster, before complete formation of gel. As the pH increases, the ionization of the carboxylic acid

groups causes maximum swelling, resulting in fewer and smaller regions of microviscosity. The rapid gel formation acts as a barrier for the release of the drug, thus prolonging the release. The release tend to be more diffusion controlled in the lower pH region (stomach), while at higher pH (intestine), the drug release mechanism is more polymer relaxation controlled [44]. Drug release rates from carbopol matrix can be affected by drug solubility, differences in the rates of hydration and swelling of the polymer hydrogel. Swelling of carbopol polymer is mainly dependent on the crosslink density, chain entanglement and crystallinity of the polymer matrix [45].

(d) Eudragit

Eudragit polymers are a series of acrylate and methacrylate polymers available in different ionic forms. Eudragit RLPO and Eudragit RSPO are fine, white powders with a slight amine-like odor [46]. These polymers referred to as ammonio methacrylate copolymers in the USPNF 23 monograph, are copolymers synthesized from acrylic acid and methacrylic acid esters. Eudragit RLPO is containing 10% of functional quaternary ammonium groups and Eudragit RSPO having 5% of functional quaternary ammonium groups. The ammonium groups are present as salts and give rise to pH independent permeability of the polymers. Both polymers are water-insoluble, and matrix prepared from Eudragit RLPO are freely permeable to water, whereas, matrix prepared from Eudragit RSPO are only slightly permeable to water [47-48].

Eudragit RLPO and RSPO provide pH-independent drug release to oral dosage forms that can be used for formulating the sustained-release dosage forms. When exposed to the dissolution medium, the solvent penetrates into the free spaces between macromolecular chains of Eudragit RLPO or RSPO. After solvation of the polymer chains, the dimensions of the polymer molecule increase due to polymer relaxation by the stress of the penetrated solvent. This phenomenon may be attributed to surface erosion or initial disaggregation of the matrix tablet prior to gel layer formation around the tablet core [47, 49].

(e) Paraffin wax

A wax matrix system is a well-developed matrix system used for sustained drug delivery because of its effectiveness, low cost, ease of manufacture, and drug stability due to the chemical inertness of wax [25]. Wax matrix dosage forms are used to embed a drug in an inert water insoluble matrix material in order to formulate

sustained or slow release formulations, and especially those containing freely water-soluble drugs such as MIL, potassium chloride and tramadol hydrochloride [50-51].

Paraffin is a purified mixture of solid saturated hydrocarbons and is obtained from petroleum or shale oil. It is translucent, odorless, tasteless, colourless, or white solid. It is slightly greasy to the touch and may show a brittle fracture. It is derived from petroleum and consists of a mixture of hydrocarbon molecules containing between twenty and forty carbon atoms. It is solid at room temperature and begins to melt above approximately 37°C (99°F). Paraffin has been used in pharmaceutical topical dosage forms such as a component of creams, suppository and ointments. In addition, Paraffin is used as a coating agent for capsules and tablets, and is used in some food applications. Paraffin as matrix forming agent and for coatings can also be used to control the drug release form dosage form [47,52].

(f) Cetostearyl alcohol

Cetostearyl alcohol is a mixture of solid aliphatic alcohols consisting mainly of stearyl ($C_{18}H_{38}O$) and cetyl ($C_{16}H_{34}O$) alcohols. It is used in cosmetics and topical pharmaceutical preparations. In topical pharmaceutical formulations, cetostearyl alcohol will increase the viscosity. The aliphatic proportion of the long chain fatty alcohols impart the cetostearyl matrix with sufficient hydrophobicity and impedes wetting of the matrix surface by dissolution fluid. This enough hydrophobicity of cetostearyl alcohol made it a good release retardant of water-soluble drug from matrix system [53].

(g) Stearic acid

Stearic acid is a saturated fatty acid with an 18-carbon chain. It is a crystalline, hard solid, somewhat glossy, white or yellowish white powder. It has been widely used in various oral and topical pharmaceutical formulations. Stearic acid is also used in cosmetics and food products. It is mainly used in oral formulations as a tablet and capsule lubricant. Literature revealed that it has also been used for controlled release tablets or pellets formulations. Hot melt granulation technique is widely used to prepare the drug-stearic acid matrix formulations. Stearic acid has been used for the controlled release as a dispersion medium of drugs in the form of microspheres made of fats and waxes using spray and congealing method [54-55].

1.6 Release kinetics and release mechanism of drugs from matrix formulations

In-vitro dissolution has been recognized as an important element in drug development. Under certain conditions, it can be used as a surrogate for the assessment of Bio-equivalence. There are several models to represent in-vitro drug release profile from dosage form. The quantitative interpretation of the values obtained in the dissolution assay is facilitated by the usage of a generic equation that mathematically translates the dissolution curve in function of some parameters related with the pharmaceutical dosage forms.

1.6.1 Model dependent in-vitro release characterization

Model dependent methods are based on different mathematical functions, which describe the dissolution profile. Once a suitable function has been selected, the dissolution profiles are evaluated depending on the derived model parameters. Several kinetic models have been proposed to describe the release characteristics of a drug from controlled release polymer matrix like zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Baker-Lonsdale, Weibull, Hopfenberg and Gompertz [56].

The following four equations hold the special position and are currently in common use due to their simplicity and applicability.

Zero order: $X = K_o t$ eq. (1)

First order: $\text{Log } X = \text{Log } X_0 - K_1 t / 2.303$ eq. (2)

Higuchi model: $Q = K_H t^{1/2}$ eq. (3)

Korsmeyer peppas model: $M_t/M_\infty = K t^n$ eq. (4)

where, X_o is initial amount of drug, X is amount of drug released at time t, M_t/M_∞ is the fraction of drug released at any time t; and K_o, K_1, K_H, and K are release rate constants for equations 1, 2, 3 and 4, respectively. In equation 4, n is the diffusional exponent indicative of mechanism of drug release. In the case of cylindrical tablets, a value of $n = 0.45$ indicates fickian or case I release; $0.45 < n > 0.89$ indicates non-fickian or anomalous release; $n = 0.89$ indicates case II release; and $n > 0.89$ indicates super case II release [57-59].

1.6.2 Model-independent in-vitro release characterization

The drug release of all the formulation and variables can be compared using the following dissolution parameters: time of 50% of total drug release (T50%), time of 80% of total drug release (T80%) and mean dissolution time (MDT).

The MDT values were calculated by the following equation:

$$\mathrm{MDT} = \frac{\sum_{j=1}^{n} tj\ \Delta Mj}{\sum_{j=1}^{n} \Delta Mj} \qquad \text{eq. (5)}$$

The similarities between two in-vitro dissolution profiles can be assessed by procedures such as difference factor (f_1) and similarity factor (f_2) .

The dissimilarity factor, (f_1) and similarity factor (f_2) were calculated as following equations:

$$f1 = \frac{\sum_{t=1}^{n} (Rt - Tt)}{\sum_{t=1}^{n} Rt} \text{ X } 100 \qquad \text{eq. (6)}$$

$$f_2 = 50 \log [\{1+1/n \Sigma (R_t - T_t)^2\}^{-0.5} \text{ x } 100] \qquad \text{eq. (7)}$$

where, n is the number of dissolution sample times, t is the time sample index, and R_t and T_t are either the individual or mean percent dissolved at each time point for the reference and test dissolution profiles, respectively [60-61].

1.7 Swelling and erosion studies

Drug release from swellable matrix tablets is strongly associated with the swelling and dissolution characteristics of the hydrophilic polymer, i.e., the formation and erosion of an outer gel layer on the matrix surface [62]. Hydrophilic polymers develop a gel layer around the tablets, which acts as a barrier to drug release by opposing penetration of water into the tablet and movement of dissolved solutes out of the matrix tablet. During the drug release process the gel layer thickness as well as its structure and composition experiences a continuous change. With time, the swollen gel layer becomes sufficiently hydrated for erosion or dissolution to take place [63]. Therefore, there has been increasing interest focused on quantitative analysis of erosion and swelling front characteristics.

1.8 Objective of present research work

The problems associated with MIL therapy, as discussed in above sections, indicated that there is a need to design a controlled release formulation of MIL, which will reduce the frequency of dosing and lower the incidence and intensity of side effects to have better patient compliance with improved therapeutic level for the treatment of depression and fibromyalgia.

As tablet is easier to manufacture, economic and well preferred, the present research work was thus planned to design once a day oral controlled release tablet of MIL using various rate controlling polymers either alone or in combination to achieve desired drug release for better outcomes.

The objectives of the present research work were decided to,

Studies on design and characterization of oral controlled release dosage form of milnacipran hydrochloride by using different polymers.

Evaluate the designed formulations for in-vitro performance, and investigate, and optimize various factors affecting the rate of drug release and product quality.

Evaluate selected formulations in-vivo and do pharmacokinetics study in animals.

Suitable and sensitive analytical methods were developed and validated for analysis of variety of samples like bulk powders, formulations, in-vitro release samples, stability samples and bio samples.

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CHAPTER 2

DRUG PROFILE- MILNACIPRAN HYDROCHLORIDE

2.1 Introduction

Milnacipran hydrochloride (MIL) is a selective serotonin and norepinephrine reuptake inhibitor. It was originally developed and manufactured by Pierre Fabre Medicament in France, and was approved in that country as an antidepressant in 1997 [1]. It has since been approved for this indication in multiple countries and currently marketed for this indication in over 45 countries worldwide including several European countries. Cypress Bioscience bought the exclusive rights for approval and marketing of the drug for fibromyalgia purpose in the United States and Canada in 2003 from the manufacturer Pierre Fabre Laboratories [2-3].

In January 2009, the U.S. Food and Drug Administration (FDA) approved MIL for the treatment of fibromyalgia, making it the third medication approved for this purpose in the United States [4].

Some of the drug information and properties are listed below:

2.2 Physical and chemical properties

Chemical name: MIL is chemically designated as (1R,2S)-rel-2(Amino-methyl)-N,N-diethyl-1-phenyl-cyclopropanecarboxamide hydrochloride and its structure is shown in Figure 2.1.

Synonyms: F-2207; Ixel; Toledomin; Dalcipran; Milnacipran Hydrochloride.

Empirical formula: $C_{15}H_{22}N_2O$. HCl

Molecular weight: 282.8

CAS No.: 101152-94-7

Melting point : 179°C

Physical description: MIL is a white to off-white, odourless, crystalline powder.

Dissociation constant (pKa): 9.65

Permeability coefficient (Log P): 1.42

Solubility: It is freely soluble in aqueous buffers over the entire physiological pH range. It is freely soluble in water, methanol, ethanol, chloroform, and methylene chloride and sparingly soluble in diethyl ether [5-6].

BCS class: Class I, highly soluble and highly permeable drug.

2.3 Pharmacological properties

2.3.1 Mechanism of action

A relationship exists between the monoamine neurotransmitters, norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) in the brain and the symptoms of major depressive disorder. MIL blocks 5-HT and norepinephrine reuptake into the neuron, thereby increasing 5-HT and NE extracellular concentrations [3,7].

MIL has no significant affinity for α- and β-adrenergic, muscarinic (M1-5), histamine (H1-4), dopamine (D1-5), opiate, benzodiazepine, or γ-aminobutyric acid (GABA) receptors. MIL has no significant affinity for Ca^{2+}, K^{+}, Na^{+} and Cl^{-} channels and does not inhibit the activity of human monoamine oxidases (MAO-A and MAO-B) or acetylcholinesterase [8-9].

One of the main differences between the various antidepressants and MIL is its equal preference and activity on the uptake of NE and 5-HT**.** The exact mechanism of the central pain inhibitory action and effectiveness in fibromyalgia symptom are unknown in humans [10-11]**.**

2.3.2 Therapeutic indications

Treatment of depression

Major Depression**,** also known as major depressive disorder or unipolar depression, is a highly debilitating disorder that has been estimated to affect up to 21% of the world population [12]. It is a CNS disorder characterised by a combination of symptoms that interfere with a person's ability to work, sleep, study, eat, and enjoy pleasurable activities [7].

For the treatment of depression, MIL has been proved clinically comparable in efficacy with TCAs (imipramine) with significantly better tolerated in depression patients. Clinical trials comparing MIL and selective serotonin reuptake inhibitors (SSRIs) concluded a superior efficacy for MIL with similar tolerability for MIL and SSRIs. A meta-analysis of a total of 16 randomized controlled trials with more than 2200 patients concluded that there were no statistically significant differences in efficacy, acceptability and tolerability when comparing MIL with other antidepressant agents [12].

Management of fibromyalgia

Fibromyalgia (FM) is a complex syndrome characterized by chronic widespread musculoskeletal pain that often co-exists with sleep disturbances, decreased physical functioning, cognitive dysfunction and fatigue.

Various clinical studies proved that in the central nervous system, both serotonin and norepinephrine have been found to play important roles in pain perception via their involvement in descending antinociceptive pathways. Thus, MIL, as a SNRI, this drug should have clinically significant analgesic effects. Clinical trials demonstrated the efficacy of MIL in the management of FM. MIL was found to be safe and well tolerated in the majority of patients. MIL was viewed as a wonderful new weapon in the fight against both depression and pain [8, 13].

Treatment of lupus

Recent studies proved that MIL is also useful against lupus. Lupus is a chronic autoimmune disease in which the immune system turns against the body and harms healthy cells and tissues. It is a rheumatic disease, which can affect many parts of the body including the joints, skin, kidneys, lungs, heart or brain. Some of the most common symptoms include extreme fatigue, painful or swollen joints, unexplained fever, skin rashes, and kidney problems. Scientific evidence indicates that lupus is caused by a combination of genetic and environmental factors. Lupus is characterized by periods of increased or intensified disease activity, called flares [14-15].

2.3.3 Tolerability and side effects

MIL has demonstrated numerous adverse reactions in human clinical trials with tolerability decreasing with an increasing dose. In the placebo controlled trials in patients with fibromyalgia, the most frequent spontaneously reported adverse events were as follows: nausea, palpitations, headache, constipation, increased heart rate and hyperhidrosis, vomiting, and dizziness [16]. Discontinuation due to adverse reactions was generally more common among patients treated with higher dose 200 mg/day compared to lower 100 mg/day. The adverse effects can originate from the fluctuation in the plasma drug concentrations of an active substance following administration. Most of the reported adverse events were reduced or disappeared with the discontinuation of treatment [17].

2.4 Pharmacokinetics

The pharmacokinetic profile of MIL is as summarized in Table 2.1 [1,5].

Absorption

MIL is well absorbed after oral administration. Absolute bioavailability is about 85-90 %. It is not affected by food intake. The peak plasma concentration is about 120 ng/ml achieved in 2 h after a single 50 mg dose. Inter-subject variability is low. Plasma concentrations are linearly proportional with dose over the range of single acute doses of 25 to 200 mg as shown in Table 2.2 [1, 2].

Distribution

Protein binding is low (13%) and not saturable. The volume of distribution of MIL is about 5 litre/kg with a total clearance of about 40 litre/hour [1].

Metabolism

MIL undergoes minimal first-pass metabolism, with approximately 55% of the drug excreted unchanged in urine. MIL is metabolized mainly by conjugation (Glucoronisation). Active metabolites have been found at very low levels without clinical relevance. Cytochrome P450 2D6 is involved in the metabolism of many psychotropic drugs and its inhibition is frequently a cause of drug-drug interactions. This enzyme has no impact on the metabolism of MIL and no oxidative metabolites of MIL have been detected in humans [1-3].

The pharmacokinetics of MIL are not modified in subjects who are deficient in the CYP2D6 isoenzyme (slow sparteine-like metabolisers). Furthermore, MIL does not interfere in-vivo with other isoenzymes of cytochrome P450 [1, 18].

Elimination

Plasma elimination half-life is about 8 hours. Elimination occurs mainly via the kidney with tubular secretion of the product in unchanged form. After repeated doses, MIL is totally eliminated in 2 to 3 days after termination of therapy. The liver and kidneys are both involved in the elimination of MIL as illustrated by renal and non-renal clearances with values of 23.8 ± 7.3 and 16.4 ± 3.1 l/h, respectively. This balance between renal and non-renal clearances may be an advantage in patients presenting with moderate renal insufficiency [3,5].

2.5 Dosage and administration

The recommended dose titration schedule for MIL is 12.5 mg once on Day 1, then 12.5 mg twice a day on Days 2-3, and then 25 mg twice a day on Days 4-7, and then 50 mg twice a day after Day 7. Recommended maintenance dose is 50 mg twice daily. In clinical trials, MIL was evaluated with a dose titration schedule. The daily dose may be increased to 200 mg (or 100 mg twice a day) based on individual response. Dosing should be adjusted in patients with severe renal impairment (Creatinine Clearance < 29 ml/min) with a reduced maintenance dose to 25 mg twice a day. Following extended use, MIL should be tapered and not abruptly discontinued. MIL may be taken with or without food, but taking it with food may improve tolerability [4, 13].

2.6 Marketed formulations

There are various brands of MIL are available with dose of 12.5 mg, 25 mg, 50 mg and 100 mg immediate release tablets or capsules as shown in Table 2.3 [19-21].

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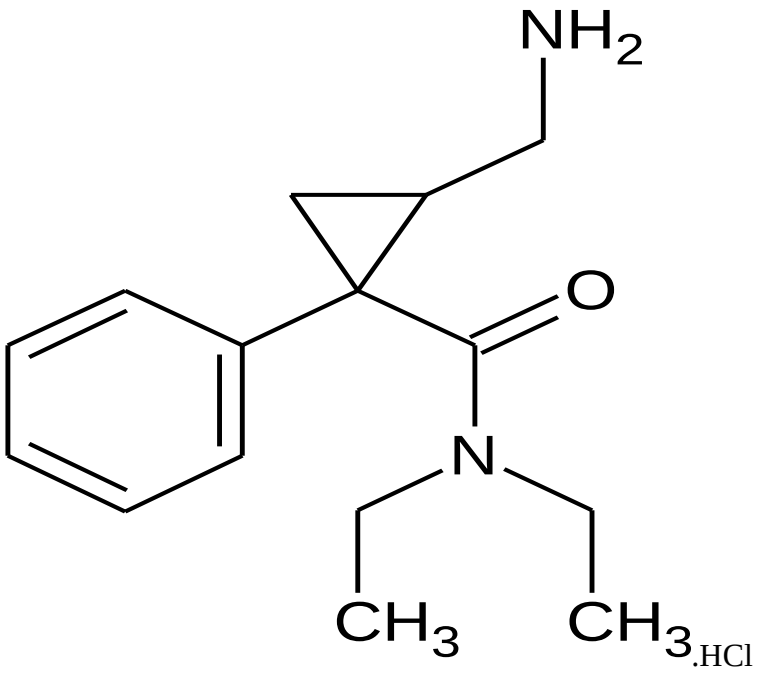

Figure 2.1: Chemical structure of milnacipran hydrochloride

Table 2.1: Pharmacokinetic parameters of milnacipran hydrochloride [1, 5]

Parameter	Value
Bioavailability	85%-90%
Volume of distribution (V_d)	400 L
T_{max}	2-4 h
Plasma protein binding	13%
Elimination	Renal Excretion
Elimination half- life ($t_{1/2}$)	6-8 h

Table 2.2: Pharmacokinetic parameters of single dose milnacipran hydrochloride obtained from clinical study in 12 healthy male volunteers [1,2]

Dose (mg)	C_{max} (ng/ml)	T_{max} (h)	AUC (ng/ml.h)	$t_{1/2}$ (h)
25	64.1	1.7	730	7.1
50	133.9	2.0	1833	8.1
100	269.0	2.1	2149	5.8
200	434.6	1.9	3895	6.3

Table 2.3: List of marketed formulations of milnacipran hydrochloride

Brand name	Formulation	Company name
Ixel	Tablet, Capsule	Pierre Fabre (European countries)
Savella	Tablet	Forest Pharmaceuticals (USA)
Toledomin	Tablet	Pierre Fabre (Japan)
Dalcipran	Tablet	Roche (Mexico)
Acmil	Tablet	Ranbaxy (India)
Milborn	Capsule	Sun Pharma (India)
Milnace	Capsule	Torrent (India)
Milza	Capsule	Intas (India)
Milpran	Capsule	Ajanta Pharma (India)

CHPATER 3

ANALYTICAL AND BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

3.1 Introduction

Analytic method development is key elements of drug discovery, development and manufacture of pharmaceuticals. Analytical methods need to be validated or revalidated prior to their introduction into routine analyses. Validation of developed method is the process used to confirm that the analytical procedure developed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results. Development and validation of simple, sensitive and economic analytical method is essential for the estimation of drug in bulk, formulations, in-vitro drug release samples, stability study, in-vivo pharmacokinetic studies, and bioavailability studies. Therefore, suitable analytical methods are needed during drug design and formulation development process. Different sensitive methods like ultraviolet-visible (UV) spectroscopic, fluorescence, high performance liquid chromatography (HPLC) and mass spectroscopy can be developed according to the need of the study.

Extensive literature survey revealed that there was no official UV-spectroscopic and LC method reported in major pharmacopeia like USP, EP, JP and BP for determination of milnacipran hydrochloride (MIL) in bulk, formulations and bio-samples. A simple UV- spectroscopic method in distilled water and derivative colorimetric method of MIL was developed by Parejiya et al [1] and Mubarakunnisa et al [2] respectively. Rao et al. also developed visible spectrophotometric method using oxidation and redox reactions [3]. Few liquid chromatographic methods are reported with different combinations of stationary and mobile phase. Puozzo C., et al. developed a HPLC method coupled with a fluorimetric detection but the samples were derivatized with fluorescamine for fluorescence detection [4]. Labat L., et al. developed by micellar electro-kinetic capillary chromatography method for separation of new antidepressants and their metabolites [5]. Patti A., et al. studied chiral determination of MIL and its 9-fluorenylmethoxycarbonyl derivative in tablet formulation on cellulose based stationary phases [6]. Mehta P., et al. and Peketi, et al. developed RP-HPLC method for analysis of MIL with sensitivity range of 5 to 50 µg/ml and 10 to 50 µg/ml respectively [7-8]. Dias C., et al, developed stability-indicating LC and a second order derivative UV spectroscopic methods with range of 20-100 µg/ml [9]. Ucakturk et al. and Lacassie et al. developed gas chromatographic methods for determination of MIL in human plasma respectively [10-11].

Above literature revealed that, reported UV methods were not developed in physiological pH or required some chemical reactions. Though several HPLC methods have been reported, but some of them are sensitive only up to microgram level or need some complex reactions or require tedious sample preparation or need sophisticated instrument or costly in determination of MIL in samples.

Thus, in the present research work, a simple, sensitive, economic and rapid spectrophotometric method was developed for the routine estimation of drug in bulk, formulations and in vitro release samples. Liquid chromatographic methods were developed for estimation of drug in formulation, stability and biomatrix samples. All developed methods were validated according to the standard guidelines [12-13]. Suitable statistical tests were performed to check validity of the developed methods [14]. These developed and validated methods were used for the estimation of MIL in bulk, formulations, in-vitro release samples, stability samples and plasma samples. It was also expected that the developed and validated methods may help the industries as well as researchers for routine determination of MIL rapidly at low cost.

3.2 Materials and methods

3.2.1 Chemicals

Pure milnacipran hydrochloride (MIL) was obtained as gift sample from Torrent Pharmaceutical limited, Ahmedabad, Gujarat, India. Acetonitrile (ACN), methanol, orthophosphoric acid, triethylamine and potassium dihydrogen orthophosphate were of HPLC grade and purchased from Merck, Mumbai, India. Hydrochloric acid and sodium hydroxide were of analytical grade and purchased from SDFCL (Mumbai, India). In-house prepared triple distilled water (TDW) was used for preparation of buffers used in spectrophotometric method. In case of HPLC method, for preparing aqueous phase, Millipore water (Millipore, USA) was used. The aqueous phase after the preparation was further passed through 0.22 µ millipore membrane filters (Millipore, USA). Commercial formulations of MIL, Milnace^{TM} tablets with labeled claim of 25 and 50 mg of MIL per tablet (Torrent Pharmaceutical Limited, Ahmedabad, India) and Milborn^{TM} capsules with labeled claim of 25 and 50 mg of MIL per capsule (Sun Pharma Limited, Ahmedabad, India) were purchased from local Indian market.

3.2.2 Equipment

For UV spectrophotometric method

A double-beam Jasco (Japan) UV-Vis-NIR spectrophotometer, model V570 connected to a computer loaded with Spectra Manager software was used for spectrophotometric method development. The UV spectrophotometer has an automatic wavelength correction with wavelength accuracy of 0.1 nm. Matched quartz cells of 10 mm path length were used in the spectrophotometric studies.

For HPLC method

The HPLC system (Shimadzu®, Japan) consisted of LC -10ATVP liquid chromatographic pump, SPD-10AVP UV-Vis detector, CTO-10ASVP column oven and SIL-HT auto sampler was used for method development. Separations were carried out on LiChrospher® 100 RP-18 analytical C_{18} column (Hibar® 250-4,6; 5µm; Merck®). Chromatographic peaks were integrated using LC-Solutions® work station loaded on a computer system. The detector was operated at a wavelength of 220 nm. The run time of the proposed assay was 15 min under isocratic elution.

3.2.3 Reagents

(a) Preparation of pH 1.2 Hydrochloric acid buffer (USP)

Hydrochloric acid buffer was prepared by dissolving 0.745 g of Potassium chloride in 50 ml of water to yield solution of 0.2M Potassium chloride. To this solution 85 ml of 0.2M hydrochloric acid solution was added and the volume was made up with water up to 200 ml to yield pH 1.2 hydrochloric acid buffer.

(b) Preparation of pH 6.8 Phosphate buffer (USP)

6.805 g of potassium dihydrogen orthophosphate was transferred carefully into a 1000 ml volumetric flask and 500 ml of TDW was added into the flask to dissolve potassium dihydrogen orthophosphate completely. Then 112 ml of 0.2M sodium hydroxide was added into the volumetric flask and the final volume was made upto 1000 ml with water.

3.3 UV spectrophotometric method for estimation of milnacipran hydrochloride in bulk and formulations

3.3.1 Experimental

(a) Optimization of media

For UV method development, various media were screened to select proper solvent to develop a suitable UV-spectrophotometric method for MIL. Criteria for the selection of media were solubility of drug, sensitivity, ease of sample preparation, economy of method and its applicability to various purposes. Drug solutions in the selected media were scanned in the range from 200-400 nm wavelengths for selecting the wavelength of analysis. Absorbance at the selected wavelength was determined and apparent molar absorbtivity and sandal's sensitivity were calculated.

(b) Analytical method development and validation

Drug stock solution was prepared with selected solvent system and different concentrations were made from stock solution for development of calibration curve. The developed method was validated for various parameters according to standard guidelines [12-13].

Specificity and selectivity of the proposed method was established by preparing drug solution in optimized media along with and without common formulation excipients. All the samples were scanned from 200 - 400 nm at speed of 200 nm/min to observe any change in the absorbance at respective wavelength and spectrum.

The limit of detection (LOD) is defined as the lowest detectable concentration of the MIL. Limit of quantification (LOQ) is defined as lowest quantifiable concentration. LOD and LOQ were calculated as 3.3 σ/s and 10 σ/s respectively. Where 'σ' is standard deviation (SD) of intercept and 's' is slope of calibration curve.

To determine the accuracy of the proposed method, different quality control (QC) levels of drug concentration (Lower quality control [LQC], Medium quality control [MQC] and Higher quality control [HQC]) were prepared independently from stock solution and analyzed.

Accuracy was assessed as the mean percentage recovery and percentage bias (% Bias = $100 \times$ [(Predicted concentration – Nominal concentration)/Nominal concentration]. Further, different concentrations of the pure drug were added to a known pre-analyzed formulation sample and analyzed using the proposed method to check the recovery. The percent analytical recovery (% Analytical recovery) of the added pure drug was calculated as, % Analytical recovery = $[(C_v - C_u)/C_a] \times 100$, where C_v is the total drug concentration measured after standard addition, C_u is the drug concentration in the formulation sample, C_a is the drug concentration added to the formulation sample.

For Precision study of the method, intra-day and inter-day precision studies were carried out by estimating the responses of three QC standards in triplicates under same experimental conditions three times on the same day and on three different days. From the results obtained, the precision was expressed as percentage relative standard deviations (% RSD) from mean intra and inter-day assays. To determine the robustness of developed method, QC concentrations were evaluated in media with change in pH by ± 0.2 unit and calculated the mean % recovery.

(c) Estimation of drug content in formulations

The proposed validated UV methods were successfully applied for the estimation of total drug content in two different brands of pharmaceutical formulations, Milborn® capsules of Sun Pharmaceuticals Limited (Baroda, India) and Milnace® tablets of Torrent Pharmaceuticals Limited (Ahmedabad, India).

3.3.2 Results and discussion

(a) Optimization of media

The composition and pH of aqueous media decided was 100 mM hydrochloric acid buffer (pH 1.2) and 100 mM phosphate buffer (pH 6.8) based on sensitivity of method, cost, ease of preparation and applicability of method to dissolution studies. The λ_{max} of MIL was found to be 220 nm in both buffer media.

(b) Calibration curve

Two different primary stock solutions of 100 µg/ml were prepared in hydrochloric acid buffer (pH = 1.2) and phosphate buffer (pH = 6.8) by dissolving 10 mg of the drug in 100 ml of the respective buffer media. Six different concentrations

were prepared in the range of 5-30 µg/ml of MIL in the respective medium for calibration curve development.

The linear regression equation obtained at 220 nm for hydrochloric acid buffer medium; absorbance = 0.0390 x concentration (µg/ml) + 0.0598 with regression coefficient of 0.9998 and for phosphate buffer was found to be absorbance = 0.0405 x concentration (µg/ml) + 0.0198 with regression coefficient of 0.9988. At all the concentration levels the SD was low and the % RSD did not exceed 1.65. Apparent molar absorbtivity of drug was found to be 1.11×10^4 in hydrochloric acid buffer medium and 1.15×10^4 in phosphate buffer medium. Sandell's sensitivity of drug was found to be 2.56×10^{-2} and 2.46×10^{-2} in hydrochloric acid buffer medium and phosphate buffer medium respectively as shown in Table 3.1. The overlaid UV spectra of MIL in hydrochloric acid buffer medium and phosphate buffer medium are shown in Figure 3.1(a) and (b).

(c) Analytical method validation

The UV absorption spectrum of MIL was not changed in the presence of common formulation excipients. There was no difference in absorbance values or spectra of drug solutions prepared from various stock solutions. Therefore the proposed method is specific and selective for MIL.

The LOD and LOQ in phosphate buffer medium were found to be 0.53 µg/ml and 1.60 µg/ml respectively and in hydrochloric acid buffer medium LOD and LOQ were found to be 0.32 µg/ml and 0.97 µg/ml respectively.

The developed method showed high and consistent absolute recoveries at all studied QC levels. All the quality control levels (LQC = 6 µg/ml, MQC = 18 µg/ml and HQC = 27 µg/ml) showed an accuracy (% Bias) ranging from -0.63 to 0.89 in hydrochloric acid buffer medium and -1.37 to 1.00 in phosphate buffer medium. The mean % recovery values were nearly 100 with low % RSD values (<1.5) indicating accuracy of the method. The results of accuracy studies as shown in Table 3.2 indicated that the proposed method can accurately measure MIL in solutions.

Precision of the proposed method was studied by evaluating repeatability and intermediate precision. The % RSD of inter-day and intraday precision was found to be not more than 1.35 in both media at all three QC levels of

concentrations as shown in Table 3.3. Low % RSD values indicated the excellent precision of the proposed method.

Variation of pH (± 0.2) did not have any significant effect on UV absorbance of MIL in both media, which confirm the robustness of the proposed method as reported in Table 3.4.

(d) Estimation of drug content in formulations

In hydrochloric acid medium the assay values of MIL for different formulations ranged from 99.77% to 100.85% with SD not more than 1.07%. In phosphate buffer the assay values of MIL for different formulations ranged from 99.41% to 100.39% with SD not more than 1.17% as given in Table 3.5. Assay values of formulations were found to be very close to the labeled claim, suggesting that the interference of excipient matrix was insignificant in the estimation of MIL by proposed methods. The estimated drug content with low values of SD establishes the precision and applicability of the proposed method.

3.4 RP-HPLC method for estimation of milnacipran hydrochloride in bulk and formulations

3.4.1 Experimental

(a) Chromatographic conditions

For HPLC method development, several mobile phases with different proportions of organic solvents (methanol and acetonitrile) and buffer (at various pH) were tested in order to achieve the best chromatographic condition. Criteria for the selection of solvent systems were solubility and stability of drug, sensitivity, ease of sample preparation, economy of method and its applicability to various purposes. Different chromatographic columns were evaluated to achieve good analyte peak parameters such as capacity factor, asymmetry and theoretical plates.

(b) Analytical method development and validation

Drug stock solution was prepared with selected mobile phase and different concentrations were made from stock solution for development of calibration curve. The developed method was validated for various parameters according to standard guidelines [12-13].

Specificity and selectivity of the proposed method were established by preparing drug solution in selected mobile phase along with and without common formulation excipients. In addition, forced degradation studies were also carried out to evaluate the specificity of the developed method in distinguishing the drug from its degradation products. Stock solutions of MIL in the mobile phase were exposed to hydrolytic, oxidative, thermal and photolytic stresses to perform forced degradation studies. The hydrolytic study was done by using 1 N HCl (acidic hydrolysis) and 1 N NaOH (basic hydrolysis) at 40°C for 6 h; and oxidation study by using 3% H_2O_2 at 40°C for 6 h. The solutions were exposed to 50°C and UV light in a UV-chamber for thermal and photolytic studies respectively for about 24 h. The solutions were diluted appropriately with the mobile phase and injected into the HPLC system for analysis. All the solutions injected were analyzed against a control solution stored at room temperature.

LOD, LOQ, accuracy and precision were determined same as discussed in section 3.3.1 (b). The chromatographic analysis was performed under different analytical conditions and the chromatographic parameters of the main peak were evaluated for studying the robustness of the method. Changes in mobile phase flow rate (1 ± 0.2 ml/min) and buffer pH (3.1 ± 0.1) were evaluated for robustness of developed method.

The stability of MIL in mobile phase was determined by injecting calibration standard (750 ng/ml) at 0, 6, 12, 24, 36 and 48 h in triplicates into the system and analyzing under the optimized conditions. The obtained results were compared with the results of fresh stock solution and % RSD was calculated.

(c) Estimation of drug content in formulations

The proposed validated LC method was successfully applied for the estimation of total drug content in two different brands of pharmaceutical formulations, Milborn® capsules of Sun Pharmaceuticals Limited (Baroda, India) and Milnace® tablets of Torrent Pharmaceuticals Limited (Ahmedabad, India).

3.4.2 Results and discussion

(a) Chromatographic conditions

In the preliminary trials, C_{18} column (Hibar® 250-4,6; 5μm; Merck®) showed good performance and was selected as the stationary phase for the reversed

phase (RP) LC method. A mixture of 25 mM phosphate buffer (pH 3.1), acetonitrile and methanol (65:25:10; v/v/v) as mobile phase provided the best chromatographic performance. Retention time and asymmetry were significantly affected by different amounts of triethylamine and column oven temperature. Column temperature at 35°C provided least peak tailing. The best results were achieved when 0.3% (v/v) of triethylamine was added to the buffer. Aliquots of 50 µl were injected into the system with mobile phase at a constant flow rate of 1.0 ml/min. The UV detector was operated at a wavelength of 220 nm. The run time of the proposed assay was 15 min under isocratic elution. A retention time of 7.7 min was observed with injections of standard solutions of MIL.

The selected solvent system has shown excellent chromatographic peak parameters such as capacity factor ($k > 2.0$), number of theoretical plates ($N > 9000$) and tailing factor ($T_f \leq 1.2$). The obtained peak parameters were well within the acceptable limits indicating the suitability of the method. Low variability in peak area and retention time were observed upon re-injection indicating that the developed method was highly suitable for estimation of MIL.

(b) Calibration curve

Primary stock solution of 100 µg/ml of MIL was prepared and then a secondary stock of 10 µg/ml was prepared by taking an aliquot from the primary stock and diluting with the mobile phase. For developing the calibration curve, different concentrations in the range of 25 – 3000 ng/ml (25, 75, 150, 250, 500, 1000, 1500 and 3000 ng/ml) were prepared. Calibration curve was plotted between peak area of MIL against the concentration of the drug. The results of regression analysis are presented in Table 3.6. The average equation for calibration curves was, Peak area = 801.1 x concentration (ng/ml) - 10768.0. The data confirm the linearity of the standard curves over the range studied (25 - 3000 ng/ml) with regression coefficient of 0.999. The overlaid chromatograph of all standard concentrations are shown in Figure 3.2.

(c) Method validation

In specificity study, interference with the estimation of MIL using the developed method was evaluated with a solution of inactive ingredients (placebo solution). The chromatogram showed absence of peaks due to inactive ingredients when standard solution of MIL and placebo were injected (Figure 3.3). A single peak

was obtained for MIL, which indicated that there was no interference from the excipients used and also from the mobile phase.

The results obtained from forced degradation studies are summarized in Table 3.7. Potential degradation products were observed in standard solutions exposed to hydrolytic stress and oxidative stress. Under acid hydrolysis, peak for degradation product was observed at a retention time of 2.68 min (Figure 3.4 a). The chromatogram of the basic hydrolysis test showed degradation peaks at 2.49 and 5.06 min (Figure 3.4 b). Major degradation peaks were observed at retention times 3.09 and 3.73 min under oxidative stress along with some minor peaks at 1.96, 4.99 and 6.02 min (Figure 3.4 c). Under thermal stress and photolytic stress, no additional peaks were detected. Retention time of all the possible degradation peaks were not matching with pure drug which confirmed the specificity of the developed method.

The LOD and LOQ of the method were found to be 7.09 and 21.50 ng/ml respectively. Hence the method was found to be sensitive for determination of MIL.

The developed method showed high and consistent absolute recoveries at all studied QC levels (LQC = 100 ng/ml, MQC = 750 ng/ml and HQC = 2000 ng/ml). The results obtained from recovery studies are presented in Table 3.8. The mean absolute recovery ranged from 98.23 to 101.76%. Additionally, the obtained recoveries were found to be normally distributed with low and uniform % RSD (≤1.44) at all QC levels.

The results of the precision studies indicated high reproducibility with the % RSD values not more than 1.65. The data obtained from precision studies are shown in Table 3.9. At all the standard concentration levels, variation observed was insignificant indicating the repeatability of the method.

The effects of variations in pH (3.1 ± 0.1) and flow rate (1± 0.2 ml/min) on the developed analytical method were evaluated in robustness study and this change did not interfere significantly with the analytical parameters. The results obtained in the new conditions were in accordance with the original results. The % RSD values for peak area was not more than 1.96 indicating the highly robust nature of the developed method.

Stock solutions of MIL in mobile phase were stable for 48 h at room temperature. The results demonstrated the stability of drug in mobile phase with variation less than ± 0.90%.

(d) Estimation of drug content in formulations

The % assay values for the estimation of MIL in different commercially and in-house formulations ranged from 99.87 to 101.01% with % RSD not more than 1.25. Assay values of formulations were found to be very close to the labeled claim, suggesting no interference of excipient matrix in the estimation of MIL by proposed method. The good recovery and low % RSD values indicate that the assay results were satisfactory, accurate and precise. The results are as summarized in Table 3.10.

3.5 RP-HPLC bioanalytical method for estimation of milnacipran hydrochloride in rabbit plasma

3.5.1 Experimental

(a) Chromatographic conditions

The HPLC system (Shimadzu®, Japan) and mobile phase was same as discussed in section 3.4.2. Flow rate was optimized in order to achieve best chromatographic parameters and separation from plasma interference.

(b) Blood samples collection

For bioanalytical method development, blood was collected from marginal ear vein of New Zealand white rabbits. Collection of blood was carried out with permission of Institutional Animal Ethics Committee (Protocol approval No. IAEC/RES/16/07). The blood was collected in eppendrof containing 10% EDTA solution. The blood was then centrifuged at 10,000 rpm for 15 min at 4°C temperature. The clear supernatant plasma was collected and stored at -20^0C till its analysis.

(c) Solid phase extraction of plasma samples

Different solid-phase cartridges and solvent systems were used to get maximum recovery of MIL from spiked plasma samples. The maximum recovery was obtained with LiChrosep® DVB HL (Merk) solid-phase cartridges with following extraction process. Each cartridge contained 30 mg of sorbent with a total reservoir of 1 ml. The cartridge was first washed with 100% methanol at 2000 rpm for 3 min. Then cartridge was conditioned with phosphate buffer (pH 4.5) at 2000 rpm for 3 min.

Plasma sample was loaded and centrifuged at 2000 rpm for 3 min. The sample was slowly passed through pre-conditioned cartridges and washed with 1 ml of 60% methanol and the final elution of the compound was done with 1 ml of mobile phase at 2000 rpm for 3 min. A 50 µl aliquot of the elute was injected into the HPLC column.

(d) Analytical method development and validation

Selectivity of the method was studied by investigating the interference from various endogenous proteins and other impurities present in the bio-matrix. Blank rabbit plasma samples collected from rabbit were compared against calibration standards for investigating interference in determination.

The LOD was determined as the lowest detectable concentration of the MIL (signal/noise=3). LOQ was determined as minimum concentration of MIL in plasma sample that can be quantified with less than 20% RSD. Accuracy and precision were determined same as discussed in analytical section.

The stability of MIL in rabbit plasma was evaluated using QC samples under different stress conditions such as freeze thaw stability, post preparative stability and long term stability.

(e) Estimation of drug concentration in rabbit plasma

The developed and validated HPLC bioanalytical method was applied to quantify MIL concentration in rabbit plasma. The experiments were conducted as per CPCSEA (Committee for Prevention, Control and Supervision of Experimental Animals) guidelines. In-vivo study was carried out on six New Zealand albino male rabbits weighing between 2.0 and 2.5 kg. After a single oral administration of MIL (tablet formulation), 2 ml of blood sample was collected from the marginal ear vein of rabbits at predetermine time-points into eppendroff containing 10% EDTA. Blood samples were collected alternatively from three rabbits out of six rabbits at each time point. The blood was immediately centrifuged for 10 min at an ambient temperature. The supernatant plasma layer was separated and stored at -20°C until analyzed. The plasma samples were treated for solid phase extraction and analyzed with developed method. A non-compartmental analysis using the WinNonLin-Professional 2.1 (WNL-Pro 2.1) computer program (Pharsight, USA) was employed for the determination of various pharmacokinetic parameters of MIL.

3.5.2 Results and discussion

(a) Chromatographic conditions

The HPLC system (Shimadzu®, Japan) and mobile phase were same as discussed in section 3.4.2. Aliquots of extracted sample were injected into the system with a constant flow rate of 0.8 ml/min of mobile phase and temperature maintained at 35^0C. The UV-detector was operated at a wavelength of 220 nm. The run time of the proposed analysis was 15 min under isocratic elution. A retention time of 8.5 min was observed with injections of standard solutions of MIL.

(b) Calibration curve

Working standard solutions in the range of 50 ng to 2000 ng/ml (50, 200, 400, 800, 1000, 1500 and 2000 ng/ml) were prepared by spiking appropriate volumes of the stock solution of MIL to rabbit plasma and treated with solid phase extraction (SPE) process.

To establish linearity, calibration plots were obtained by analysing working standards of MIL in rabbit plasma extracted by SPE technique. The results of regression analysis are as shown in Table 3.11. At all the concentration levels, the standard deviation was low and the % RSD did not exceed 6.5. Overlaid chromatograms of blank plasma and plasma standard (600 ng/ml) are shown Figure 3.5. The linearity range in the selected mobile phase was found to be 50–2000 ng/ml. According to a linear regression analysis, the slope and intercept were found to be 126.0 and 9869.0, respectively with a regression coefficient (r^2) value of 0.9998. The extraction recovery of MIL from the spiked rabbit plasma samples were within 93.24 to 96.45% with % RSD at each concentration level less than 5.43. Thus, the proposed solid phase extraction technique was found to be accurate and precise with high recovery values.

(c) Analytical method validation

In Figure 3.5, Blank plasma sample showed absence of any interference at the retention time of the drug. Thus, the proposed method is specific and selective for the estimation of MIL in rabbit plasma. A detection limit (LOD) of 15 ng/ml was obtained at a signal-to-noise ratio of 3:1 The mean concentration of three independent samples of 25 ng/ml, calculated using calibration equation was found to be 24.31 ng/ml

with % RSD value of 8.96. Hence, the concentration of 25 ng/ml was considered as limit of quantification (LOQ) for the proposed method.

All three quality control samples LQC = 100 ng/ml, MQC = 600 ng/ml, and HQC = 1200 ng/ml showed an accuracy (% bias) ranging from -0.69 to 1.44. The high mean percent recovery values (99.31 to 101.44 %) and low SD values (± 2.05 to 7.38) established the accuracy of the method as shown in Table 3.12. In repeatability study, % RSD values for intra-day and inter-day variations were not more than 4.96 at all the different levels of concentrations. Acceptable low % RSD values indicated the excellent repeatability and intermediate precision of the method as shown in Table 3.13.

The stability of MIL in rabbit plasma was evaluated using QC samples under different stress conditions and the results obtained are shown in Figure 3.6. In freeze thaw stability (-20^{0}C to 25°C), no significant degradation of MIL was observed up to two cycles over a period of two days. The deviation from the zero time concentration was found to be less that 11.10% at the end of two freeze thaw cycles as shown in Figure 3.6 (a). In post-preparation stability study of the processed samples, MIL was found to be stable for 24 h, with a maximum deviation of 9.92% from the zero time concentration as shown in Figure 3.6 (b). In long-term stability studies, MIL was found to be stable for 7 days when stored at –20^{0}C. The deviation in recoveries after analysis at 7 days of sample preparation was found to be within acceptable limits as shown in Figure 3.6 (c). The results of this study indicated that storage temperature of – 20^{0}C was adequate for storing the plasma samples for at least 7 days.

(d) Estimation of drug concentration in rabbit plasma

The developed method was applied to quantify MIL concentration in pharmacokinetic study carried out on rabbits. The mean plasma concentration versus time profile following a single oral administration of MIL to rabbits is presented in Figure 3.7. Various other pharmacokinetic parameters have been summarized in Table 3.14. The data showed rapid release and absorption of MIL giving C_{max} of 1075.25 ± 103.92 ng/ml within 1 ± 0.15 h (T_{max}). The $AUC_{0-\infty}$ and MRT of the IR tablet were found to be 6152.17 ± 235.18 ng.h/ml and 4.95 ± 0.15 h respectively.

3.6 Conclusions

As objective were to develop suitable and sensitive analytical methods, UV spectroscopic methods, liquid chromatographic methods for bulk drug, aqueous matrix and biological matrix were developed and successfully validated. The developed UV methods were found to be simple, rapid and more economical and suitable for routine analysis for the estimation of MIL in bulk, formulations, in vitro release samples. Developed reverse phase stability indicating analytical and bio-analytical HPLC method for the estimation of MIL in bulk, formulations and rabbit plasma was found to be specific, simple and highly sensitive. In addition, HPLC method was successfully employed for in-vivo pharmacokinetic investigations of formulation. The developed method can be utilized for in-vivo characterization of various modified release formulation in rabbit before the human studies. Thus, above developed and validated methods not only can help laboratory based work but will help the industries as well for their routine and frequent analytical work for sensitive determination of MIL rapidly at low cost.

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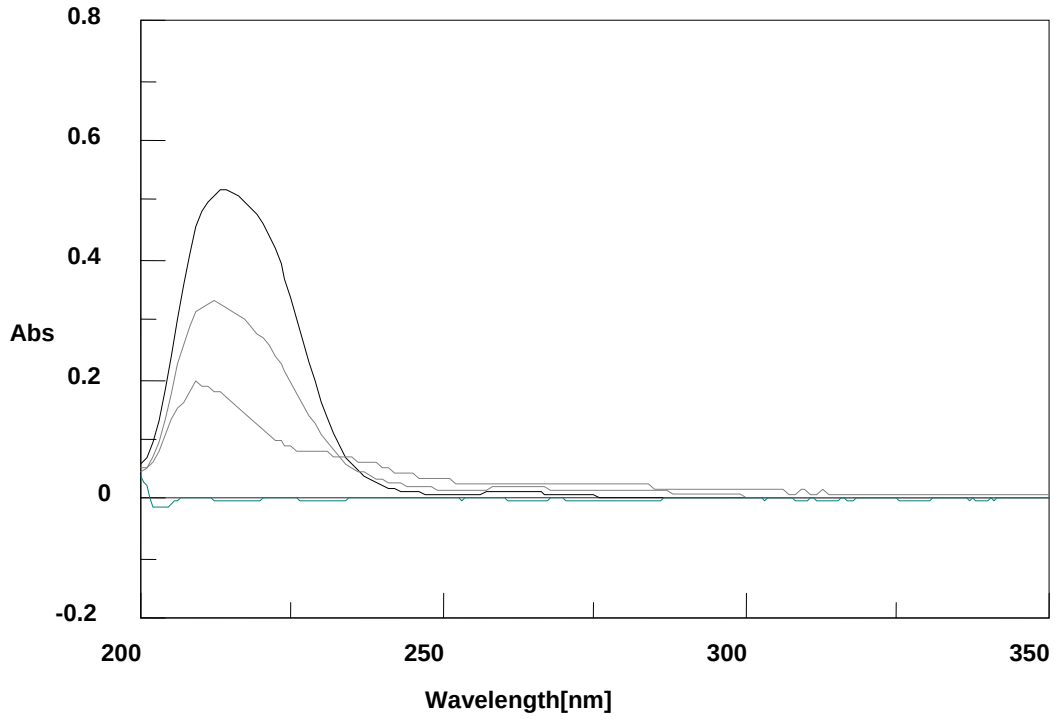

Figure 3.1 (a): Overlaid UV absorption spectra of different concentrations of MIL in pH 6.8 phosphate buffer.

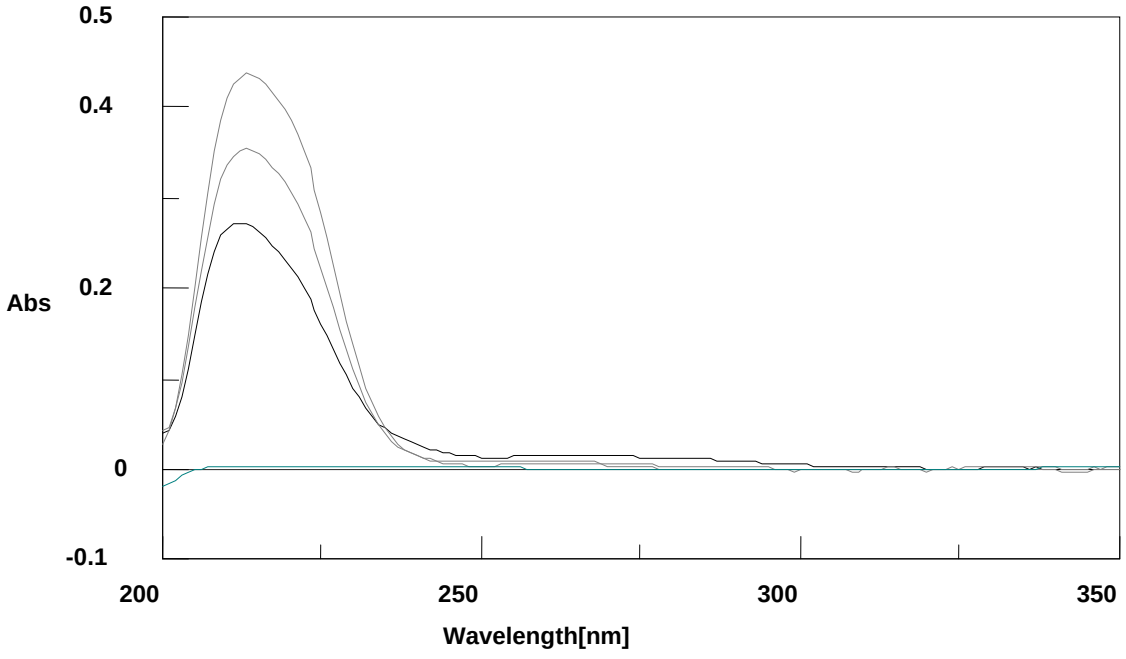

Figure 3.1 (b): Overlaid UV absorption spectra of different concentrations of MIL in pH 1.2 Hydrochloric acid buffer.

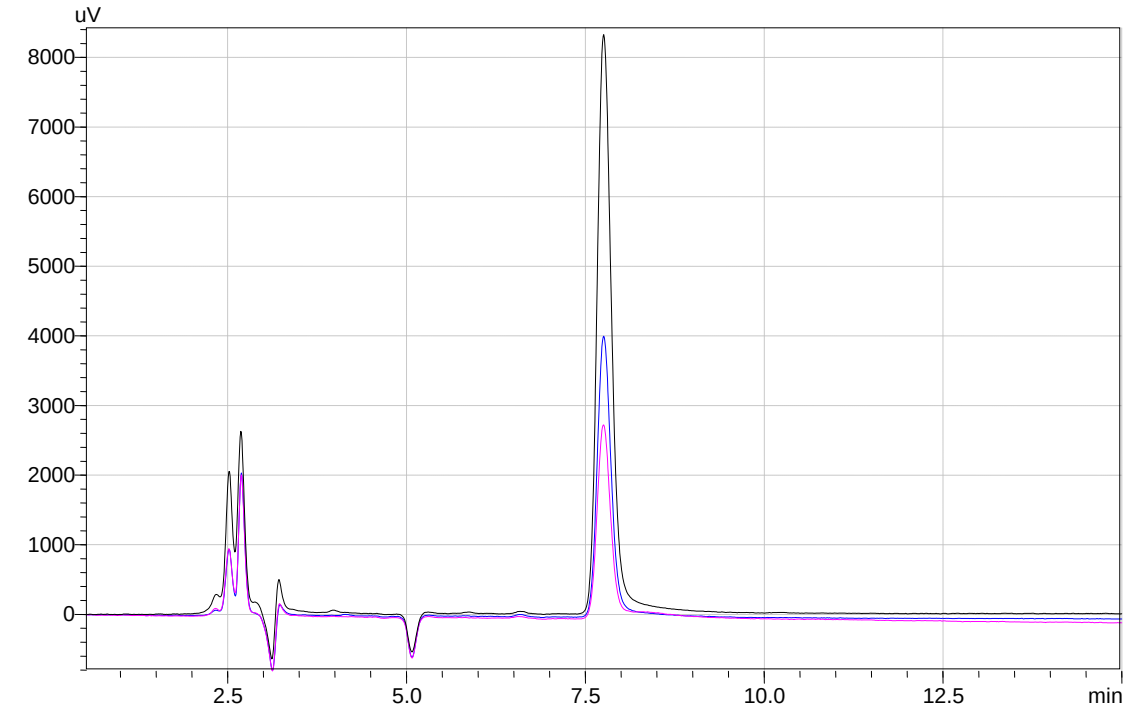

Figure 3.2: Overlay of RP-HPLC chromatograms of calibration standard concentrations of MIL.

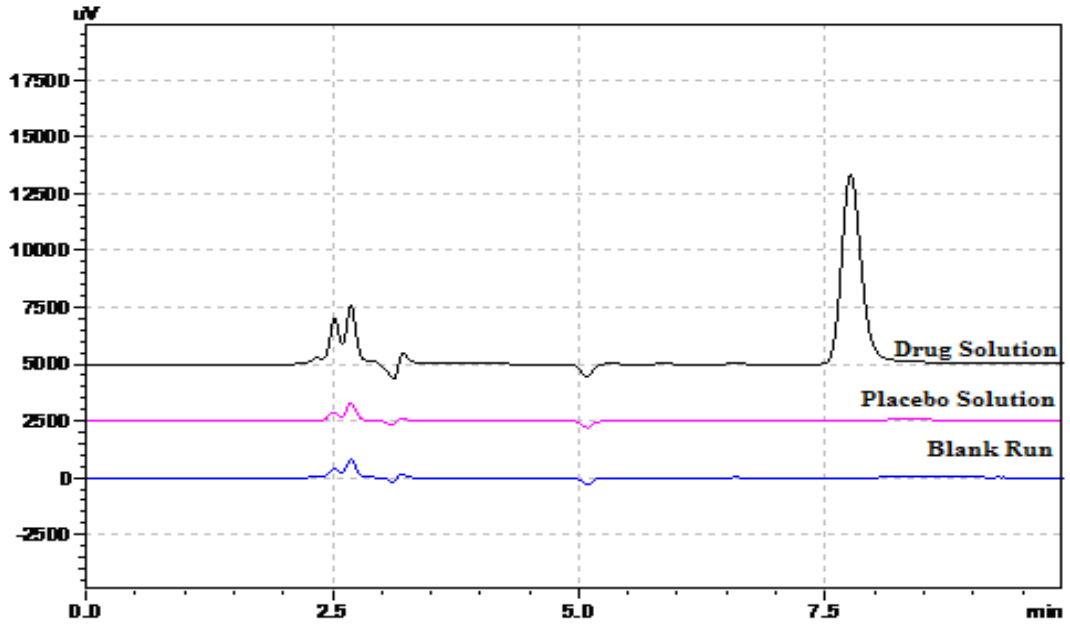

Figure 3.3: HPLC chromatogram of MIL, placebo solution and mobile phase.

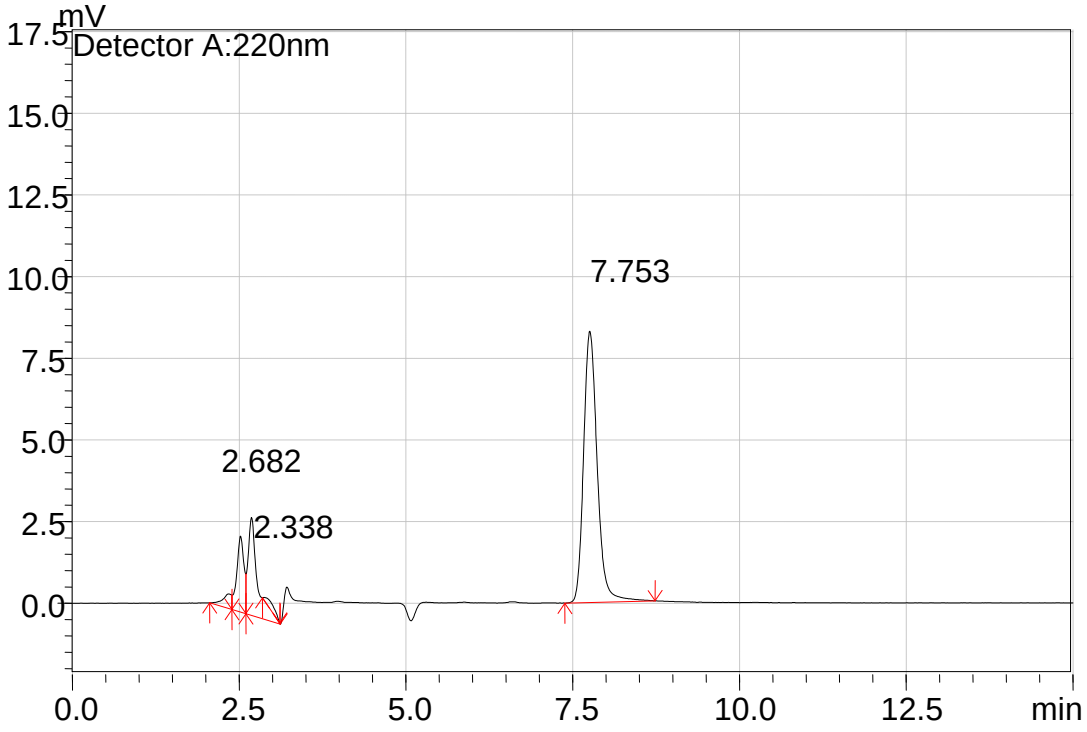

Figure 3.4 (a): Chromatogram of acid hydrolysis forced degradation study of MIL.

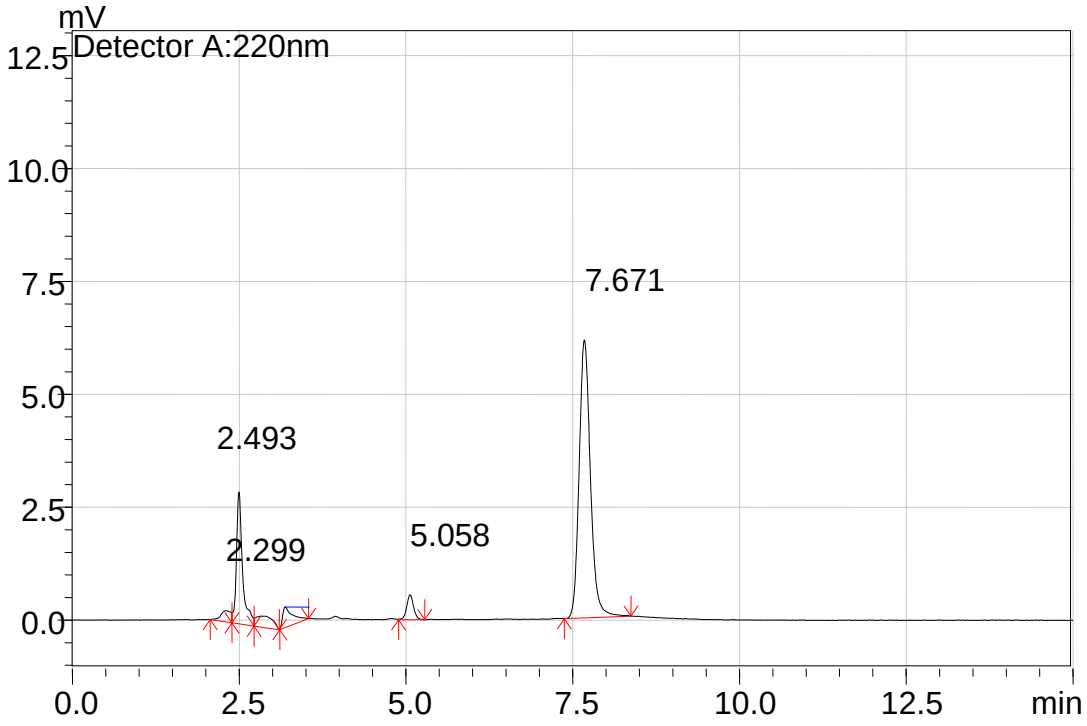

Figure 3.4 (b): Chromatogram of Base hydrolysis forced degradation study of MIL.

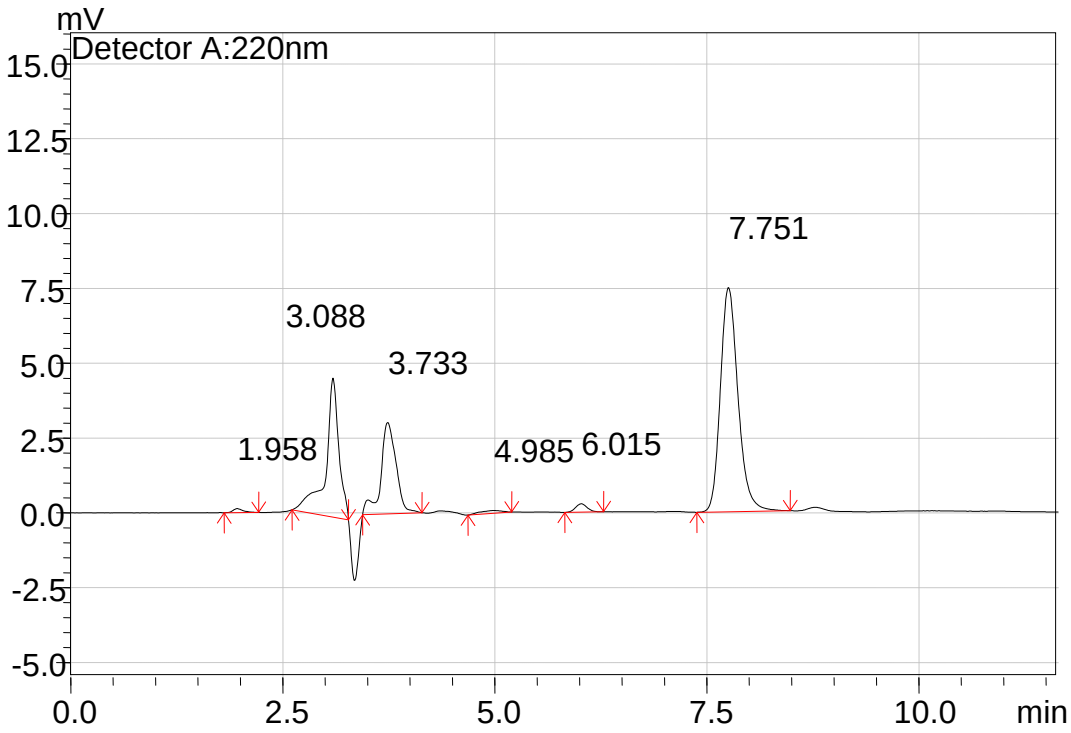

Figure 3.4 (c): Chromatogram of oxidative forced degradation study of MIL.

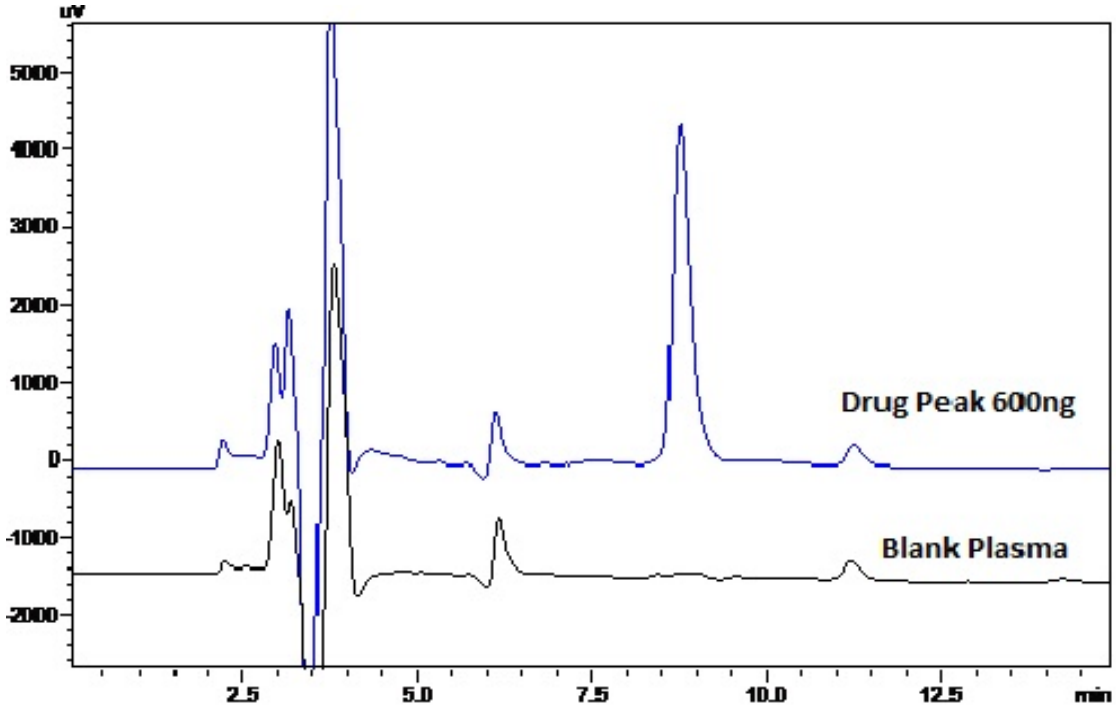

Figure 3.5: Overlaid Chromatograph of MIL in plasma and blank plasma.

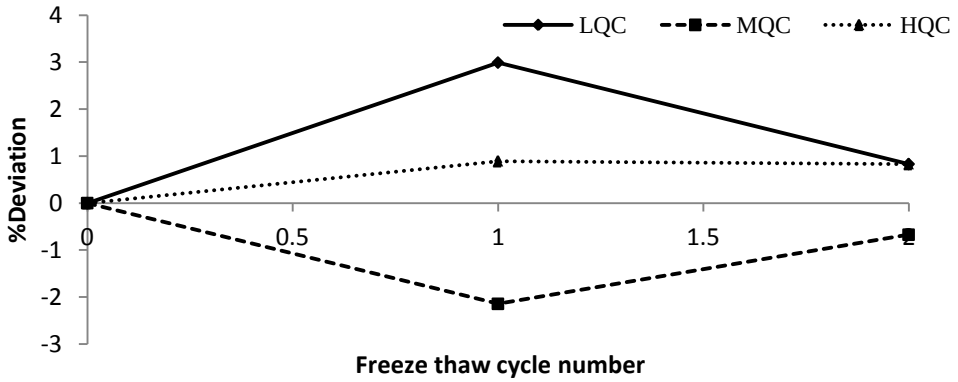

Figure 3.6 (a)

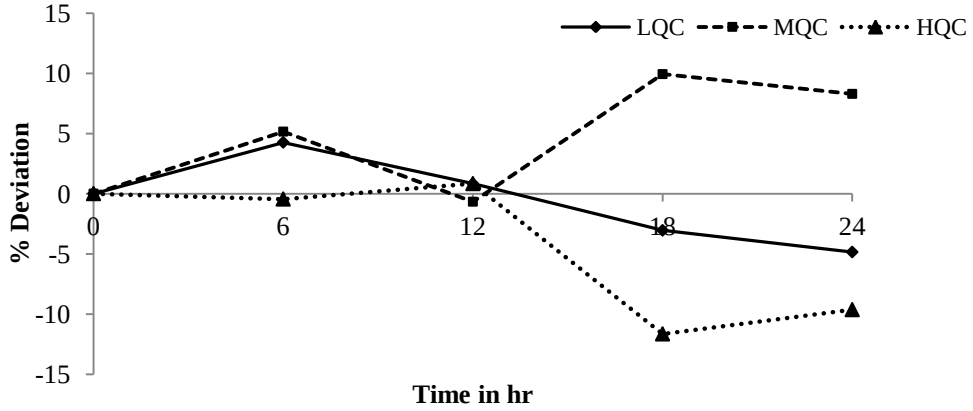

Figure 3.6 (b)

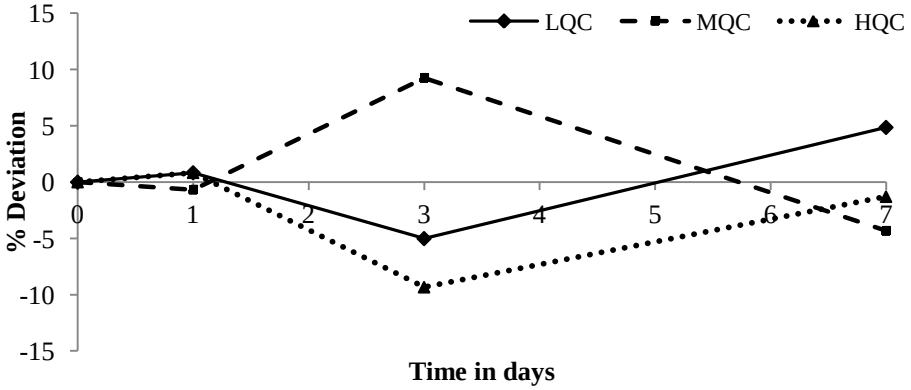

Figure 3.6 (c)

Figure 3.6: Stability study of MIL in rabbit plasma (a) freeze thaw stability; (b) post preparation stability; (c) long term stability.

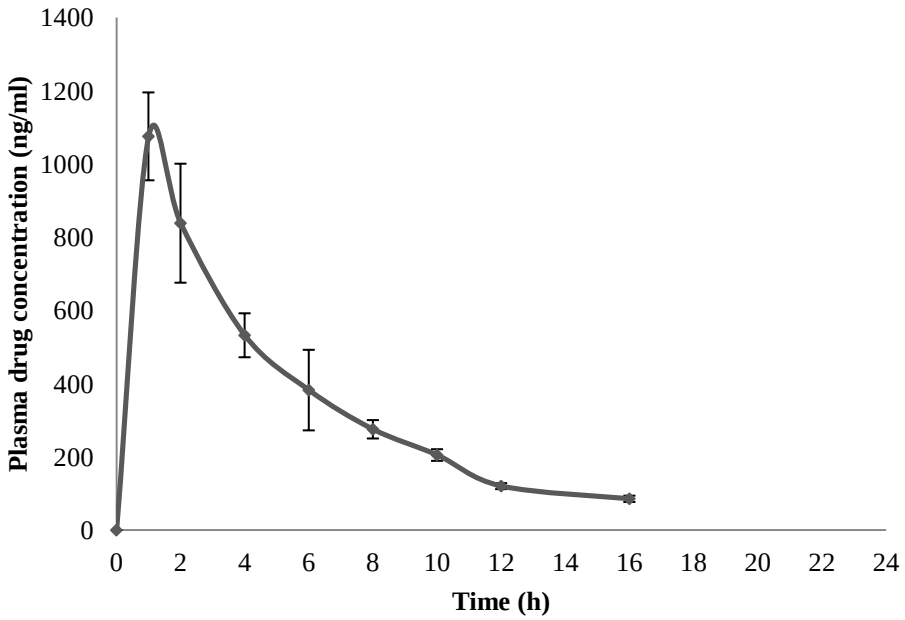

Figure 3.7: Mean plasma concentration-time profile of MIL after single oral dose in rabbits.

Table 3.1: Results of calibration curve and linearity of UV spectroscopic method for determination of MIL

Parameter	pH 1.2 Hydrochloric acid buffer	pH 6.8 Phosphate buffer
Apparent molar absorptivity (l /mol cm)	1.11×10^{4}	1.15×10^{4}
Sandell's sensitivity ($\mu g/cm^2/0.001A$)	2.56×10^{-2}	2.46×10^{-2}
Slope ± SD	$0.04 \pm 0.28 \times 10^{-2}$	$0.04 \pm 0.30 \times 10^{-2}$
Intercept ± SD	$0.06 \pm 0.62 \times 10^{-2}$	$0.02 \pm 0.46 \times 10^{-2}$
Regression coefficient (r^2)	0.9998	0.9988
Linearity (µg/ml)	5 to 30	5 to 30
Limit of detection (µg/ml)	0.32	0.53
Limit of quantification (µg/ml)	0.97	1.60

Table 3.2: Results of accuracy study for UV spectroscopic method

Concentration levels	Predicted conc. (µg/ml)			Mean % recovery	% Bias
	Range	Mean ± SD	% RSD		
pH 1.2 Hydrochloric acid buffer					
LQC	5.82 - 6.09	5.99 ± 0.09	1.50	99.94	-0.17%
MQC	17.96 - 18.27	18.16 ± 0.11	0.59	100.87	0.89%
HQC	26.64 - 26.98	26.83 ± 0.10	0.38	99.38	-0.63%
pH 6.8 Phosphate buffer					
LQC	5.96 - 6.17	6.06 ± 0.07	1.21	101.03	1.00%
MQC	17.98 - 18.19	18.07 ± 0.06	0.32	100.39	0.39%
HQC	26.47- 26.80	26.63± 0.08	0.29	98.62	-1.37%

Table 3.3: Results of precision study for UV spectroscopic method

Concentration levels	Intra-day Repeatability % RSD			Inter-day repeatability % RSD
	Day1	Day2	Day3	
pH 1.2 Hydrochloric acid medium				
LQC	1.33	0.98	1.30	1.35
MQC	0.84	0.84	0.52	0.72
HQC	0.79	0.58	0.43	0.64
pH 6.8 Phosphate buffer medium				
LQC	1.11	1.17	0.87	1.33
MQC	0.77	0.46	0.37	0.59
HQC	0.83	1.02	0.75	0.84

Table 3.4: Results of Robustness study for UV spectroscopic method

Concentration levels	Mean % Recovery ± SD	
	Hydrochloric acid medium pH 1.4	Phosphate buffer medium pH 7
LQC	99.15 ± 0.99	100.20 ± 1.48
MQC	101.92 ± 0.71	100.94 ± 0.54
HQC	100.13 ± 0.50	98.34 ± 0.31

Table 3.5: Determination of MIL content in formulations using UV spectroscopic method

Commercial products	pH 1.2 Hydrochloric acid medium		pH 6.8 Phosphate buffer medium	
	Amount found	% Assay	Amount found	% Assay
Milnace Tablet 50 mg	49.89 ± 0.16	99.77 ± 0.32	49.70 ± 0.50	99.41 ± 1.00
Milborn capsule 50 mg	50.42 ± 0.54	100.85 ± 1.07	50.20 ± 0.59	100.39 ± 1.17

Table 3.6: Results of calibration curve and linearity of RP-HPLC method

Parameters	Value
Linearity Range (ng/ml)	25 to 3000
Regression coefficient (r^2)	0.9999
Slope ± SD	801.1 ± 3.13
Intercept ± SD	-10768.0 ± 1722.54
Limit of detection (ng/ml)	7.09
Limit of quantification (ng/ml)	21.50

Table 3.7: Results of forced degradation study for RP-HPLC method

Stress condition	Degradation (%)	Retention times of degraded products (min)
Acid hydrolysis (1 N HCl at 40°C)	5.65	2.68
Base hydrolysis (1 N NaOH at 40°C)	10.50	2.49, 5.06
Oxidation (3% H_2O_2 at 40°C)	3.84	3.09, 3.73
Thermal stress (50^oC)	No degradation	Not observed
Photo-degradation (UV chamber)	No degradation	Not observed

Table 3.8: Results of recovery study of RP-HPLC method

Concentration levels	Measured Conc. (ng/ml) ± SD; % RSD	Mean % Recovery	% Bias
LQC	101.70 ± 1.47; 1.44	101.76	1.76
MQC	733.56 ± 4.34; 0.59	98.23	1.77
HQC	2034.34 ± 3.75; 0.18	101.64	1.64

Table 3.9: Results of precision study of RP-HPLC method

Concentration levels	Intra-day precision % RSD	Inter-day precision % RSD		
		Day-1	Day-2	Day-3
LQC	1.65	1.03	0.57	0.35
MQC	1.22	0.60	0.13	0.24
HQC	0.12	0.19	0.22	0.11

Table 3.10: Determination of MIL content in formulations using RP-HPLC method

Commercial products	Amount found	% Recovery	% Bias
Milborn capsule 25 mg (Sun Pharma. India)	25.25 ± 0.30 mg	101.01 ± 1.20	1.01
Milnace Tablet 25 mg (Torrent Pharm. Ltd. India)	24.96 ± 0.18 mg	99.87 ± 0.72	0.13

Table 3.11: Results of calibration curve and validation study of RP-HPLC bioanalytical method

Parameters	Values
Linearity range (ng/ml)	50 to 2000
Slope (± SD)	126 (± 126.33)
Intercept (± SD)	9869 (± 291.46)
Regression coefficient (r^2)	0.9998
% Extraction recovery ± SD	93.24 ± 9.24 to 96.45 ± 2.84
Limit of detection (ng/ml)	15
Limit of quantification (ng/ml)	25

Table 3.12: Results of accuracy study of RP-HPLC bioanalytical method

Concentration levels	Concentration (ng/ml)	Mean % recovery	% Bias
	Mean ± SD ; % RSD		
LQC	109.56 ± 7.38 ; 3.45	101.44	1.44
MQC	571.46 ± 5.38 ; 3.47	99.31	-0.69
HQC	1271.01 ± 2.05 ; 3.32	101.15	1.15

Table 3.13: Results of precision study of RP-HPLC bioanalytical method

Concentration levels	Intra-day precision % RSD	Inter-day precision % RSD		
		Day-1	Day-2	Day-3
LQC	3.58	1.06	4.96	3.31
MQC	2.70	3.98	1.90	2.94
HQC	1.60	2.04	4.61	3.74

Table 3.14: Pharmacokinetic parameters of MIL determined in rabbits using RP-HPLC bioanalytical method

Pharmacokinetic Parameters	Values
Maximum drug concentration in plasma (C_{max})	1075.25 ± 103.92 ng/ml
Time to reach maximum drug concentration (T_{max})	1.00 ± 0.15 h
Elimination half-life ($t_{1/2}$)	3.43 ± 0.23 h
Area under the curve $(AUC)_{(0-\infty)}$	6152.17 ± 235.18 ng.h/ml
$AUMC_{(0-\infty)}$	38857.27 ± 2578.32 $ng.h^2/ml$
Mean residence time (MRT)	4.95 ± 0.15 h

CHAPTER 4

PREFORMULATION STUDIES

4.1 Introduction

Preformulation study is a regulatory requirement and the first step in the rational development of dosage form of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug candidate alone and in combination with excipients [1]. A comprehensive preformulation study helps in understanding the physicochemical properties of the drug molecule and help in several decisions. It provides the foundation for development of a robust dosage form that can sustain the rigors of processing and storage condition [2].

The overall objective of preformulation study is to generate experimental information useful for development of stable, effective and bioavailable dosage form, which can be mass-produced. Efforts spent on preformulation provide cost savings in the long run, by reducing challenges during formulation development and post marketing problems. Therefore, the goals of preformulation study are (a) to establish the necessary physicochemical parameters of new drug substance, (b) to determine its stability profile, (c) to establish its physical characteristics and (d) to establish its compatibility with common excipients used for formulation [2-3].

Physicochemical properties of milnacipran hydrochloride (MIL) like solubility, dissociation constant and partition coefficient have been reported in the literature which were determined with simulation software [4]. The extensive literature survey revealed that the solubility and stability in different pH solutions ranging from 1.2 to 10.0 and partition coefficient for octanol/water system were not reported. Though some of preformulation studies like bulk characteristics, melting point and general solubility have been reported [4]. Since the current research endeavor aims at designing oral controlled release formulations, the pH solubility profiles and pH stability profiles were required to be established with the pH range encountered in the gastro intestinal tract (pH range: 1.2 to 8.0). The solid-state stability of MIL was studied in the presence of different polymers and excipients, which were planned to be used in the design of oral controlled tablet formulations. DSC and FTIR studies of various drug-excipient mixtures were carried out for compatibility study.

4.2 Materials

Pure milnacipran hydrochloride was obtained as gift sample from Torrent Pharmaceutical limited, Ahmedabad, Gujarat, India. The polymers like

hydroxypropyl methylcellulose (HPMC) [15000 cPs (METHOCEL K15M Premium) and 100000 cPs (METHOCEL K100M Premium)], ethyl cellulose (ETHOCEL^{TM} Standard Premium, 10 cPs) and sodium carboxy methyl cellulose (NaCMC) were obtained as gift samples from IPCA Laboratories, Mumbai, India. Eudragit RSPO obtained as gift samples from Dr Reddy laboratory Hyderabad, India. Carbopol 971P NF obtained as gift sample from Lubrizol, Mumbai, India. All other chemicals used were of analytical grade and purchased from Qualigens, Mumbai. Triple distilled water (TDW) from all quartz glass apparatus was used for the preparation of various aqueous phases used in the different studies.

4.3 Equipment/Instruments

A five-digit analytical balance (Mettler Toledo, Switzerland) was used for all weighing purposes. The pH were determined with pH meter (Eutech pH meter, Mumbai, India) equipped with a combined glass electrode. Rotary flask shaker (REMI Instruments, India) and vortex mixer (Spinix, India) were used for solubility analysis. Stability studies were carried out in humidity and temperature control cabinet (Thermolab, India and Wadegati, India). Bulk density and tapped density were determined with Thermonic (Campbell, India) densitometer. Thermal analysis was performed using heat flux type differential scanning calorimeter - DSC-60 (Shimadzu, Japan) with TA-60WS thermal analyzer (integrating software: TA-60WS collection monitor, version 1.51; analysis software: TA-60; temperature range: -150° to 600^{o}C; heat flow range: ± 40 mW; temperature program rate: 0 to 99^{o}C/min; atmosphere: nitrogen at 40 ml/min). Drug-excipient compatibility study was also carried out using Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu, Japan; model: IR Prestige 21; model software: IR Solutions, version 1.0). Thin layer chromatographic (TLC) plates were checked in UV Fluorescence chamber (Superfit, India). Analytical instruments mentioned in chapter 3 were used for all sample analysis.

4.4 Reagents

(a) Preparation of buffered solutions

Different buffer systems with 0.1 M strength ranging from 1.2 to 10 pH were prepared according to the procedures given in USP [5]. TDW was used as the solvent in all the cases.

(b) Preparation of unbuffered solutions

Unbuffered solutions of pH ranging from 1.2 to 10 were prepared using variable volumes of 0.1 N NaOH and 0.1 N HCl solutions. TDW was used as the solvent in all the cases.

(c) Preparation of 0.2 M sodium hydroxide

0.80g of sodium hydroxide was transferred carefully into a 100 ml volumetric flask and 50 ml of TDW was added into the flask to dissolve sodium hydroxide completely. The final volume was made up to 100 ml with TDW [5].

(d) Preparation of pH 6.8 phosphate buffer

6.81g of potassium dihydrogen orthophosphate was transferred carefully into a 1000 ml volumetric flask and 500 ml of TDW was added into the flask to dissolve potassium dihydrogen orthophosphate completely. Then 112 ml of 0.2 M sodium hydroxide was added into the volumetric flask and the final volume was made upto 1000 ml with TDW as given in USP [5].

4.5 Experimental

Various analytical methods were used for analysis during preformulation study. UV spectrophotometric method (chapter 3) was used for analysis of all samples in solubility, partition coefficient determination and stability studies. Thin layer chromatographic method was also used for solid state and solution state stability studies. The TLC plates were eluted using mixture of acetonitrile, water and ammonia (6:0.6:1.6) (v/v/v) as mobile phase for assessing the stability of the drug.

4.5.1 Physical characterization

Physical appearance, melting point and hygroscopic studies were performed for physical characterization of pure MIL. Color and odor of MIL was determined with visual observation. Melting point of MIL was determined by capillary method using digital auto melting point apparatus (Labtronics, India). Hygroscopicity study of pure MIL was performed as per standard procedure given in European Pharmacopoeia [6]. A 5 g MIL was placed in glass petridish and exposed to 80 ± 2% relative humidity condition in desiccator (previously saturated) for 24 h. The percentage increase in mass was determined and results interpreted as per limit given in European Pharmacopoeia.

4.5.2 Bulk characteristics

The bulk characteristics of MIL were determined with various parameters such as bulk density, tapped density, porosity, compressibility index, Hausner ratio and angle of repose.

Bulk density, tapped density and angle of repose were determined by standard procedure using standard density apparatus and fixed funnel method respectively [7]. The compressibility index or Carr's index (%) and the Hausner's ratio were calculated using following equations:

Compressibility index (%) = (Tapped density - Bulk density) * 100 / Tapped density

Hausner's ratio = Tapped density / Bulk density

4.5.3 Determination of solubility

Solubility of MIL was determined by thermodynamic solubility method i.e. saturation shake flask method [8-9] over the pH range 1.2 - 10 and distilled water at 37° ± 1°C. The shake-flask method proposed by Higuchi and Connors [8] is the most reliable and widely used for solubility study. The solubility of MIL was determined in pH 1.2, 5.0, 6.8, 7.4 and 10 buffers and unbuffered solutions. These solubility experiments were typically conducted over 48 h in different buffer and unbuffered solutions at above pH range. In solubility studies, excess amount of MIL was added to vials containing distilled water/unbuffered/ buffered pH solutions. The sample vials were agitated for 48 h using shaker maintained at 37° ± 1^{o}C. In all cases, samples were withdrawn in triplicate at predetermined time points and analyzed with UV-spectroscopic method after appropriate dilution.

4.5.4 Determination of apparent partition coefficient and partition coefficient

The octanol-water partition coefficient is one of the most commonly reported physicochemical properties of drugs and widely used as a measure of lipophilicity [10]. MIL is a weak basic drug and its ionization in water depends upon pKa and on the pH of the aqueous phase. Apparent partition coefficient (P_{app}) or distribution coefficient of MIL was determined in n-octanol/water, n-octanol/pH 6.8 buffer, chloroform/water and chloroform/pH 6.8 buffer systems by shake flask method [11]. The organic phase (n-octanol and chloroform) was presaturated with aqueous phase (water and buffers) separately for 24 h at room temperature (25° ± 2^{o}C). The

presaturated organic phase and aqueous phase were separated by separating funnel and used for determining the P_{app}.

To 50 ml of presaturated organic phase, 50 ml of presaturated aqueous phase (50 µg/ml of MIL) was added and kept for shaking on rotary flask shaker at room temperature. The initial concentration of the drug in aqueous phase was determined using a validated UV-Spectroscopic method. Aqueous phase was separated and samples were withdrawn at 24 and 48 h of shaking. Samples were diluted appropriately and analyzed. The entire experiment was carried out in triplicates for in n-octanol/water, n-octanol/pH 6.8 buffer, chloroform/water and chloroform/pH 6.8 buffer systems. P_{app} was calculated using the equation given below and Log P_{app} was then calculated by taking logarithm to base 10 of P_{app}.

$$P_{app} = C_o / C_a = (S_i - S_f) / S_f$$

Where, S_i = initial amount of the drug in aqueous phase and S_f = final amount of the drug in aqueous phase. The obtained Log P_{app} value was used for mathematically determination of Partition coefficient (P) or Log P using below equation [11]:

$$\text{Log } P_{app} = \text{Log } P - \text{Log } [1 + 10^{(pKa-pH)}]$$

4.5.5 Stability studies

(a) Solution state stability studies

The stability of drugs in solution state is affected not only by their chemistry, but also by their environment, such as temperature (ambient or accelerated), light and pH. The solution state stability of drug will provide useful information for analytical method development, selection of dissolution medium, formulation design and process selection [12]. No report has been found on solution state stability of MIL. Therefore, solution state stability for MIL was carried out in different pH solutions at various storage conditions.

Solution state stability of MIL in various buffer and unbuffered solutions was studied at room temperature. MIL was dissolved in various buffer and unbuffer pH solutions (pH 1.2, 2.0, 3.0, 4.0, 5.0, 6.8, 7.4, 8, 9, 10) to get the concentration of 20 µg/ml. To determine the thermal stability and photo stability, 20 µg/ml solution of MIL was made with distilled water in glass container and exposed to different temperature conditions like controlled room temperature (CRT): 25 ± 2^{o}C,

accelerated temperature condition (AT): 40 ± 2^{o}C, high temperature : 60 ± 2^{o}C and natural sunlight.

The entire study was done in triplicate. Samples were collected at different time points and were analyzed by validated UV-spectroscopic method. At different time points the solutions were spotted on a TLC plate eluted using mixture of acetonitrile, water and ammonia (6:0.6:1.6) (v/v/v) as mobile phase for assessing the stability of the drug. MIL dissolved in distilled water was used as control in all stability studies.

(b) Solid state stability and compatibility study

The study of drug–excipient compatibility represents an important phase in the preformulation stage for the development of all dosage forms. In fact, potential physical and chemical interactions between drug and excipients can affect the physicochemical nature, the stability and bioavailability of drugs and consequently, their therapeutic efficacy and safety. At early stage of product development, drug–excipient compatibility testing helps in the selection of excipients, process and packaging materials that increases the probability of developing a stable dosage form [13]. Several analytical techniques are available for study the drug/ excipient interaction. Differential scanning calorimetry (DSC) is one of the well-developed thermal techniques used in detection of incompatibilities in drug/excipient interactions. Infrared spectroscopy, TLC and HPLC studies are well-established non-thermal methods that have been used widely to indicate the drug/excipient interactions [14].

Solid-state stability of MIL and compatibility with selected excipients were studied, which were planned to be used in the preparation of oral controlled release tablets. Various formulation excipients such as dibasic calcium phosphate (DCP), microcrystalline cellulose (MCC), HPMC (15000 and 100000 cPs), ethylcellulose, sodium carboxy methylcellulose (NaCMC), carbopol, Eudragit RSPO, paraffin wax, stearic acid, cetosteryl alcohol, PVP K30, talc and magnesium stearate were used in the study.

The solid admixtures of the drug with each excipient (1:1 and 1:2) were prepared by geometric mixing, filled in the vials, and labeled. These samples were stored at different storage conditions like long term stability condition or controlled room temperature (CRT: 25 ± 2^{o}C/60 ± 5% RH), at accelerated condition (AT: 40 ±

2^{o}C/75 ± 5% RH) and refrigerated condition (FT: 5 ± 2^{o}C) as per ICH stability guidelines. At predetermined time intervals, samples (in triplicates) were withdrawn and characterized for physical observations, drug content and impurities detection. Drug content was analyzed by UV-spectroscopic method and impurities were detected with TLC method.

To study the compatibility of MIL with various excipients, the solid admixtures were characterized using DSC and FTIR immediately and every 3 months for a period of one year. In case of DSC study, a weighed quantity (approx 4 mg) of the solid admixture was taken and sealed in standard aluminum pan with lid. The temperature range of measurement was 30 to 300^{o}C with a heating rate of 10^{o}C/min. Nitrogen gas was purged at a flow rate of 40 ml/min to provide the inert environment. Similarly, thermograms were also recorded for pure drug as well as pure excipients.

FTIR study was also carried out for pure MIL, individual excipient and physical mixture of MIL with excipient. A small quantity (5 to 10 mg) of the solid admixture was taken in a mortar and pulverized with dried potassium bromide. After thorough mixing a small quantity of the mixture was taken in FTIR sample holder and the spectrum was recorded. FTIR spectrum was also recorded for pure drug as well as pure excipients. For DSC and FTIR study, samples were withdrawn from long term stability condition at predetermined time intervals.

4.6 Results and discussion

4.6.1 Physical characterization

MIL appeared as white to off white in color and odorless powder. Melting point was found to be 179° ± 0.5°C. For hydroscopic study, MIL was kept for 24 h in 80% RH condition and weight gain was measured. The percentage weight gain was found to be 0.15 which indicated the non-hygroscopic property of MIL (Table 4.1).

4.6.2 Bulk characteristics

Bulk density was characterized with 10 g of MIL in 100 ml of glass measuring cylinder. The untapped volume (Bulk volume) was found to be 31 ml. The tapped volume was evaluated as per USP procedure using densiometer. The bulk density and tapped density were found to be 0.33 and 0.47 g/ml respectively. The compressibility index (%) and the Hausner's ratio were calculated and found to be

29.78 and 1.42 respectively. Angle of repose and flow rate were carried out with funnel method and found to be 32° and 1.62 g/min respectively (Table 4.1).

High values of compressibility index (more than 25%), Hausner's ratio (more than 1.2), Angle of repose (more than 25°) and very slow flow rate of MIL indicated that MIL has poor compressibility and poor flow.

4.6.3 Determination of solubility

The solubility of MIL at 37 ± 2^{o}C in TDW and various buffered and unbuffered solutions of pH ranging from 1.2 to 10 is given in Table 4.2. MIL was found to be highly soluble in the pH ranging from 1.2 to 10 in both buffered as well as unbuffered systems. The solubility values of MIL in buffered systems in pH 1.2 to 10 ranged from 1.92 ± 0.23 to 2.10 ± 0.05 g/ml, indicating no significant difference in the solubility of MIL with the change in pH. The solubility values of MIL in unbuffered systems in pH 1.2 to 10 ranged from 1.93 ± 0.24 to 2.05 ± 0.04 g/ml, indicating no significant change in the solubility. The solubility of MIL in TDW was found to be 1.98 ± 0.14 g/ml. The solubility results of MIL in different pH solutions indicated that MIL was highly soluble (approx 2 gm/ml) at all pH solutions and there was no influence of pH on solubility.

4.6.4 Determination of partition coefficient

The apparent partition coefficient (P_{app}) of MIL was determined in n-octanol/water, n-octanol/ pH 6.8 phosphate buffer, chloroform/water and chloroform/pH 6.8 phosphate buffer by shake flask method. No significant difference was observed in the P_{app} values of MIL in n-octanol/water and n-octanol/pH 6.8 phosphate buffer systems. Log of partition coefficient (Log P) was calculated using value of Log P_{app}. Log P values of MIL in n-octanol/water and n-octanol/pH 6.8 phosphate buffer at 48 h were found to be 2.06 and 1.96 respectively. The Log P values of MIL in chloroform/water and chloroform/pH 6.8 buffer systems at 48 h were found to be 2.12 and 1.99 respectively as presented in Table 4.3. The reported computational value of Log P is 1.72. This difference between computational and experimental value of Log P value may be attributed to experimental value and sensitivity of the method for determination of Log P (shake flask method and UV spectroscopic analysis). Log P value of MIL confirmed its high permeability and rapid in-vivo absorption.

4.6.5 Stability studies

(a) Solution state stability studies

Solution state solubility of MIL was determined in various buffer and unbuffer solutions. The log percent remaining to be degraded (% RTD) versus time profiles for MIL in various buffered and unbuffered systems are given in Figure 4.1 and Figure 4.2 respectively. First order kinetics was observed for the degradation of MIL in various buffered and unbuffered systems. High regression coefficient ($r^2 \approx 1$) established linear relationship between log % RTD versus time. First order degradation rate constants obtained from the slope were used to determine $t_{90\%}$ (time taken for 90% of the drug remaining to be degraded) at various pH values (Table 4.4).

No significant difference was observed in the degradation of MIL in various buffered and unbuffered systems. The degradation rate constant (K_{deg}) values obtained were ranging from 4.26×10^{-4} to 4.82×10^{-4} h^{-1} and 4.44×10^{-4} to 5.24×10^{-4} h^{-1} in buffered and unbuffered pH systems respectively. The $t_{90\%}$ values obtained were ranging from 9.10 to 10.31 days and 8.38 to 9.90 days in buffered and unbuffered pH systems respectively.

The retention factor (R_f) of MIL was found to be 0.63 in TLC analysis using mixture of acetonitrile, water and ammonia (6:0.6:1.6) (v/v/v) as mobile phase. There was no difference found in the R_f values of freshly prepared pure drug solution and drug in various buffered and unbuffered pH systems at different time intervals. Only one spot corresponding to the R_f of MIL was observed under UV light of 220 nm wavelength in various buffered and unbuffered pH systems.

In thermal stability at various temperature conditions, the log % RTD versus time profiles were linear for all the plots indicating first order kinetic as presented in Figure 4.3. The first order degradation rate constants obtained from the slope of the curves were used to determine $t_{90\%}$ and $t_{50\%}$ at various temperature conditions (Table 4.5). The degradation rate constant was found to be dependent on the temperature condition. The degradation rate constant (K_{deg}) value was lesser (5.63×10^{-4} h^{-1}) at room temperature (25°C) and higher (9.58×10^{-4} h^{-1}) at 60°C. The $t_{90\%}$ values decreased with increase in temperature condition. The $t_{90\%}$ value was found to be 7.79 days at 25°C and 4.58 days at 60°C. In the TLC studies, only one spot corresponding to

the R_f of MIL was observed under UV light of 220 nm wavelength for all the samples, indicating the stability of MIL at these temperature conditions.

(b) Solid state stability studies

The results of solid sate stability indicated that the solid admixtures of MIL with various excipients showed good stability with drug content values ranging from 99.15 to 101.05% with a maximum SD of 1.35 during the storage period. The degradation kinetics of pure MIL and solid admixtures of MIL with various excipients are given in Table 4.6. At refrigerated condition (FT: 5 ± 2^{o}C), pure MIL and the solid admixtures of MIL with various excipients were found to be stable for the entire period of study (12 months). The log % RTD versus time plots for pure MIL and the solid admixtures were linear indicating first order kinetics with high regression coefficient ($r^2 \approx 1$). The degradation rate constant for pure MIL was found to be 14.55×10^{-4} and 44.50×10^{-4} month^{-1} at CRT and AT respectively. The $t_{90\%}$ values for pure MIL at CRT and AT were found to be 72.40 and 23.68 months respectively.

The degradation rate constant (K_{deg}) values for the solid admixtures stored at CRT conditions were ranging from 15.43×10^{-4} to 18.12×10^{-4} month^{-1}. The $t_{90\%}$ values were ranging from 58.14 to 68.29 months. The K_{deg} values for the solid admixtures stored at AT conditions were ranging from 25.88×10^{-4} to 48.59×10^{-4} month^{-1}. The $t_{90\%}$ values were ranging from 21.69 to 40.71 months. MIL was found to be stable both alone as well as in solid admixtures for at least a period of 12 months when stored at CRT conditions and at least for a period of 6 months when stored at AT conditions. MIL pure and solid admixtures were also evaluated for impurity using TLC method. There was no significant change observed in R_f (0.63) for all samples stored at CRT and AT up to 12 months and 6 months respectively.

(c) Compatibility studies of milnacipran hydrochloride in presence of excipients

Characterization with DSC was carried out for pure MIL, pure excipients and solid admixtures of MIL with various excipients mixed in the ratio of 1:1. The DSC was used in order to detect the formulation incompatibilities due to drug-excipient interaction. Any abrupt or drastic change in the thermal behavior of either the drug or excipient may indicate a possible drug-excipient interaction. The thermograms of pure drug, pure excipients, physical mixture of drug and excipients are shown in

Figure 4.4 - 4.12. The peak temperature and enthalpy values (ΔH (J/g)) for drug, excipient and drug-excipient mixture are summarized in Table 4.7.

The DSC thermogram of pure MIL showed a sharp melting endotherm at 179.80 ^{o}C with a normalized energy of 47.55 J/g. In the DSC thermograms of HPMC (15000 and 100000 cPs), EC, CP, DCP, PVP K-30 and talc, no endothermic peaks were observed. The thermograms of solid admixtures of MIL with various excipients characterized after 6 months and 1 year of storage, also had shown similar endothermic peak at approximately 179-180^{o}C with almost the same normalized energy (values ranging from 35.23 to 44.68) (Table 4.7), indicating that MIL was unaffected in the presence of various excipients selected for the study.

A slight change in peak shape and peak position (shifting to higher or lower temperature) was observed in some solid admixtures particularly in NaCMC and stearic acid admixtures with MIL.

The DSC thermogram of the physical mixture of MIL with NaCMC indicated the shifting of MIL melting point peak. This may be due to the structural transitions of the polymer chains in the NaCMC at high temperature. Similar result was also reported by Lojewska et al and Wei Li et al, which confirmed that the transition might be probably related to the partial oxidation of the OH groups on the polymer chains [15-16].

The DSC thermogram of stearic acid admixture with MIL showed shifting of melting endothermic peak of MIL toward lower value i.e. 168°C. The reason for this may be due to formation of eutectic mixture of MIL and stearic acid. Gordon et al. and Wong et al. reported similar observations with regard to DSC thermograms of ibuprofen-stearyl alcohol and ibuprofen-cetostearyl alcohol mixtures respectively [17-18]. Both studies attributed the observed changes in the thermograms to the formation of eutectic mixtures between drug and wax. Therefore, it is possible to deduce that observed changes in the thermograms of the present study may be due to the formation of a eutectic mixture between the drug - stearic acid. There was no significant change found in FTIR, TLC and assay studies of NaCMC and stearic acid admixtures with MIL which assured the compatibility of these excipients with MIL.

The data obtained from the compatibility studies of MIL with various excipients by DSC was further supported by FTIR studies. The FTIR spectrum of pure

MIL and solid admixtures of MIL with various excipients are given in Figure 4.13 - 4.15. The FTIR spectrum of solid admixtures of MIL with various excipients characterized after 6 months and 1 year of storage. The characteristic peak of amide carbonyl group at 1614 cm^{-1}, aromatic C-H stretching at 3059 cm^{-1} and N-H stretching of amine group at 3153 cm^{-1}, were present in all the spectrum, indicated the stable nature of MIL in the solid admixtures.

FTIR spectra of all compatibility samples showed that the characteristic bands of MIL were not altered in binary mixtures indicating no interactions between MIL and the selected excipients. Similar results were obtained when the samples were analyzed after 1 year of storage at CRT condition.

DSC and FTIR studies of MIL and solid admixtures of the drug established the stable nature and compatibility of drug with various excipients at least for a period of 1 year when stored at CRT conditions.

4.7 Conclusions

Study of physical characteristics indicated non-hygroscopic and poor flowing properties of MIL. Solubility studies in various buffered and unbuffered pH systems showed that MIL is highly soluble at all pH ranged 1.2 to 10. MIL followed first order degradation kinetics in solution state with good stability over the entire pH range. Solid state stability studies showed that MIL was stable and compatible with various excipients for sufficient time period. DSC and FTIR studies had further supported the data obtained in solid state stability studies. The results of preformulation studies can be helpful in selection of compatible excipients, moisture level in final product and process for design and development of oral controlled tablets formulations of MIL.

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Table 4.1: Physical and bulk characteristics of MIL

Parameters	Characterization
Physical appearance	White to off white odorless crystalline powder
Bulk density	0.33 gm/ml
Tapped density	0.47 gm/ml
Carr's index (%)	29.78
Hausner's ratio	1.42
Angle of repose	32°
Flow rate	1.62 gm/min
Hygroscopicity	Non hygroscopic
Melting point	179° ± 0.5 °C

Table 4.2: Results of solubility analysis of MIL at different pH

pH	Solubility in gm/ml, mean ± SD	
	Buffered solutions	**Unbuffered solutions**
TDW		1.98 ± 0.14
1.2	1.92 ± 0.23	1.95 ± 0.15
4.5	1.93 ± 0.12	1.93 ± 0.24
6.8	1.94 ± 0.20	1.96 ± 0.20
7.4	2.08 ± 0.08	2.01 ± 0.05
10	2.10 ± 0.05	2.05 ± 0.04

Table 4.3: Determination of apparent partition coefficient and partition coefficient of MIL

Partition system	Apparent partition coefficient (P_{app} ± SD)	% RSD	Log P
n-Octanol / Water	0.231 ± 0.02	1.72	2.06
n-Octanol / pH 6.8 phosphate buffer	0.368 ± 0.02	0.71	1.96
Chloroform / Water	0.263 ± 0.11	0.90	2.12
Chloroform / pH 6.8 phosphate buffer	0.391 ± 0.02	0.68	1.99

Table 4.4: pH stability data of MIL in buffered and unbuffred solutions

	Buffer solutions			Unbuffer solutions		
pH	**K_{deg} (10^{-4} h^{-1})**	**r^2**	**$t_{90\%}$ (days)**	**K_{deg} (10^{-4} h^{-1})**	**r^2**	**$t_{90\%}$ (days)**
1.2	4.26 ± 0.02	0.9881	10.31	4.44 ± 0.04	0.9881	9.90
2.0	4.30 ± 0.03	0.9842	10.22	4.51 ± 0.02	0.9649	9.73
3.0	4.39 ± 0.03	0.9715	9.99	4.57 ± 0.02	0.9765	9.60
4.0	4.50 ± 0.02	0.9780	9.75	4.66 ± 0.03	0.9599	9.43
5.0	4.49 ± 0.04	0.9863	9.76	4.69 ± 0.02	0.9767	9.35
6.8	4.60 ± 0.02	0.9712	9.54	4.86 ± 0.04	0.9885	9.04
7.4	4.69 ± 0.03	0.9954	9.36	4.86 ± 0.02	0.9725	9.03
8.0	4.71 ± 0.02	0.9857	9.32	5.01 ± 0.02	0.9453	8.76
9.0	4.81 ± 0.03	0.9833	9.14	5.12 ± 0.03	0.9498	8.58
10.0	4.82 ± 0.02	0.8599	9.10	5.24 ± 0.04	0.9817	8.38

Table 4.5: Thermal stability data of MIL aqueous solution at different temperature conditions

Storage conditions	**K_{deg} (10^{-4} h^{-1})**	**r^2**	**$t_{90\%}$ (days)**	**$t_{50\%}$ (days)**
Room temperature	5.63 ± 0.01	0.9945	7.79	51.25
Accelerated condition	6.43 ± 0.03	0.9971	6.83	44.91
60°C temperature	9.58 ± 0.04	0.9965	4.58	30.14
Natural light exposure	6.46 ± 0.01	0.9962	6.80	44.73

Table 4.6: Stability data of MIL in different solid admixtures stored at controlled room temperature and accelerated temperature conditions

Storage conditions	Controlled Room temperature 25 ± 2°C/60 ± 5% RH			Accelerated temperature 40 ± 2°C/75 ± 5% RH		
Samples	K_{deg}* 10^{-4} $month^{-1}$	r^2	$t_{90\%}$ months	K_{deg}* 10^{-4} $month^{-1}$	r^2	$t_{90\%}$ months
Pure MIL	14.55	0.9945	72.40	44.50	0.9843	23.68
MIL+ HPMC 15K	16.83	0.9694	62.60	33.78	0.9891	31.19
MIL+ HPMC 100K	16.47	0.9680	64.00	46.20	0.9986	22.81
MIL+ NaCMC	16.81	0.9745	62.68	48.59	0.9982	21.69
MIL+ Carbopol	17.46	0.9650	60.37	42.72	0.9866	24.67
MIL + Paraffin wax	17.13	0.9743	61.50	44.38	0.9699	23.75
MIL+ Stearic acid	18.12	0.9543	58.14	44.86	0.9863	23.49
MIL+ EC	18.06	0.9722	58.36	45.42	0.9849	23.20
MIL+ Eudragit RSPO	16.01	0.9901	65.84	43.18	0.9610	24.40
MIL+ PVP K-30	16.72	0.9907	63.03	47.51	0.9776	22.18
MIL+ DCP	17.18	0.9921	61.34	33.67	0.9857	31.30
MIL + Mg Stearate	15.43	0.9926	68.29	25.88	0.9855	40.71
MIL + Talc	16.58	0.9847	63.55	35.08	0.9775	30.04

* K_{deg} is first order degradation rate constant.

Table 4.7: Thermal properties of MIL and solid admixture of MIL with excipients during compatibility study

Sample	Onset (^{o}C)	Peak (^{o}C)	Endset (^{o}C)	Heat (J/g)
Pure MIL	176.55	179.80	184.13	-47.55
Na CMC	139.66	147.97	153.54	-38.17
Paraffin	56.64	63.57	67.03	-103.00
Stearic acid	55.03	57.42	69.08	-70.86
Mg stearate	102.30	121.40	127.50	- 48.35
MIL + HPMC 15K	175.09	178.31	181.95	-41.07
MIL + HPMC 100K	177.26	179.25	183.88	-44.68
MIL + NaCMC	164.98	170.40	174.00	-41.18
MIL + Carbopol 971	177.68	179.83	183.42	-35.23
MIL + Ethyl cellulose	176.61	179.80	183.17	-42.54
MIL + Eudragit RSPO	175.38	178.78	183.26	-39.23
MIL + Paraffin wax	172.41	177.38	180.39	-42.88
MIL + Stearic acid	160.50	168.03	172.79	-40.75
MIL + PVP K30	177.34	179.25	183.85	-43.79
MIL + DCP	175.93	178.94	182.78	-40.10
MIL + Mg. stearate	177.67	179.83	183.52	-38.02
MIL + Talc	176.77	178.95	182.90	-43.45

Table 4.8: Wavelength attribution of IR spectrum of MIL

Wavelength (cm^{-1})	Attribution
3153	N-H stretching of amine
3059	aromatic C-H stretching
2968, 2935 and 2802	methyl and methylene symmetrical and asymmetrical stretching and vibration
1614	amide carbonyl stretching
1454	Symmetrical methyl bending.
736 and 698	Mono substituted aromatic ring

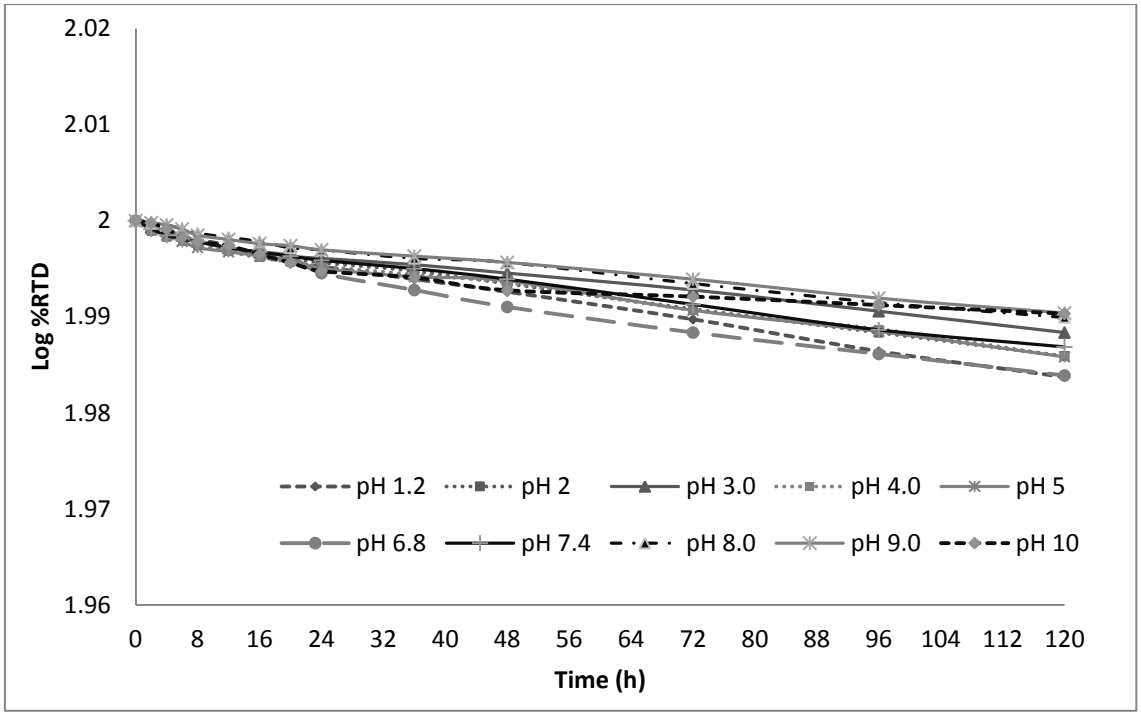

Figure 4.1: Solution state stability analysis of MIL in buffer solutions.

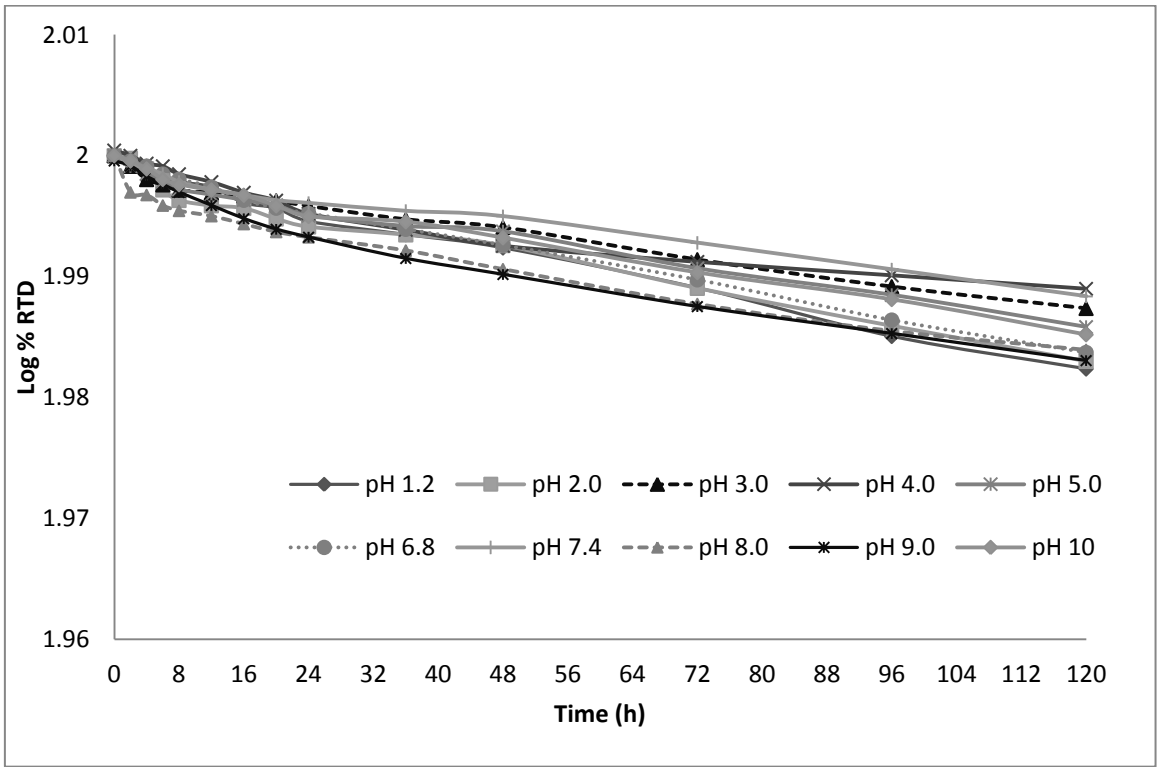

Figure 4.2: Solution state stability analysis of MIL in unbuffer solutions.

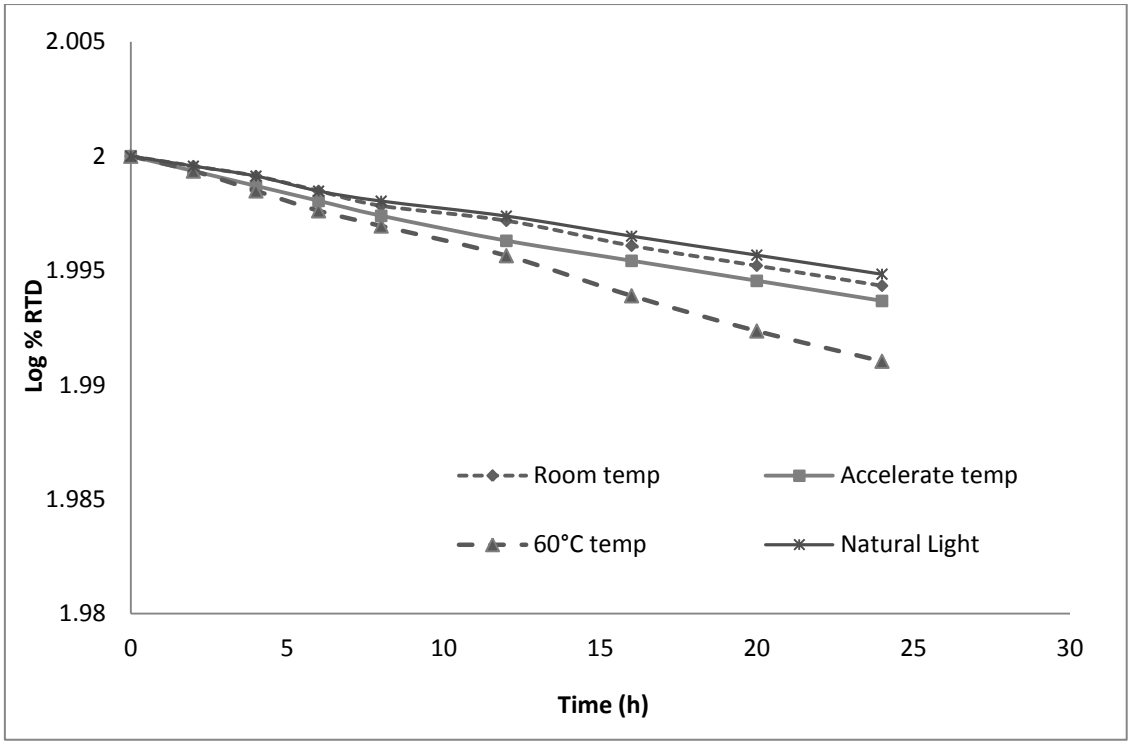

Figure 4.3: Solution state stability analysis of MIL in different thermal conditions.

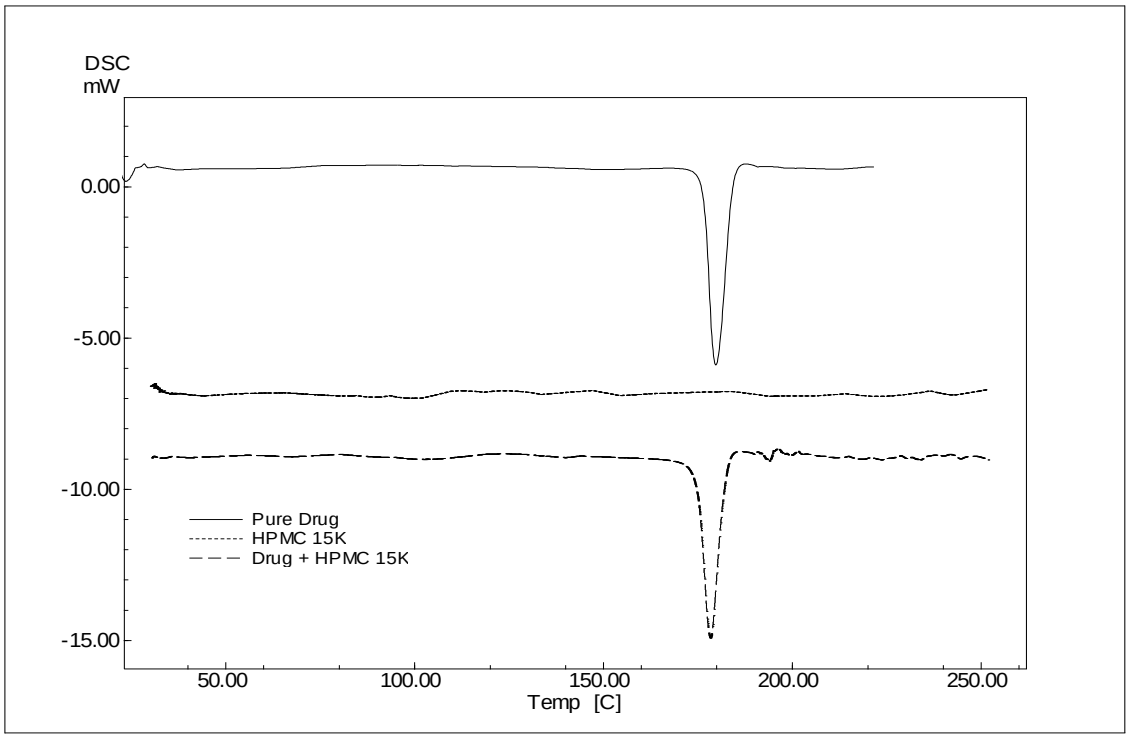

Figure 4.4: DSC thermograms of MIL, HPMC 15K and mixture of both.

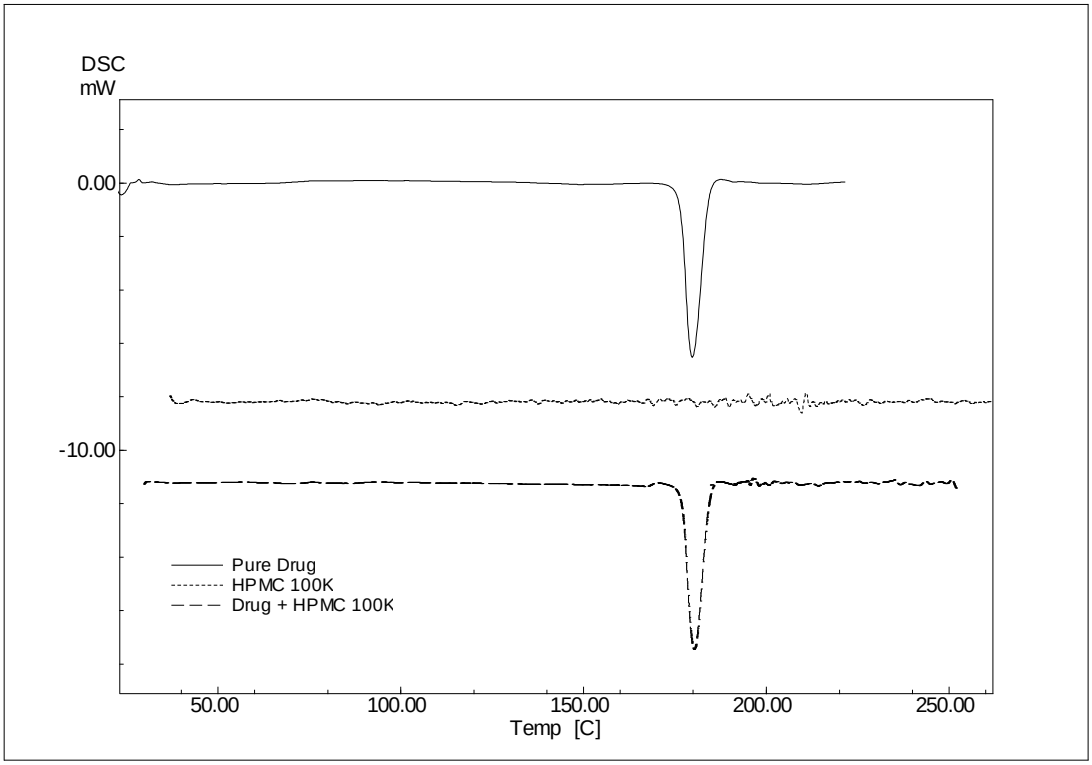

Figure 4.5: DSC thermograms of MIL, HPMC 100K and mixture of both.

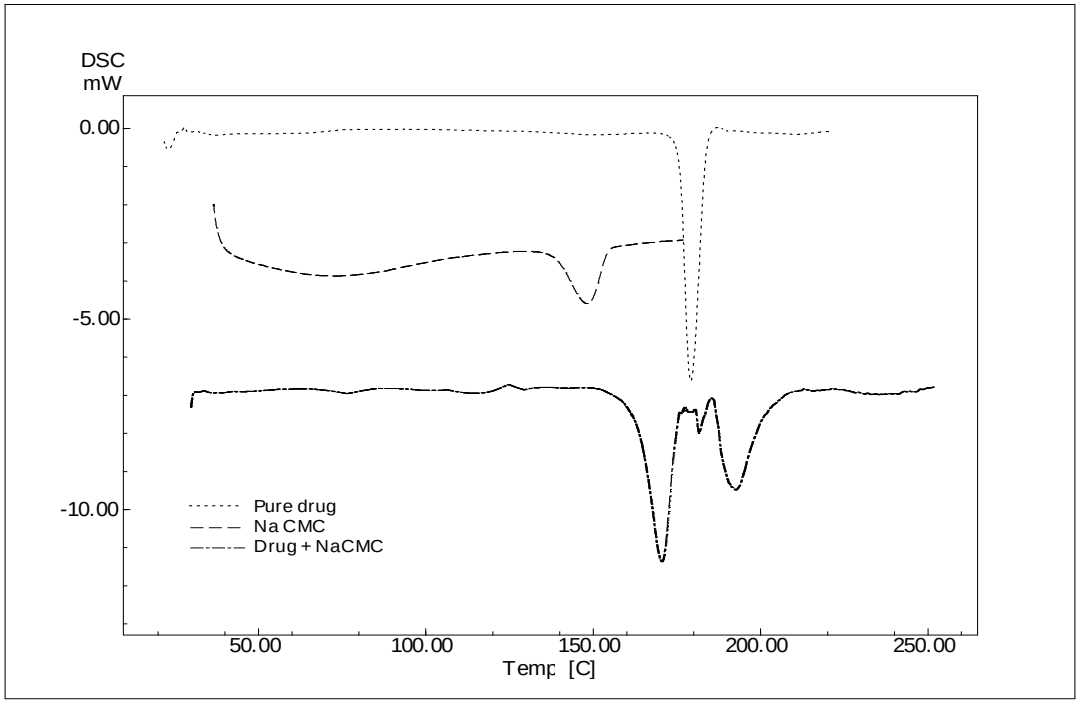

Figure 4.6: DSC thermograms of MIL, Sodium CMC and mixture of both.

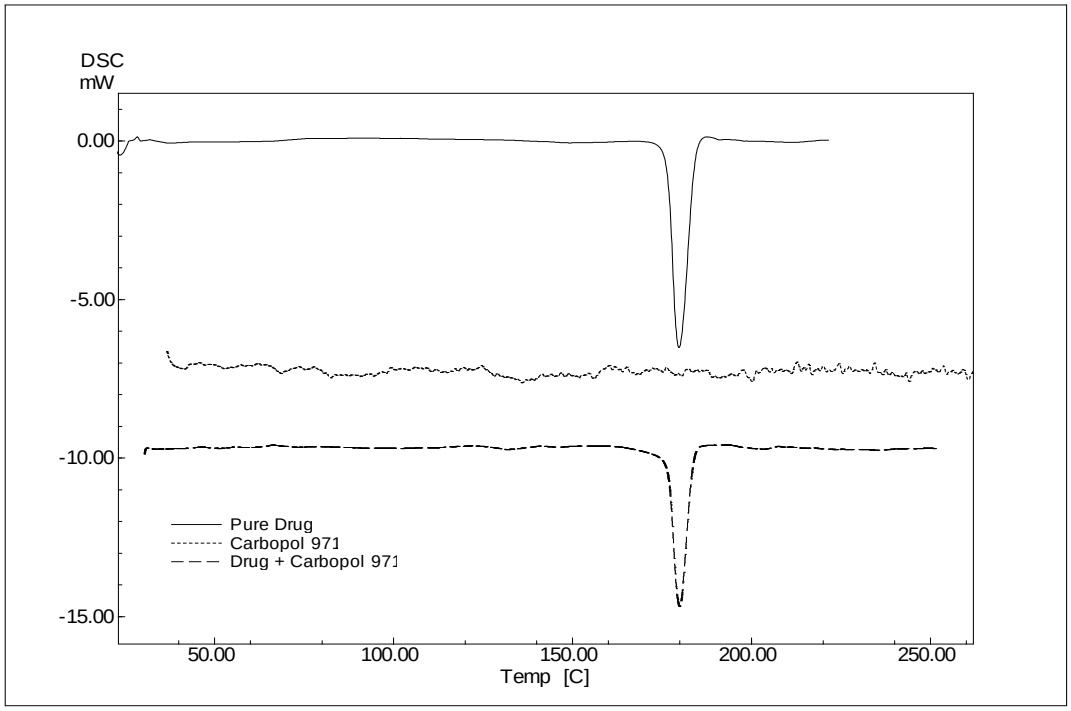

Figure 4.7: DSC thermograms of MIL, carbopol 971 and mixture of both.

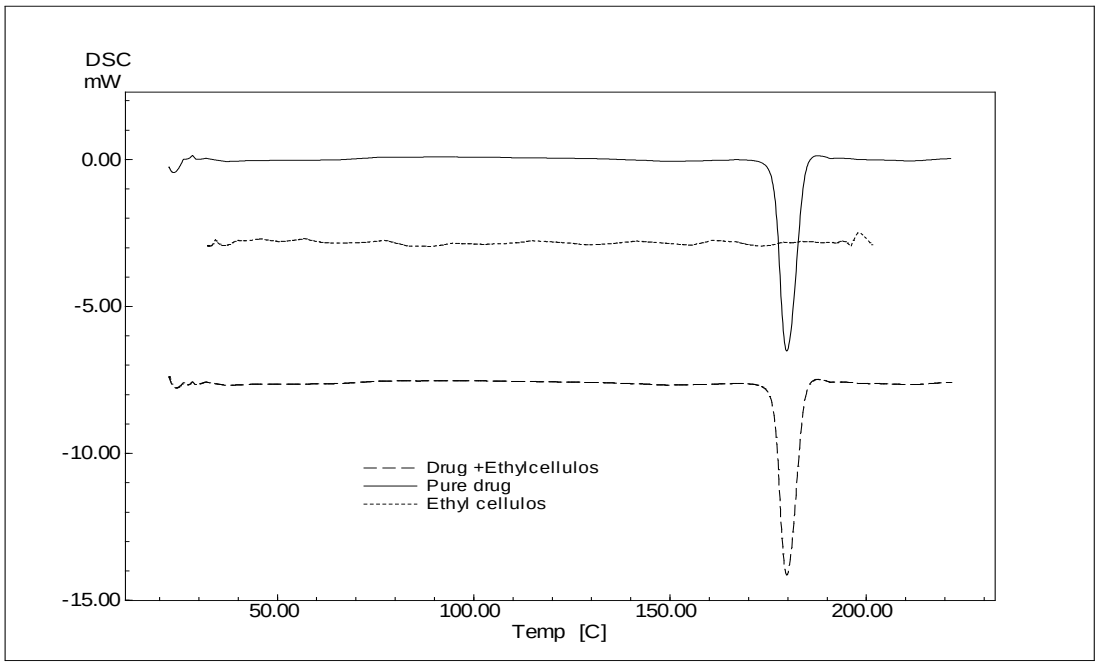

Figure 4.8. DSC thermograms of MIL, ethyl cellulose and mixture of both.

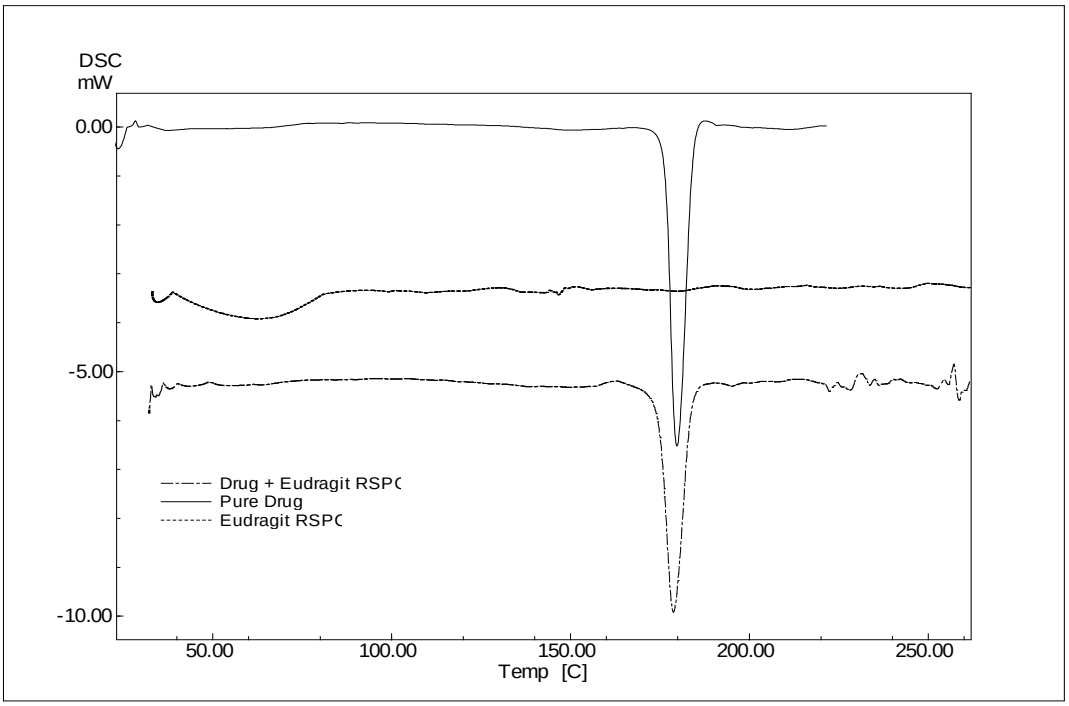

Figure 4.9: DSC thermograms of MIL, Eudragit and mixture of both.

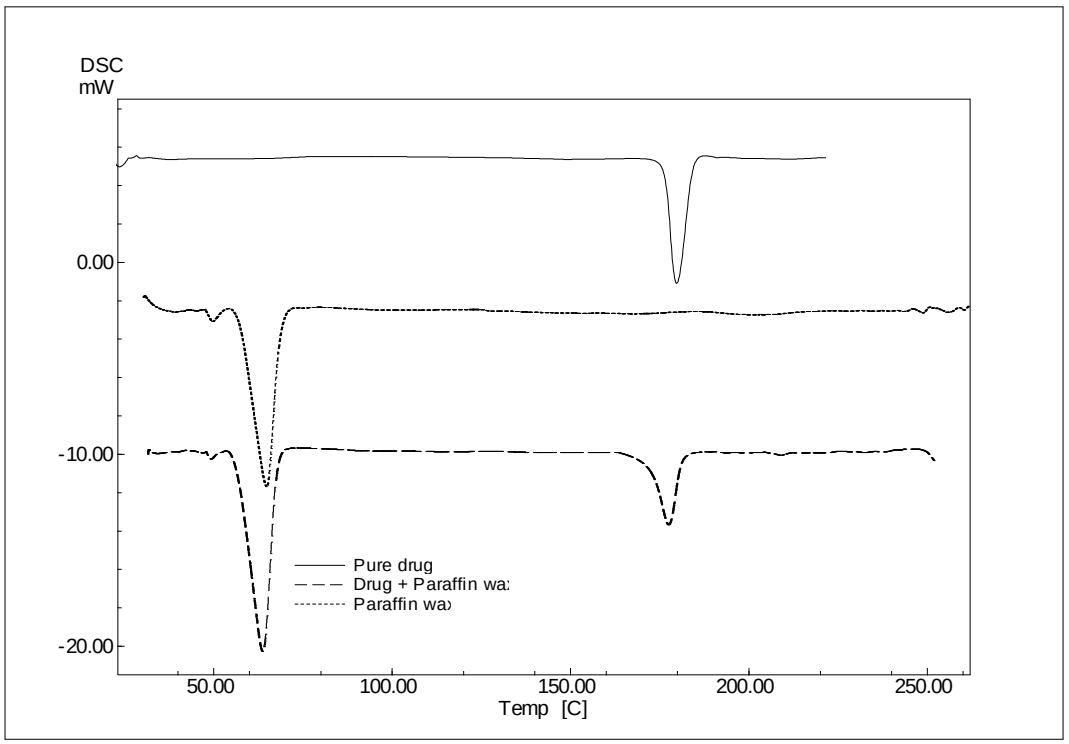

Figure 4.10: DSC thermograms of MIL, paraffin wax and mixture of both.

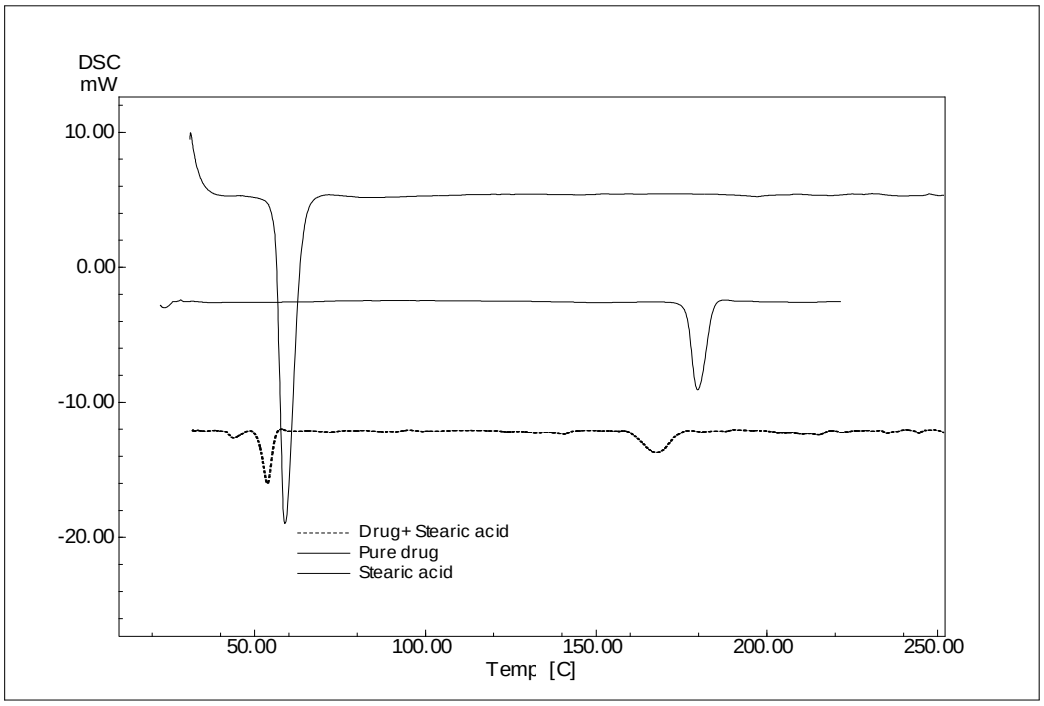

Figure 4.11. DSC thermograms of MIL, stearic acid and mixture of both.

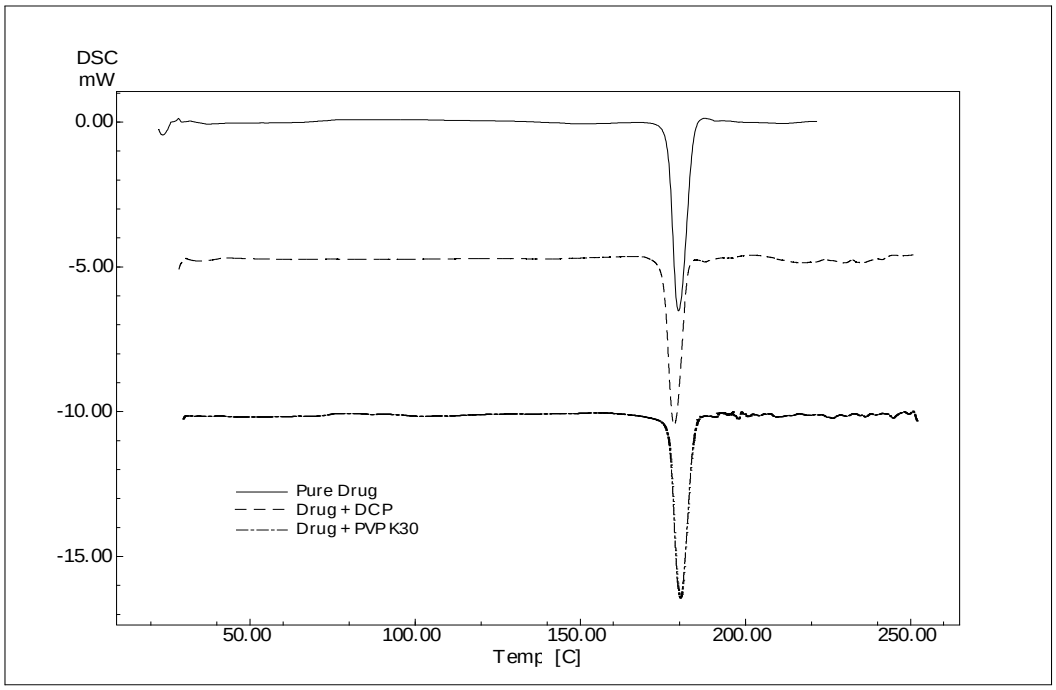

Figure 4.12: DSC thermograms of MIL and mixture of MIL with DCP and PVP K-30.

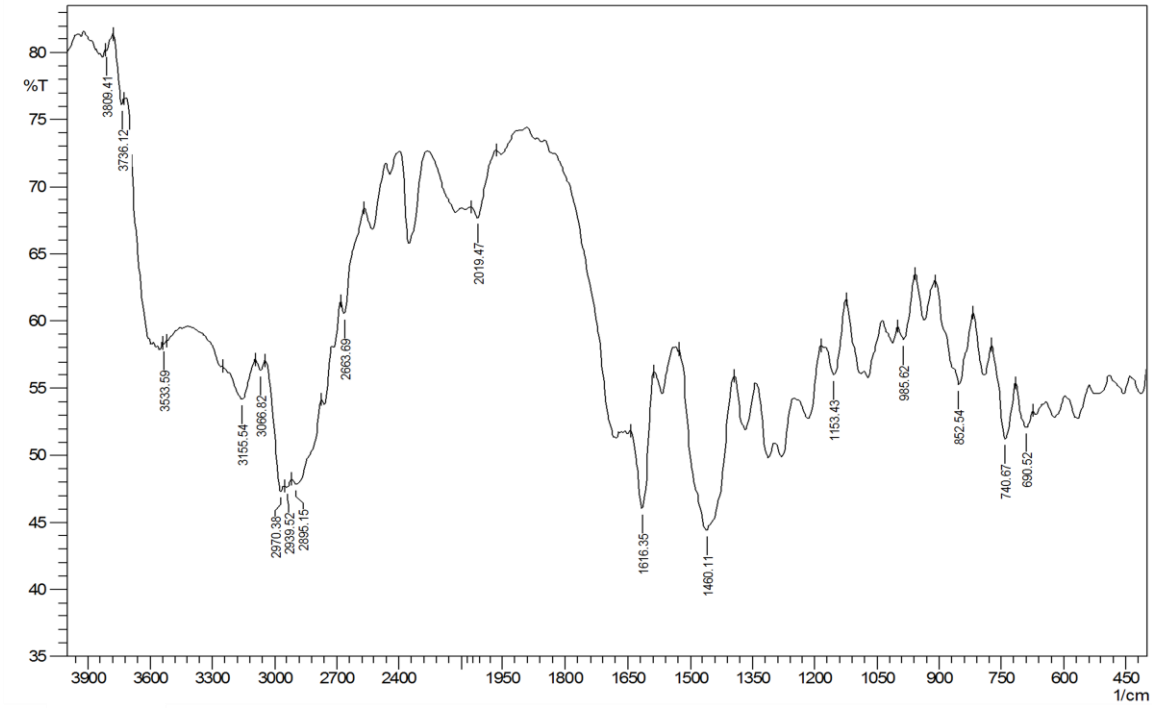

Figure 4.13: FTIR spectra of pure MIL.

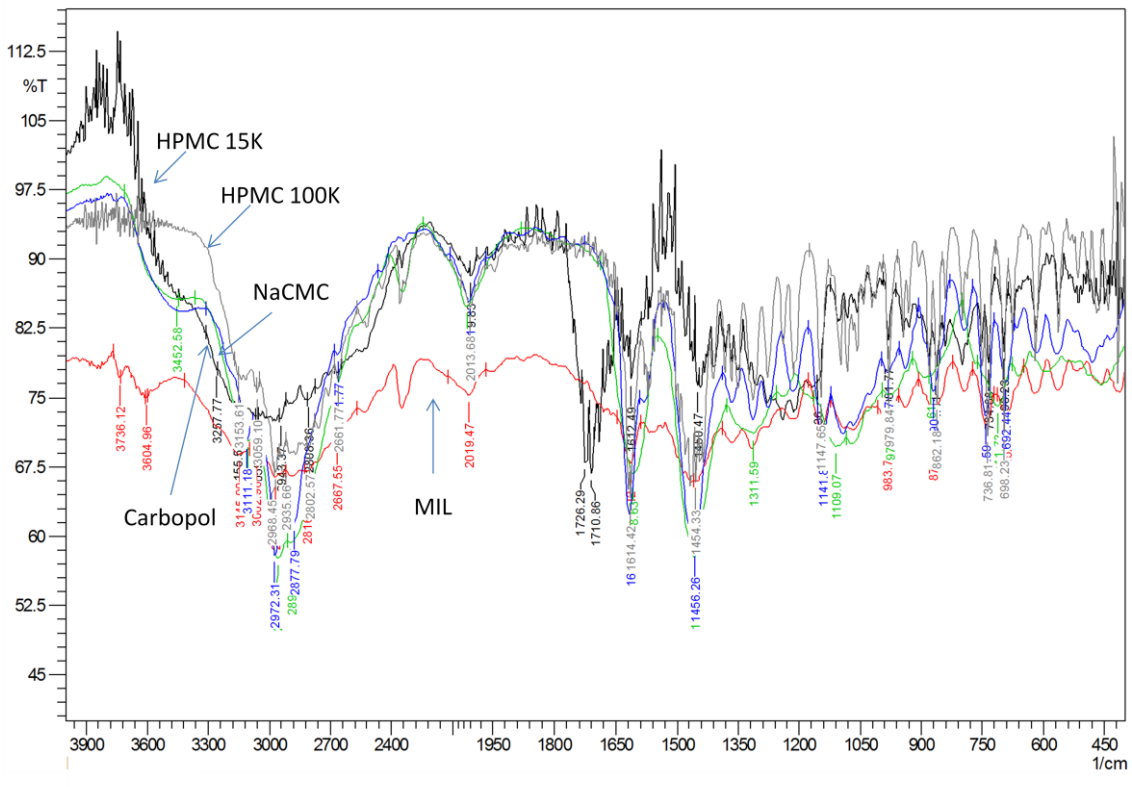

Figure 4.14: FTIR spectra of MIL with hydrophilic polymers (HPMC 15K, HPMC 100K, carbopol, sodium CMC).

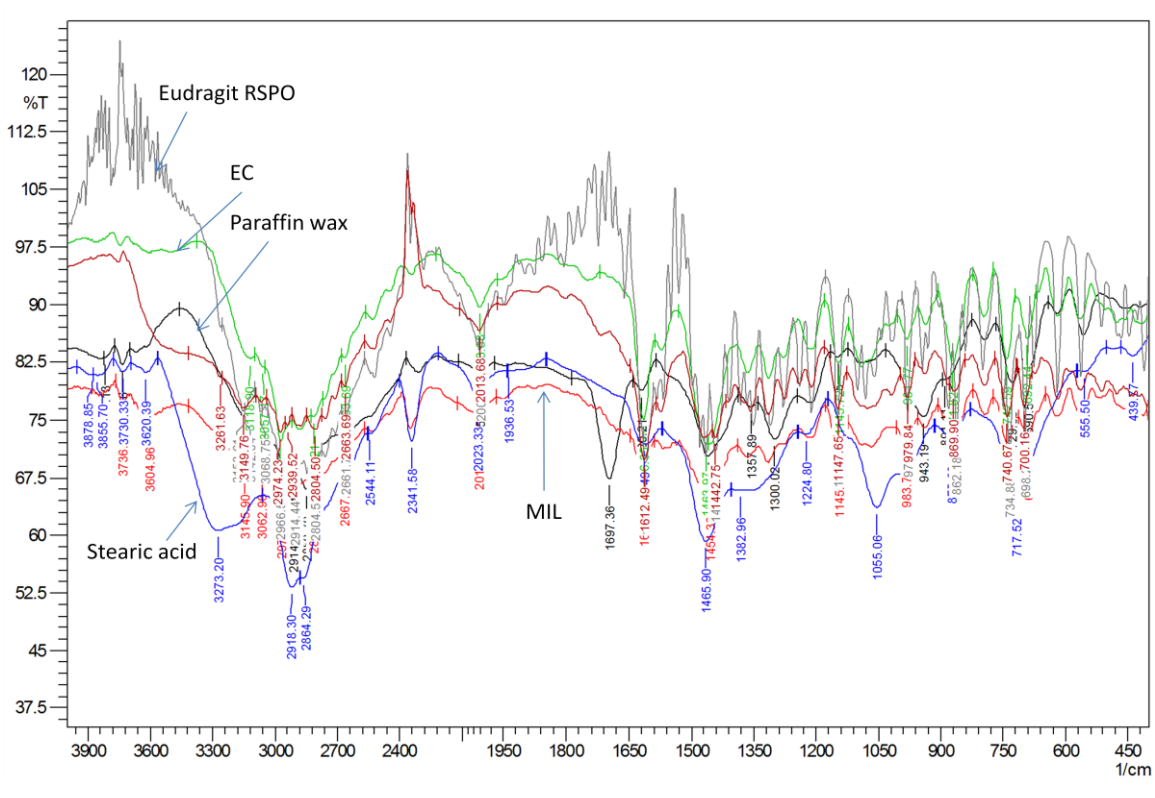

Figure 4.15: FTIR spectra of MIL with Hydrophobic Polymers (Ethyl cellulose, Eudragit RSPO, paraffin wax, cetostearyl alcohol and stearic acid)

CHAPTER 5

FORMULATION DEVELOPMENT AND IN-VITRO CHARACTRERIZATION

5.1 Introduction

Milnacipran hydrochloride (MIL) is commercially available as immediate release (IR) tablets and capsules formulations. Frequent dosing requirement due to its short elimination half life and number of associated side effects like nausea, vomiting, constipation, etc make it poor patient compliance and let to discontinuation [1-2]. Thus, there was an opportunity to formulate controlled release (CR) formulation of MIL, which will reduce the frequency of dosing, the incidence and intensity of side effects with better patient compliance with improved and economic therapy for the treatment of depression and fibromyalgia [3].

Very few works have been reported on CR formulation of MIL. Parejiya et al. worked on 'Tab in Tab' type controlled release formulation [4] and formulated a controlled release osmotic tablet of MIL [5]. These reported approaches were tedious, costly and various critical process parameters were involved. Moreover, as they were manufactured by complicated process, so there may be possibility of varying drug release rate at commercial production scale. Thus, there was a need of simple, economic, scalable and reproducible process for controlled release formulation of MIL, which can be scaled up at industrial level.

The use of polymeric matrices has become extremely popular in controlling the release rate of drugs from solid dosage forms. For simple and economic process development, these systems have been preferred [6-7]. Therefore, matrix based CR tablet formulations were decided for this project due to economic and easy process as well as the high reproducibility.

In the present study, two main approaches were selected (i) hydrophilic matrix based CR tablets (ii) multi granules based CR tablets using hydrophilic and hydrophobic polymers.

For the design of hydrophilic matrix CR formulation, rapid hydrating and swelling polymers were used as release retardant. Early preformulation studies revealed that MIL was highly water soluble drug (more than 1000 mg/ml at 27°C) at all pH range. For such very highly soluble drug, quick gel forming agent was required. A hydrophilic polymer can be suitable for such a polymeric matrix formulation, which hydrate immediately as it comes in contact with water [6-8]. A hydrophilic matrix based system is a homogeneous dispersion of drug molecules within a skeleton in

which one or several hydrophilic polymer incorporated that swells upon contact with water. The mechanisms of drugs release are complex and involve different processes: the entry of the aqueous medium into the matrix, swelling of the matrix, dissolution of the drug in the medium, diffusion of the drug through the gel layer, and erosion of the swelled matrix [8-9].

In this project water-soluble cellulose ethers like hydroxypropyl methylcellulose (HPMC), Sodium carboxymethylcellulose (NaCMC) and water insoluble cross-linked acrylic polymer carbopol were used as release retardant for hydrophilic matrix based CR preparation. Literature revealed that these polymers have been gained popularity in the formulation of oral formulations, due to their good swelling properties, their non-toxic nature, their ability to accommodate a large percent of drug and negligible influence of the processing variables on drug release rates [10-13].

Hydroxypropyl methylcellulose is the first choice for formulation of hydrophilic matrix system, providing robust mechanism, available in wide range of viscosity grades, nonionic nature, consistent reproducible release profiles, cost effectiveness and utilization of conventional equipment and methods [14-15]. Sodium carboxymethylcellulose, an anionic polymer is also one of the dominant hydrophilic carriers used in matrix tablet formulations [16-17]. Carbopol is a cross-linked polymer of acrylic acid with a high molecular weight that forms a hydrogel in aqueous solutions depending on the degree of hydration of the carboxyl group in carbopol. It is readily hydrated, absorb water and swell quickly up to 1000 times their volume to form a gel when exposed to pH environment above 4. Even though it is highly hydrophilic, it's largely used in controlled release dosage form because of its insoluble nature in water [18-19]. Among various grades of carbopol, 971P is highly retardant carbomer resin.

Some literature study revealed that a combination of hydrophilic and hydrophobic polymers in a matrix could be suitable for controlling the drug release for prolong time [20-21]. Hydrophilic polymers have advantage of rapid hydration and formation of viscous gel layer to restrict the drug percolation [8] whereas hydrophobic polymers not only act as water repellent surface but also provide several advantages, ranging from good stability at varying pH values and moisture levels [20]. Thus, to combine the advantages of both hydrophilic and hydrophobic retardant in single dosage form, combination of both types of granules in a compact mass can be excellent to

extend the drug release for sufficient time period at low concentration of polymers [21]. Therefore, in the present work, simple, economic, scaleable and reproducible multi granules based controlled release tablet formulation was designed using combination of hydrophilic and hydrophobic granules.

The adjustment of the polymer concentration, the viscosity character and the addition of different types and levels of excipients to matrix can modify the drug release rate. The main challenge in the formulation of these systems lies in achieving a suitable rate of drug release to obtain therapeutic plasma drug level over the intended time period. Accordingly, the biopharmaceutical and pharmacokinetic aim must be attained with the available technological resources. Thus, a deep knowledge of the factors affecting the release rate of drug is crucial for the correct technological development of sustained release systems.

However, by thorough study, no literature has been found on polymeric matrix based CR tablets of MIL prepared using HPMC 15K, HPMC 100K, carbopol, NaCMC and wax as retardant materials. Multi granules approach was first time used for MIL in this study. Moreover, study of formulation variables on drug release from hydrophilic matrix and multi granules compact of MIL was also studied in present work. In addition, various model dependent and model independent approaches for in-vitro characterization of designed formulations of MIL were used in the present work.

In this chapter, studies involving design and in-vitro characterization of matrix embedded and multi granules based oral CR tablets of MIL were presented. Matrix embedded CR tablets of MIL were prepared using various polymers either alone or in combination by wet granulation process. Physical characterization of the developed formulations was done by various quality control tests. The effect of formulation variables like polymer proportion, viscosity of hydrophilic polymer, compression force, agitation speed and pH of dissolution medium on in-vitro release characteristics were also studied with various dissolution parameters. Stability of the developed formulations was assessed at various temperature and humidity conditions. Batch reproducibility of the developed formulations was also assessed.

5.2 Materials

Pure milnacipran hydrochloride was obtained as gift sample from Torrent Pharmaceutical limited, Ahmedabad, Gujarat, India. The polymers like

hydroxypropyl methylcellulose 15000 cPs (HPMC 15K : Methocel K15M Premium) and 100000 cPs (HPMC 100K : Methocel K100M Premium)], ethyl cellulose (Ethocel^{tm} Standard Premium, 10 cPs) and sodium carboxy methyl cellulose were obtained as gift samples from IPCA Laboratories, Mumbai, India. Hydrophobic polymer Eudragit RSPO obtained as gift samples from Dr Reddy's laboratory Hyderabad, India. Carbopol obtained as gift sample from Lubrizol, Mumbai, India. All other chemicals used were of analytical grade and purchased from Qualigens, Mumbai.

5.3 Equipment/Instruments

A 10-station tablet compression machine (Rimek rotary tabletting machine, Mini Press I, Ahmedabad, India) using round, flat face, beveled edge punches of 7 and 9 mm was used for manufacturing the tablets. Standard screw gauge was used for measuring the thickness and diameter of the tablets. A five digit analytical balance (Mettler Toledo, Switzerland) was used for all the weighing purposes. Friability of the designed tablets was determined in a friability tester (Campbell Electronics, Mumbai, India). In-vitro release studies were carried out using USP Type II dissolution apparatus (Dissolution Tester (USP), TDT-08L, Electrolab, Mumbai, India). Tablet hardness was determined using a Monsanto tablet hardness tester (Campbell Electronics, Mumbai, India). The pH was determined on a pH meter (Eutech pH meter, Mumbai, India) equipped with a combined glass electrode. Humidity and temperature control cabinets (Thermolab, India and Wadegati, India) were used for stability studies of developed formulations. Analytical instruments mentioned in chapter 3 were used for all sample analysis.

5.4 Methods

5.4.1 Formulation of controlled release matrix tablets of milnacipran using hydrophilic polymers

Matrix embedded CR tablet formulations of MIL were prepared using various proportions of hydrophilic release retardant polymers HPMC 15K or HPMC 100K or NaCMC or carbopol 971P alone or in combinations as presented in Table 5.1 (a), (b) and (c). Di basic calcium phosphate (DCP) was used as diluent. DCP, an insoluble filler, was used to improve compressibility and to control initial burst release. The tablets were manufactured by wet granulation process using polyvinyl pyrrolidone (PVP) K-30 binder in isopropyl alcohol as granulating solvent. The drug, polymer and

DCP (passed through 30# mesh) were mixed uniformly and granulated with binder solution and dried in a tray drier at 50°C for 20-25 min. The dried granules were sized through mesh 20#. The final granules were blended with talc and magnesium stearate and compressed on 10-station tablet compression machine using round, flat face, and beveled edge punches of 9-mm diameter at different compression forces. Three batches were prepared for each formulation with each tablet containing 100 mg MIL.

5.4.2 Formulation of multi granules based controlled release tablets of milnacipran hydrochloride

Controlled release tablets of MIL were also formulated with various proportions of hydrophilic polymer granules and hydrophobic wax or polymeric granules as shown in Table 5.2 (a) and (b). Both type the granules were prepared separately as given below:

Preparation of hydrophilic polymer granules:

Hydrophilic granules were prepared by wet granulation method. Drug was mixed uniformly with hydrophilic polymer and DCP then granulated with PVP K-30 using isopropyl alcohol as granulating solvent. The mass was dried and sieved through 16-mesh size.

Preparation of hydrophobic wax granules:

Hydrophobic wax granules were prepared by melting stearic acid and paraffin wax at 80°- 85°C in a water bath. Uniform mixture of drug and DCP was added to molten wax with continuous agitation. The molten mass was allowed to cool at room temperature. The congealed solid mass was then sieved through 16-mesh size.

Preparation of hydrophobic polymeric granules:

These granules were prepared by wet granulation process. Drug was mixed uniformly with diluent DCP and hydrophobic polymer ethyl cellulose and Eudragit RSPO then granulated with PVP K-30 using isopropyl alcohol as granulating agent. The mass was dried and sieved through 16 mesh.

The final granules were blended with talc and lubricated with magnesium stearate. Then these granules were compressed on 10-station tablet compression machine using round, flat face, beveled edge punches of 9-mm diameter at

different compression forces. Three batches were prepared for each formulation with each tablet containing 100 mg MIL.

5.4.3 Physical characterization of the designed tablets

Various quality control test like weight variation, thickness, hardness, friability were carried out for physical characterization of prepared tablets as per Indian Pharmacopeia [22]. The weight variation was determined by taking weight of 20 tablets using an electronic balance. The weight data were analyzed for percent variation and mean weight. The crushing strength of tablets was determined using Monsanto hardness tester. Friability was determined by testing 10 tablets in a friability tester for 4 min at 25 rpm (100 revolutions).

5.4.4 Drug content

The drug content of the manufactured tablets of each batch was determined in triplicate. For each batch, 20 tablets were taken, weighed and finely powdered. An accurately weighed quantity of this powder was taken and suitably dissolved in pH 1.2 buffer, and analyzed using UV spectrophotometric method reported in chapter 3.

5.4.5 In-vitro release study

In-vitro drug release test (six replicates) of designed formulations was carried out with USP Dissolution Testing Apparatus type II Paddle. The dissolution test was performed using 900 ml of 0.1N HCl (pH 1.2) and pH 6.8 phosphate buffer at 37 ± 0.2°C and 50 rpm. At predetermined time points (1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h) 10 ml of the dissolution samples were withdrawn from the dissolution jars and replaced with fresh dissolution medium. The samples were suitably diluted and analyzed with UV spectroscopic method reported in chapter 3. Percentage cumulative drug release (% CDR) was calculated for data analysis.

5.4.6 In-vitro release characterization

The drug release of all the formulations and variables were analyzed and tried to fit with various release kinetic models such as zero order, first order, higuchi and *korsmeyer-peppas* model. Drug release were also compared with model independent dissolution parameters like time for 50% drug release (T50%), time for 80% drug release (T80%) and mean dissolution time (MDT) as discussed in Chapter 1.

5.4.7 Effect of various formulation and dissolution parameters on drug release

Formulations were also prepared and evaluated for study the effect of various formulation and dissolution media variables. The following variations in tablet formulae and dissolution parameters were done and their effect on in-vitro release rate, release mechanism and dissolution characteristics (T50%, T80% and MDT) were studied.

(a) Effect of polymer proportions

CR release tablets were prepared with 50, 100, 150 and 200 mg of HPMC 15K, HPMC 100K and NaCMC. In the case of carbopol based formulations, tablets were formulated with 10, 25, 50, 75 and 100 mg of carbopol 971P. For multi granules based formulations, tablets were prepared with only hydrophilic granules, only hydrophobic granules and combination of hydrophilic and hydrophobic in the proportion of 70:30 and 50:50 respectively.

(b) Effect of polymer viscosity

Two different viscosity grade HPMCs, 15K and 100K were used in the present investigation to study the effect of viscosity on drug release. In case of carbopol matrix, carbopol 971P and 974P were used to study the effect of viscosity of polymer on drug release. In case of multi granules based CR tablets, HPMC 4K, HPMC 15 and HPMC 100K were used.

(c) Effect of compression force

For this study, formulations were compressed at three different compression force levels required to produce tablets of about 4, 7 and 10 kg/cm^2.

(d) Effect of pH of dissolution media

In-vitro dissolution study of selected formulations was carried out in two different dissolution medium pH 1.2 and pH 6.8.

(e) Effect of agitation speed

For this study, in-vitro dissolution study of selected formulations was performed at three different agitation speed 50, 100 and 150 rpm of paddle.

5.4.8 Swelling and erosion studies

Swelling and erosion studies of the matrix tablets were carried out under conditions identical to those described for the dissolution testing. After keeping first 2 h in 0.1 N HCl and then in pH 6.8phosphate buffer, the tablets were removed, gently wiped with a tissue paper to remove surface water.

Water uptake and mass loss were determined gravimetrically according to the following equations:

$$\text{Degree of swelling or water uptake} = \frac{\text{Wet weight - Original dry weight}}{\text{Original dry weight}}$$

Matrix erosion studies were performed by a method similar to those of Roy and Rohera [23]. After the swelling studies, the wet samples were then dried in an oven at 80°C for 24 h time period, allowed cooling in desiccator and finally weighed until constant weight was achieved. The experiment was performed in triplicate for each time point. The tablet erosion at different times was estimated from the following equation:

$$\text{Erosion (\% mass loss)} = \frac{\text{Original weight - Remaining dry weight}}{\text{Original weight}}$$

5.4.9 Batch reproducibility and stability on storage

In order to test the reproducibility and robustness of technology of the optimized formulation, three separate batches of optimized formula were prepared with previously discussed granulation and tableting processes. These three batches were tested for physical properties and drug release. Release profile of each batch was compared with one another by means of the f_2 similarity factor.

To study the effect of storage conditions on stability and release profile of designed formulations, the tablets of all the formulations were sealed in airtight cellophane packets and stored at 5°±3°C, 25°±2°C/60±5% RH (long term storage condition) and 40°±2°C/75±5% (Accelerated storage condition) as per ICH guidelines [24]. Physical characteristics and in-vitro drug release behavior of the formulations were studied up to 12 months for determining the effect of storage.

5.5 Results and discussion

5.5.1 Physical characterization of the designed tablets

Compressed tablets were evaluated for their physical characterization. All physical quality parameters of the designed CR formulations of MIL were found to be within the acceptance limit [22]. The quality control tests such as hardness (between 3.5 -10.5 kg/cm^2), friability (not more than 0.7% w/w), weight variation (not more than ± 2.5 %) and content uniformity (98.0% to 102.0%) of all tablet formulations were found to be satisfactory and reproducible as observed from the data in Table 5.3 (a), (b) (c) and Table 5.4. The results indicated that the wet granulation method was an acceptable method for preparing good quality matrix tablets of MIL.

5.5.2 In-vitro drug release studies

5.5.2.1 Formulation of controlled release matrix tablets of milnacipran hydrochloride using hydrophilic polymers

Effect of various formulation parameters on drug release were studied as follows:

(a) Effect of polymer proportion and viscosity of polymers

Formulations prepared with HPMC

Controlled release matrix tablets were prepared with different viscosity grade of HPMC (15K and 100K) using different polymer proportions. A plot of cumulative percent drug released versus time for HPMC 15K matrix tablet formulations (H15K-1, H15K-2, H15K-3 and H15K-4 containing 50, 100, 150, 200 mg of HPMC 15K respectively) and HPMC 100K matrix tablet formulations (H100K-1, H100K-2, H100K-3 and H100K-4 containing 50, 100, 150, 200 mg of HPMC 100K respectively) are shown in Figure 5.1 and 5.2. Various release parameters were evaluated as presented in Table 5.5.

Release profiles of all the tablet matrices indicated that the release was significantly dependent on the proportion of polymer used. As the polymer level increased from 50 mg to 200 mg the release rate decreased.

In case of matrices that contained HPMC 15K, the initial release for first two hours varied between 25% and 52% depending on polymer proportion. The release of the drug extended from 10 h to 16 h, as polymer concentration increased from 50 to 200 mg in tablets. Among these formulations, release rate was fastest from formulation

containing 50 mg of HPMC 15K with a release rate constant K value of 41.86 $h^{-0.37}$. The release rate was slowest for formulation containing 200 mg of HPMC 15K with K value of 21.84 $h^{-0.54}$. The MDT, T50% and T80% values increased from 2.81 to 5.74 h; 4.69 to 7.70 h and 6.85 to 12.32 h respectively as HPMC 15K proportion increased from 50 mg to 200 mg in the formulations.

In case of formulations containing HPMC 100K, the initial release for first two hours varied between 25% and 46% depending on HPMC 100K polymer proportion, but the release was found more controlled in later stages in the tablets with a higher proportion of the polymer as indicated in Figure 5.2. A pattern of decreased release rate and extended drug release time was observed with increased polymer proportions. The release of the drug extended from 12 h to 24 h when polymer concentration increased from 50 to 200 mg in formulations. Among HPMC 100K formulations, release rate was fastest from formulation containing 50 mg of polymer with K value of 33.92 $h^{-0.419}$. The release rate was slowest for formulation containing 200 mg polymer with K value of 19.08 $h^{-0.538}$. Dissolution parameters such as MDT, T50% and T80% values increased from 3.29 to 7.21 h; 4.28 to 9.62 h and 7.51 to 15.39 h respectively as HPMC 100K proportion increased from 50 mg to 200 mg in the formulations as presented in Table 5.5.

All the HPMC 15K and 100K formulations were found to be following *korsmeyer-peppas* release model with regression value 0.9709 to 0.9997. The release exponent 'n' of *korsmeyer-peppas* model inferred that release from the formulation with low polymer concentration (50 mg to 100 mg) was dependent on the diffusion of drug through polymeric matrix where as at high polymer concentration the release was dependent on both drug diffusion as well as polymer relaxation [25-26].

Results of above study indicated that increase in polymer ratio in polymeric matrix resulted in the decrease in the drug release rates. The reason may be, as the proportion of these polymers in the matrix increased, there was an increase in the amount of water uptake and proportionally greater swelling leading to a thicker gel layer with a longer diffusional path. This could have caused a decrease in the effective diffusion coefficient of the drug and therefore a reduction in the drug release rate [27-28]. The reason for initial high drug release can be due to high diffusivity of drug and lag time of polymer hydration in dissolution fluid. Initially, drug close to matrix surface

might be released before the surrounding polymer reached to polymer disentanglement or swelling [29].

The effect of viscosity of HPMC (15K vs 100K) on the drug release from formulations containing the same proportion of polymer (200 mg as representative) is shown in Figure 5.3. As the viscosity of HPMC increased from 15000 cps to 100000 cps (H15K to H100K), the release rate extended from 10 h to 24 h. A significant difference was observed for all dissolution parameters when compared HPMC 15K (H15K-1 to H15K-4) with HPMC 100K (H100K-1 to H100K-4) with same proportion of polymers.

This can be justified as the polymer of higher viscosity induces greater chain entanglement than a polymer of lower viscosity. Higher viscosity grades are fast hydrating and form a mechanically stable gel layer. Fast hydrating polymers show rapid gel development, limiting initial dose dumping from a matrix and extending the period of release [30]. In addition, presence of drug and other excipients alters the hydration rate of polymers. A mechanically stable gel layer provides a more tortuous and resistant barrier to diffusion, resulting in a slow release [31-32]. Therefore, it is time taking to pass a molecule through such viscous hydrogel. The release rate was faster with lower viscosity grades of HPMC, probably owing to less polymer entanglement and less gel strength and to the larger effective molecular diffusional area at lower viscosity as compared with higher viscosity grades of HPMC [33-34].

Formulations prepared with NaCMC alone

In-vitro release studies of formulations CMC-1, CMC-2, CMC-3 and CMC-4 containing 50, 100, 150 and 200 mg of NaCMC respectively indicated that increasing the concentration of the polymer in the matrix lead to slower drug release as presented in Figure 5.4.

Matrix that contained lower concentrations of NaCMC tended to release the drug in shorter time periods. The initial release for first two hours varied between 35% and 55% depending on polymer proportion, but the release was found more controlled in later stages in the tablets with a higher proportion of the polymer. The initial burst effect was probably due to the fact that the gel layer, which controls the release of the drug, needs some time to become effective [35] . The release of the drug extended from 10 h in the case of 50 mg (CMC-1) to 20 h in the case of 200 mg (CMC-

4) NaCMC concentration. The MDT values were found to be 2.61, 3.12, 3.78 and 4.66 h respectively for formulations CMC-1 to CMC-4. The T50% and T80% values increased from 1.63 to 3.13 h and 5.17 to 9.51 h respectively as NaCMC increased from 50 mg to 200 mg in the formulations. The release rate also decreased with increase in polymer concentration in formulations as shown in Table 5.6. The release exponent 'n' values of *korsmeyer-peppas* model for NaCMC based formulations indicated that the release was predominantly dependent on drug diffusion through polymer.

The high water solubility and diffusivity of drug and lag time of polymer hydration in dissolution fluid might be reasons for high drug release in first few hours. Literature also revealed that NaCMC has comparatively less gel strength than high viscosity grade of HPMC, which resulted in poor retardation of high soluble drug MIL with alone NaCMC polymer [36].

Formulations prepared with combinations of NaCMC and HPMC 15K

Combinations of HPMC 15K and NaCMC polymers in different proportions are shown in Table 5.1 (b). A plot of cumulative percent drug released versus time of MIL from formulations H15K/CMC-1, H15K/CMC-2, H15K/CMC-3 and H15K/CMC-4 containing combinations of HPMC 15K and NaCMC are shown in Figure 5.5. Various release parameters were determined as shown in Table 5.6.

The release rate decreased and the drug release extended as increased in the total polymer proportion (NaCMC + HPMC) from 100 mg to 200 mg in the matrix as presented in Figure 5.5. Table 5.6 also illustrated that dissolution parameters such as MDT, T50% and T80% values increased from 4.38 to 6.90 h; 2.38 to 5.23 h and 8.93 to 13.60 h respectively as total polymer proportion increased from 100 mg to 200 mg in matrices. This can justified as the proportion of these polymers in the matrix increased, there was an increase in the amount of water uptake and proportionally greater swelling leading to a thicker gel layer with a longer diffusional path. This could cause a decrease in effective diffusion of drug and therefore reduction in drug release rate.

The release rate was greatly influenced by proportion of NaCMC and HPMC 15K in the matrix. As the proportion of NaCMC increased from 50 mg to 100 mg with constant 50 mg of HPMC 15K polymer (H15K/CMC-1 vs H15K/CMC-2) in the matrix, the MDT, T50% and T80% values increased from 4.38 to 5.02 h; 2.38 to

2.79 h and 8.93 to 10.95 h respectively. Similar results were observed when HPMC 15K proportion increased from 50 to 100 mg. Interestingly, it was observed that the amount of HPMC 15K played a dominant role in these mixtures for release retardation. The MDT, T50% and T80% values increased from 4.38 to 6.21 h; 2.38 to 4.40 h and 8.93 to 12.62 h respectively as HPMC 15K proportion increased from 50 mg to 100 mg (H15K/CMC-1 and H15K/CMC-3).

Above study indicated that mixing of two cellulose ethers polymers, ionic and non-ionic, for the formulation of hydrophilic matrices, a valuable decrease in drug release rate can be achieved. NaCMC has been reported to have synergistic hydrogen-bonding interactions with HPMC [37-38]. The addition of a non-ionic cellulose like HPMC to NaCMC matrix increases the gel viscosity. This was attributed to the strong hydrogen bonding between the carboxyl groups on NaCMC and the hydroxyl groups on HPMC, leading to strong cross-linking between the two polymers [39].

The release rate was faster with NaCMC alone, probably owing to less polymer entanglement and less gel strength and also to the larger effective molecular diffusional area as compared with matrix of polymer mixture [40-41].

Release of all the formulations was found to fit with *korsmeyer-peppas* model with regression value 0.9854 to 0.9960. The release exponent 'n' indicated that release from the formulation with low polymer concentration (100 mg to 150 mg) was dependent on the diffusion of drug through polymeric matrix where as at high polymer concentration (200 mg) the release was dependent on both drug diffusion as well as polymer relaxation.

Formulations prepared with carbopol

In-vitro release profile of MIL from matrices CBL-1, CBL-2, CBL-3, CBL-4 and CBL-5 containing 10, 25, 50, 75 and 100 mg of carbopol 971P are shown in Figure 5.6. The initial release for the first two hours varied between 35% and 80% depending on polymer proportion. The release of the drug extended from 6 hours in the case of 10 mg (CBL-1) to 24 hours in the case of 100 mg (CBL-5) polymer concentration. The release was fastest (K value 59.53 $h^{-0.30}$) with 10 mg and slowest (K value 25.95 $h^{-0.37}$) with 100 mg of carbopol proportion in matrix. The MDT, T50% and T80% values increased from 1.42 to 6.17 h; 0.56 to 5.57 h and 2.64 to 19.81 h

respectively as carbopol 971P proportion increased from 10 mg to 100 mg in the formulations as shown in Table 5.7. The drug release was found to be best fit (r^2 > 0.9874) with *korsmeyer-peppas* model for study the release mechanism. The release exponent '*n'* values for all the formulation (0.30 to 0.37) indicated that release from the formulation was dependent on the diffusion of drug through polymeric matrix.

The results indicated that drug release was dependent on polymer proportion in tablets. An increase in polymer level, increases the viscosity of the gel layer and thus increases the diffusional path length. This could decrease the diffusion co-efficient of drug result in a reduction in drug release. The release was better controlled with low amount of carbopol as compare to HPMC and NaCMC. This can be explained with carbopol nature. Carbopol polymers have been known for their excellent selling properties. These polymers rapidly hydrate, absorb water, and swell quickly up to 1000 times of their volume. Thus, when carbopol matrix came in contact with release media a stable hydrated gel was formed that controlled the drug release. Due to the crosslinked nature of the polymer, the hydrogel was not simple entangled chains of polymer but discrete microgels made up of many polymer particles, in which the drug was dispersed. It has been also proved that drug release from such microgel depends upon drug solubility. Highly water-soluble drugs released mainly by diffusion [42-43]. The results of studied formulation were also following diffusion mechanism.

For study the effect of viscosity, carbopol 974P (75 mg) was used as matrix polymer using composition of CBL-4 formulation. The viscosity of carbopol 974P is more than 971P due to high cross linking. The release rate was found to be faster with carbopol 974P than carbopol 971P as shown in Figure 5.7. Release rate constants of *korsmeyer-peppas* model were found to be 36.78 $h^{-0.33}$ and 32.38 $h^{-0.35}$ for formulation containing carbopol 974P and carbopol 971P respectively. The MDT, T50% and T80% values decreased from 5.36 to 5.08 h; 3.48 to 2.57 h and 13.41 to 10.85 h as higher viscosity polymer used (Table 5.7). The above observation with polymer viscosity was opposite as compare to HPMC polymers. The reasons can be explained as follow. Upon hydration, carbopol 974P with high cross linked density formed non-uniform gel, consist of micro and macro viscosity region. This non-uniformity attributed to the higher number of channels present in their gel structure. Carbopol 971P with low crosslink density formed a uniform gel and this homogenous

gel structure offered high resistance to drug diffusion compared to grades with high degree of cross-linking [43-44].

(b) Effect of compression force on drug release

Formulations prepared with HPMC

The effect of compression force on the drug release was studied by preparing tablets using the same polymer proportion (100 mg) of HPMC 15K (H15K-2) and HPMC 100K (H100K-2) but with different compression forces to get tablets with different hardness level of 4, 7 and 10 kg/cm^2.

In case of formulations with HPMC 15K, the effect of compression force was more pronounced. Matrix with lower compression force (4 kg/cm2) sustained the drug release only up to 10 h. Tablets with 7 and 10 kg/cm^2 hardness extended the drug release up to 12 h and 20 h respectively as shown in Figure 5.8. The drug release from formulations prepared with low compression force (4 kg/cm^2) was found to be significantly much faster than compared to formulations prepared with higher compression forces. In addition, the MDT, T50% and T80% values also increased significantly as compression force increased from 4 to 10 kg/cm^2 as presented in Table 5.8. The values of release exponent 'n' suggested that drug release was predominantly diffusional controlled (n < 0.45) at all compression force as shown in Table 5.8. The f_2 (similarity factor) values were found to be 45.33 and 47.86 when drug release compared for tablets compressed at 7 kg/cm^2 with tablets compressed at 4 and 10 kg/cm^2 respectively. The f_2 values also proved significant difference between formulations of variable hardness.

In case of formulations with HPMC 100K matrix, tablets with lower compression force (4 kg/cm2) sustained the drug release only up to 12 h. Tablets with 7 and 10 kg/cm^2 hardness extended the drug release up to 16 h and 24 h respectively as presented in Figure 5.9. Data compiled in Table 5.8 indicated the similar pattern of observation as compression force increased from 4 kg/cm^2 (lower compression force) to 10 kg/cm^2 (higher compression force) the MDT, T50% and T80% values increased markedly. At higher compression force (10 kg/cm^2) release mechanism was found to be anomalous or non-Fickian diffusion (n > 0.45) while at lower compression force release was found to be predominantly diffusional controlled (n< 0.45). This may be due to enhanced entanglement and rigidity of matrix at increased compression force. The f_2

values were found to be 48.54 and 42.60 when drug release compared for tablets compressed at 7 kg/cm^2 with tablets compressed at 4 and 10 kg/cm^2 respectively. The f_2 values also proved significant difference between formulations of variable hardness.

Formulations prepared with sodium CMC

The effect of compression force was studied with formulation CMC-2 containing 100 mg of NaCMC alone and formulation H15K/CMC-1 containing mixture 50 mg of NaCMC and 50 mg of HPMC 15K. Compression force was varied to get three different hardness levels, 4, 7 and 10 kg/cm^2.

In case of formulation CMC-2, the release rate decreased with increase in compression force as shown in Figure 5.10. At lower compression force (hardness 4 kg/cm^2) the release rate was higher and complete release was occurred within 10 h with release rate (K) of 46.83 $h^{-0.34}$. Tablets with 7 and 10 kg/cm^2 compression force extended the drug release up to 12 h and 20 h with release rate of 37.75 $h^{-0.410}$ and 32.80 $h^{-0.40}$ respectively. Dissolution parameters like MDT, T50% and T80% values increased from 2.38 to 4.66 h; 1.21 to 2.90 h and 4.75 to 9.50 h respectively as hardness increased from 4 to 10 kg/cm^2 (Table 5.9). The f_2 values were found to be less than 50 (49.7 and 47.5) which also confirmed the significant variation in drug release with varied compression force.

In case of formulation H15K/CMC-1 (NaCMC/HPMC 15K matrix), as shown in Figure 5.11, there was a significant difference in release profiles among formulations compressed at different hardness levels. The release was extended up to 12 h, 20 h and 24 h with formulations compressed at 4, 7 and 10 kg/cm^2 respectively. The release rate decreased with increase in compression force of NaCMC/HPMC 15K matrix tablets. The release rate constants, obtained from *korsmeyer-peppas* empirical equation, for the formulations with different hardness levels, 4, 7 and 10 kg/cm^2 were found to be 42.75 $h^{-0.0.36}$, 36.70 $h^{-0.356}$ and 27.88 $h^{-0.42}$ respectively. Table 5.9 indicated that dissolution parameters such as MDT, T50% and T80% values increased from 2.83 to 6.05 h; 1.54 to 3.99 h and 5.70 to 12.15 h respectively as hardness increased from 4 to 10 kg/cm^2. In addition, the f_2 values were found less than 50 (48.9 and 48.6) when drug release compared for tablets compressed at 7 kg/cm^2 with tablets compressed at 4 and 10 kg/cm^2 respectively. This also confirmed the influence of compression force on drug release.

Formulations prepared with carbopol

The effect of compression force on the drug release was studied with formulation CBL-4 but with different compression forces to get tablets with different hardness levels, low hardness: 4, optimal hardness: 7 and high hardness: 10 kg/cm^2.

Figure 5.12 illustrated the effect of compression force on release profile of MIL from carbopol matrix tablets. The release profiles were found to be similar (f_2 > 50) among batches compressed at the three compression forces. It was observed that tablets compressed at hardness 4 showed an initial burst effect due to a partial initial disintegration, however, once the polymer was swollen the dissolution profiles became similar to those tablets compressed at 7 hardness. Although compression force is a statistically significant factor in tablet hardness, its effect on drug release from carbopol tablets was minimal between short hardness difference (2-3 kg/cm^2). It could be assumed that carbopol produces highly hydrated matrix which was independent on the initial porosity, thus the compression force seems to have little influence on drug release [45-46]. Therefore, the independence of the drug release from carbopol matrix tablets with respect to the compression force was again proved the reported investigations.

However, a change in compression force from 4 to 10 kg/cm^2 indicated significant change in drug release. A high difference in compression force (approx 6 kg/cm^2) caused significant change in matrix porosity. The release rate decreased with increase in compression force from carbopol matrix tablets. The MDT, T50% and T80% values increased from 4.83 to 5.92 h, 2.18 to 4.97 h and 10.40 to 17.54 h respectively as compression force increased from 4 kg/cm^2 (lower compression force) to 10 kg/cm^2 (higher compression force) as shown in Table 5.10.

Results of compression force effect on hydrophilic matrices (HPMC, NaCMC and carbopol polymers) indicated that as the hardness of the tablet was increased, the release rate decreased. This can be explained as, at higher compression force porosity decreased, resulting in an increase in binding surfaces and thus a harder tablet. The decrease in porosity also results in a more tortuous pathway result in longer pathway for drug release [34]. The drug release was found to be faster at lower compression forces than at higher ones because of the relatively larger matrix porosity or more interparticulate voids in compact mass, which allowed greater penetration of

dissolution fluid into the matrix, thus enhancing polymer erosion and drug dissolution [47].

(c) Effect of pH of dissolution media on drug release

Formulations prepared with HPMC

The effect of dissolution media (pH 1.2 and 6.8) on the drug release was studied with formulations containing 100 mg of HPMC 15K (H15K-2) and HPMC 100K (H100K-2) as shown in Figure 5.13 and 5.14 respectively. In-vitro drug release data as reported in Table 5.8, indicated that there was no significant difference observed in dissolution parameters such as MDT, T50% and T80% in different dissolution media. The f_2 was found to be more than 50, also indicated the similarity of drug release from HPMC matrix in different dissolution media.

The similarity in drug release profiles can be explained by pH independent solubility of MIL and HPMC. MIL has been discussed as highly soluble at all pH. HPMC is a cellulose derivative with methoxyl and hydroxypropyl substituents on a β-o-glucopyranosyl ring backbone, is very resistant to changes in pH or ionic content of the medium. At pH values from 2 to 13, HPMC is relatively stable. Various similar studies on hydrophilic matrices formulated with HPMC also proved that the pH of the medium had no effects on the rheological characteristics of the HPMC gel [48-49].

Formulations prepared with sodium CMC

The effect of pH of dissolution media (pH 1.2 and 6.8) on the drug release from polymeric matrix prepared with 100 mg polymer proportion in CMC-2 and H15K/CMC-1, were performed in pH 1.2 and pH 6.8 buffer medium as shown in Figure 5.15 and 5.16. Various model independent dissolution parameters were also analyzed for study the effect of pH of dissolution medium as shown in Table 5.9. The similarity factor was found to be 54.23 for CMC-2 formulation and 77.61 for H15K/CMC-1 formulation which indicated similarity between release profile at pH 1.2 and 6.8 dissolution media. The drug release rate of CMC-2 in 0.1 N HCl dissolution medium was faster (K value 36.41 $h^{-0.46}$) than in the pH 6.8 phosphate buffer (K value 28.36 $h^{-0.53}$). The retardation in the drug release in the pH 6.8 phosphate buffer could be explained by the ionization of the carboxyl groups of NaCMC and swelling at this pH.

In case of formulation containing both NaCMC and HPMC 15K, the release rate was not significantly affected by pH of dissolution medium because of strong and stable gel formed by HPMC 15K. It has been reported that the hydrophilic matrices formulated with HPMC had no effect of pH of medium on the rheological characteristics of the gel due to pH independent swelling of HPMC [49-50].

Formulations prepared with carbopol

The effect of pH of dissolution media (pH 1.2 and 6.8) on the drug release from polymeric matrix were performed on CBL-4 formulation. Release was significantly different in both media as indicated in Figure 5.17. The drug release rate in pH 1.2 was faster (K value 34.98 $h^{-0.38}$) than in the pH 6.8 phosphate buffer (K value 24.41 $h^{-0.40}$). Release parameters reported in Table 5.10 indicated that dissolution parameters such as MDT, T50% and T80% values increased from 4.16 to 6.22 h; 2.55 to 5.93 h and 8.76 to 19.04 h respectively as pH of dissolution medium changed from 1.2 to 6.8. The similarity factor was found to be 35.43 ($f_2 < 50$) also indicated release profile was significantly affected by pH of dissolution medium.

The difference in the dissolution profiles of carbopol based formulations was due to the difference in the ionization of carbopol in different dissolution media. The carboxylic groups of carbopol backbone ($pK_a = 6.0$) ionized very little in acidic media and formed larger regions of microviscosity resulting in faster penetration of solvent into the glassy core. So the swelling and diffusional path length of the carbopol matrix was lesser in pH 1.2. At pH 6.8, the carboxylic groups of carbopol were ionized and repel each other causing maximum swelling, resulting in fewer and smaller regions of microviscosity. Since the diffusional path length was increased, the dissolution rate decreased. Therefore, the drug release was fastest in pH 1.2 and slowest in pH 6.8. [51-52].

(d) Effect of agitation speed

Formulations prepared with HPMC

Formulations H15K-2 and H100K-2 containing 100 mg of HPMC 15K and HPMC 100K respectively were used for this study. In-vitro release studies were carried out at three different stirring speeds 50, 100 and 150 rpm. The dissolution profiles at different rpm for HPMC 15K and HPMC 100K are shown in Figure 5.18 and 5.19 respectively.

In case of HPMC 15K matrix, when agitation speed increased from 50 to 150, the release rate increased. The MDT, T50% and T80% decreased significantly from 3.62 to 2.44 h; 2.19 to 1.23 and 7.40 to 4.81 h respectively as presented in Table 5.8. The f_2 values were found to be 72.47 and 45.66 when formulations compared for 50 v/s 100 rpm and 50 v/s 150 rpm respectively. The similarity factor indicated that release profiles were not significant different ($f_2 > 50$) when rpm changed from 50 to 100 rotations but at higher agitation speed (150 rpm) release profile was found to be dissimilar with 50 rpm.

Similar results were observed for HPMC 100K matrix. As agitation speed increased from 50 to 150 for HPMC 100K formulation the dissolution parameters such as MDT, T50% and T80% decreased significantly from 4.34 to 2.96 h; 2.86 to 1.69 and 8.50 to 5.70 h respectively as compiled in Table 5.8. The similarity factor values were found to be 76.09 and 46.21 when formulations compared between 50 vs 100 rpm and 50 vs 150 rpm respectively.

Formulations prepared with sodium CMC

Figure 5.20 and 5.21 illustrate the effect of stirring speed on MIL release from the designed CR matrix with NaCMC alone (CMC-2) and binary polymeric matrix of NaCMC and HPMC15K (H15K/CMC-1).

In case of NaCMC matrix (CMC-2), the release rate of MIL was enhanced as stirring speed increased from 50 to 150 rpm due to the polymer dissolution rate and external mass transfer increased with hydrodynamic stress. Investigations reported in Table 5.9 showed that the release rate constant increased and the MDT, T50% and T80% values decreased as stirring speed increased from 50 to 150 rpm. The f_2 values were found to be 61.66 and 40.71 when drug release compared for 50 v/s 100 rpm and 50 v/s 150 rpm respectively. There was no significant difference in drug release observed at 50 and 100 rpm.

In case of formulation prepared with NaCMC and HPMC 15K polymers (H15K/CMC-1), The release rate constant of *korsmeyer-peppas* model increased from 36.70 $h^{-0.356}$ to 41.37 $h^{-0.40}$ as stirring speed increased from 50 to 150 rpm. The MDT, T50% and T80% decreased from 4.38 to 2.56 h; 2.38 to 1.60 and 8.93 to 5.11 h respectively with increased agitation speed as shown in Table 8. The release profiles

were found to be similar (f_2 = 72.77) at 50 and 100 paddle rpm and dissimilar (f_2 = 45.14) between 50 and 150 paddle rpm.

Formulations prepared with carbopol

For study the effect of agitation speed, dissolution of CBL-4 formulation was performed with 50, 100 and 150 paddle rpm. The dissolution profiles at different rpm are shown in Figure 5.22. The release rate constant increased from 32.38 $h^{-0.35}$ to 41.25 $h^{-0.34}$ as stirring speed increased from 50 to 150 rpm. The MDT, T50% and T80% decreased significantly from 5.36 to 3.55 h; 3.48 to 1.77 and 13.41 to 7.18 h respectively with increased stirring speed as shown in Table 5.10. The f_2 values were found to be 57.75 and 42.16 when drug release compared between 50 v/s 100 rpm and 50 v/s 150 rpm respectively. There was no significant difference in drug release observed at 50 to 100 rpm.

The outcomes of effect of agitation speed on hydrophilic matrices (HPMC, NaCMC and carbopol polymers) indicated that there was no significant difference in drug release observed at 50 v/s 100 rpm for all formulations. This showed that matrices made of high viscosity polymers were less susceptible to erosion as a result of them having a higher intrinsic water holding capacity. However, there was significant difference in drug release observed for 50 v/s 150 rpm. The reasons for above variations can be explained as follow. The diffusion layer thickness decreased at high agitation speed resulting in increased mass transport from the matrix surface [53]. Increased drug release can also be attributed to the increased rate of detachment of polymer chains away from the matrix surface, as the stirring rate increased. At high stirring speed (150 rpm), the stagnant boundary layer disturbed and caused high rate of diffusion of drug. Above results suggested that the drug release from designed matrix would not be changed significant at minor agitation (50 to 100 rpm) due to physical agitation and probably peristaltic movement in the gastrointestinal tract [54-55].

5.5.2.2 Multi granules controlled release tablets of milnacipran hydrochloride

Two types of multi granules formulations were prepared.

(i) Multi granules controlled release tablets of MIL prepared with hydrophilic polymeric granules and hydrophobic wax granules (MG-1)

(ii) Multi granules controlled release tablets of MIL prepared with hydrophilic and hydrophobic polymeric granules (MG-2)

(a) Effect of polymer proportion on drug release

The in-vitro drug release profiles of formulations F-1 to F-5 (MG-1) and F-6 to F-10 (MG-2) containing various proportion of hydrophilic and hydrophobic polymeric granules are shown in Figure 5.23 and 5.24 respectively. The initial release for first two hours varied between 22% and 38% for MG-1 formulations and between 21% and 35% for MG-2 formulations.

Data reported in Table 5.11 showed that the MDT, T50% and T80% increased as hydrophobic content increased from 0 to 100% in formulations. The values of release exponent *n* increased (0.38 to 0.57) as the proportion of hydrophobic retardant increased in tablets. So, it can be inferred that the influence of polymer relaxation/erosion on the mechanism of drug release increased with increase in hydrophobic proportion [56].

All the dissolution parameters indicated that hydrophobic wax or hydrophobic polymer was playing a major role in controlling the drug release. The release of MIL from the combinations got more retarded than that of alone hydrophilic content, it may be due to higher lipophilicity offered by combination [57-58]. This can be attributed to the slower penetration of dissolution medium in matrices due to increased liophilicity of matrix [59-60]. Further, penetration of solvent molecule was hindered due to formation of gel layer of hydrophilic part leading to the slow percolation of drug for a prolonged period [27].

(b) Effect of viscosity of hydrophilic granules

The effect of polymer viscosity on drug release was studied with formulation F-3 and F-8 containing 50% proportion of HPMC hydrophilic granules. Three formulations containing same portion of HPMC but different viscosity grades 4K, 15K and 100K were evaluated for in-vitro drug release behavior. Plot of percent cumulative release vs time for various grade of HPMC are shown in Figure 24 and 29 for MG-1and MG-2 respectively..

As the viscosity of HPMC was increased from 4K to 100K in the formulations, the release rate extended from 6 h to 24 h as shown in Figure 5.25 and 5.26. In case of F-3 (MG-1) formulation, the release rate constant of *korsmeyer-peppas* model was found to be 58.68 $h^{-0.298}$, 36.83 $h^{-0.386}$ and 21.80 $h^{-0.496}$ for formulations containing 4K, 15K and 100K respectively which indicated that the release rate

decreased as viscosity of polymer increased. In addition, MDT, T50% and T80% also extended from 1.55 to 7.02 h; 0.58 to 5.33 h and 2.83 to 13.73 h respectively as higher viscosity polymer used in formulations as shown in Table 5.12. Similar relation of viscosity of polymer with drug release parameters were observed for F-8 (MG-2) formulation as presented in Table 5.12.

The f_2 values were found to be 18.10 for F-3 and 19.95 for F-8 when formulations compared for HPMC 4K vs 100K. When release profile compared for HPMC 15K vs 100K, the f_2 values were found to be 34.30 for F-3 and 30.17 for F-8, also indicated that the viscosity of polymer significantly influence the drug release from designed formulations.

Results of above study illustrated that the release rate was faster with lower viscosity grades of HPMC, probably owing to less polymer entanglement and less gel strength and also to the larger effective molecular diffusional area at lower viscosity as compared with higher viscosity grades of HPMC [29-31].

(c) Effect of compression force

The effect of compression force on the drug release was studied with formulation F-3 of MG-1 and F-8 of MG-2 compressed at different compression forces to get tablets with different hardness levels, 4, 7 and 10 kg/cm^2.

In case of F-3 (MG-1), The release rate decreased with increase in compression force as shown in Figure 5.27. The release of the drug was found to be significantly faster for formulation compressed at low compression force (4 kg/cm^2) than formulation compressed at higher compression force. The MDT, T50% and T80% values increased from 4.10 to 8.26 h; 3.14 to 7.59 h and 7.67 to 17.56 h respectively as compression force increased from 4 to 10 kg/cm^2 as given in Table 5.12. The similarity factor (f_2) was found to be 40.82 for release profiles compared for tablets compressed at 4 vs 7 kg/cm^2 and 49.43 for tablets compressed at 7 vs 10 kg/cm^2 indicated that the compression force significantly influence the drug release from formulations.

In case of F-8 (MG-2), the release rate decreased with increase in compression force as shown in Figure 5.28. The drug release from formulations compressed at low compression force (4 kg/cm^2) was found to be significantly much faster than formulation compressed at higher compression force. In addition, other

dissolution parameters such as MDT, T50% and T80% increased as compression force increased from 4 to 10 kg/cm^2. The similarity factor (f_2) was found to be 30.40 and 49.66 when release profiles compared for tablets compressed at 4 vs 7 kg/cm^2 and 7 vs 10 kg/cm^2 respectively.

Above results indicated, the compression force significantly influenced the drug release from formulations. Slow drug release at higher compression force might be due to increase in bonding surface area and apparent density of compact powder mass of tablets. The drug release was found to be faster at lower compression forces than at higher ones because of the relatively larger matrix porosity of the tablet, which allowed greater penetration of dissolution fluid into the matrix, thus enhancing polymer disentanglement and drug dissolution [27, 29].

(d) Effect of pH of dissolution medium

The effect of pH of dissolution media on the drug release was performed with formulation F-3 of MG-1 and F-8 of MG-2 in pH 1.2 and 6.8 dissolution media at 50 rpm.

Figure 5.29 and 5.30 illustrated that there was no significant difference observed in release profile at pH 1.2 and 6.8 buffer for both the formulations. All the dissolution parameters presented in Table 5.12 also indicated the similarity between release parameters. Further, the *f2* values were found to be 86.65 for F-3 and 82.69 for F-8 indicated the similarity of release profile in different dissolution medium.

Above similarity in release profiles can be justified with pH independent nature of MIL and release retardants. Hydrophobic wax and hydrophobic polymers (ethyl cellulose and eudragit RSPO) used in the formulations were found to be water insoluble and having pH independent dissolution. In hydrophilic part, HPMC was also found to very resistant to changes in pH or ionic content of the medium [29]. Thus, release profiles were not affected by ionic content of dissolution media.

(e) Effect of agitation speed

For study the effect of agitation speed, dissolution of formulation F-3 of MG-1 and F-8 of MG-2 was performed at three different stirring speed 50, 100 and 150 paddle rpm.

In case of F-3 (MG-1), Figure 5.31 indicated that as the rpm increased from 50 to 150 the drug release increased from the formulations. When agitation speed or paddle rpm increased 50 to 150, the MDT, T50% and T80% decreased from 7.02 to 3.54 h; 5.33 to 2.27 and 13.74 to 6.86 h respectively (Table 5.12). The similarity factor (f_2) was found to be 49.73 and 33.38 when release profiles compared between 50 vs 100 rpm and 50 vs 150 rpm, indicated the significant difference in drug release at different rotation speed.

In case of F-8 (MG-2), the results shown in Figure 5.32 supported the observation of MG-1. Decrease in the values of MDT, T50% and T80% with increase in agitation speed confirmed that agitation speed influence the drug release from matrix as presented in Table 5.12. In addition, f_2 values were was found to be 40.28 and 32.88 for release profiles when compared for 50 vs 100 rpm and 50 vs 150 rpm, also confirmed the above observations.

The observed variation in drug release with rpm, might be due to the difference in the hydrodynamic stress around the surface of tablets undergoing dissolution. At low agitation (50 rpm) there was slow fluid motion and formation of stable stagnant layer surrounding the tablets. This restricted the quick entry of fluid and also the release of drug out of the tablet. However, as rpm increased (100 and 150 rpm) there was greater fluid flow that resulted in increased attrition of the tablet matrix with fluid and disturbed the stagnant layer around the tablets. This could cause in higher drug release [29, 61].

5.5.3 Swelling and erosion studies

The results obtained from the swelling and erosion studies of CR matrix tablets of MIL prepared using various polymers HPMC 15K, HPMC 100K, NaCMC and carbopol at same amount 100 mg in formulations are presented in Figure 5.33 and 5.34 respectively.

In carbopol based formulations % swelling was found to higher and the % erosion was found to be lesser than compared to HPMC or NaCMC formulations for the entire duration of study. Previous investigators have also noted that carbopol forms mechanically strong matrices at low concentrations due to the chemically crosslinked structure of the polymer that swells, but does not dissolve fast in water [62]. The % erosion was found to be higher in NaCMC formulations than compared to carbopol and

HPMC formulations because of which faster drug release (drug release extended up to 12 h) achieved in NaCMC formulations than compared to HPMC formulations (drug release extended up to 16 h).

Swelling of the matrix, indicated by the transition of the polymer from the glassy to the rubbery state, is an important parameter in the determination of the release characteristics of the matrix system. It was observed from the swelling and erosion studies that the %swelling and %erosion of the matrix tablets was totally dependent on the viscosity of the polymer used. The % swelling increased with increase in polymer viscosity, while % erosion decreased with increase in polymer viscosity. This can be explained as following. As the polymer matrix becomes hydrated, the mobility of the polymer chains increase, thereby increasing the hydrodynamic volume of the polymer compact, which allows the compact to swell. As polymer chains become more hydrated and the gel becomes more dilute, the disentanglement concentration may be reached, i.e., the critical polymer concentration below which the polymer chains disentangle and detach from a gelled matrix. These events result in simultaneous swelling, dissolution, and erosion. High molecular weight polymers showed significantly greater swelling and less erosion than lower molecular weight polymers [63]. Carbopol has highest molecular weight and high crosslinked polymer showed maximum swelling and least erosion. Similarly, HPMC 100K showed higher swelling than HPMC 15K and NaCMC due to its higher molecular weight. This was because higher viscosity grades HPMC have higher and faster water absorption capacities and tend to swell rapidly than compared to the lower viscosity grades [64]. Moreover, the matrix formed by higher viscosity grades HPMC would have more gel strength than the one formed by lower viscosity grades because of which the erosion would be lesser. Due to these reasons the diffusional path length increased and the diffusion coefficient of the drug through the matrix decreased as the viscosity grade of HPMC was increased. The results obtained from these swelling and erosion studies further support the data obtained in effect of viscosity of HPMC on drug release studies, where it was observed that for the same proportion of polymer, the drug release rate decreased with increase in viscosity of HPMC used in the formulation [39-40].

The drug release mechanism also confirmed that swelling played dominant role in drug release than erosion. These swelling and erosion studies have

provided necessary information in understanding the effect of type of polymer on drug release from CR matrix tablets.

5.5.4 Batch reproducibility and stability on Storage

There was no significant change observed in the physical and chemical characteristics of all the formulations during their reproducibility studies. No significant difference was observed in the drug release profile and release kinetic of different batches of each CR formulation of MIL, indicating the reproducibility of designed formulations. The results of stability studies carried out for the selected formulations at different conditions of temperature and humidity are given in Table 5.13. At refrigerated condition (5 ± 2 ^{o}C), all the selected formulations were found to be stable for the entire period of study (24 months). The drug content in triplicate was determined for each formulation by UV-spectroscopic method as discussed in chapter 3. The degradation rate constants at different storage conditions and corresponding $t_{90\%}$ values were determined as listed in Table 5.13. The selected formulations degradation were found to follow first order as plot of log percent drug remaining to be degraded (log % RTD) versus time were linear indicating first order kinetics ($r^2 > 0.9756$).

At long term storage condition (25 ± 2 ^{o}C/60 ± 5% RH), K_{deg} values for MIL in various formulations ranged from 1.78 X 10^{-3} month^{-1} to 2.39 X 10^{-3} month^{-1} and $t_{90\%}$ values ranged from 43.96 to 59.05 months. The maximum degradation for MIL was found for formulations prepared using carbopol and the minimum degradation was found for formulations prepared using HPMC 100K. All the formulations were stable for entire study duration (24 months) with no apparent change in physical characteristics.

At accelerated storage condition (40 ± 2 ^{o}C/75 ± 5% RH), K_{deg} values for MIL in various formulations ranged from 3.50 X 10^{-3} month^{-1} to 6.45 X 10^{-3} month^{-1} and $t_{90\%}$ values ranged from 16.34 to 30.10 months. The maximum degradation for MIL was found for formulations prepared using NaCMC. The minimum degradation for MIL was found for formulations prepared using HPMC 15K. All the formulations were stable for entire study duration (6 months) with no apparent change in physical characteristics.

It was observed that with raise in temperature (from 25 ± 2 ^{o}C to 40 ± 2^{o}C) and relative humidity (60 ± 5% RH to 75 ± 5% RH), the K_{deg} values increased and $t_{90\%}$ values decreased in case of all the polymeric formulations. This might be due to the increased frequency of collisions between the reacting drug molecules at higher temperature condition (according to Arrhenius theory) and presence of moisture could accelerate the degradation.

The in-vitro release profiles of all the formulations stored at long-term storage and accelerated storage condition were compared with their initial release profiles with f_2 factor values. The f_2 factor values in all the cases found to be more than 75 indicating that designed CR tablets of MIL were significantly similar with initial samples. Thus, release characteristics of designed formulations were not significantly altered during stability studies.

5.6 Conclusions

In the present study, hydrophilic matrix based controlled release tablets of MIL were designed and formulated by wet granulation method. Novel multi granule based controlled release tablet formulations of MIL were also designed using hydrophilic and hydrophobic granules. The designed matrix tablets showed good physical properties indicating that the method of preparation of formulation is suitable and acceptable for manufacturing of good quality and reproducible matrix tablets of MIL. The designed formulations were novel, easy, economic and reproducible novel drug delivery systems. Various dissolution parameters like T50%, T80% and MDT were used to study the effect of formulation variables like polymer proportion, polymer viscosity, compression force and pH of dissolution medium on drug release. The designed formulations were able to prolong the drug release up to 16-20 h. The designed formulations were mostly found to be stable in suggested storage conditions. These designed CR formulations can overcome the disadvantages associated with conventional formulations of MIL.

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Table 5.1 (a): Formulation component of designed controlled release tablets of MIL with HPMC 15K and HPMC 100K polymers

Ingredients	**Drug**	**HPMC 15K**	**HPMC 100K**	**DCP**	**PVP K-30**	**Talc**	**Mg stearate**	**Tablet Wt**
Batch No	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)
H15K-1	100	50		200	15	10	5	380
H15K-2	100	100		150	15	10	5	380
H15K-2/4	100	100		150	15	10	5	380
H15K-2/10	100	100		150	15	10	5	380
H15K-3	100	150		100	15	10	5	380
H15K-4	100	200		50	15	10	5	380
H100K-1	100		50	200	15	10	5	380
H100K-2	100		100	150	15	10	5	380
H100K-2/4	100		100	150	15	10	5	380
H100K-2/10	100		100	150	15	10	5	380
H100K-3	100		150	100	15	10	5	380
H100K-4	100		200	50	15	10	5	380

Table 5.1 (b): Formulation component of designed controlled release tablets of MIL with NaCMC and HPMC polymers

Ingredients	**Drug**	**NaCMC**	**HPMC 15K**	**DCP**	**PVP K-30**	**Talc**	**Mg stearate**	**Tablet Wt**
Batch No	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)
CMC-1	100	50		200	15	10	5	380
CMC-2	100	100		150	15	10	5	380
CMC-2/4	100	100		150	15	10	5	380
CMC-2/10	100	100		150	15	10	5	380
CMC-3	100	150		100	15	10	5	380
CMC-4	100	200		50	15	10	5	380
H15K/CMC-1	100	50	50	150	15	10	5	380
H15K/CMC-1/4	100	50	50	150	15	10	5	380
H15K/CMC-1/10	100	50	50	150	15	10	5	380
H15K/CMC-2	100	100	50	100	15	10	5	380
H15K/CMC-3	100	50	100	100	15	10	5	380
H15K/CMC-4	100	100	100	50	15	10	5	380

Table 5.1 (c): Formulation component of designed controlled release tablets of MIL with carbopol 971P polymer

Ingredients	Drug	CBL	DCP	PVP K-30	Talc	Mg stearate	Tablet Wt
Batch No.	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)
CBL-1	100	10	160	15	10	5	300
CBL-2	100	25	145	15	10	5	300
CBL-3	100	50	120	15	10	5	300
CBL-4	100	75	95	15	10	5	300
CBL-4 (CBL 974P)	100	75	95	15	10	5	300
CBL-4/4	100	75	95	15	10	5	300
CBL-4/10	100	75	95	15	10	5	300
CBL-5	100	100	70	15	10	5	300

Table 5.2 (a): Formulation component of multi-granules based controlled release tablets of MIL

	MG-1			**MG-2**	
Ingredients (mg)	**Hydrophilic part**	**hydrophobic part**	**Ingredients**	**Hydrophilic part**	**hydrophobic part**
	Part A	Part B		Part A	Part B
Drug	100	100	Drug	100	100
HPMC 100K	100	_	HPMC 100K	100	_
DCP	100	100	DCP	100	100
PVP-30	20	_	PVP-30	20	_
Stearic Acid	_	60	Eudragit RSPO	_	60
Paraffin wax	_	60	Ethyl Cellulose	_	60
Talc	10	10	Talc	10	10
Mg stearate	10	10	Mg stearate	10	10

Table 5.2 (b): Fraction compositions of multi-granules based controlled release tablets of MIL

Batch No. (For MG-1)	Fraction of part A	Fraction of part B	Tablet wt (mg)
F-1	100	0	340
F-2	70	30	340
F-3	50	50	340
F-3/H4K	50	50	340
F-3/H15K	50	50	340
F-3/4	50	50	340
F-3/10	50	50	340
F-4	30	70	340
F-5	0	100	340
Batch No. (For MG-2)	**Fraction of part A**	**Fraction of part B**	**Tablet wt (mg)**
F-6	100	0	340
F-7	70	30	340
F-8	50	50	340
F-8/H4K	50	50	340
F-8/H15K	50	50	340
F-8/4	50	50	340
F-8/10	50	50	340
F-9	30	70	340
F-10	0	100	340

Table 5.3 (a): Physical characterizations of designed controlled release tablets of MIL with HPMC polymers

Formulation	Weight variation	Thickness	Hardness	Friability	Assay
	(%)	(mm)	kg/cm^2	(% w/w)	(%)
H15K-1	± 1.31	4.90 ± 0.03	7.20 ± 0.30	<0.50	98.85 ± 0.74
H15K-2	± 1.48	4.92 ± 0.02	7.10 ± 0.20	<0.50	99.25 ± 1.20
H15K-2/4.0	± 2.32	5.00 ± 0.04	4.10 ± 0.30	<0.70	100.08 ± 0.50
H15K-2/10.0	± 1.43	4.82 ± 0.04	10.20 ± 0.40	<0.40	99.87 ± 0.65
H15K-3	± 0.48	4.92 ± 0.03	7.10 ± 0.20	<0.50	100.12 ± 1.05
H15K-4	± 1.76	4.91 ± 0.02	7.00 ± 0.40	<0.50	99.75 ± 0.97
H100K-1	± 1.97	4.90 ± 0.05	7.10 ± 0.30	<0.50	98.52 ± 0.98
H100K-2	± 1.52	4.90 ± 0.02	7.20 ± 0.20	<0.50	101.05 ± 1.19
H100K-2/4.0	± 0.53	4.98 ± 0.03	4.00 ± 0.20	<0.60	100.17 ± 1.30
H100K-2/10.0	± 2.17	4.81 ± 0.04	10.10 ± 0.30	<0.40	99.75 ± 0.73
H100K-3	± 2.00	4.91 ± 0.02	7.20 ± 0.20	<0.50	100.3 ± 0.57
H100K-4	± 1.80	4.91 ± 0.02	7.10 ± 0.30	<0.50	99.68 ± 0.83

Weight variation and thickness: mean of 20 tablets with SD, hardness: Mean of 10 tablets with SD, Assay: Mean of triplicate with SD.

Table 5.3 (b): Physical characterizations of designed controlled release tablets of MIL with NaCMC and HPMC polymers

Formulation	Weight variation	Thickness	Hardness	Friability	Assay
	(%)	(mm)	kg/cm^2	(% w/w)	(%)
CMC-1	± 1.51	4.92 ± 0.04	7.20 ± 0.30	<0.50	99.15 ± 1.45
CMC-2	±0.52	4.92 ± 0.04	7.00 ± 0.30	<0.50	98.75 ± 1.82
CMC-2/4.0	±1.60	5.00 ± 0.03	4.10 ± 0.20	<0.60	99.92 ± 0.52
CMC-2/10.0	±1.21	4.83 ± 0.04	10.00 ± 0.30	<0.30	99.68 ± 1.61
CMC-3	±1.23	4.93 ± 0.05	7.10 ± 0.25	<0.50	100.22 ± 0.54
CMC-4	±0.80	4.93 ± 0.03	7.20 ± 0.20	<0.50	100.75 ± 0.34
H15K/CMC-1	±1.20	4.92 ± 0.02	7.00 ± 0.20	<0.50	99.52 ± 1.25
H15K/CMC-1/4.0	±1.32	5.00 ± 0.05	4.00 ± 0.30	<0.50	99.44 ± 0.70
H15K/CMC-1/10.0	±0.90	4.82 ± 0.04	10.10 ± 0.20	<0.40	100.02 ± 1.20
H15K/CMC-2	±0.75	4.92 ± 0.03	7.10 ± 0.30	<0.50	100.2 ± 1.05
H15K/CMC-3	±1.30	4.93 ± 0.02	7.00 ± 0.20	<0.50	100.04 ± 0.53
H15K/CMC-4	±1.10	4.93 ± 0.03	7.00 ± 0.30	<0.50	99.58 ± 0.32

Weight variation and thickness: mean of 20 tablets with SD, hardness: Mean of 10 tablets with SD, Assay: Mean of triplicate with SD

Table 5.3 (c): Physical characterizations of designed controlled release tablets of MIL with carbopol polymer

Formulation	Weight variation	Thickness	Hardness	Friability	Assay
	(%)	(mm)	kg/cm^2	(% w/w)	(%)
CBL-1	± 1.40	3.52 ± 0.02	7.10 ± 0.20	<0.50	99.17 ± 0.85
CBL-2	± 1.20	3.50 ± 0.03	7.10 ± 0.30	<0.50	98.57 ± 1.40
CBL-3	± 0.87	3.51 ± 0.02	7.00 ± 0.20	<0.50	100.65 ± 1.12
CBL-4	± 0.74	3.51 ± 0.03	7.10 ± 0.30	<0.50	100.08 ± 1.15
CBL-4/ CBL 974P	± 1.50	3.50 ± 0.04	7.10 ± 0.30	<0.50	101.05 ± 1.30
CBL-4/4.0	± 2.40	3.60 ± 0.05	4.20 ± 0.20	<0.60	99.87 ± 1.72
CBL-4/10.0	± 1.90	3.40 ± 0.04	10.10 ± 0.40	<0.30	99.55 ± 1.35
CBL-5	± 1.31	3.52 ± 0.04	7.00 ± 0.20	<0.50	99.73 ± 1.27

Weight variation and thickness: mean of 20 tablets with SD, hardness: Mean of 10 tablets with SD, Assay: Mean of triplicate with SD

Table 5.4: Physical characterizations of multi-granules based controlled release tablets of MIL

Formulation	Weight variation	Thickness	Hardness	Friability	Assay
	(%)	(mm)	kg/cm^2	(% w/w)	(%)
F-1	± 0.85	5.05 ± 0.02	7.10 ± 0.20	<0.40	99.85 ± 2.50
F-2	±0.92	5.10 ± 0.02	7.00 ± 0.30	<0.50	100.25 ± 1.82
F-3	±1.25	5.20 ±0.03	7.00 ± 0.30	<0.50	101.02 ± 1.57
F-3/H4K	±0.85	5.20 ± 0.04	7.10 ± 0.30	<0.60	99.72 ± 0.90
F-3/H15K	±2.00	5.20 ± 0.03	7.00 ± 0.40	<0.50	100.95 ± 1.30
F-3/4.0	±0.85	5.30 ± 0.03	4.20 ± 0.20	<0.60	100.45 ± 0.70
F-3/10.0	±1.35	5.10 ± 0.04	10.10 ± 0.30	<0.40	100.83 ± 1.47
F-4	±1.65	5.30 ± 0.03	7.10 ± 0.30	<0.50	98.95 ± 0.93
F-5	±1.74	5.30 ± 0.04	7.00 ± 0.40	<0.60	99.82 ± 2.05
F-6	± 2.37	5.00 ± 0.02	6.90 ± 0.30	<0.30	100.24 ± 1.10
F-7	±1.75	5.00 ± 0.03	7.10 ± 0.30	<0.30	101.35 ± 1.83
F-8	±1.07	5.20 ± 0.03	7.00 ± 0.30	<0.40	99.63 ± 0.92
F-8/H4K	±1.15	5.25 ± 0.04	7.00 ± 0.30	<0.50	100.12 ± 1.20
F-8/H15K	±1.60	5.25 ± 0.03	7.00 ± 0.30	<0.50	98.63 ± 1.50
F-8/4.0	±1.55	5.35 ± 0.02	4.30 ± 0.30	<0.70	100.25 ± 1.65
F-8/10.0	±0.90	5.20 ± 0.03	10.20 ± 0.30	<0.30	99.90 ± 1.42
F-9	±0.85	5.30 ± 0.02	7.20 ± 0.30	<0.40	99.55 ± 1.73
F-10	±1.12	5.30 ± 0.04	7.40 ± 0.20	<0.40	100.52 ± 0.89

Weight variation and thickness: mean of 20 tablets with SD, hardness: Mean of 10 tablets with SD, Assay: Mean of triplicate with SD.

Table 5.5: In-vitro release characterizations of designed controlled release tablets of MIL prepared with HPMC polymers

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
H15K-1	0.9910	41.86	0.37	2.81	1.61	5.70
H15K-2	0.9871	36.97	0.39	3.62	2.19	7.40
H15K-3	0.9891	27.48	0.49	4.55	3.39	8.85
H15K-4	0.9709	21.84	0.54	5.68	4.63	11.06
H100K-1	0.9997	33.92	0.43	3.29	2.29	6.22
H100K-2	0.9949	31.78	0.43	4.34	2.86	8.50
H100K-3	0.9789	24.25	0.49	5.68	4.29	11.05
H100K-4	0.9850	19.08	0.54	7.21	6.01	14.40

K : dissolution rate constant of korsmeyer-peppas model, n is release exponent, MDT (mean dissolution time): Mean of 6 tablets with maximum SD within ± 0.20 h , T50% (time where 50% drug released from formulation) : Mean of 6 tablets with maximum SD within ± 0.15 h. T80% (time where 80% drug released from formulation): Mean of 6 tablets with maximum SD within ± 0.15 h.

Table 5.6: In-vitro release characterizations of designed controlled release tablets of MIL prepared with NaCMC and HPMC polymers

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
CMC-1	0.9960	40.93	0.41	2.61	1.63	5.17
CMC-2	0.9952	37.75	0.41	3.12	1.98	6.24
CMC-3	0.9900	33.65	0.42	3.78	2.58	7.75
CMC-4	0.9854	30.85	0.42	4.66	3.13	9.51
H15K/CMC-1	0.9890	36.70	0.36	4.38	2.38	8.93
H15K/CMC-2	0.9884	35.17	0.34	5.02	2.79	10.95
H15K/CMC-3	0.9854	25.83	0.45	6.21	4.40	12.62
H15K/CMC-4	0.9907	22.20	0.49	6.90	5.23	13.60

K : dissolution rate constant of korsmeyer-peppas model, n is release exponent, MDT (mean dissolution time): Mean of 6 tablets with maximum SD within ± 0.20 h , T50% (time where 50% drug released from formulation) : Mean of 6 tablets with maximum SD within ± 0.15 h. T80% (time where 80% drug released from formulation): Mean of 6 tablets with maximum SD within ± 0.15 h.

Table 5.7: In-vitro release characterizations of designed controlled release tablets of MIL prepared with carbopol polymer

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
CBL -1	0.9901	59.53	0.30	1.42	0.56	2.64
CBL -2	0.9943	48.01	0.30	2.70	1.14	5.36
CBL -3	0.9794	37.04	0.36	4.24	2.33	8.75
CBL -4	0.9874	32.38	0.35	5.36	3.48	13.41
CBL-4/ CBL 974P	0.9800	36.78	0.33	5.08	2.57	10.85
CBL -5	0.9948	25.95	0.37	6.17	5.57	19.81

K : dissolution rate constant of korsmeyer-peppas model, n is release exponent, MDT (mean dissolution time): Mean of 6 tablets with maximum SD within ± 0.20 h , T50% (time where 50% drug released from formulation) : Mean of 6 tablets with maximum SD within ± 0.15 h. T80% (time where 80% drug released from formulation): Mean of 6 tablets with maximum SD within ± 0.15 h.

Table 5.8: Results of effect of various formulation parameters on drug release from matrix tables of MIL prepared with HPMC.

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
H15K-2	0.9871	36.97	0.39	3.62	2.19	7.40
H15K-2/4.0	0.9966	48.41	0.32	2.48	1.11	4.84
H15K-2/10.0	0.9885	28.87	0.43	5.38	3.58	10.66
H15K-2/pH 6.8	0.9987	34.09	0.44	3.62	2.40	7.00
H15K-2/rpm 100	0.9973	37.92	0.40	3.26	1.99	6.42
H15K-2/rpm 150	0.9972	46.50	0.35	2.44	1.23	4.81
H100K-2	0.9949	31.78	0.43	4.34	2.86	8.50
H100K-2/4.0	0.9994	42.90	0.34	3.14	1.56	6.16
H100K-2/10.0	0.9845	22.57	0.49	6.65	5.05	13.14
H100K-2/pH 6.8	0.9955	32.18	0.42	4.35	2.82	8.55
H100K-2/rpm 100	0.9867	34.98	0.40	4.01	2.46	8.01
H100K-2/rpm 150	0.9954	40.84	0.39	2.96	1.69	5.70

K : dissolution rate constant of korsmeyer-peppas model, n is release exponent, MDT (mean dissolution time): Mean of 6 tablets with maximum SD within ± 0.20 h , T50% (time where 50% drug released from formulation) : Mean of 6 tablets with maximum SD within ± 0.15 h. T80% (time where 80% drug released from formulation): Mean of 6 tablets with maximum SD within ± 0.15 h.

Table 5.9: Results of effect of various formulation parameters on drug release from matrix tables of MIL prepared with NaCMC and HPMC.

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
CMC-2	0.9952	37.75	0.41	3.12	1.98	6.24
CMC-2/4.0	0.9937	46.83	0.34	2.38	1.21	4.73
CMC-2/10.0	0.9815	32.80	0.40	4.66	2.90	9.50
CMC-2/pH 1.2	0.9906	36.41	0.46	2.79	1.99	5.49
CMC-2/pH 6.8	0.9890	28.36	0.53	3.67	2.91	7.04
CMC-2/rpm 100	0.9879	44.87	0.34	2.63	1.37	5.38
CMC-2/rpm 150	0.9862	50.15	0.35	1.89	0.99	3.75
H15K/CMC-1	0.9890	36.70	0.35	4.38	2.38	8.93
H15K/CMC-1/4.0	0.9937	42.75	0.36	2.83	1.54	5.70
H15K/CMC-1/10.0	0.9788	27.88	0.42	6.05	3.99	12.15
H15K/CMC-1/pH 1.2	0.9634	37.37	0.36	4.05	2.26	8.43
H15K/CMC-1/pH 6.8	0.9831	34.70	0.37	4.65	2.66	9.40
H15K/CMC-1/rpm 100	0.9856	36.61	0.39	3.64	2.23	7.44
H15K/CMC-1/rpm 150	0.9910	41.37	0.40	2.56	1.60	5.11

K : dissolution rate constant of korsmeyer-peppas model, n is release exponent, MDT (mean dissolution time): Mean of 6 tablets with maximum SD within ± 0.20 h , T50% (time where 50% drug released from formulation) : Mean of 6 tablets with maximum SD within ± 0.15 h. T80% (time where 80% drug released from formulation): Mean of 6 tablets with maximum SD within ± 0.15 h.

Table 5.10: Results of effect of various formulation parameters on drug release from matrix tables of MIL prepared with carbopol

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
CBL-4	0.9874	32.38	0.35	5.36	3.48	13.41
CBL-4/ CBL 974P	0.9800	36.78	0.33	5.08	2.57	10.85
CBL-4/4.0	0.9892	39.56	0.30	4.83	2.18	10.40
CBL-4/10.0	0.9930	27.49	0.37	5.92	4.97	17.54
CBL-4/pH 1.2	0.9716	34.98	0.38	4.16	2.55	8.76
CBL-4/pH 6.8	0.9920	24.41	0.40	6.22	5.93	19.04
CBL-4/rpm 100	0.9943	38.73	0.31	5.04	2.27	10.32
CBL-4/rpm 150	0.9934	41.25	0.34	3.55	1.77	7.18

K : dissolution rate constant of korsmeyer-peppas model, n is release exponent, MDT (mean dissolution time): Mean of 6 tablets with maximum SD within ± 0.20 h , T50% (time where 50% drug released from formulation) : Mean of 6 tablets with maximum SD within ± 0.15 h. T80% (time where 80% drug released from formulation): Mean of 6 tablets with maximum SD within ± 0.15 h.

Table 5.11: In-vitro release characterizations of multi granules based controlled release tablets of MIL

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
F-1	0.9869	32.25	0.39	4.88	3.11	10.51
F-2	0.9937	26.66	0.44	5.94	4.31	11.56
F-3	0.9957	21.80	0.49	7.02	5.33	13.73
F-4	0.9988	17.64	0.54	8.31	6.77	16.03
F-5	0.9990	14.14	0.57	8.95	9.11	20.72
F-6	0.9849	31.33	0.38	5.44	3.35	11.26
F-7	0.9918	25.39	0.44	6.68	4.60	13.23
F-8	0.9951	22.08	0.48	7.13	5.52	14.72
F-9	0.9967	20.12	0.48	7.69	6.65	17.68
F-10	0.9952	14.47	0.55	8.26	9.48	22.23

K : dissolution rate constant of korsmeyer-peppas model, n is release exponent, MDT (mean dissolution time): Mean of 6 tablets with maximum SD within ± 0.20 h , T50% (time where 50% drug released from formulation) : Mean of 6 tablets with maximum SD within ± 0.15 h. T80% (time where 80% drug released from formulation): Mean of 6 tablets with maximum SD within ± 0.15 h.

Table 5.12: Results of effect of various formulation parameters on drug release from multi granules based controlled release tables of MIL

Formulation	korsmeyer-peppas model			MDT	T50%	T80%
	r^2	K (% h^{-n})	n	(h)	(h)	(h)
F-3	0.9957	21.80	0.49	7.02	5.33	13.73
F-3/H4K	0.9999	58.68	0.29	1.55	0.58	2.83
F-3/H15K	0.9854	36.83	0.39	3.68	2.21	7.46
F-3/4.0	0.9990	27.35	0.53	4.10	3.14	7.67
F-3/10.0	0.9838	16.06	0.56	8.26	7.59	17.56
F-3/pH 6.8	0.9902	22.78	0.48	6.84	5.09	13.46
F-3/rpm 100	0.9922	29.51	0.42	5.26	3.47	10.50
F-3/rpm 150	0.9985	35.34	0.42	3.54	2.27	6.86
F-8	0.9951	22.08	0.48	7.13	5.52	14.72
F-8/H4K	0.9994	55.46	0.33	1.68	0.73	3.08
F-8/H15K	0.9954	39.01	0.38	3.31	1.91	6.46
F-8/4.0	0.9950	36.29	0.42	3.31	2.14	6.52
F-8/10	0.9860	16.21	0.54	7.97	7.32	18.96
F-8/pH 6.8	0.9926	22.57	0.47	6.97	5.27	14.09
F-8/rpm 100	0.9897	28.08	0.48	4.51	3.32	8.81
F-8/rpm 150	0.9941	32.99	0.46	3.52	2.47	6.85

Table 5.13: Stability data of designed controlled release tablets of MIL stored at long term storage conditions and accelerate storage conditions

Formulations	Long term storage condition (25 ± 2 °C/60 ± 5% RH)			Accelerated storage condition (40 ± 2 °C/75 ± 5% RH)		
	K_{deg} X 10^{-3} ($Month^{-1}$)	r^2	$t_{90\%}$ (months)	K_{deg} X 10^{-3} ($Month^{-1}$)	r^2	$t_{90\%}$ (months)
H15K-3	2.02	0.9741	52.07	3.50	0.9918	30.10
H15K-4	2.01	0.9721	52.36	3.72	0.9745	28.33
H100K-3	1.78	0.9655	59.05	4.72	0.9874	22.32
H100K-4	1.90	0.9788	55.47	4.49	0.9746	23.47
CMC-3	2.12	0.9849	49.75	5.20	0.9849	20.28
CMC-4	2.15	0.9780	48.95	6.45	0.9977	16.34
H15K/CMC-3	2.05	0.9852	51.54	4.38	0.9993	24.07
H15K/CMC-4	1.92	0.9852	54.81	4.70	0.9977	22.41
CBL-3	2.19	0.9800	48.18	4.49	0.9855	23.47
CBL-4	2.40	0.9842	43.96	4.61	0.9705	22.88
F-2	2.23	0.9849	47.18	4.84	0.9746	21.79
F-3	2.27	0.9780	46.46	4.42	0.9746	23.83
F-7	2.08	0.9802	50.74	4.84	0.9855	21.79
F-8	2.15	0.9802	49.11	5.07	0.9855	20.80

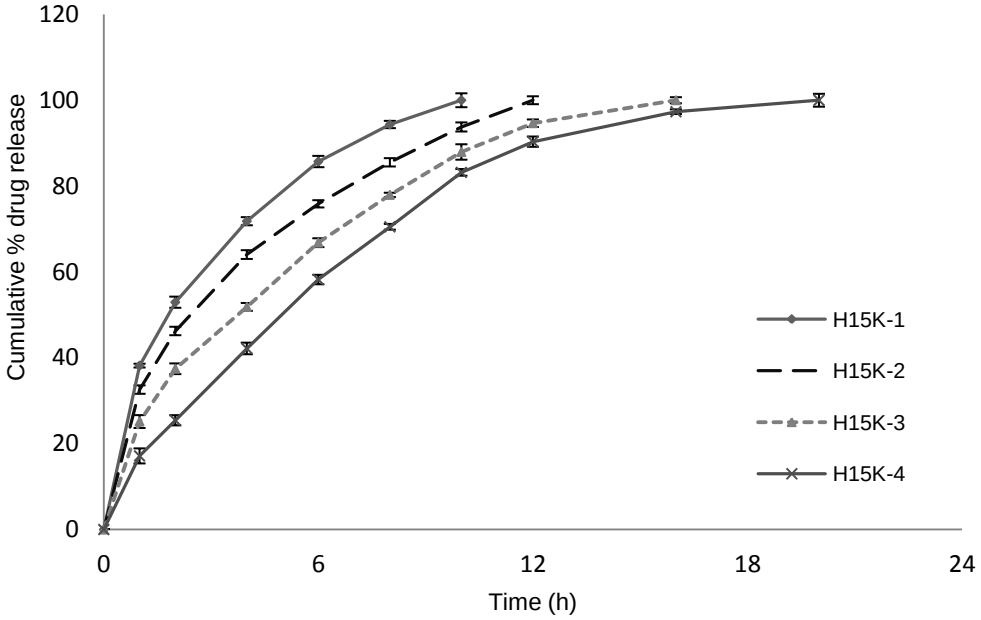

Figure 5.1: Comparative in-vitro release profile of MIL from designed controlled release tablets containing varying proportions of HPMC 15K polymer. Each data point represents the mean of 6 tablets with SD.

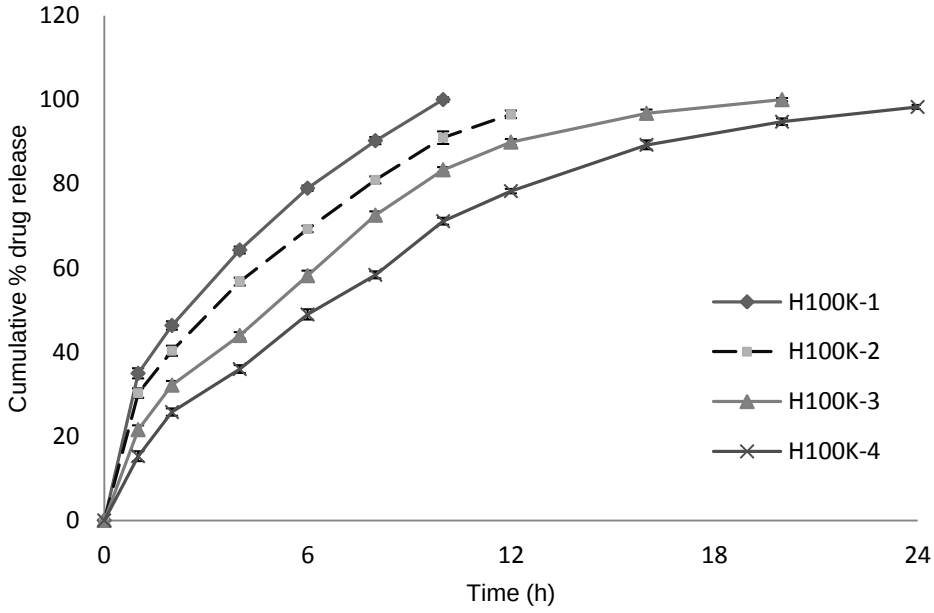

Figure 5.2: Comparative in-vitro release profile of MIL from designed controlled release tablets containing varying proportions of HPMC 100K polymer. Each data point represents the mean of 6 tablets with SD.

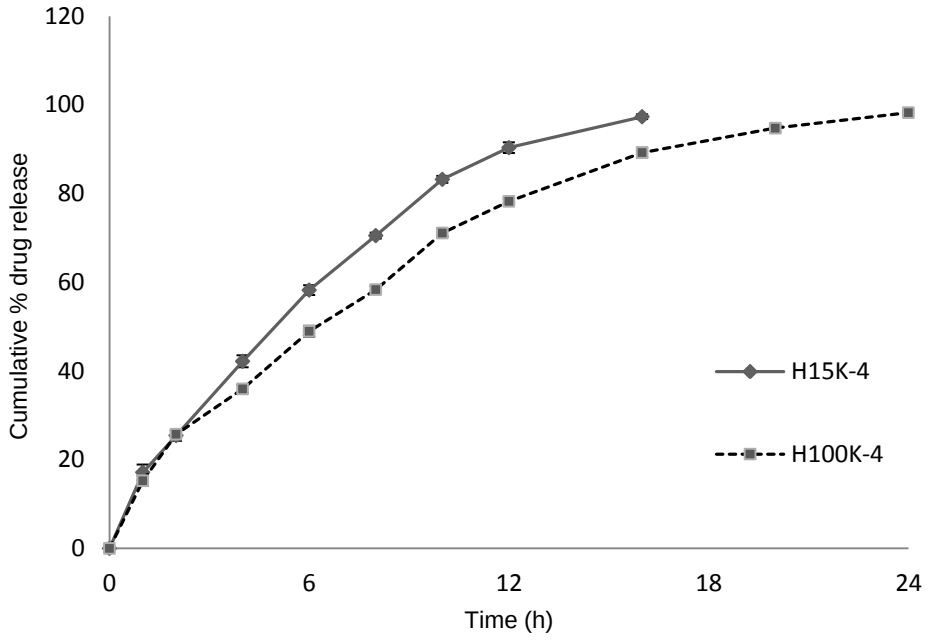

Figure 5.3: Effect of viscosity of HPMC polymer on MIL release from controlled release tablets. Each data point represents the mean of 6 tablets with SD.

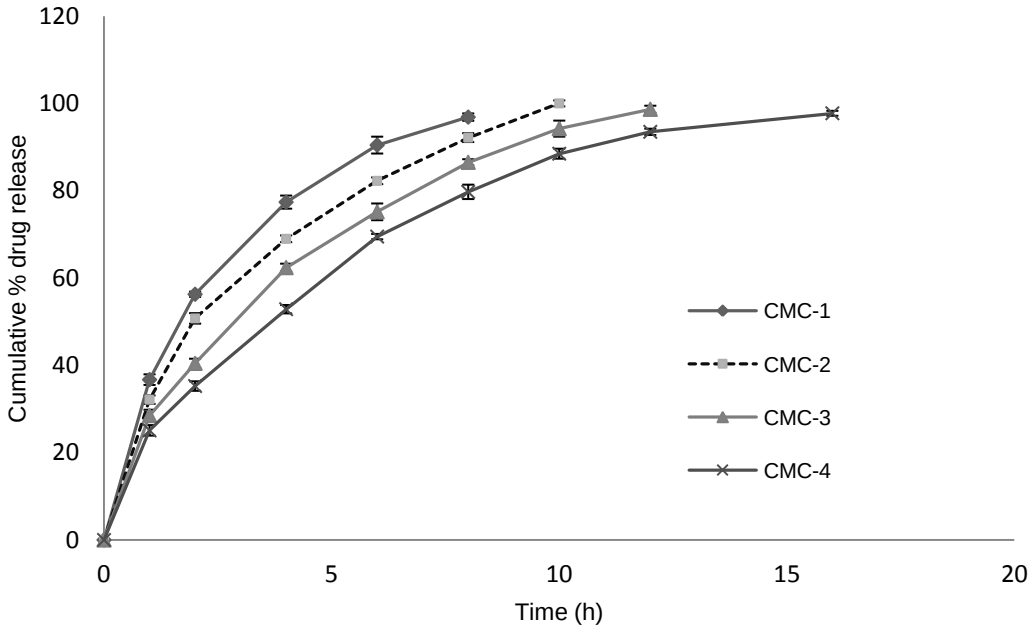

Figure 5.4: Comparative in-vitro release profile of MIL from designed controlled release tablets containing varying proportions of NaCMC polymer. Each data point represents the mean of 6 tablets with SD.

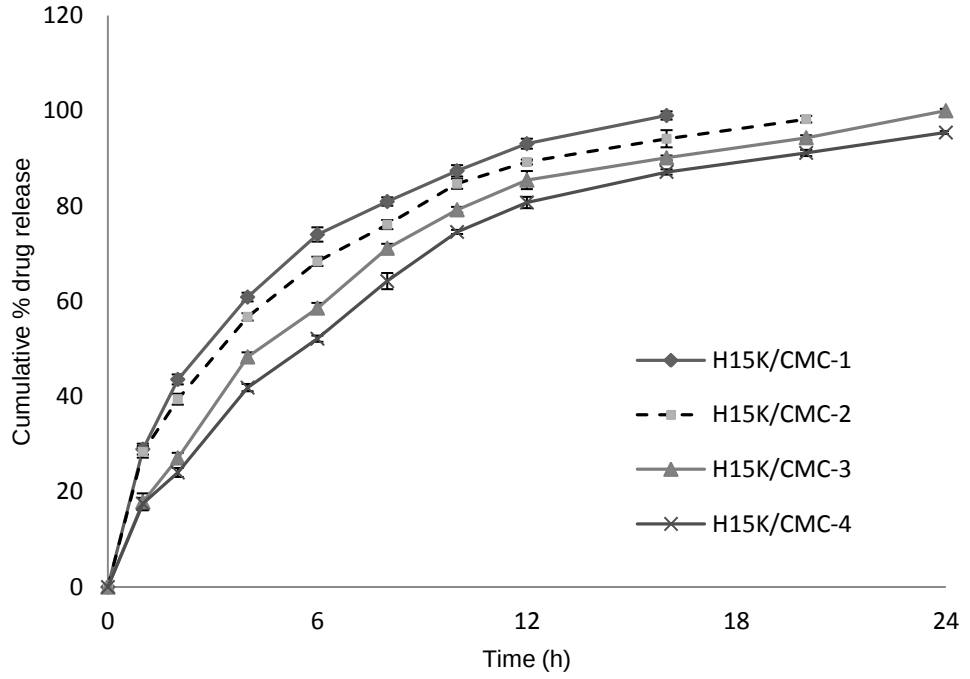

Figure 5.5: Comparative in-vitro release profile of MIL from designed controlled release tablets containing varying proportions of NaCMC and HPMC 15K polymers. Each data point represents the mean of 6 tablets with SD.

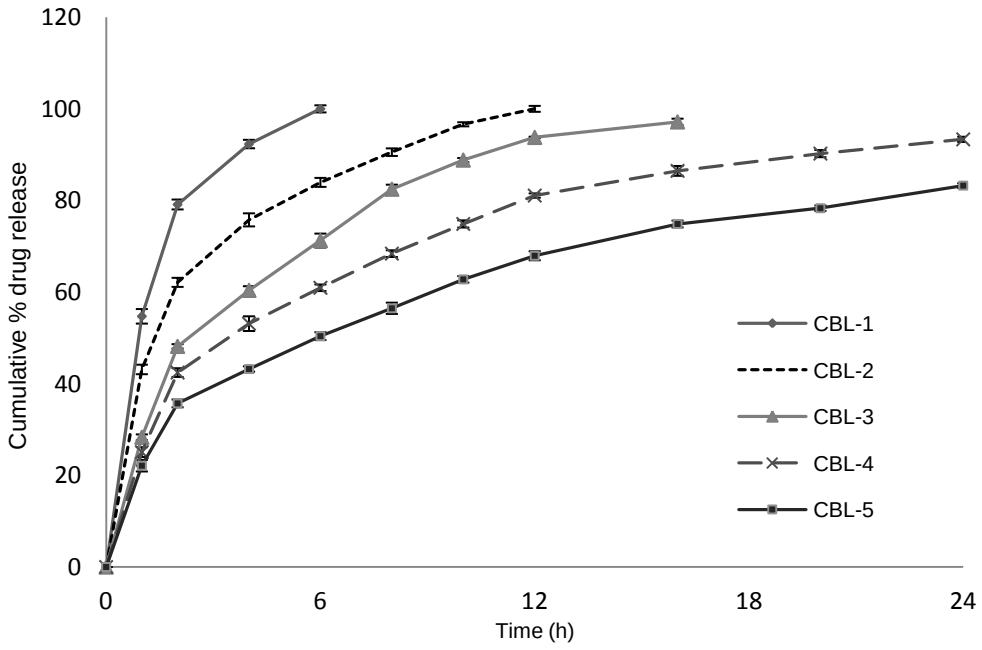

Figure 5.6: Comparative in-vitro release profile of MIL from designed controlled release tablets containing varying proportions of carbopol 971P polymer. Each data point represents the mean of 6 tablets with SD.

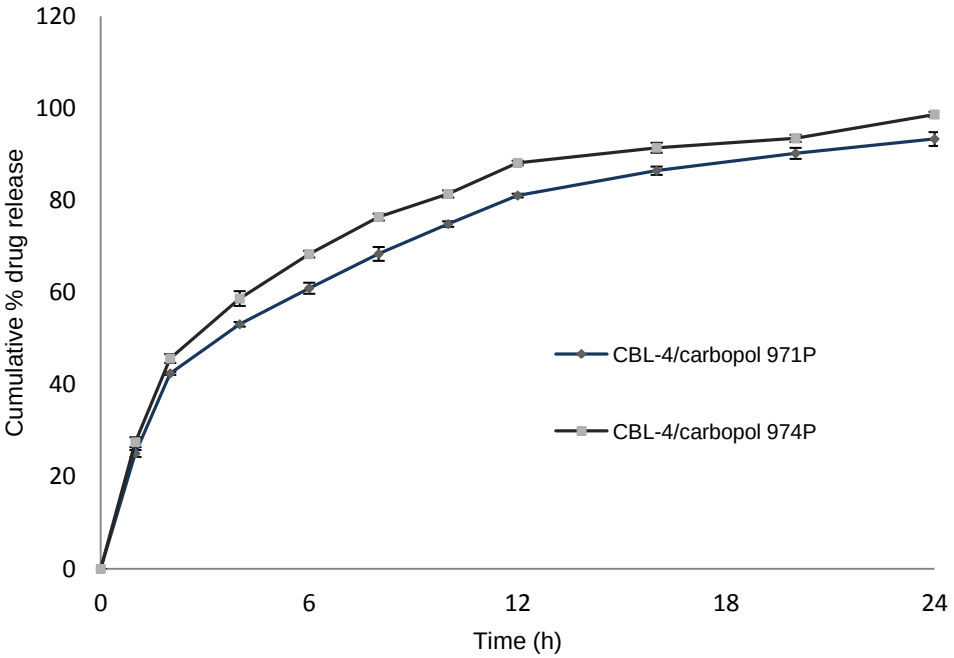

Figure 5.7: Effect of viscosity of carbopol polymer on MIL release from controlled release tablets. Each data point represents the mean of 6 tablets with SD.

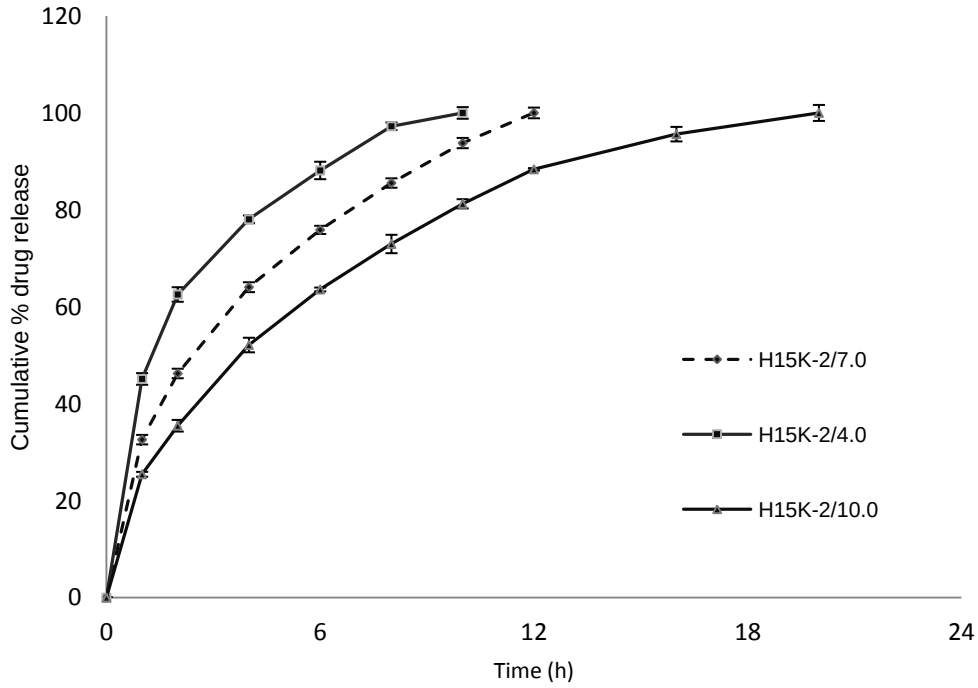

Figure 5.8: Effect of compression force on MIL release from controlled release tablets containing HPMC 15K polymer. Each data point represents the mean of 6 tablets with SD.

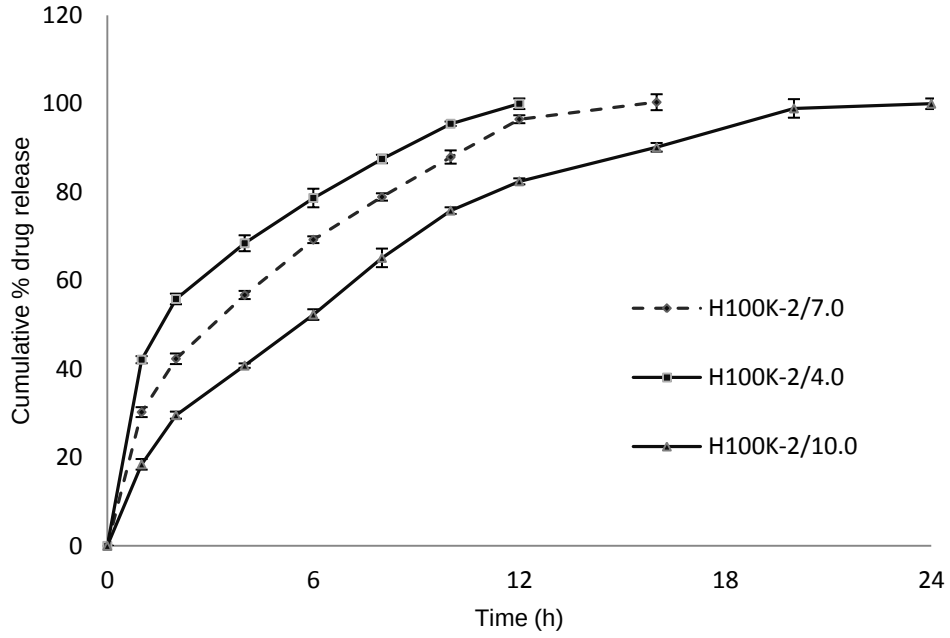

Figure 5.9: Effect of compression force on MIL release from controlled release tablets containing HPMC 100K polymer. Each data point represents the mean of 6 tablets with SD.

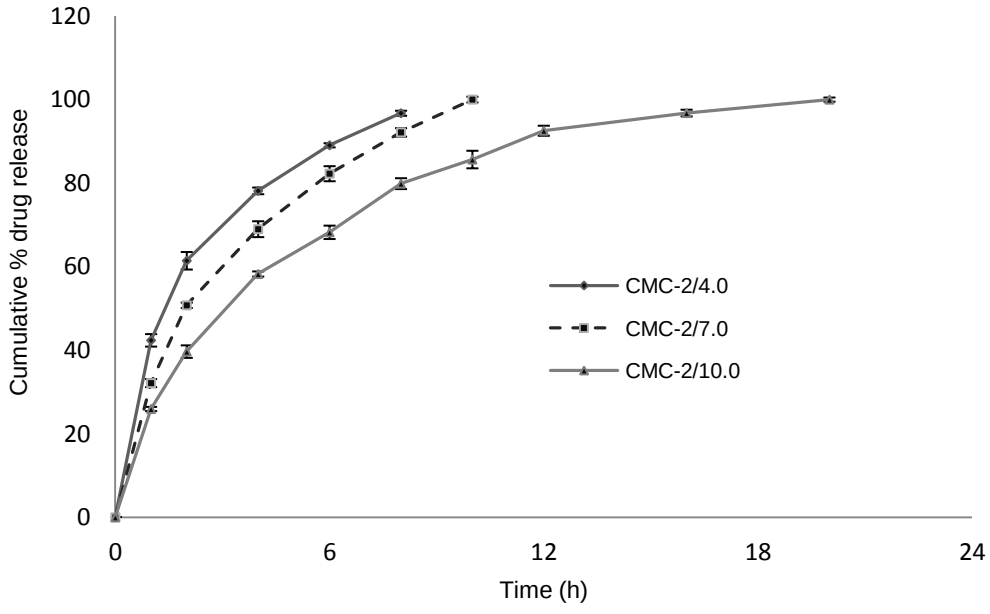

Figure 5.10: Effect of compression force on MIL release from controlled release tablets containing NaCMC polymer. Each data point represents the mean of 6 tablets with SD.

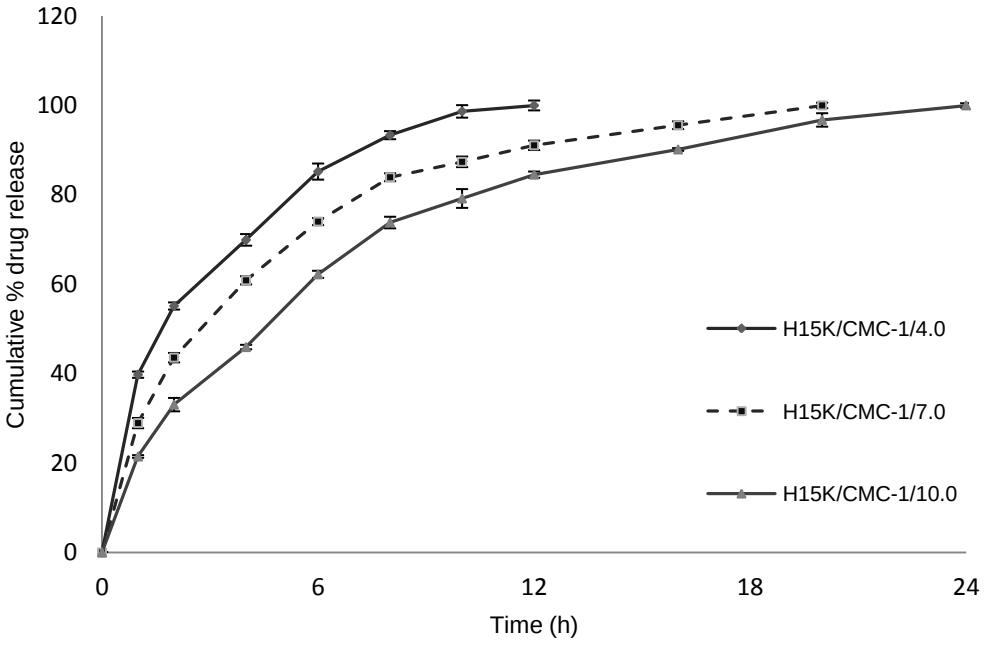

Figure 5.11: Effect of compression force on MIL release from controlled release tablets containing NaCMC and HPMC 15K polymers. Each data point represents the mean of 6 tablets with SD.

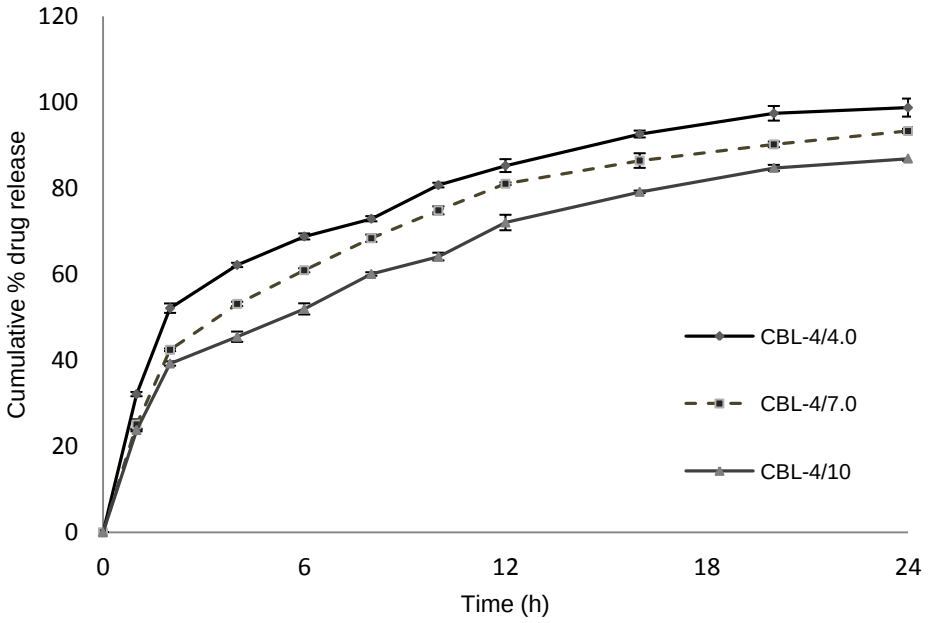

Figure 5.12: Effect of compression force on MIL release from controlled release tablets containing carbopol 971P polymer. Each data point represents the mean of 6 tablets with SD.

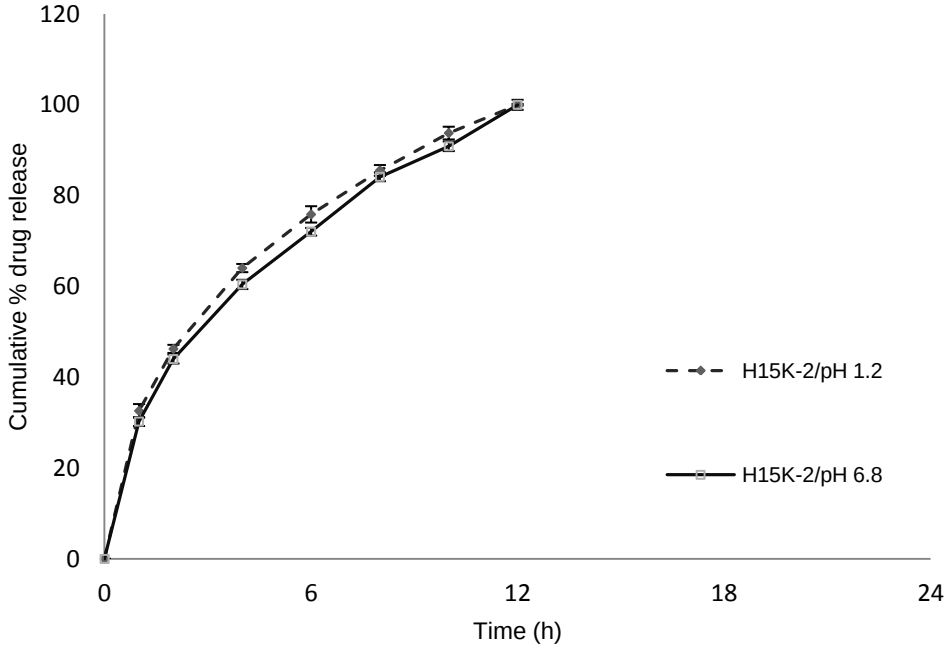

Figure 5.13: Effect of pH of dissolution medium on MIL release from controlled release tablets containing HPMC 15K polymer. Each data point represents the mean of 6 tablets with SD.

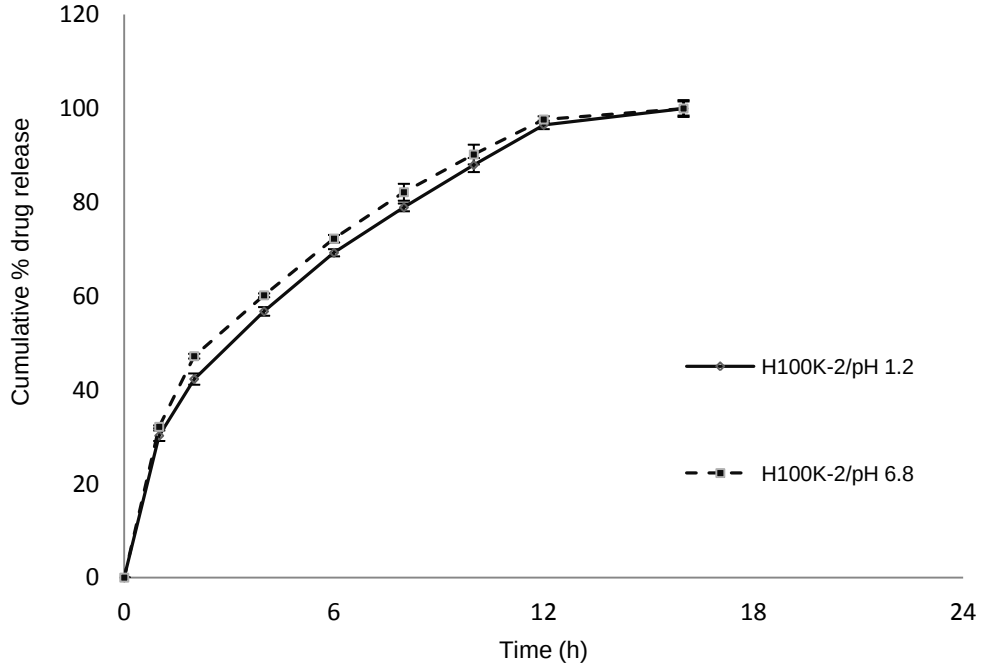

Figure 5.14: Effect of pH of dissolution medium on MIL release from controlled release tablets containing HPMC 100K polymer. Each data point represents the mean of 6 tablets with SD.

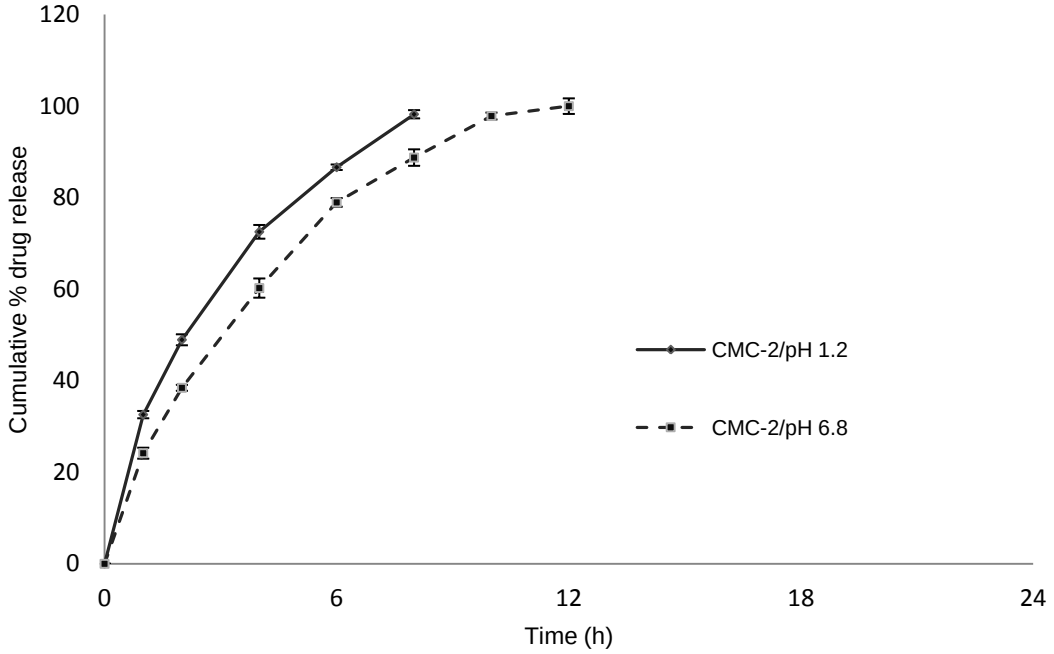

Figure 5.15: Effect of pH of dissolution medium on MIL release from controlled release tablets containing NaCMC polymer. Each data point represents the mean of 6 tablets with SD.

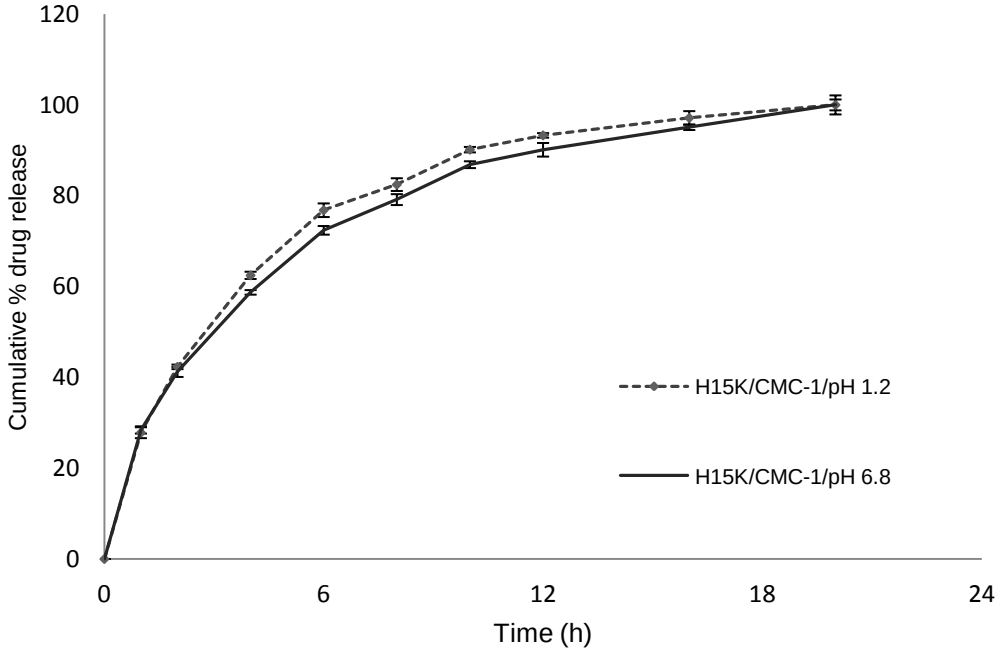

Figure 5.16: Effect of pH of dissolution medium on MIL release from controlled release tablets containing NaCMC and HPMC 15K polymers. Each data point represents the mean of 6 tablets with SD.

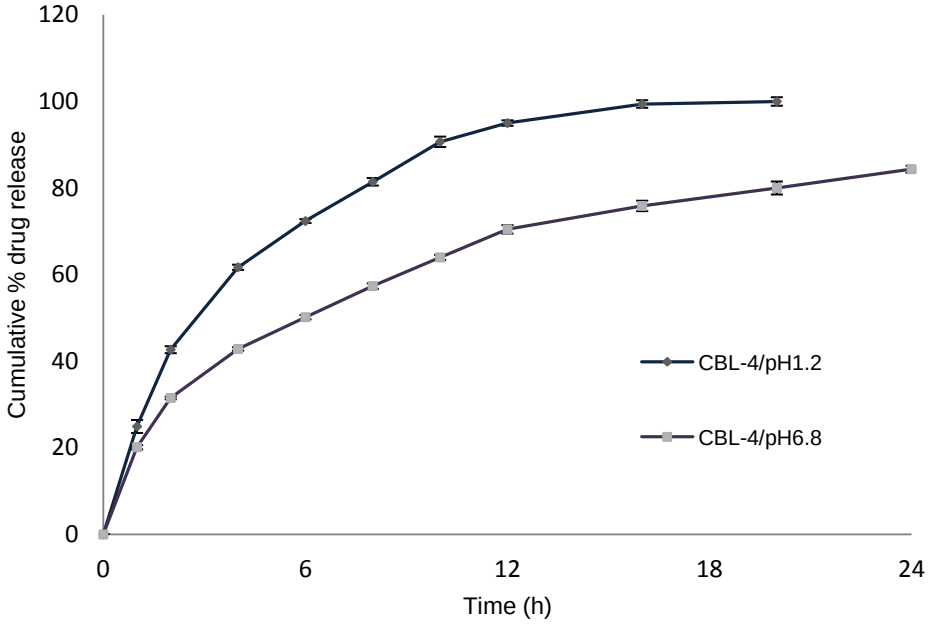

Figure 5.17: Effect of pH of dissolution medium on MIL release from controlled release tablets containing carbopol 971P polymer. Each data point represents the mean of 6 tablets with SD.

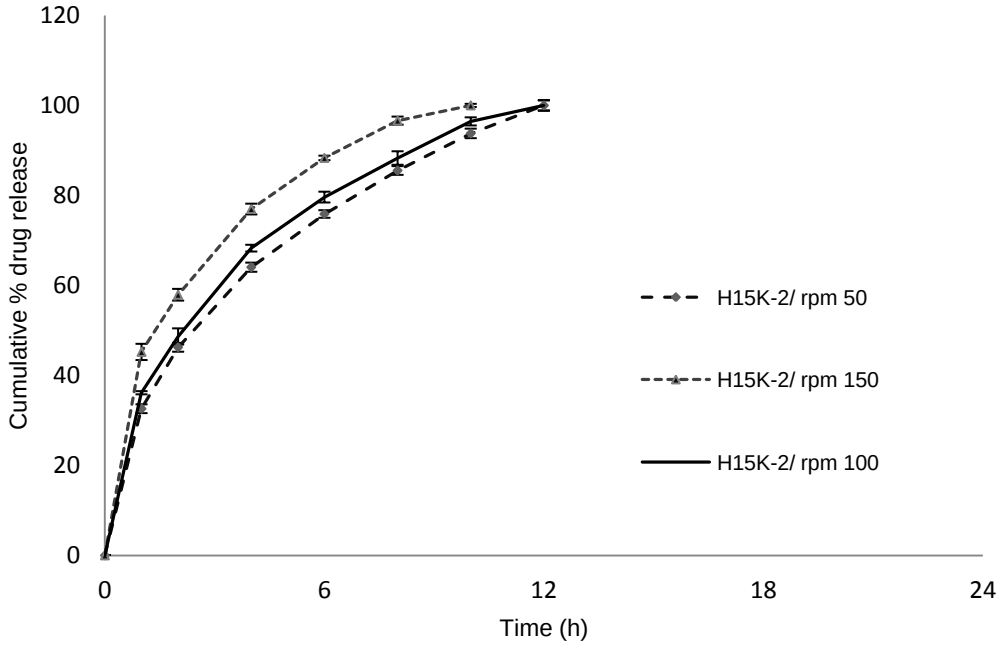

Figure 5.18: Effect of agitation speed on MIL release from controlled release tablets containing HPMC 15K polymer. Each data point represents the mean of 6 tablets with SD.

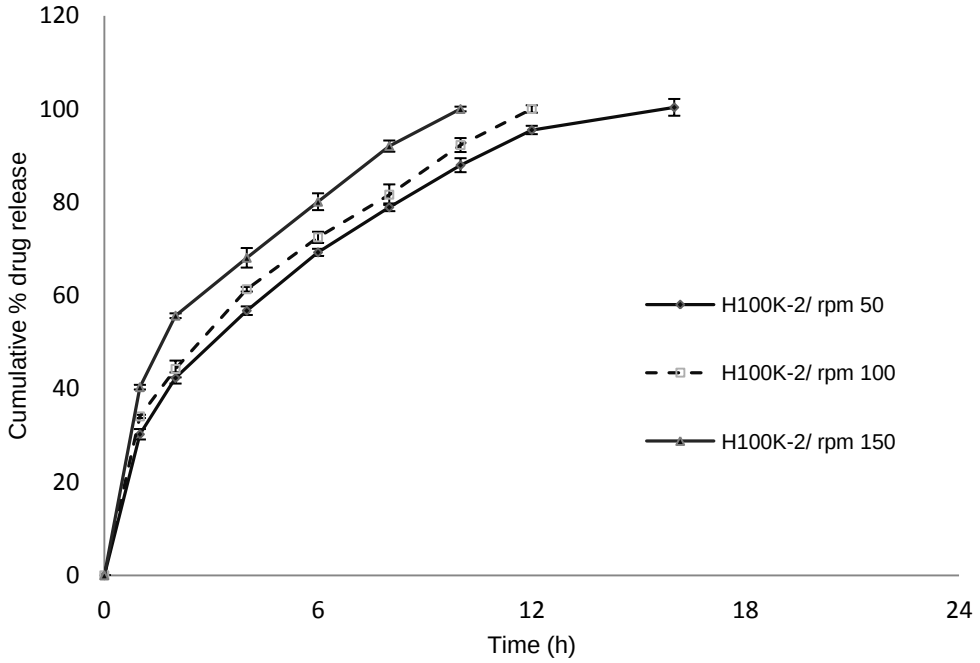

Figure 5.19: Effect of agitation speed on MIL release from controlled release tablets containing HPMC 100K polymer. Each data point represents the mean of 6 tablets with SD.

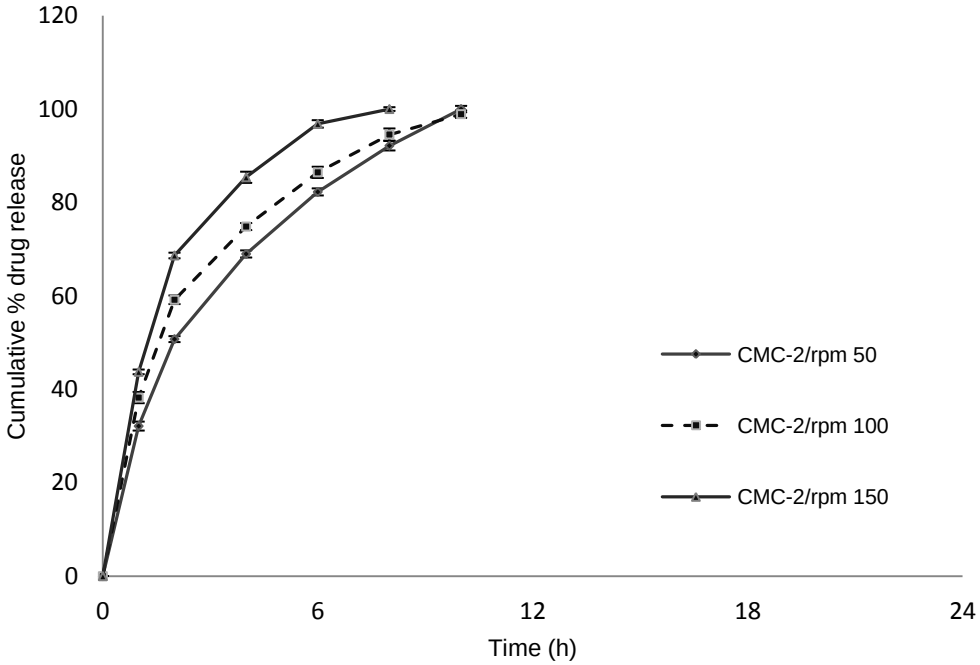

Figure 5.20: Effect of agitation speed on MIL release from controlled release tablets containing NaCMC polymer. Each data point represents the mean of 6 tablets with SD.

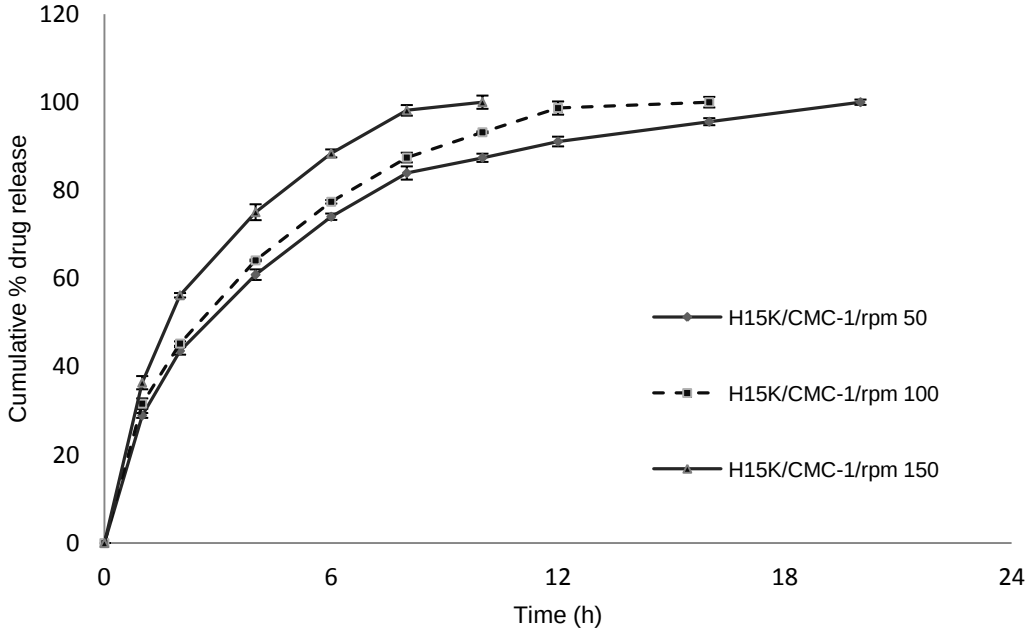

Figure 5.21: Effect of agitation speed on MIL release from controlled release tablets containing NaCMC and HPMC 15K polymers. Each data point represents the mean of 6 tablets with SD.

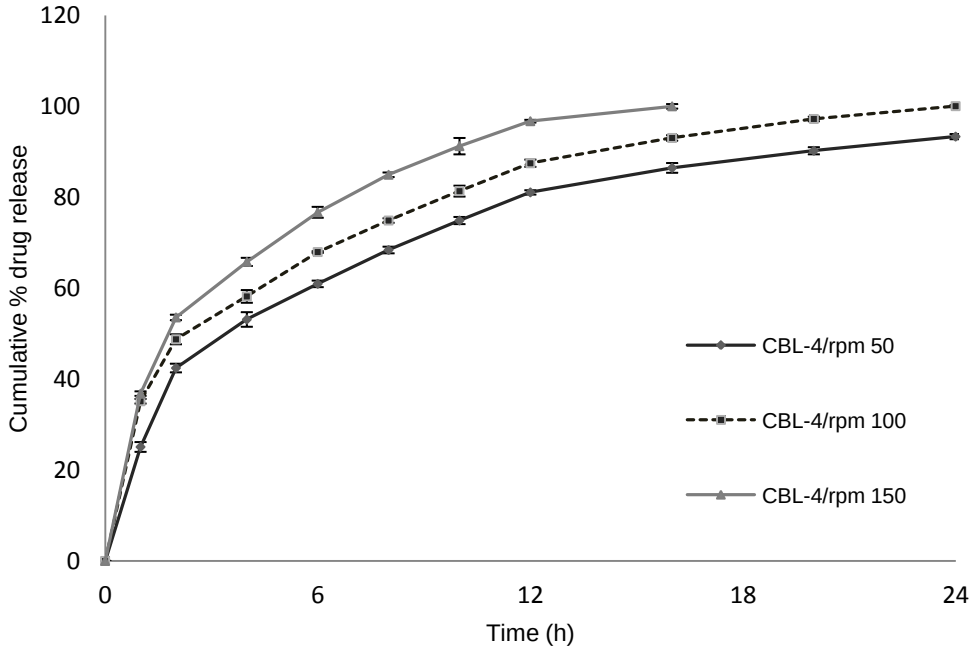

Figure 5.22: Effect of agitation speed on MIL release from controlled release tablets containing carbopol 971P polymer. Each data point represents the mean of 6 tablets with SD.

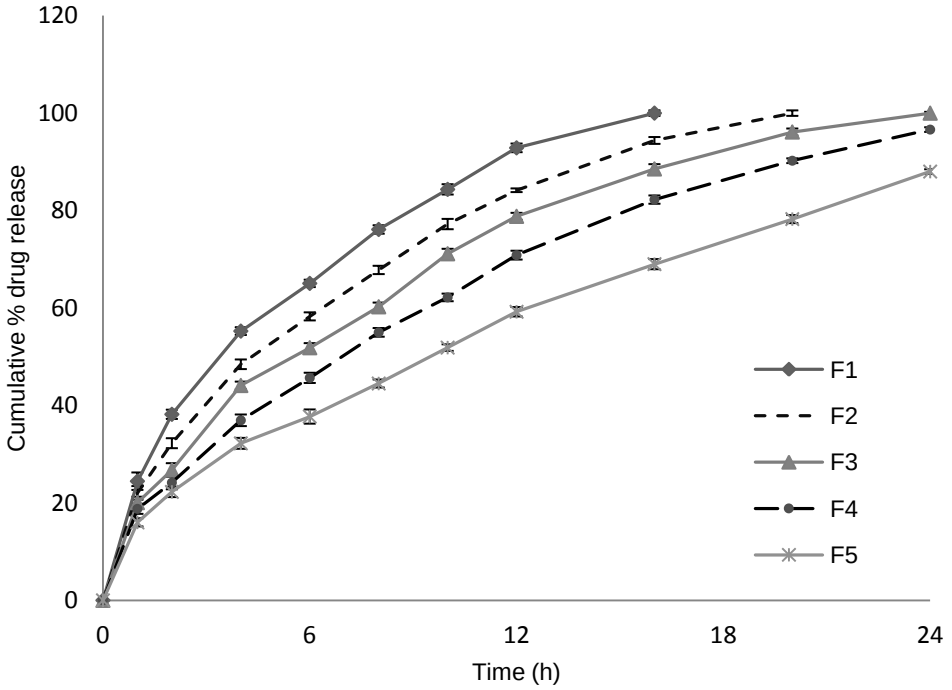

Figure 5.23: Comparative in-vitro release profile of MIL from multi-granules based controlled release tablets containing varying proportions of hydrophilic polymeric and hydrophobic wax granules (MG-1). Each data point represents the mean of 6 tablets from with SD.

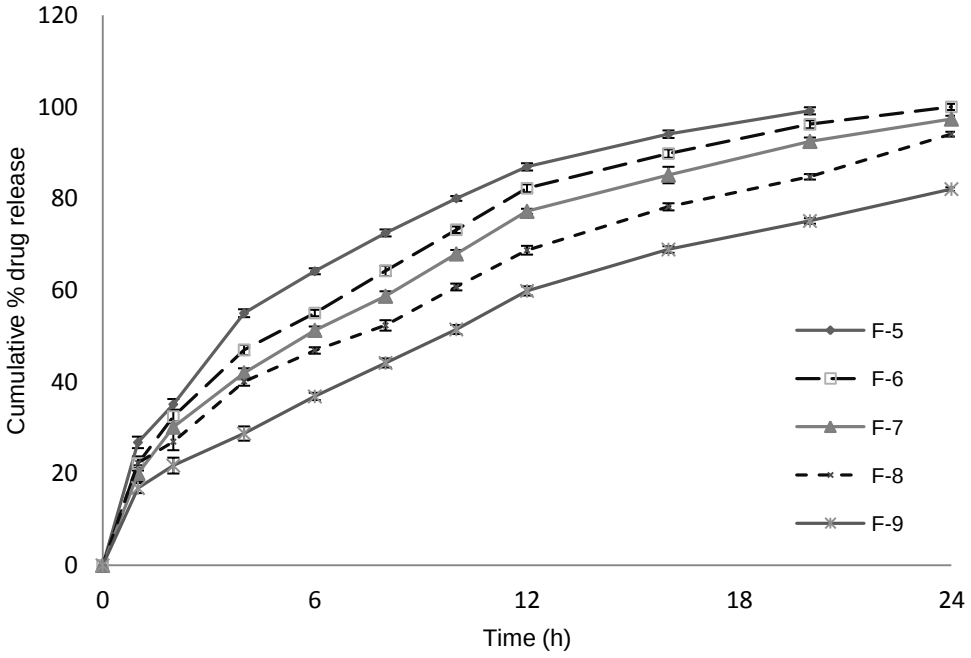

Figure 5.24: Comparative in-vitro release profile of MIL from multi-granules based controlled release tablets containing varying proportions of hydrophilic and hydrophobic polymeric granules (MG-2). Each data point represents the mean of 6 tablets with SD.

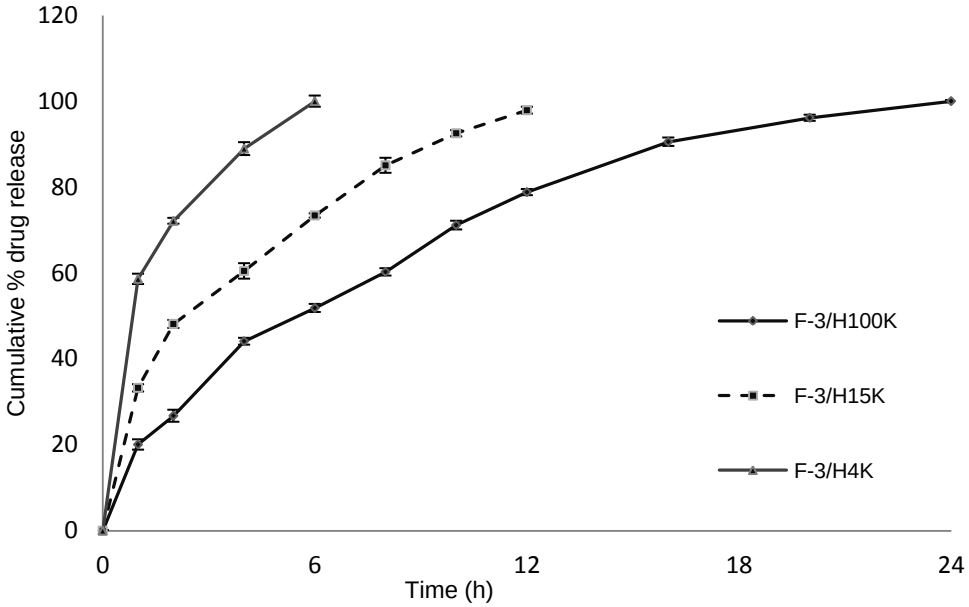

Figure 5.25: Effect of viscosity of HPMC polymer on MIL release from multi-granules based controlled release tablets (MG-1). Each data point represents the mean of 6 tablets with SD.

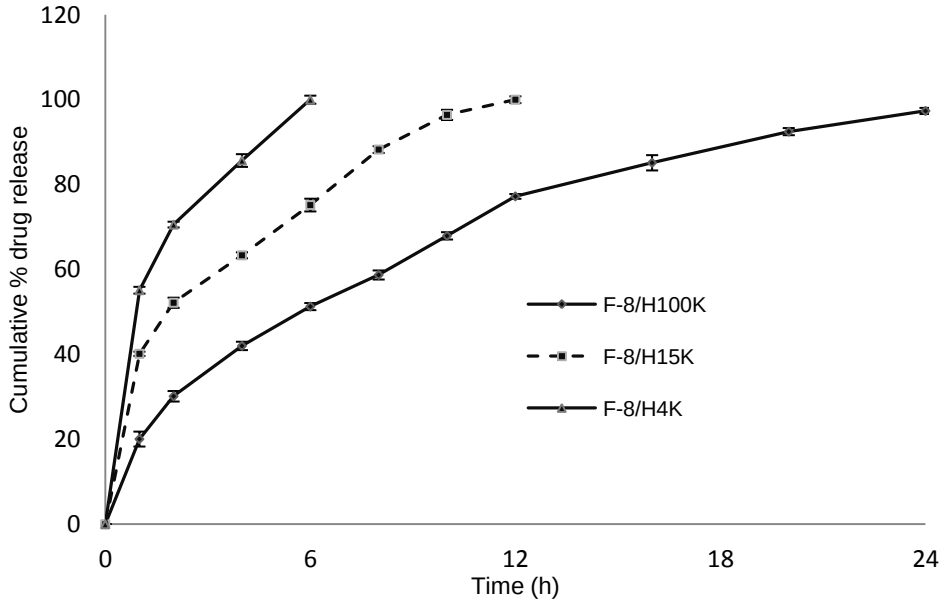

Figure 5.26: Effect of viscosity of HPMC polymer on MIL release from multi-granules based controlled release tablets (MG-2). Each data point represents the mean of 6 tablets with SD.

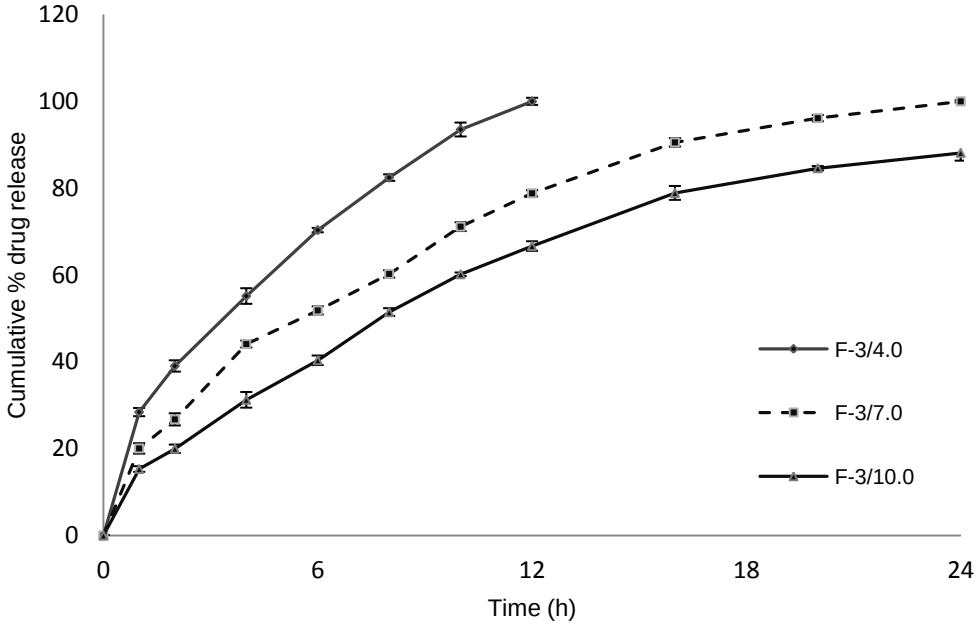

Figure 5.27: Effect of compression force on MIL release from multi-granules based controlled release tablets (MG-1). Each data point represents the mean of 6 tablets with SD.

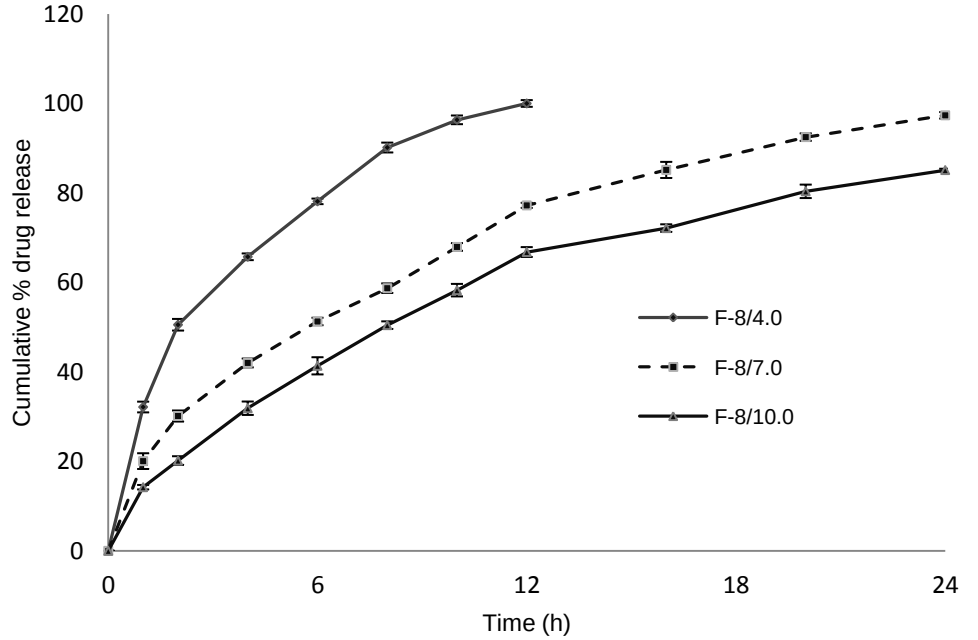

Figure 5.28: Effect of compression force on MIL release from multi-granules based controlled release tablets (MG-2). Each data point represents the mean of 6 tablets with SD.

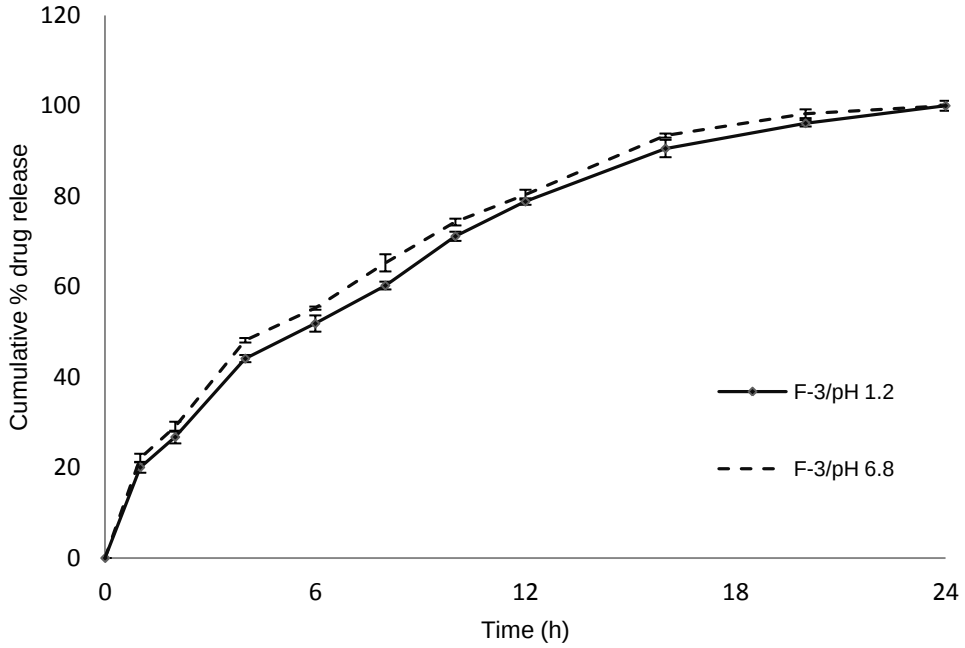

Figure 5.29: Effect of pH of dissolution medium on MIL release from multi-granules based controlled release tablets (MG-1). Each data point represents the mean of 6 tablets with SD.

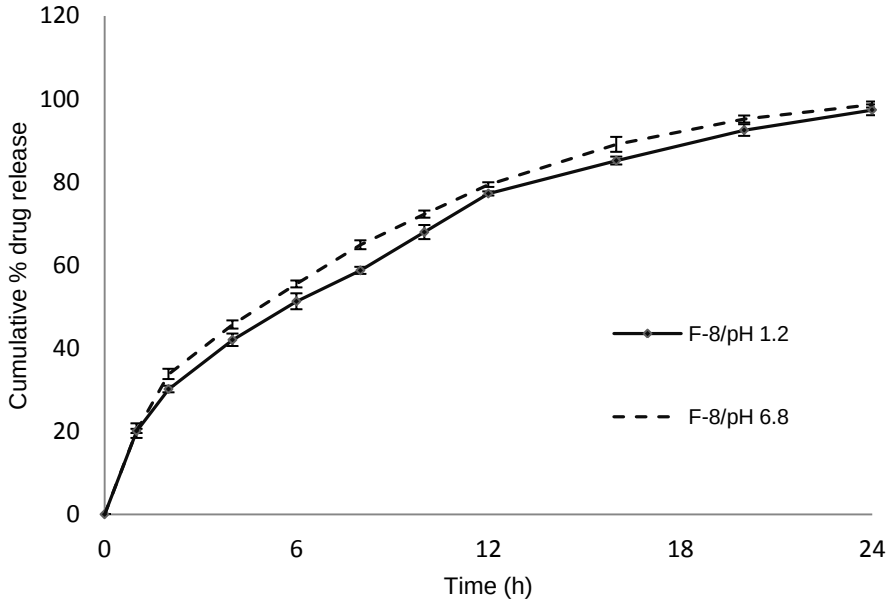

Figure 5.30: Effect of pH of dissolution medium on MIL release from multi-granules based controlled release tablets (MG-2). Each data point represents the mean of 6 tablets with SD.

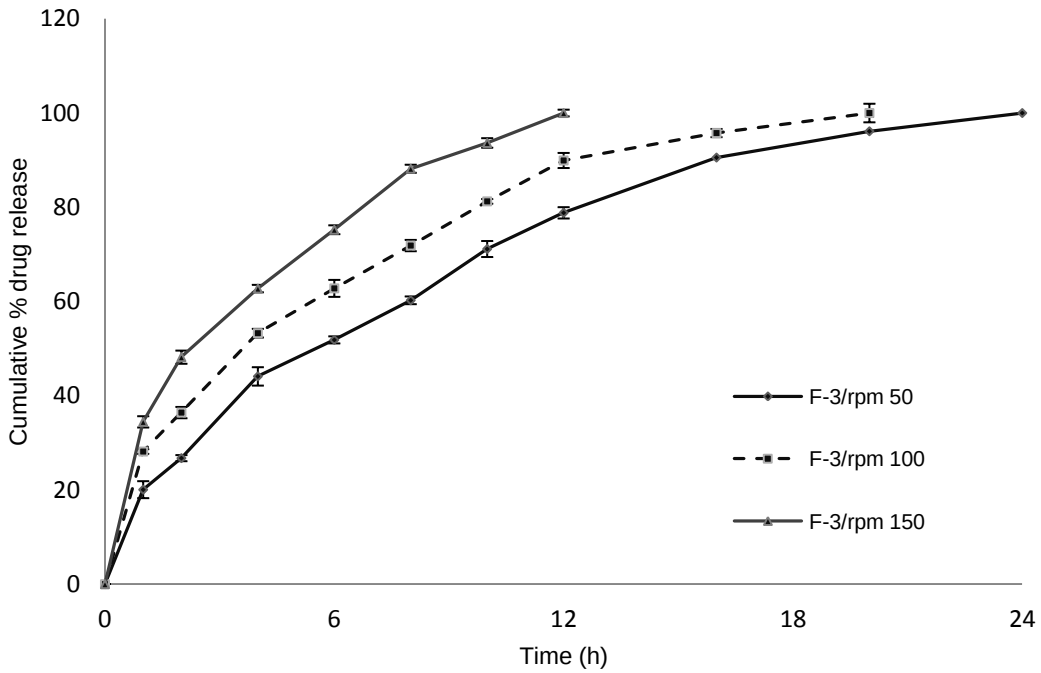

Figure 5.31: Effect of agitation speed on MIL release from multi-granules based controlled release tablets (MG-1). Each data point represents the mean of 6 tablets with SD.

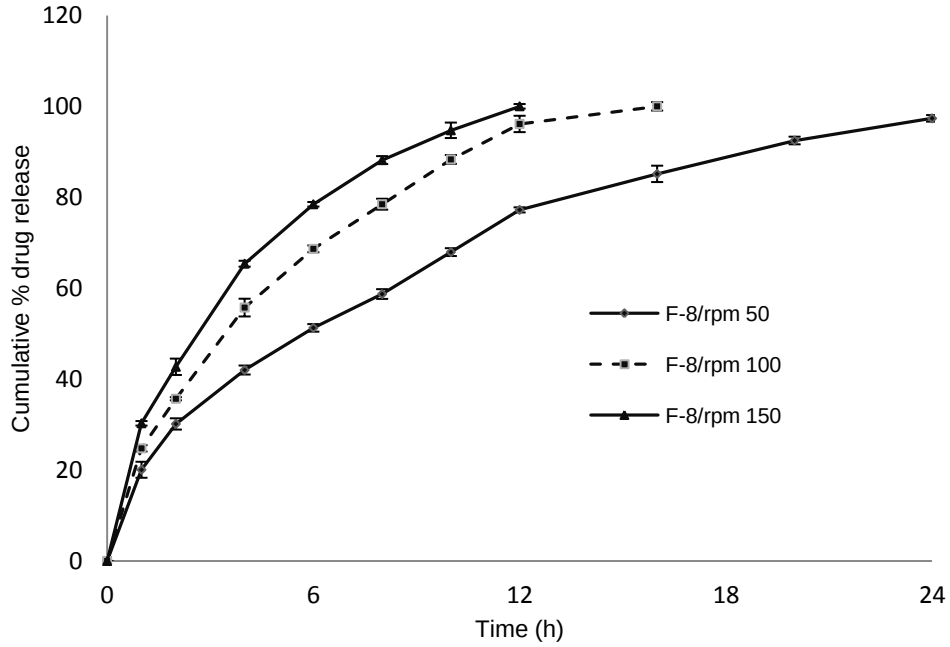

Figure 5.32: Effect of agitation speed on MIL release from multi-granules based controlled release tablets (MG-2). Each data point represents the mean of 6 tablets with SD.

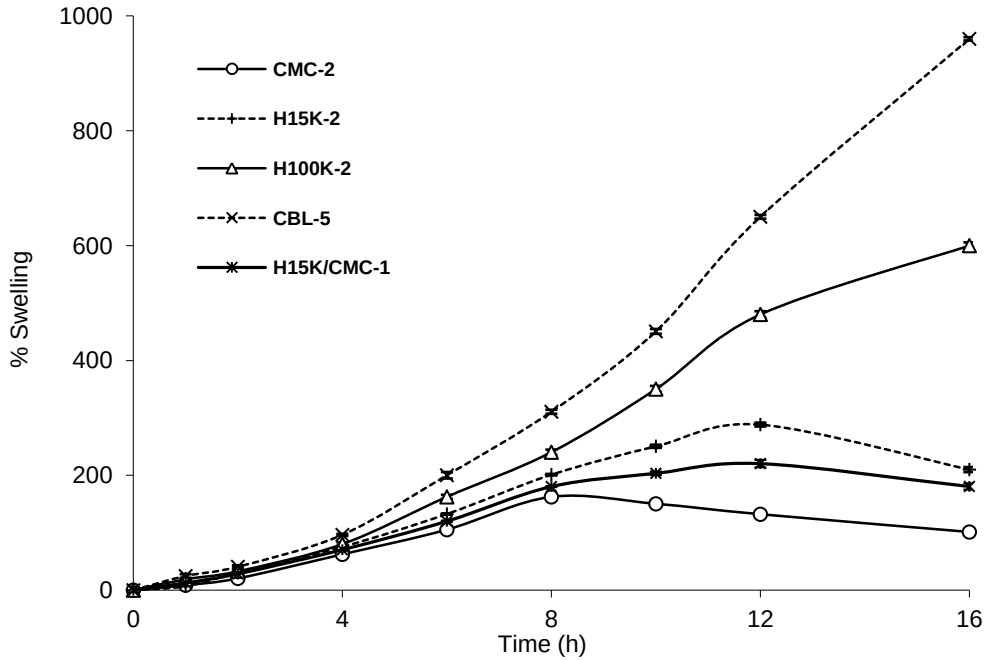

Figure 5.33: Swelling studies of controlled release matrix tablets of MIL formulated with different hydrophilic polymers HPMC 15K, HPMC 100K, NaCMC and carbopol separately. Each data point represents the mean of 6 tablets with SD.

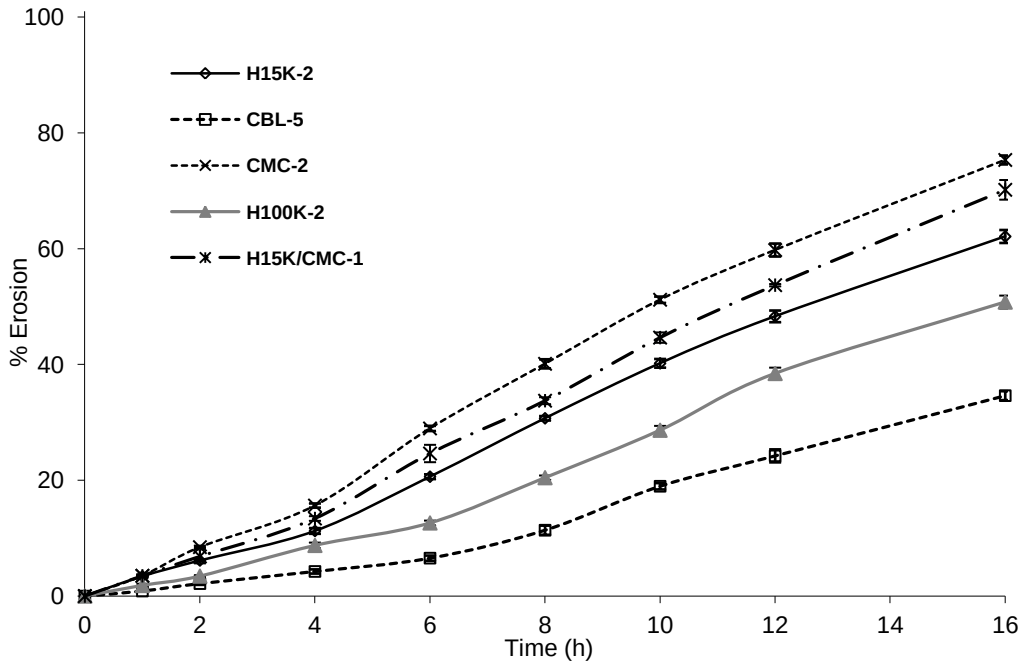

Figure 5.34: Erosion studies of controlled release matrix tablets of MIL formulated with different hydrophilic polymers HPMC 15K, HPMC 100K, NaCMC and carbopol separately. Each data point represents the mean of 6 tablets with SD.

CHAPTER 6

PHARMACOKINETIC AND BIOAVAILABILITY STUDIES

6.1 Introduction

For any new formulation designed, it is necessary to do bioavailability and pharmacokinetic studies in human to know plasma profile, extent and rate of availability It is more so for controlled release products. But initial study in suitable animal model can help optimization of formulation and prediction of outcome in human being.

For any designed formulation, it is also essential to do bioavailability and pharmacokinetic study, to predict the therapeutic efficacy. As in this work, it is proposed to develop controlled release oral dosage form of milnacipran hydrochloride (MIL), the designed formulations are required to evaluate in animal model for in-vivo release performance, extent and rate of absorption and other pharmacokinetic characters.

In the present study, in-vitro evaluation study helped to identify few formulations suitable for expected efficacy and thus required in-vivo evaluation. These selected formulations were administered to rabbits to study their in-vivo performance.

6.2 Experimental

6.2.1 Preparation of formulations for animal study

Based on in-vitro release studies (T50% : 4 ± 1 h and T80% : 12 ± 2 h) hydrophilic polymeric matrix formulations H15K-3, H100K-3, CMC/H15K-3 and multi granules polymeric matrix formulation F-7 were made of suitable dose (25 mg) for pharmacokinetic study in rabbits. It was ensured that the designed low dose formulations produced same characters and release profile as higher dose products.

6.2.2 In-vivo study in rabbits

In vivo study for each selected formulation was carried out on six New Zealand albino male rabbits weighing between 2.0 and 2.5 kg. The animals were kept in individual cages and maintained at 25° C for 10 days prior to experiment. Standard diet and water ad libitum were given to them. All experiments have been performed according to guidelines of the Institutional Animal Ethics Committee (Protocol approval No. IAEC/RES/16/07) at BITS, PILANI, India. The experiments were conducted as per CPCSEA (Committee for Prevention, Control and Supervision of Experimental Animals) guidelines. All studies were performed after keeping the

rabbits on fast overnight. The oral administration of tablets (IR or selected CR matrix tablets) was done by opening the mouth of rabbits using a specially designed restrainer and placing the tablet carefully at the end tongue to ensure proper administration of the tablet.

6.2.3 Sample preparation

Blood samples were collected in triplicate (sampling from three rabbits at each time point alternatively from a group of six rabbits) from marginal ear vein of rabbits at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h post dosing of formulation using a 21 G needle in clean and dry centrifuge tubes. Blood samples were also collected from all the rabbits just before the administration of the tablets. The blood samples were processed as suggested in chapter 3. The processed samples were analyzed using in-house developed bioanalytical HPLC method as described in chapter 3.

6.2.4 Data analysis

The plasma drug concentration at various time points was determined and subjected to non-compartmental analysis using WinNonlin Standard edition, Version 2.1 software (WinNonlin Scientific Consultants, USA) to obtain various pharmacokinetic parameters like C_{max} (Maximum serum concentration), T_{max} (Time taken to reach maximum concentration), area under the curve (AUC) and mean residence time (MRT). The elimination half-life was determined based on the last four time points in the elimination phase obtained for each tablet. Relative bioavailability (F_r) values for the CR matrix tablets were determined as the ratio of $AUC_{(0-\infty)}$ of CR matrix tablet to the $AUC_{(0-\infty)}$ of IR tablet. The differences in various PK parameters were evaluated statistically by one way ANOVA.

6.2.5 In-vitro in-vivo correlations (IVIVC) analysis

Level A IVIVC is the most informative and very useful from a regulatory perspective since it represents a point-to-point relationship between in-vitro dissolution and the in-vivo input rate of the drug from the dosage form [1]. In-vitro dissolution data and obtained plasma drug levels are then treated mathematically to determine whether a correlation exists. Mostly such correlation can usually be expected when drug release from formulation is governing the subsequent absorption kinetics in the body [2]. This is an essential design element for a modified-release dosage form. Level A IVIVC for MIL formulations was established by plotting the fraction drug

dissoluted $(F)_d$ of optimized formulation versus the fraction drug absorbed $(F)_a$ in-vivo. Fraction dissolved values were taken from in-vitro release data and fraction absorbed was determined by the Wagner–Nelson method using the following equation:

$$(F)_a = C_p + k_e * AUC_{0-t} * 100 / k_e * AUC_{0-\infty}$$

where $(F)_a$ is the fraction of drug absorbed at any time t, C_p is the drug plasma concentration at same time t, k_e is the elimination rate constant, AUC_{0-t} and $AUC_{0-\infty}$ are areas under the curve between time zero and time t and between time zero and infinity, respectively. Linear regression analysis was applied to fit the data and regression coefficient (r) was calculated to evaluate the robustness of IVIVC [3-4].

6.3 Results and discussion

The mean plasma concentration of MIL following oral administration of above formulations is shown in Figure 6.1. The extent of absorption is a key characteristic of a drug formulation, and therefore the AUC is an important parameter for analysis in a comparative bioavailability study. Further, C_{max}, T_{max}, $t_{1/2}$, and MRT were also determined as they are also important pharmacokinetic parameters for comparison as shown in Table 6.1.

As illustrated in Figure 6.1, the plasma concentration–time profiles of MIL explicitly indicated that in all CR formulations, plasma profile extended to 24 hrs in comparison to 15 hrs for IR tablets suggesting extended and controlled release and absorption of MIL from the designed formulations. Remarkable differences in plasma drug profile were observed between IR and different CR formulations, expressed by lower C_{max} and delayed T_{max} values for CR tablets, but with longer plasma profile.

The absorption of MIL from IR tablets were found to be rapid with high C_{max} (1075.25 ng/ml) value attained at 1 h post administration but the plasma concentrations of MIL declined rapidly. The C_{max} values of H15K-3, H100K-3, CMC/H15K-3 and F-7 were found to be 727.55, 652.23, 641.69 and 640.58 ng/ml respectively which were found to be significantly ($p < 0.5$) lower than that produced by IR tablets but extended for longer time. The time to reach peak plasma concentration of CR formulations found to be between 6-8 h, more than immediately release tablets, due to the lower rate of drug release in case of CR formulations. From the profiles it can be predicted that onset of action may not be delayed.

The mean AUC value for the prepared IR formulation was found to be 6152.17 ng.h/ml and for CR formulations were found to be between 10940.14 to 11731.49 ng.h/ml. There was statistically significant difference ($p < 0.5$) between AUC values for the IR and CR formulations. This Enhanced T_{max} (6-8 h compared to 1 h) and longer plasma drug profile indicated the controlled release characteristic of the designed CR formulations. Another indication of controlled drug delivery of the prepared formulations is the increase in mean residence time (MRT) and absorption rate (C_{max} /AUC) [5-6]. The MRT was found to be doubled compared to IR tablet and absorption rate was also significantly lower for the CR tablets (0.065 h versus 0.18 h for IR) .

Plasma drug levels were converted to fraction drug absorbed using Wagner–Nelson method and correlated with fraction drug released in-vitro. Level A correlation (IVIVC) was observed applied for all the CR formulations. The regression coefficient was found in the range of 0.9747 to 0.9974, which indicated a good correlation between in-vitro release and in-vivo absorption of MIL from designed CR formulations as shown in Figure 6.2. This correlation can be further used for various modification in formulations.

Based on in-vitro and in-vivo outcomes, it could be concluded that administration of designed CR tablets, the drug plasma level was markedly lower and maintained for 24 h. This extended and lower MIL plasma level was expected to reduce the frequency of dosing and intensity of the previously mentioned side effects associated with the immediate release formulations.

6.4 Conclusions

In-vivo performance of different optimized controlled release formulations of MIL studied in rabbits against an immediate release formulation of MIL confirmed controlled release character of the designed formulations. The delayed T_{max}, prolonged half life and reduced C_{max} indicated slow and prolonged in-vivo release and absorption of MIL from CR tablets. A good Level A IVIVC was observed for developed CR formulations.

References

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2. Devane, J., Butler, J., 1997. The impact of in vitro-in vivo relationships on product development. Pharm. Tech., 21, 146-159.
3. Jaber, E., 2006. In vitro - in vivo correlation: from theory to applications. J. Pharm. Pharmaceut. Sci., 9, 169-189.
4. Skelly, J., Amidon, G., Barr, W., Benet, L., Carter, F., Robinson, J., Shah, V., Yacobi, A., 1990. Testing and correlation for oral controlled/modified-release dosage forms. Pharm. Res., 7, 975–982.
5. Saleh, A., Al-Suwayeh, Mohammed, H., 2009. In vitro and in vivo evaluation of sustained release hydralazine hydrochloride tablets prepared by thermal granulation technique. Aust. J. Basic & Appl. Sci., 3, 2866-2875.
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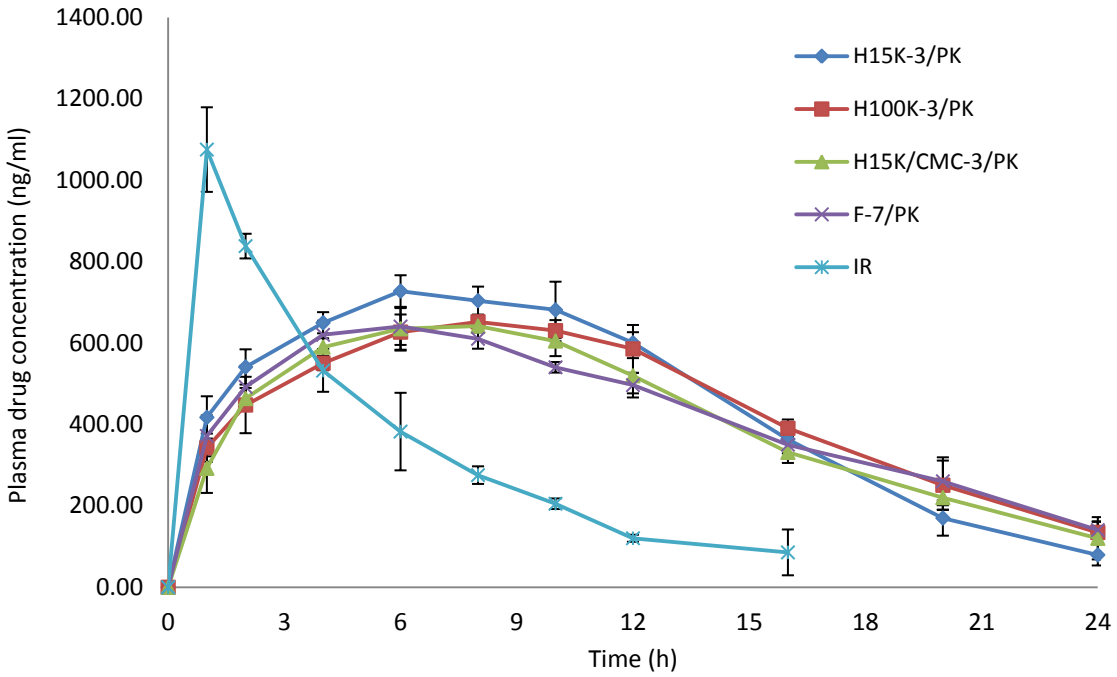

Figure 6.1: Comparative in-vivo plasma drug concentration profile of immediate release and controlled release tablets of MIL in rabbit.

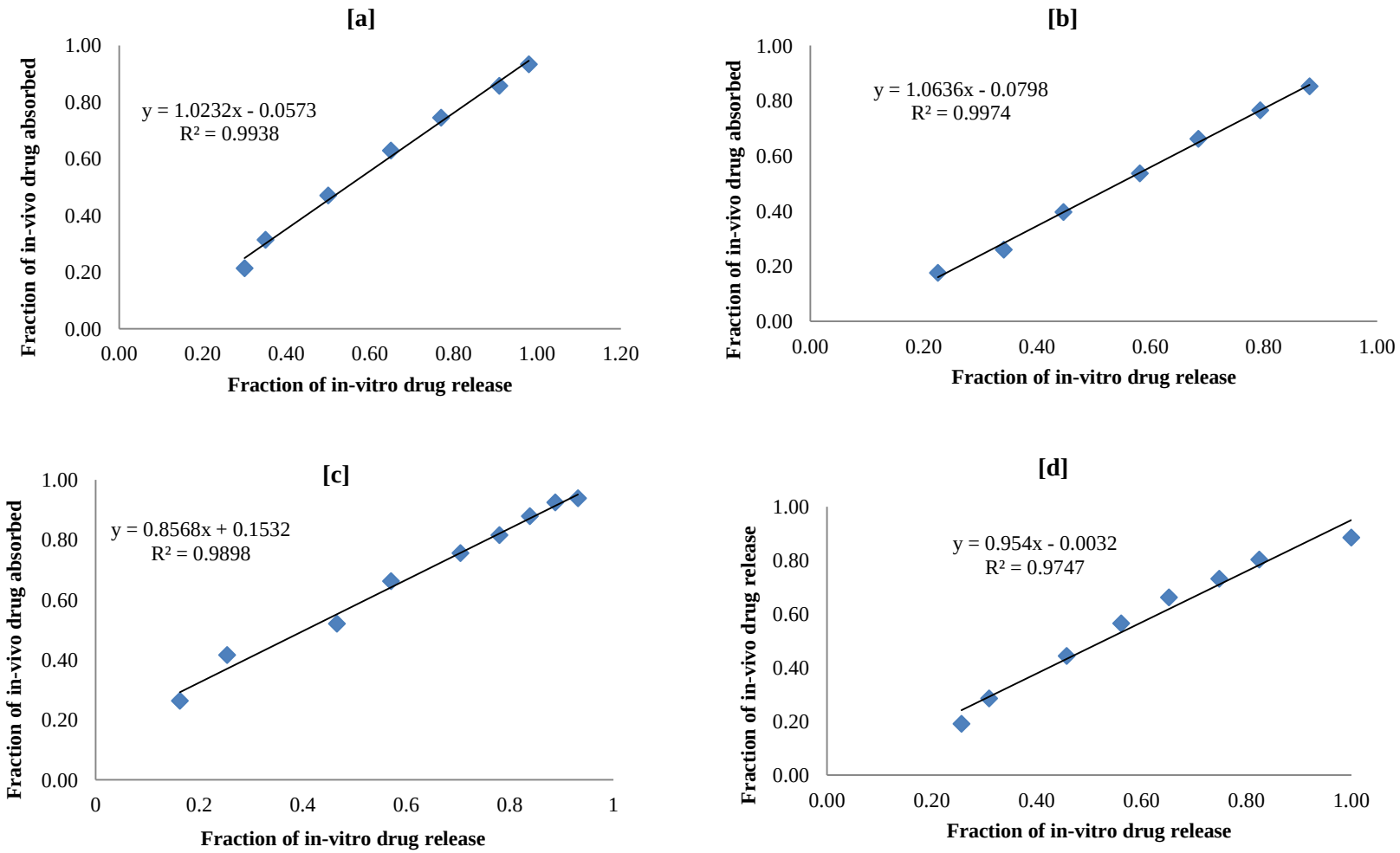

Figure 6.2: Level A IVIVC of designed CR formulations of MIL. (a) H15K-3, (b) H100K-3, (c) H15K/CMC-3 and (d) F-7.

Table 6.1: Summary of pharmacokinetic parameters obtained following the oral administration of single dose of immediate release (IR) tablet and selected CR matrix tablets of MIL in rabbits.

Parameters	IR Tablets	H15K-3	H100K-3	H15K/CMC-3	F-7
C_{max} (ng/ml)	1075.25 ± 103.92	727.55 ± 38.97	652.23 ± 17.32	641.69 ± 15.59	640.58 ± 45.23
T_{max} (h)	1 ± 0.15	6.00	8.00	8.00	6.00
$AUC_{(0-\infty)}$ (ng.h/ml)	6152.17 ± 235.18	11268.22 ± 354.77	11634.42 ± 480.96	10940.14 ± 297.35	11731.49 ± 421.38
$AUMC_{(0-\infty)}$ (ng.h^2/ml)	38857.27 ± 2578.32	115412.80 ± 6574.45	145028.87 ± 7254.93	132129.79 ± 6879.65	158896.86 ± 8547.58
MRT (h)	4.95 ± 0.15	9.51 ± 0.34	10.38 ± 0.23	10.03 ± 0.18	10.14 ± 0.37
Elimination half-life (h)	3.43 ± 0.23	6.59 ± 0.42	7.19 ± 0.65	6.95 ± 0.50	7.03 ± 0.71
Relative bioavailability (F_r)	-	1.83	1.89	1.78	1.91

CHAPTER 7

SUMMARY AND CONCLUSIONS

7.1 Summary and conclusions

Milnacipran hydrochloride (MIL) is well used drug for the treatment of depression and fibromyalgia. The objective of present study was to design matrix embeded oral controlled release (CR) formulation, using various hydrophilic and hydrophobic polymers, to overcome the problems associated with multi dose conventional dosage form of MIL for long term therapy and for better patient compliance.

Controlled release tablets were prepared by different approaches using different polymers, individually or mixture. All the designed formulations were evaluated for their drug content, weight variation, hardness, friability and in vitro release profile. Based on in vitro evaluation, selected formulations were used to do bioavailability and pharmacokinetic studies in animal model.

Preformulation studies indicated that MIL was non-hygroscopic, poor flowing and highly soluble between pH 1.2 to 10. Solid state stability studies showed that MIL was stable and compatible with various excipients for sufficient time period. In addition, DSC and FTIR studies also indicated the compatibility of MIL with excipients used for controlled release formulations. Controlled release formulations were prepared using hydrophilic polymers (HPMC 15K, HPMC 100K, sodium CMC, carbopol) alone or in combination using wet granulation process. Multi granules based CR tablets were prepared with mixing the granules of hydrophilic polymers and hydrophobic polymers in different proportions. The quality control parameters of all the formulations were found to be satisfactory and within the official pharmacopoeial limits suggesting used process can produce good quality products.

In-vitro drug release studies of designed formulations were carried out in USP type II apparatus using suitable media. The effect of various formulation variables such as polymer type, polymer proportion, polymer viscosity and compression force; and effect of dissolution factors like pH of dissolution media and agitation speed on the in-vitro drug release were assessed using dissolution parameters.

In-vitro release profile and dissolution parameters indicated that the release was significantly dependent on the proportion and nature of polymer used. Hydrophilic polymer matrices that contained lower concentrations of polymer tended to release the drug quickly and at higher polymer concentration release extended up to

24 h. Results also indicated that as viscosity of polymer increased, the release rate extended significantly.

The drug release was found to be much faster for tablets compressed at lower compression force (4 kg/cm^2) than the formulations compressed at higher compression forces (7 kg/cm^2 and 10 kg/cm^2). Tablets prepared with carbopol showed insignificant difference in release at varied compression force due to formation of highly hydrated matrix which was independent on hardness of tablet.

Use of hydrophilic matrices, HPMC and NaCMC, indicated that drug release was not affected by pH of dissolution fluid. It was observed that the drug release from carbopol matrices was dissolution medium-dependent due to the anionic nature of carbopol. Agitation speed from 50 to 100 rpm had no significant effect on drug release but at higher rpm (150 rpm) significant effect on drug release was observed.

In case of multi granules based CR tablets, all the dissolution parameters indicated that hydrophobic wax proportion was playing a major role in controlling the drug release. The release of MIL from the combinations got more retarded than that of formulations using single hydrophilic polymer. It may be due to higher lipophilicity offered by combination of waxes results in slower penetration of dissolution medium in matrices. All the designed CR formulations were found to be stable for entire stability study duration with no apparent change in physical characteristics and in-vitro release behavior.

In-vivo studies of selected CR formulations in rabbits demonstrated prolong release as plasma concentration found to be sustained up to 20-24 h. Remarkable differences in plasma drug profile were observed between immediate release and designed CR formulations. The delayed t_{max}, prolonged plasma residence time, reduced C_{max} and extended plasma concentration indicated slow and prolonged in-vivo release and absorption of MIL from CR tablets. Thus, it can be postulated that designed CR formulations can overcome the disadvantages associated with conventional tablets of MIL.

The present study suggested that the designed CR formulations are promising for commercialization. The method used for manufacturing was found to be

relatively simple and can easily be adopted in conventional formulation manufacturing units on a commercial scale.

7.2 Future perspectives

- As future perspective of present work, study the effect of process scale-up on formulation characters and release profile need to be studied.
- There is scope for making formulations using other type of polymers, singly and in combinations. Various formulation parameters such as effect of drug loading, polymer particle size, tablets geometric, etc also need to be studied further.
- In the present work, selected controlled release formulations were evaluated in animal model for in-vivo performance. However, further studies of the developed delivery systems need to be carried out in human subjects to establish clinical effectiveness of the designed formulations.
- Designed formulation need to be evaluated further for therapeutic efficacy with pharmacodynamic study.

APPENDIX

List of Publications and Presentations

Publications

1. Singhvi, G., Kalantre, P., Dhoot, H., Saha, R.N., 2013. Spectrophotometric determination of nor-epinephrine serotonin reuptake inhibitor (snri) drug milnacipran in pure and in dosage forms. Asian Journal of Chemistry, 25, 3682-3686.
2. Singhvi, G., Gampa, G., Saha, R.N., 2013. Development and validation of a stability indicating liquid chromatographic method for the determination of milnacipran in bulk and its formulations. Current Pharmaceutical Analysis, 9, 191-198.
3. Singhvi, G., Shah, A., Yadav, N., Saha, R.N., 2014. Study the effect of formulation variables on drug release from hydrophilic matrix tablets of milnacipran and prediction of in-vivo plasma profile. Pharm. Dev. Technol., 19, 708-716.
4. Singhvi, G., Shah, A., Nalla, S.R., Saha, R.N., 2014. Comparative pharmacokinetic evaluation of controlled release matrix tablets of milnacipran Hydrochloride in rabbit. Drug delivery letters, 4, 21-25.
5. Singhvi, G., Parmar, N., Patel, N., Saha, R.N., 2014. Novel multi-granules controlled release tablets of milnacipran: design with simplex lattice technique, in-vitro characterization and pharmacokinetic predictions. Journal of Young Pharmacist, 6, (3), 24-31.
6. Singhvi, G., Shah, A., Yadav, N., Saha, R.N., 2014. Prediction of *in vivo* plasma concentration–time profile from *in vitro* release data of designed formulations of milnacipran using numerical convolution method. Drug Dev. Ind. Pharm. In press, (doi:10.3109/03639045.2013.850706)
7. Singhvi, G., Shukla, V.K., Ukawala, R., Gampa, G., Saha, R.N., 2013. Development of a new, rapid and sensitive HPTLC method for estimation of Milnacipran in bulk, formulation and compatibility study. Arabian Journal of Chemistry. In press (doi:10.1016/j.arabjc.2013.09.004).

Paper Presentations at National and International conferences

1. Singhvi, G., Saha, R.N., Dhoot, H., Shekar, V. Development and validation of rapid and simple UV- spectrophotometric method for estimation of milnacipran hydrochloride in bulk and formulation. Contemporary Trends in Biological and Pharmaceutical Research (CTBPR), 2011, Pilani, India.
2. Singhvi, G., Sharma, S., Goyal, M., Gampa, G., Saha., R.N. Design and characterization of hydrophilic and hydrophobic matrix based sustained release tablet of SNRI drug candidate. 3rd world congress on Bioavailability and Bioequivalence, pharmaceutical R&D summit, 2012, Hyderabad, India.
3. Singhvi, G., Yadav, N., Shukla, V.K., Saha, R.N. Study of formulation variables influencing the drug release from hydrophilic matrix tablets of milnacipran. 13th International symposium of Controlled Release Society Indian Chapter on Advances in Technology and Business potential of new drug delivery systems, 2013, Mumbai, India.

Biography of Dr. Ranendra N. Saha

Dr. Ranendra N. Saha is Shri B K Birla & Shrimati Sarala Birla Chair Professor (Senior Professor of Pharmacy) and presently Director of BITS Pilani Dubai Campus. He has been HoD, Dean and Deputy Director at BITS Pilani. He completed his Bachelor of Pharmacy and Master of Pharmacy from Jadavpur University, Kolkata and Ph.D. from BITS, Pilani. In 2011 he has been awarded *Shri B. K. Birla and Shrimati Sarala Birla Chair Professorship* at BITS Pilani for his contributions in teaching and research. He has vast experience in the field of Pharmacy especially in Pharmaceutics, novel drug delivery systems and Pharmacokinetics. He received "*Pharmacy Professional of the Year 2013*" Award given by Indian Association of Pharmaceutical Scientists and Technologists. He is also recipient of "*The Best Pharmacy Teacher Award*" for the year 2005, awarded by Association of Pharmaceuticals Teachers of India (APTI), in recognition of his contribution in teaching and research in the field of Pharmacy. He visited several countries on invitation and Visiting Professor to Kathmandu University, Nepal.

He has more than 33 years of teaching, research and administrative experience. He has supervised large number of doctoral, postgraduate and undergraduate students. He has published book, several book chapters, research articles in renowned journals and presented papers in conferences in India and abroad. He has successfully completed several government and industry sponsored projects. Dr. Saha has developed commercial products for industries, transferred technologies to industries and filed patents. He is member of advisory board and selection committee member of a number of Universities in India and abroad.

Biography of Mr. Gautam Singhvi

Mr. Gautam Singhvi has completed his Bachelor of Pharmacy from Lachoo memorial college of Sc. and Tech., Jodhpur, Rajasthan university in the year of 2005 and Master of Pharmacy from Bharathidasan University, Tiruchirappalli in 2007. He served for Torrent Research Centre, Ahmedabad for nearly one and half years in formulation development department. He joined as lecturer in Department of Pharmacy, BITS Pilani in 2008. He registered in doctoral program of BITS Pilani in 2008. He has published research articles in renowned journals and presented papers in conferences.