Studies on Oral Controlled Release Formulations of Rifampicin and Isoniazid

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on Oral Controlled Release Formulations of Rifampicin and Isoniazid**" and submitted by **HIREMATH PRAVEEN SANGAMESH**, ID No. 2000PHXF022 for award of Ph. D. Degree of the Institute, embodies original work done by him under my supervision.

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With love

To

My Wife, Daughter,

Parents and In-laws

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol / Abbreviation	Meaning
"	Inches
#	Number
%	Percentage
% RSD	Percentage relative standard deviation
% w/v	Percentage weight by volume
α	Alpha
γ	Gamma
λ_{max}	Wavelength of maximum absorption
λ_{data}	Wavelength of actual estimation
nmol/ml	Nanomole per milliliter
ng	Nanogram
ng/ml	Nanogram per milliliter
~	Approximately
<	Less than
=	Equals to
>	Greater than
µg	Microgram
µg/ml	Microgram per milliliter
µm	Micrometer
21 CFR Part 11	Title 21 Code of Federal Regulations described by US FDA
3-FRSV	3-formyl rifamycin SV
AIDS	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
BCS	Biopharmaceutical classification system
BP	British Pharmacopoeia
C.V.	Coefficient of variation
C-18	Octa decyl silane column packing

Class-I drug	Drug with high solubility and high permeability as per BCS
cm	Centimeter
cm^2	Square centimeter
C_{max}	Maximum concentration
CNS	Central nervous system
Cp,dis	Polymer disentanglement concentration
cPs	Centipoises
CRT	Controlled room temperature (25 ± 2 °C and 60 ± 5% RH)
D	Actual diffusion coefficient in the release media
D*	Apparent diffusion coefficient in the hydrated gel layer
D1	First derivative absorbance (amplitude) value
DC	Direct compression or directly compressed
DNA	Deoxyribose nucleic acid
DOTS	Directly observed treatment shortcourse
e.g.	For example
EC	Ethyl cellulose
et al	And co-workers
f_2 factor	Similarity factor value for drug release profiles
F_{Calc}	Calculated F-value
F_{Crit}	Critical F-value (F statistics table)
FDC	Fixed dose combination drugs for the treatment of TB
FFBE	Flat faced bevel edges
FT	Freeze condition (5 ± 2 °C)
F-value	The measurement of distance between individual distributions
g	Gram
GIT	Gastrointestinal tract
GMP	Good manufacturing practice
h	Hour
H/INH	Isoniazid

HCl	Hydrochloric acid
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
h^{-n}	Per hour raised to the power 'n' (the diffusional exponent)
HPC	Hydroxypropyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
HQC	Higher quantification concentration
i.e.	That is
ICH	International Conference on Harmonization
IP	Indian Pharmacopoeia
IR	Infra red
IUATLD	International Union Against Tuberculosis and Lung Diseases
K	Release rate constant
kDa	Kilo Dalton
K_{deg}	First order degradation rate constant
kg	Kilogram
kg/cm^2	Kilogram per square centimeter
KH_2PO_4	Potassium dihydrogen orthophosphate
KOH	Potassium hydroxide
LAM	Lipoarabinomannam
L-HPC	Low substituted (low molecular weight) HPC
LOD	Limit of detection
LOQ	Limit of quantitation
LQC	Lower quantification concentration
M	Molar
M. tuberculosis	*Mycobacterium tuberculosis*
MC	Methyl cellulose
mg	Milligram
mg/kg	Milligram per kilogram

MIC	Minimum inhibitory concentration
min	Minute
ml	Milliliter
ml/min	Milliliter per minute
mM	Millimolar
mm	Millimeter
mm^2	Square millimeter
mPas	Milli Pascal
MQC	Medium quantification concentration
MS	Mean sum of squares
Mt / M∝	Fraction of the drug released at any time 't'
mV	Millivolts
MW	Molecular weight
n	Diffusional exponent, indicative of the release mechanism
N	Normality of a solution
NaCl	Sodium chloride
NaH_2PO_4	Sodium dihydrogen orthophosphate
NaOH	Sodium hydroxide
NCE(s)	New chemical entity(s)
NDDS	Novel drug delivery system
NIR	Near Infrared
nm	Nanometer
NME	New molecular entity
NSAID(s)	Nonsteroidal anti-inflammatory drug(s)
°C	Degrees Centigrade
OCRS(s)	Oral controlled release system(s)
p/p-value	Probability of observing a test statistic
PEO	Polyethylene oxide
pH	Negative logarithm of hydrogen ion concentration
PHEMA	polyhydroxyethylmethyl acrylate
pKa	Negative logarithm of acid dissociation constant

PLG	Poly (DL-lactide-co-glycolide)
PLS	Partial least square
PO_4	Phosphate buffer
r	Correlation coefficient
R	Rifampicin
RH	Relative humidity
RNA	Ribose nucleic acid
RP	Reverse phase
rpm	Revolutions per minute
S.D.	Standard deviation
SC	Standard concave
SE	Standard error
sec(s)	Second(s)
SGF	Simulated gastric fluid (without pepsin)
SIFsp	USP Simulated intestinal fluid (without pancreatin)
SLS	Sodium lauryl sulphate
SS	Sum of the squares
τ	Tortuosity factor
$t_{1/2}$	Half life
$t_{50\%}$	Time taken for 50% drug release
$t_{90\%}$	Time to reach 90% potency
TB	Tuberculosis
TNF-α	Tumor necrosis factor - alpha
USFDA	Food and Drug Administration, United States of America
USP	United States Pharmacopoeia
UV	Ultra violet
vs.	Versus
WHO	World Health Organization
ZCP	Zero crossing point

SUMMARY

For centuries tuberculosis (TB) has remained a complex socio-economic problem that impedes human development. Apart from annual death toll of two million, people of developing and poor countries are paying intangible economic, psychological and social costs in terms of manpower loss, pain, suffering, grief and discrimination. Although current chemotherapeutic agents for tuberculosis treatment are therapeutically effective and well tolerated, a number of problems still remain. The treatment of tuberculosis is burdensome. Tuberculosis requires multidrug therapy for prolonged period of time, which are generally associated with incidence of toxic or side effects, thus resulting in poor patient compliance and discontinuation of the therapy. The poor bioavailability of the rifampicin and isoniazid from conventional fixed dose combination (FDC) formulations is another major drawback in the treatment of tuberculosis. Consequently, attempts are being made either to develop alternative drugs or to reduce toxicity and improve bioavailability of already existing drugs by developing novel drug delivery systems. Finding more effective drugs requires exhaustive in put in terms of money and time with involvement of tough process. Further more, there is no surety that after all these efforts the invented new drugs will be therapeutically efficient, safe and more promising than the existing drug candidates. Therefore, the best alternative is better utilization of the existing drugs at their existing dose levels for the efficient and effective treatment of tuberculosis by designing alternative better delivery systems. This cost effective alternative will especially be very useful in many Third world countries like India as the tuberculosis considered to be a serious threat, particularly to poor people. This goal could be achieved by development of oral controlled release formulations for rifampicin and isoniazid, alone and in combination. These formulations will not only overcome the bioavailability problems associated with these drugs but also will minimize the potential adverse effects and will enhance the patient compliance as the amount and number of doses will decrease.

The objective of the present research endeavor was to design the controlled release (C.R.) formulations of rifampicin and isoniazid, two important first line anti-tubercular drugs commonly used in the treatment of the mycobacterium tuberculosis infection. The present

work aimed at the design of C.R. formulations of rifampicin and isoniazid, alone and in combination using different hydrophilic and hydrophobic polymers, alone and in combinations. The designed formulations were evaluated for their in vitro characteristics like, physical properties and in vitro release studies.

To support various activities like, pure drug analysis, assay of the designed C.R. formulations, in vitro release studies of C.R. formulations, and analysis of stability study samples; simple, rapid, sensitive, and accurate UV visible spectrophotometric methods have been selected or developed for the estimation of rifampicin and isoniazid, alone and in their combination. A spectrophotometric method has been selected for the estimation of rifampicin. For isoniazid estimation and simultaneous estimation of rifampicin and isoniazid, new methods have been developed. The selected or developed methods were validated statistically according to standard guidelines (USP 2000 and ICH guidelines 1996) and by recovery studies.

Further for proper formulation development, preformulation studies were carried out. The drug excipient compatibility (solid state stability) studies were carried out for both the drugs (rifampicin and isoniazid) with various prospective polymers/additives at various storage conditions (as per ICH 1996 guidelines) and the drugs found to be stable in presence of most of the additives and polymers. Similarly, the solution state stability studies for the drugs have been carried out at 37 ± 2 °C in various buffered and unbuffered pH solutions. Both rifampicin and isoniazid showed pH dependent stability (in solution), rifampicin degradation was higher at low (1.2) and high (7.4) pH conditions and was comparatively more stable around pH 5.0, isoniazid was more stable at lower pH value (1.2) and degradation rate was higher at pH 2.0 above which isoniazid stability again increased. The solubility studies of rifampicin were carried out in various buffered and unbuffered pH solutions. It was observed that the rifampicin was more soluble at lower pH (1.2) and increase in the pH decreased the solubility up to pH 6.8 beyond which solubility further increased due to zwitterionic nature of the rifampicin with two pKa values. The detailed solubility studies for isoniazid were not done as isoniazid is reported to be Class-I drug according to biopharmaceutical classification system. The studies for dissolution method development/selection were carried out for both rifampicin and isoniazid. For rifampicin, the reported dissolution medium, pH 7.4 phosphate buffer with

0.02% w/v of ascorbic acid was selected and validated in our laboratory conditions. For isoniazid, pH 7.4 phosphate buffer was selected as dissolution medium and validated. For rifampicin and isoniazid combined C.R. formulations, the dissolution media used were simulated gastric fluid (SGF, pH 1.2) for first two hours followed by simulated intestinal fluid USP (SIFsp, pH 6.8) further up to 24 h, and the method was validated.

The C.R. formulations of rifampicin and isoniazid (alone and in combination) have been formulated by matrix embedded technology using different hydrophilic and hydrophobic polymers like, hydroxypropyl methylcellulose (HPMC) of different viscosity grades, hydroxypropyl cellulose (HPC), Carbopol 934P, Eudragit L100-55, ethyl cellulose (EC) and their combinations. The effect of following parameters on the in vitro drug release characteristics has been studied;

- Polymer type
- Polymer ratio
- Polymer viscosity
- Compression force
- Method of granulation
- Dissolution methodology
- Hydrodynamic conditions in release studies
- Addition of Eudragit L100-55
- Change in the formulation/processing technology
- Addition of hydrophobic polymer EC in hydrophilic polymer matrices
- Presence of one drug on other drug's release when present in the same polymer matrix
- Change in the release medium and its volume
- Segregation of rifampicin and isoniazid in two separate layers as bilayer C.R. release matrix tablets

The release profiles were analyzed by various release kinetics models like, zero order release model, first order release model, Higuchi's square root kinetics model and Korsmeyer-Peppas model. Release profiles were further analyzed for f_2 (similarity) factor values to assess the similarity between the release profiles.

The designed matrix tablets of rifampicin and isoniazid, alone and in combination, showed good physical properties with very low weight variation, high degree of content uniformity and low friability indicating that the methods of preparation of formulations were reproducible and acceptable methods. The tablet manufacturing method was relatively simple and can be easily adopted in conventional tablet manufacturing units in industries on a commercial scale. All the parameters studied found to have significant influence on the release profiles of rifampicin and isoniazid from the matrix tablet formulations. The duration of rifampicin and isoniazid release could be extended from 4 h to 24 h by varying the polymer type, polymer ratio, polymer viscosity and processing techniques. The nature of the rifampicin release was found to be zero order or first order or Higuchi's square root kinetics depending upon the polymer type, polymer ratio and processing technique. The isoniazid release profiles found to follow Higuchi's square root kinetics or first order in all formulations. Increase in the polymer ratio decreased the release in case of both drugs. Increase in the polymer viscosity decreased the release in case of rifampicin, but in case of isoniazid beyond 4000 cPs (HPMC K4M) increase in the viscosity did not significantly affect the release. Rifampicin formulations were more sensitive to the effect of compression force. Among rifampicin formulations, the effect of compression force was more pronounced in case of formulations with low viscosity HPMC and HPC. The rifampicin release was faster from the directly compressed tablets than from the IPA granulated formulations. The rifampicin release was higher in case of USP type II (paddle) method compared USP type I (basket method). The effect of hydrodynamic conditions on rifampicin release was observed only in case of formulations with low viscosity HPMC. The release of isoniazid was higher in 0.1 N HCl compared to pH 7.4 phosphate buffer. The isoniazid release was faster from the IPA granulated formulations than from the directly compressed tablets in Carbopol 934P formulations. Addition of the Eudragit L100-55 decreased the release of isoniazid in both SGF and SIFsp. But the presence of Eudragit L100-55 resulted in the decrease in the rifampicin release in SGF and increase in the rifampicin release in SIFsp. In case of combined C.R. formulations, the release of rifampicin and isoniazid was not affected by the change in the release media from formulations with HPMC, where as, formulations with HPC were sensitive to the change in the release media. The variation in the

formulation processing technique, in terms of granulating the rifampicin along with the isoniazid instead of adding rifampicin extragranularly, found to have profound significance on the release profiles of both rifampicin and isoniazid in both HPC and HPMC. Release of both rifampicin and isoniazid decreased when the rifampicin was added intragranularly. The presence of both rifampicin and isoniazid in a single C.R. matrix tablets found to have mutual influence on their release profiles. In presence of isoniazid, rifampicin release rate increased and in presence of rifampicin, isoniazid release rate found to decrease. Bilayered tablets made using HPC and Eudragit for rifampicin and HPMC-Eudragit combination for isoniazid produced good release rate for both the drugs.

Three batches of each formulation were made and the formulations were evaluated for their physical properties and in vitro release characteristics. Very good batch reproducibilities were observed. The low standard deviation values for all physical properties like, drug content, friability, weight variation and hardness from three different batches prepared separately showed excellent reproducibility. Significantly similar release profiles of rifampicin and isoniazid from all batches proved the reproducibility of release profiles.

The two best formulations from each batch were selected for stability studies as per ICH guidelines. The rifampicin and isoniazid (in combination) were found to be stable at controlled room temperature (CRT) in all polymer formulations. Degradation rate was dependent on the temperature and humidity. Thus, aqueous granulation was avoided and wet granulation, using IPA, or direct compression was used in the manufacturing rifampicin and isoniazid matrix tablets. These methods were found to be significantly beneficial in obtaining stable C.R. matrix tablets. The similarity factor found to be high in all cases indicating that there were no significant change in rifampicin and isoniazid release profiles on 6 months storage.

Further in vivo absorption and bioavailability studies are required to be done to establish utility of the designed formulations and establish in vitro-in vivo correlation.

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Chapter 1

Introduction

1.1. Tuberculosis (TB)

Tuberculosis, caused by Mycobacterium tuberculosis (*M. tuberculosis*), remains the world's leading infectious killer disease and considered to be a foremost cause of death due to a single microorganism (Shishoo et al. 2001). It has probably killed 100 million people over the past 100 years, although a cure was available in the second half of the 20th century (Iseman 2000). Since the time immemorial, it has been a scourge to the mankind (Panchagnula et al. 2001). Fossil bones dating back to 8000 BC provide the earliest evidence of TB in man and animals. Globally, it has been estimated that one in every three people is infected with TB, and approximately 3 million die from it each year (Peloquin and Berning 1994, Pitchenic and Fertel 1992, Stead 1997). Taking in to the consideration the severity and spread of the disease, in 1993 World Health Organization (WHO) declared TB as a 'global emergency' as more than 1900 million people are infected with this organism (Singh et al. 2001). Each year 9-10 million people suffer from this disease worldwide and WHO projects that by 2020, another 200 million individuals to become sick, and 70 million to die from TB. Furthermore, the impending HIV pandemic has increased morbidity and mortality due to TB (Ramachandran et al. 2003, Sriram and Yogeswari 2003, Perlman 1999). By 2020, TB and HIV infection together are expected to account for 90% of adult deaths from infectious diseases (Baris 2000.). The disease spreads rapidly because of urban migration, over crowding and lack of sanitary facilities, which are more prominent in developing countries or poor countries like India. The problem in India is extremely greater as it is estimated that India accounts for one fourth of global TB burden. India has an estimated 14 million TB cases to which about 2 million are added every year. Each year TB kills 5 lakh people in India, more than 1350 every day and nearly 1 every minute (Govt. of India 1999).

There were an estimated 8–9 million new cases of tuberculosis in 2000, fewer than half of which were reported; 3–4 million cases were sputum-smear positive, the most infectious form of the disease (Corbett et al. 2003). Most cases (5–6 million) are in people aged 15–49 years. Sub-Saharan Africa has the highest incidence rate (290 per 100 000 population), but the most populous countries of Asia have the largest numbers of cases: India, China, Indonesia, Bangladesh, and Pakistan together account for more than half the global burden. 80% of new cases occur in 22 high-burden countries.

The global tuberculosis caseload appears to be growing slowly. Case numbers have declined more or less steadily in western and central Europe, North and South America, and the Middle East. By contrast, there have been striking increases in countries of the former Soviet Union and in sub-Saharan Africa (WHO 2003).

Tuberculosis rates have increased in the former Soviet Union because of economic decline and the general failure of tuberculosis control and other health services since 1991 (Shilova and Dye 2001). Periodic surveys have shown that more than 10% of new tuberculosis cases in Estonia, Lativia, and some parts of Russia are multi-drug resistant, i.e., resistant to at least isoniazid and rifampicin, the two most effective antitubercular drugs (WHO 2000a). However, resistance is a byproduct of tuberculosis resurgence in these countries, not the primary cause of it. HIV infection accounts for much of the recent increase in the global tuberculosis burden. Worldwide, an estimated 11% of new adult tuberculosis cases in 2000 were infected with HIV, with wide variations among regions: 38% in sub-Saharan Africa, 14% in more developed countries, and 1% in the Western Pacific Region. Rates of HIV infection among patients with tuberculosis have so far remained below 1% in Bangladesh, China, and Indonesia. The increase in tuberculosis incidence in Africa is strongly associated with the prevalence of HIV infection. Rates of HIV infection among tuberculosis patients are correspondingly high, exceeding 60% in Botswana, South Africa, Zambia, and Zimbabwe. About two million people died of tuberculosis in 2000; about 13% of these people were also infected with HIV (Corbett et al. 2003).

1.1.1. Chemotherapy of Tuberculosis

Mycobacteria are aerobic, non-spore forming, nonmotile bacilli with a waxy coat that causes them to retain the red dye when treated with acid in the acid-fast stains. Two species of mycobacterium cause tuberculosis: M. tuberculosis and M. bovis (Mycobacterium bovis). M. tuberculosis is transmitted by inhalation of infected droplets coughed or sneezed in to the air by a patient with tuberculosis.

The goals of treatment are to ensure cure without relapse, to prevent death, to stop transmission, and to prevent the emergence of drug resistance. M tuberculosis can remain dormant for long periods. The number of tubercle bacilli varies widely with the type of

lesion, and the larger the bacterial population, the higher the probability that naturally resistant mutants are present even before treatment is started (David 1970). Long-term treatment with a combination of drugs is required (Fox et al. 1999). Treatment of active tuberculosis with a single drug should never be attempted, and a single drug should never be added to a failing regimen (Iseman 2000).

Almost all recommended treatment regimens have two phases (American Thoracic Society 2003) on the basis of extensive evidence from controlled clinical trials. There is an initial intensive phase designed to kill actively growing and semidormant bacilli. This action shortens the duration of infectiousness with rapid smear and culture conversion after 2–3 months of treatment, in most cases (80–90%) (Girling 1989). At least two bactericidal drugs, isoniazid and rifampicin, are necessary in the initial phase. Pyrazinamide given in the initial intensive phase allows the duration of treatment to be reduced from 9 to 6 months, but it offers no benefit if given past the second month to patients with drug susceptible tuberculosis (Hong Kong Chest Service/British Medical Research Council 1991). The addition of ethambutol benefits the regimen when initial drug resistance may be present or the burden of organisms is high.

Drugs used in the treatment of tuberculosis can be divided in to two major categories; first line and second line drugs. "First line" agents combine the greatest level of efficacy with an acceptable degree of toxicity. These include, isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin (Mandell and Petri Jr 2001). The large majority of patients could be treated successfully with these drugs. Excellent results for patients with non-drug-resistant tuberculosis can be obtained with a 6-month course treatment; for the first 2 months, isoniazid, rifampicin and pyrazinamide are given, followed by isoniazid and rifampicin for the remaining 4 months. Administration of rifampicin in combination with isoniazid for 9 months also is effective therapy for all forms of disease caused by strains of M. tuberculosis susceptible to both the agents. In areas where primary resistance to isoniazid occurs, therapy usually is initiated with four drugs – rifampicin, isoniazid, pyrazinamide, and ethambutol (or streptomycin) until sensitivity tests are completed. Occasionally, however, because of microbial resistance, it may be necessary to resort to "second line anti-tubercular" drugs in addition, so that treatment may be initiated with 5 to 6 drugs. This category of agents include ofloxacin, ciprofloxacin,

ethionamide, aminosalicylic acid, cycloserine, amikacin, kanamycin, and capreomycin (Iseman 2000).

M. tuberculosis is a formidable organism having a sturdy wall, which is impervious to most antibiotics. Because of slow division of organism, no course of treatment of less than 6 months duration is effective in smear positive patients. Unfortunately, no drug that significantly improves treatment outcome or shortens the duration of treatment is likely to be available in the near future. Therefore, the only available option is to make use of existing drugs effectively and rationally. As treatment of TB always requires multi-drug therapy, to reduce the emergence of drug resistant strains, WHO has formulated a standard Short Course Chemotherapy regimens consisting of essential first-line anti-TB drugs. Treatment regimens have an initial (intensive) phase lasting for 2 months and a continuation phase for next four months. In the intensive phase, there is a rapid killing of tubercle bacilli; infectious patients become non-infectious within 2 weeks and symptoms improve. For sterilizing effects, in the continuation phase, fewer drugs are necessary for a longer period of time.

1.1.1.1. Directly Observed Treatment Shortcourse (DOTS)

Several studies have shown that reliable prediction of which patients will take all prescribed medication by themselves is not possible (Fox 1993); only direct observation can ensure that all drugs are taken. Directly observed treatment, in which a trained observer personally observes each dose of medication being swallowed by the patient, can ensure high rates of treatment completion, reduce development of acquired drug resistance, and prevent relapse (Weis et al. 1994). Non-adherence to tuberculosis treatment is known to have been common ever since the advent of chemotherapy in the 1950s. Thus, most tuberculosis treatment trials since that time have been carried out with direct observation.

Anti-tuberculosis drug therapy is the only effective means for the treatment of active disease. Single drug therapy is associated with a substantial relapse rate and development of initial drug resistance quickly. Therefore, a patient with active disease should receive treatment at least with three drugs to rapidly decrease the bacillary population and to prevent the emergence of drug resistance. Short course chemotherapy is the most

effective way and DOT improves compliance and ensures effectiveness of drug treatment (Bhagi 2001).

1.1.1.2. Fixed dose combination (FDC) tablets in the management of TB

Despite the availability of highly effective drugs for TB, curing rate still remains low, since patients do not take prescribed medication with sufficient regularity and duration to achieve a complete cure. A major threat of patient non-compliance is an increased risk of developing acquired drug resistance (Kochi 1993). To avoid the problem of further creation and propagation of multi-drug resistant (MDR) TB due to non-compliance, WHO and international union against tuberculosis and lung diseases (IUATLD) recommended the use of FDC formulations in place of single drug formulations (WHO 1999). Deviations from such regimens result in increased cost and risks of side effects, or decreased chance of cure, or both. FDC formulation thus is a combination of two or more first line anti-TB drugs namely rifampicin, isoniazid, pyrazinamide and ethambutol in a fixed proportion in a single dosage form that evolved from the fact that TB always require multi-drug treatment (Blomberg and Fourie 2003).

FDC therapy has some advantages like (Joint Tuberculosis Committee of the British Thoracic Society 1998, Mitchison 1998, WHO 1999, Hong Kong Chest Service/British Medical Research Council 1989, Uplekar and Shepard 1991),

1) Reduced risk of emergence of drug resistant strains
2) Less risk of medication errors
3) Dosage adjustment according to patient need
4) Simplify drug supply management, shipping and distribution
5) FDC tablets minimize the risk of theft and misuse of rifampicin for conditions other than tuberculosis
6) Better management of DOTS using FDCs and better patient compliance

1.1.2. Problems associated with conventional therapy

1.1.2.1. Toxic or adverse effects

Chronic diseases like tuberculosis require multidrug therapy for prolonged period of time, which are generally associated with incidence of toxic or side effects that result in poor patient compliance and discontinuation of the therapy (Prabhakaran et al. 2004). A major threat of patient non compliance is an increased risk of developing acquired drug resistance (Ain et al. 2002). Presently available drugs, especially rifampicin and isoniazid, are associated with incidence of adverse/ toxic effects, which necessitates the cessation of therapy.

Most of the toxic/ adverse effects of anti-tubercular drugs are dependent on both the dose and administration interval. Common adverse effects of antitubercular drugs include severe gastrointestinal disturbances like nausea, anorexia, abdominal pain, vomiting and diarrhoea. Cutaneous reactions and severe hypersensitivity reactions like immune thrombocytopenia, haemolytic anaemia and/ or acute renal failure are also reported. Influenza like syndrome characterized by fever, chills myalgias and arthralgias are other adverse effects. Chronic peripheral neuropathy and other CNS toxic effects are also common (Burman et al. 2001, Ross and Horne 1990).

Another serious and life threatening adverse effect associated with anti-TB therapy is toxic hepatitis. Anti-tubercular drug induced hepatitis is one of the most prevalent drug-induced liver injuries in most of the countries (Pande et al. 1996, Parthasarathy et al. 1986). The incidence is typically between 1% to 36%, and mortality cases are not rare. Isoniazid is the major drug incriminated in anti-tubercular drug induced hepatotoxicity (Black et al. 1975). It has been observed that concomitant administration of rifampicin and isoniazid, which is the main goal of the FDC formulations, further increase the risk of hepatitis (Steele et al. 1991, Malcom et al. 1991, Lesobre et al. 1969). There has been a serious concern that the incidence of hepatitis with jaundice may be much greater (approximately 10%) in Indian patients receiving this combination. It has been speculated that acetyl isoniazid, the main metabolite of isoniazid, undergoes conversion into monoacetyl hydrazine whose metabolites are hepatotoxic. These enzymatic reactions may be enhanced by rifampicin, which is a powerful inducer of liver enzymes. Another

hypothesis postulates formation of hydrazine and isonicotinic acid as a result of the metabolism of the isoniazid, the former being hepatotoxic. This reaction is believed to be enhanced by enzyme induction mediated by rifampicin (Ross and Horne 1990). One more report says that rifampicin administration enhances the isoniazid hepatotoxicity probably because of tissue deficiency of vitamin B_6 aggravated by the loss of pyridoxal hydrazone of isoniazid (Gronhagen-Riska et al. 1978).

1.1.2.2. Improper bioavailability

Lower bioavailability of drugs from conventional formulations has been a matter of serious concern and remains as the major cause for the treatment failure. Still today the treatment outcome of tuberculosis is questionable due to variable bioavailability of drugs. Several reports suggest that the bioavailability problems associated with drugs mainly due to improper delivery of drugs from the formulations (Fox 1990, Zak et al. 1981, McIlleron et al. 2002, Shishoo et al. 2001, Eidus and Hodgkin 1975, Jalsenjak et al. 1980).

Bioavailability problem associated with rifampicin

One of the key issues that hindered the widespread use of FDCs for the treatment of tuberculosis (TB) is the concern about the bioavailability of rifampicin (Fox 1990). It was considered that the variable bioavailability of rifampicin was largely confined to FDC formulations; however, reduced plasma concentrations following administration of rifampicin-only formulations were also reported by Zak et al as early as 1981 (Zak et al. 1981). In recent years, the problem of bioavailability associated with generic formulations of rifampicin was again highlighted by McIlleron et al, who found that two rifampicin capsule formulations showed reduced blood concentrations and were responsible for the failure of TB treatment (McIlleron et al. 2002). In this regard, reduced blood concentrations from 'rifampicin-only' formulations indicate that rifampicin bioavailability problems not only associated with FDC formulations but also with single component formulations. This is a matter of serious concern because of the small therapeutic margin of around 10% between the prescribed dose (10 mg/kg) and the minimum dose (9 mg/kg) required for therapeutic action (Long et al. 1979).

It has been recently proposed by Shishoo et al (Shishoo et al. 2001) that, the problem of poor bioavailability of rifampicin from the formulations is due to increased decomposition in acidic pH conditions of the stomach. Degradation of rifampicin is pH dependent, in acidic medium it hydrolyzes to 3-formyl rifamycin SV (3-FRSV) and, it undergoes oxidation in alkaline medium to form inactive quinone derivative i.e., rifampin quinone. 3-FRSV precipitates in acidic conditions. The aldehyde (3-FRSV) shows high antimicrobial activity in vitro but is poorly absorbed and inactive in vivo (USP DI 1996). Therefore, formation of 3-FRSV in the acidic environment of the stomach leads to a decrease in the amount of rifampicin available for absorption, and thus, can be an important factor affecting bioavailability of rifampicin. Prankerd et al examined the stability of rifampicin over a wider range of acidic pH values (Prankerd et al. 1992). The kinetic study of rifampicin degradation in acidic mediums (ranging from pH 1.08 to 5.0) suggested that the rate of degradation was maximum at pH 1.08 and decreases as much as 0.5 log units over the pH range of 1 to 4.3. The mechanism of rifampicin degradation was found to be fast reversible hydrolytic cleavage at its azomethine bond followed by slower secondary reactions. Because of the reversible nature of the initial reaction step, the overall loss of rifampicin from solution was dependent on its initial concentration. Thus, degradation of rifampicin in acidic pH of the stomach is dependent on the initial amount of rifampicin released in the stomach.

Much concern has been expressed about the poor bioavailability of rifampicin from FDC products containing isoniazid. Accordingly, the WHO and IUATLD have issued a joint statement that anti-tubercular FDC formulations should only be used in National Tuberculosis Programmes if the bioavailability of at least the rifampicin component has been demonstrated (Singh et al. 2000b). There are few reports in the literature, which proved that the degradation of rifampicin in acidic conditions gets enhanced in presence of isoniazid (Jindal et al. 1994, Shishoo et al. 1999). The extent of degradation of rifampicin, in the absence and presence of isoniazid, has been determined in 0.1 N HCl and simulated gastric fluid (SGF) at 37 °C in 45 min (USP dissolution test conditions) (Mariappan et al. 2000, Singh et al. 2000a, Singh et al. 2000b). Apparently, rifampicin alone decomposes to an average extent of 6.33%, while in presence of isoniazid decomposition increases to an average extent of 16.32%. Seifart et al reported a similar

conclusion that the degradation of rifampicin is enhanced in presence of isoniazid (Seifart et al. 1991). Similar degradation trend was reported by Shishoo et al (Shishoo et al. 1999). They reported a 15.9% and 8.7% decrease in rifampicin concentration in the presence and absence of isoniazid. As the average gastric emptying time varies from 15 min to 3 h, study has been done to analyze the extent of degradation of rifampicin and isoniazid in 0.1 N HCl at 37° C in presence of each other (Singh et al. 2000a). The decomposition of rifampicin in the presence of isoniazid at 15 min was 8.5%, increasing to 50% after 3 h. The relative decrease in isoniazid in the same period was 1.8 and 10.3% respectively. These data indicated that the decomposition of rifampicin is accelerated approximately threefold in the presence of isoniazid. Isoniazid decomposes to a much lesser extent, accounting to approximately one-fifth of decomposition of rifampicin.

The reported literatures support the fact that in vivo degradation of the rifampicin is a major factor contributing to its poor bioavailability from FDC formulations containing rifampicin and isoniazid. Among the various physical/ chemical factors that can possibly be the reasons for the poor/ variable bioavailability of rifampicin, one that explains most of the issues related to this typical problem is the rapid decomposition of the rifampicin in situ in stomach acidic conditions, which will be further enhanced in presence of isoniazid. The acidic decomposition of rifampicin in situ also explains as to why the products with satisfactory dissolution show poor rifampicin bioavailability and vice versa. It is because stomach decomposition comes in between drug dissolution and its absorption. Therefore, it is not wrong to say that the decomposition of rifampicin in stomach before absorption is a strong contributing factor for the treatment failure and emergence of drug resistance. It is expected that the drug decomposition, on administration in empty stomach, might range from 20 to 50%, which means a reduction in the dose from about 10 to 12 mg/kg to as low as 5 to 6 mg/kg body weight. It was indicated by Long et al (Long et al. 1979) that a decrease in the dose of rifampicin below 9 mg/kg of body weight results in loss of therapeutic efficacy.

Bioavailability problem associated with isoniazid

Apart from severe toxicity problems, conventional formulations of isoniazid are also associated with bioavailability problems. Hereditary differences have an important

bearing on the acetylation and inactivation of the isoniazid and consequently on the response of individual to this drug (Eidus and Hodgkin 1975). Patients taking isoniazid have been classified as either rapid or slow acetylators of the drug (Jalsenjak et al. 1980). Isoniazid undergoes appreciable presystemic metabolism in the wall of the small intestine and liver, resulting in the concentrations in the plasma of rapid acetylators which are half of those in slow acetylators after normal dose of 300 mg of the drug (Eidus and Hodgkin 1975). In such cases, it is not possible to maintain the therapeutically effective concentration (above the MIC) of the drug for the sufficient duration of time until next dose is administered. Such sub therapeutic concentrations might be severely dangerous and can lead to treatment failure and further more, encourage the development of isoniazid resistant strains of M. tuberculosis.

1.1.2.3. Enzyme inducing activity of rifampicin

One more drawback of rifampicin containing conventional anti-tubercular formulations is enzyme-inducing activity of rifampicin. A potent enzyme inducer rifampicin stimulates metabolism of other co-administered drugs in the liver. It induces the metabolic enzymes, particularly CYP3A, in the gut and liver (Burman et al. 2001). Depending on the drug and metabolic pathway induced, the extent of CYP3A induction by rifampicin can be increased by increasing the dose and decreased by extending the administration interval for equivalent dosages (Keung et al. 1999). Rifampicin induction is often maximized at the standard 600 mg daily dose because of its potent inductive capability. Many rifampicin inductive reactions cause therapeutically relevant decrease in plasma concentrations of co-administered drugs (Venkatesan 1992).

Rifampicin also stimulates its own metabolism in the liver (Douglas and McLeod 1999). On repeated administration of rifampicin, its plasma levels are found to decrease. Thus, the bioavailability of rifampicin reported to be decreased from 93% after the first single oral dose to 68% after 3 weeks of oral therapy (Loos et al. 1985). The plasma levels of rifampicin remain same whether administered as a FDC or as single component formulations of the respective drugs, administered simultaneously (Ellard et al. 1986).

Enzyme inducing activity of rifampicin also results in the increased risk of hepatotoxicity caused by isoniazid. As already mentioned, acetyl isoniazid, the main metabolite of

isoniazid, undergoes conversion into monoacetyl hydrazine whose metabolites are hepatotoxic. These enzymatic reactions may be enhanced by rifampicin, which is a powerful inducer of liver enzymes. It was also mentioned that the rifampicin enzyme induction enhances the formation of hydrazine and isonicotinic acid as a result of the metabolism of the isoniazid, the former being hepatotoxic (Ross and Horne 1990).

1.1.3. Need for oral controlled release formulations of anti-tubercular drugs

One area for the future application of controlled-release technology is in the treatment of the chronic microbial disease tuberculosis (Batyrbekov et al. 1997). Although current chemotherapeutic agents for tuberculosis treatment are therapeutically effective and well tolerated, a number of problems remain. The treatment of tuberculosis is burdensome, extends over long periods and requires continuous and repeated administration of large drug doses. Thus, traditional drug chemotherapy has many serious limitations, which are already discussed in previous sections. Poor patient compliance is the single most common reason for the failure of chemotherapy of tuberculosis (Fox 1983). One useful method to ensure compliance in tuberculosis patients is to supervise the administration of drugs, which is not always practical. An alternative approach is to administer the drugs in carriers/delivery systems that release drugs in a sustained manner at therapeutic concentrations over a specified period of time. This strategy helps to improve patient compliance in terms of reducing the dosage frequency, and may also minimize the risk of emergence of drug-resistant mutants and potential toxicity (Ain et al. 2003).

1.1.3.1. Need for oral controlled release formulations of rifampicin

Rifampicin treatment has been associated with several adverse actions like heartburn, epigastric distress, cramps, diarrhoea, anorexia, nausea, vomiting, 'flu like syndrome', and other CNS side effects (Madhan Kumar and Panduranga Rao 1997). Prolonged exposure to rifampicin treatment is reported to cause hepatitis, jaundice, and even death (Scheuer et al. 1974). These toxic/ adverse effects could be minimized to a greater extent by formulating the drug in to controlled release formulations. Such formulation would avoid the toxic effects by reducing drug blood fluctuations.

Rifampicin has got very short half life, its half life ranges from 1.5 to 4.5 h (mean 3 h). Repeated daily dosage leads to more rapid elimination of the drug as manifested by a reduction in its half life (Nitti et al. 1972). Its half life decreases from 3.4 h, from the first day of administration to as low as 2.1 h on 14th day (Immanuel et al. 1985). As this drug is administered once daily, apparently it becomes extremely difficult to maintain the therapeutically effective blood concentrations for the entire treatment period with the conventional formulations. There are due chances that, the drug concentrations might fall below the MIC in the later hours. Controlled release formulations will overcome this problem by maintaining the therapeutically effective concentrations of the drug well above the MIC for prolonged period of time necessary for therapeutic success.

Bioavailability problems associated with conventional rifampicin therapy is one more important cause that prompts to develop controlled release formulations. The degradation of the rifampicin in acidic pH of the stomach is dependent on the initial amount of rifampicin released in the stomach. One interesting observation has been reported in case of conventional rifampicin formulations (Shishoo et al. 1999). In case of formulations having faster release rate of rifampicin in dissolution medium, more amount of rifampicin is released in short time and gets exposed to the acidic environment of the stomach for longer time. Thus, such formulations show higher rifampicin concentrations for initial short time interval in the dissolution medium followed by decrease in the concentrations in the later hours. On the other hand, for the formulation showing slower initial release of rifampicin, lesser amount rifampicin is available for degradation in the acidic medium and hence, shows higher concentrations of rifampicin as the time progresses. Thus, by incorporating in to controlled release formulations, the degradation of rifampicin can possibly be reduced much lower extent. As it has been mentioned that there is a small therapeutic margin (around 10%) exists between the prescribed dose (10 mg/kg) and the minimum dose (9 mg/kg) required for therapeutic action for rifampicin (Long et al. 1979), reduction in the degradation would improve the therapeutic success rates by enhancing the bioavailability.

Rifampicin bioavailability problems are also due to induction of its own metabolism (auto induction). Based on literature reviews, it can be said that the enzyme inducing activity of rifampicin is dose/ concentration dependent. Thus, if less amount of rifampicin

is released at a particular time, fewer amounts will further be available for stimulating its own metabolism in the liver. Hence, by formulating in to controlled release formulations, the concentration dependent enzyme inducing activity and auto induction of its own metabolism could be decreased.

Even though it is well known fact that, rifampicin is absorbed from the stomach rapidly and completely (Acocella 1978), for several diseases, e.g. duodenal tuberculosis, rifampicin needs to be released directly into the small intestine and not before in order to show a therapeutic effect against the infection (Nowak et al. 1994). In such cases a carrier matrix with controlled release of rifampicin in the small intestine would be helpful in the effective treatment of the diseases (Clausen and Bernkop 2001). Apart form the targeted release in the intestine, such formulations also help to increase the bioavailability of the rifampicin because rifampicin is known to be rapidly absorbed from the intestine and the absorption rate increases with the time (Acocella 1978).

In the last several years, different types of controlled release formulations of rifampicin have been developed to improve the clinical efficacy of the drug and patient compliance that can be summarized as follows. Rifampicin tablets were formulated with ethylene-vinyl acetate copolymer as a matrix material (Rao et al. 2001a). Modified pulsnicap technique was used to formulate rifampicin controlled release formulations using different hydrophilic polymers (Seshasayana et al. 2001). Rifampicin controlled release tablets were formulated using chitosan and matrices were cross-linked by formaldehyde vapors (Rao and Murthy 2000). Release of rifampicin from ethylcellulose coated nonpareil beads was studied (Rao and Murthy 2002). In vitro in vivo correlation studies have been carried out for ethylcellulose coated nonpareil beads of rifampicin in healthy human volunteers (Rao et al. 2001b). Respirable microspheres of rifampicin have been formulated using poly (lactide-co-glycolide) (Hara and Hickey 2000). Sustained release solid dispersions of rifampicin were formulated using Eudragit S100 and Eudragit RL100 by coevaporation method (Ammar and Khalil 1997). Rifampicin-loaded poly (D,L-lactide)/ poly (ethylene golycol) (PDLLA/ PEG) copolymer microspheres as an injectable drug delivery systems have been reported (Celikkaya et al. 1996). Rifampicin-loaded poly (D,L-lactide) (PDLLA) microspheres were prepared by a modified solvent evaporation method (Denkbas et al. 1995). Mucoadhesive matrix-tablets of rifampicin

were formulated with thiolated polymers (Bernkop et al. 2000). Multiple emulsions containing rifampicin were prepared and evaluated for in vitro and in vivo performance (Nakhare and Vyas 1997). Implantable polymeric drug delivery devices of rifampicin were formulated using polydimethylsiloxane (Schierholz 1997). Subdermal implants incorporating rifampicin in pure and microencapsulated forms with biodegradable material were used as a new drug delivery system in experimental tuberculosis of guinea pigs (Mathur et al. 1985). Directly compressible rifampicin tablets were formulated using polymethacrylic acid (PMAA) and starch compositions for the site specific delivery of rifampicin to the intestine (Clausen and Bernkop 2001). Rifampicin loaded biodegradable microspheres were formulated using polylactide (PLA) and PLGA polymers by solvent evaporation technique (Bian et al. 1999).

Injectable drug delivery systems necessitate hospitalization of the patients and are painful that may lead to patient non compliance and treatment interruption. Implantable delivery systems are associated with surgical procedures, which also require hospitalization and are accompanied with severe pain. Also, in most of the above reported cases, methods of preparation of formulations were tedious and required additional treatments like sintering or exposure to formaldehyde vapors to achieve the desired release. On extensive literature survey no report has been found on the use of hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), Carbopol, Eudragit L100-55 polymers as matrix forming materials for the development of oral controlled release formulations of rifampicin.

1.1.3.2. Need for oral controlled release formulations of isoniazid

One of the major drawbacks in the use of isoniazid for the treatment of tuberculosis is the severe toxic/adverse effects associated with it (Schaberg et al. 1996, Burman et al. 2001). It has been reported that the isoniazid is a major drug incriminated in anti-tubercular drug induced hepatotoxicity (Black et al. 1975, Wong et al. 2000, Pessayre et al. 1977). It has been mentioned that most of the toxic/ adverse effects of anti-tubercular drugs are dose dependent. These severe toxic effects lead to discontinuation of the therapy because of the lack of patient compliance. In such conditions administration of isoniazid as a controlled release formulations results in the reduced drug blood fluctuations that lead to

decrease in the toxic/adverse effects as therapeutically effective and safe drug blood concentrations are maintained for the entire duration of the treatment.

Improper bioavailability from conventional isoniazid formulations is another factor that demands the development of controlled release formulations. It has been found that the absorption of isoniazid from controlled release matrix formulations continues throughout enteric tract and is as complete as that from conventional formulations (Eidus and Hodgkin 1975). High doses of isoniazid could now be administered without encountering the toxic reactions because of the delayed absorption from the controlled release formulations. These controlled release formulations not only optimize the drug blood levels in fast acetylators but also minimize the first pass metabolism of isoniazid to its derivatives. It has been reported that all of the metabolites of isoniazid are essentially devoid of antitubercular activity, but some of them strongly contribute toward hepatotoxicity. In such case, apart from the improved optimization of the drug blood levels, controlled release formulations also help to minimize hepatotoxic adverse effects.

There are several reports in the literature, which substantiated the need for the controlled release formulations of isoniazid that can be summarized as follows. A new isoniazid formulation was designed for moderately fast and fast acetylators of isoniazid (Eidus and Hodgkin 1975). It has been reported that the best results were obtained with the formulation containing 37% free isoniazid and 63% matrix component (Eidus and Hodgkin 1975). Sustained release microcapsules of isoniazid were formulated using ethyl cellulose polymer and in vitro release studies have been done (Jalsenjak et al. 1980). Isoniazid/PLGA matrices were formulated by solvent casting (low density foam) and dry-mixing methods in a view to extend and maintain drug blood levels for the required period of time and to enhance the patient compliance (Gangadharam et al. 1994). Injectable PLG microparticles of isoniazid were formulated and in vitro and in vivo release from different formulations was examined (Dutt and Khuller 2001). Controlled release isoniazid tablets were formulated using three different matrix materials to attain in fast inactivators sustained blood concentrations similar to those produced by conventional isoniazid tablets in slow acetylators during chemotherapy (Bulut et al. 1989). Poly(acryloyl-L-proline methyl ester)-based hydrogels containing 1 and 5% of a crosslinking agent were studied as drug delivery systems for isoniazid (Caliceti et al.

2001). Microcapsules of isoniazid were prepared by phase separation coacervation process using ethyl cellulose as coating polymer (Barik et al. 2001). Isoniazid albumin microspheres were prepared evaluated (Bosela et al. 1998). Implant (depot) isoniazid formulations were developed using PLGA polymer (Gangadharam et al. 1999). Controlled release implantable isoniazid formulations have been developed using PLGA polymer as a rod (Gangadharam et al. 1994). The suitability of a slow-release matrix preparation of isoniazid for use in once-weekly chemotherapy has been investigated in South Indian patients (Parthasarathy et al. 1986).

Injectable drug delivery systems necessitate hospitalization of the patients and are painful that may lead to patient non compliance and treatment interruption. Implantable delivery systems are associated with surgical procedures, which also require hospitalization and are accompanied with severe pain. On extensive literature survey no report has been found on the use of hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), Carbopol, Eudragit L100-55 polymers as matrix forming materials for the development of oral controlled release formulations of isoniazid.

1.1.3.3. Need for oral controlled release formulations of combination drugs rifampicin and isoniazid

Fixed dose combination combines the most effective drugs in one formulation this minimizes the adaptation of monotherapy by patients and hence reduces the chances of developing drug resistances. However, these conventional formulations have been associated with many serious drawbacks, like, the development of microbial drug resistance, improper bioavailability of drugs and, toxico-allergic side effects. These problems can be alleviated by the use of controlled-release drug delivery systems (Batyrbekov et al. 1997).

Treatment with rifampicin and isoniazid is associated with incidence of severe adverse/ toxic effects, which has been already discussed. There also exists a report, which says that the adverse effects of anti-tubercular drugs (rifampicin and isoniazid) were observed in combination tablet that were absent in separate formulations of these drugs (Kingston et al. 2001). In case of multi-drug combination formulations the incidence of toxic effects would be higher as individual drug's toxicity will contribute towards total toxicity. It is a

known fact that the hepatotoxicity, a life threatening toxic effect, would be more pronounced when isoniazid and rifampicin were administered together. Guidelines also suggest that the tuberculosis patient have to consume rifampicin and isoniazid combination tablet on an empty stomach (in the fasting state). This reduces the patient compliance because of gastric irritation associated with it (Panchagnula et al. 2003). Consequently, attempts are being made either to develop alternative drugs or to reduce toxicity of already existing drugs. The prospect of finding newer and more effective drugs similar to the existing ones is small because it requires exhaustive in put in terms of money and time. Further more, there is no surety that after all these cumbersome efforts the invented new drugs will be therapeutically efficient. Therefore, the best alternative is better utilization of the existing drug combinations at their existing dose levels for the efficient and effective treatment of tuberculosis. This goal could be achieved by the controlled delivery of the drug combinations, which reduces the toxic adverse effects of the drugs, improve bioavailability and hence improve the patient compliance. The enzyme inducing activity of rifampicin, which is also a concentration dependent phenomenon, would be minimized when given in the form of controlled release formulation, this in turn reduces the potentiation of hepatotoxic adverse effects of isoniazid.

The poor bioavailability of the rifampicin in presence of isoniazid from combined formulations is another major draw back that necessitates the development of oral controlled release formulations for rifampicin and isoniazid combination.

Some combined controlled release formulations of rifampicin and isoniazid have been reported in the literature and are summarized as follows. Poly (DL-lactide-co-glycolide) nanoparticles based sustained drug delivery systems have been developed for rifampicin, isoniazid and pyrazinamide (Pandey et al. 2003a). Alginate hydrogel microparticles were developed for oral controlled delivery of antitubercular drugs isoniazid, rifampicin and pyrazinamide alone and in combination (Ain et al. 2003). Poly (DL-lactide-co-glycolide) (PLG) nanoparticles encapsulating rifampicin, isoniazid and pyrazinamide have been developed to improve the patient compliance (Pandey et al. 2003b). Isoniazid and rifampicin encapsulated lung specific stealth liposomes have been formulated to improve

patient compliance and to reduce cost, dosage and toxic effects of the therapy (Labana et al. 2002). Inhalable biodegradable microparticles containing isoniazid, and rifampicin were prepared and tested (Sharma et al. 2001). PLG microparticles were developed as injectable drug delivery systems for isoniazid and rifampicin to improve the compliance of tuberculosis therapy (Dutt and Khuller 2001a). Comparative evaluation of two types of controlled release drug delivery systems, liposomes and PLG microparticles, has been carried out in vitro and in vivo for antitubercular drugs rifampicin and isoniazid (Dutt and Khuller 2001b). Implantable drug delivery systems based on polyurethane biodegradable polymer have been developed for antitubercular drugs to minimize the drug resistance and to reduce the toxico-allergic side effects (Batyrbekov et al. 1997). An oral controlled release formulation has been developed based on PLG microparticles for the delivery of isoniazid, rifampicin, and pyrazinamide either individually or in combination (Ain et al. 2002). A colloidal dosage form for the oral delivery of rifampicin and isoniazid in combination was developed with the aid of artificial neural network (ANN) data modeling (Kustrin et al. 2003). Sustained release capsular systems of rifampicin and isoniazid, (an osmotically regulated multi-drug oral delivery system) comprising asymmetric membrane coating and dense semipermeable membrane coating were developed to reduce the potential side effects and enhance the patient compliance associated with the multidrug therapy of tuberculosis. (Prabhakaran et al. 2004).

A number of polymer based drug delivery systems have been reported (as discussed in previous paragraph) for antitubercular drugs. The most widely used once are those of PLG polymers in the form of films, fibers, implants, rods, microparticles, nanoparticles, liposomes and etc. Liposomes, since their discovery, have not only been recognized as useful models of biological membranes but also as a unique biocompatible vehicle for drug delivery (Oerkins et al. 1993, Lasic 1993). However, liposomes suffer from certain limitations, mostly of a technical nature, such as the lack of reproducibility of the preparations, the limited stability of the drug–carrier complex during storage and potential changes in the structural organization of the lipid bilayer induced by certain drug molecules (Bermudez et al. 1999). Studies performed with liposomal forms of antitubercular drugs showed that the bilayer composition is critical for targeting liposomes and for obtaining drug–liposome stable formulations (Bermudez et al. 1999,

Deol, et al. 1997). Moreover, the targeting of drugs by lipid vesicles requires a complete understanding of the physicochemical characteristics of the drug–liposome system in order to predict their behavior and stability in vivo (Rodrigues et al. 2003). It is a very difficult task to manufacture or produce the liposomes in a large scale, which is another major drawback. Implantable formulations require surgical procedures for their insertion that necessitates hospitalization of the patients and are also painful. Injectable drug delivery systems like implantable systems also require hospitalization of the patients and are painful that leads to patient non compliance and treatment interruption. A step further in the development of these drug delivery systems was the use of PLG polymers or alginates as oral drug delivery vehicles. The formulations could now be administered via a more acceptable and tolerable route, i. e., orally, exhibiting a controlled release of drugs. These systems could offer alternative treatment regimens for effective and efficient treatment of TB with improved compliance as well as lower probability of development of multidrug resistant strains of *M. tuberculosis*. Furthermore, reduction in the incidence of toxic side effects and provision of self-medication overall could lead to treatment adherence and therapeutic success. Till date only few oral controlled release formulations for rifampicin and isoniazid combination have been reported. Most of them are microparticultate and nanoparticulate drug delivery systems and only one recent article was found in the literature related to osmotically regulated oral controlled release systems for rifampicin and isoniazid combination. On extensive literature survey no report has been found in the area of polymer matrix tablet based oral drug delivery systems for rifampicin and isoniazid combination.

1.2. Oral Controlled Release Formulations

1.2.1. Conventional oral drug delivery systems and their limitations

For many decades treatment of an acute or chronic disease has been mostly accomplished by delivery of drugs to patients using conventional dosage forms like tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols, and injectables. Even today these conventional drug delivery systems are the primary pharmaceutical products commonly seen in the prescription and over-the-counter drug marketplace. Oral drug

delivery has been known for decades as the most widely utilized and convenient route of drug administration compared to all other routes. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration, cost, as well as the traditional belief that by oral administration the drug is as well absorbed as the food stuffs that are ingested daily (Chien 1992). Pharmaceutical products designed for oral delivery and currently available on the prescription and over-the-counter markets are mostly designed for immediate release of drug for rapid absorption. Conventional multidose therapies for long duration of action are not without problems. The problems are, continuous peak and trough profiles of the drug blood levels with the brief optimum therapeutic drug blood level, frequent administration of the dosage forms, adverse/side effects, etc (Ainaoui and Vergnaud 2000). The fluctuating drug levels in blood and tissues lead to a variable influence on the disease treatment (Conard et al. 1982) and are related to an excessive use of the drug that lead to unnecessary wastage of drug. As the drug concentrations remain higher for long duration there are increased chances of incidence of toxic or side effects that necessitates discontinuation of the treatment. If the drug concentrations come below minimum effective concentrations for a prolonged period of time, or therapy is discontinued, these might result in emergence of drug resistant strains in case of antibiotic treatment. This is another major threat in the treatment of chronic diseases like tuberculosis, malaria, diabetes, hypertension and AIDS. Increased frequency of administration is another drawback in case of conventional medications (Singh and Agarwal 2002). The number of tablets/dosage forms per day definitely has a significant influence on the patient compliance and adherence to the treatment (Panchagnula et al. 2001). Less the frequency of administration more will be the patient compliance and treatment adherence.

Apart from these drawbacks conventional systems also suffer from many other problems. Rifampicin degrades in acidic stomach conditions, for such drugs immediate release drug delivery systems will lead to maximum degradation in the stomach and decreased bioavailability (Shishoo et al. 2001). However, making controlled release, moving down the gastrointestinal tract (GIT) would lead to higher bioavailability. Some of the nonsteroidal anti-inflammatory drugs and antibiotics produce severe irritation, discomfort and gastro intestinal intolerance when administered as immediate release dosage forms.

Some drugs (Ellard et al. 1972) undergo gut metabolism and first pass metabolism in the liver producing hepatotoxic metabolites. For such drugs, immediate release formulations could lead to decreased bioavailability and increased side effects.

1.2.2. Oral controlled release formulations and their advantages

The focus of pharmaceutical research is being steadily shifted from the development of new chemical entities to the development of novel drug delivery systems of existing drug molecules to maximize their effectiveness in terms of therapeutic action and patent protection (Parakh et al. 2003). The revolution in oral drug delivery systems has been made possible by the development of the two areas of knowledge: pharmacokinetics and pharmaceutical technology. Controlled release drug delivery systems offer important advantage over traditional dosage forms in diseases requiring prolonged durations of therapy. The development of an improved pharmaceutical dosage form, such as an oral controlled release preparation, should be based upon the pharmacokinetic and pharmacodynamic properties of the drug. In addition, issues such as minimization of adverse drug reactions, reduction in the repeated fluctuations of serum and tissue concentrations, prevention of the development of resistant organisms (in the case of antimicrobial therapy), patient compliance factors and overall treatment cost must be considered (Hoffman et al. 1998).

Controlled release drug delivery systems growing at a rate faster than the total pharma business worldwide (Sen 2004). Drug delivery companies and their pharmaceutical industry partners are poised to reap the rewards of the multibillion-dollar drug delivery market, which is forecast to grow from US $50 billion in 2000 to US $100 billion by 2005-2006 (Das and Das 2003, Sen 2004). Oral controlled release systems (OCRS) constitute the largest proportion (almost 60%) of the total drug delivery market and are expected to grow at 9% or more every year through 2007. The driving forces behind this booming market can be divided into two main groups: patient-related factors and market-driven factors.

1.2.2.1. Patient related factors/ advantages

Avoiding peak and trough profiles of multidose therapy by maintaining steady and therapeutically effective levels of the drug over a prolonged time period is beneficial for patient compliance. This results in the effective therapeutic efficacy and better management of the disease, with reduction in adverse side effects and improvement in tolerability. Drug plasma levels are maintained at a constant level or within a narrow window with no alternative peak and trough profiles and with AUC of plasma concentration versus time curve comparable with total AUC from multiple dosing with immediate release dosage forms. The enhanced patient compliance is also due to reduction in dosing frequency and reduction in the number of dosage units to be administered. Reduction in total healthcare cost could probably be the important factor especially in poor or developing countries like India.

1.2.2.2 Market-driven factors/ advantages

Drug delivery is a valuable drug lifecycle management tool. The most important force driving the growth and viability of the pharmaceutical industry, the regular introduction of new chemical entities (NCEs), is currently weak. Expenses accrued from drug development are hitting the roof, and true innovation is at an all-time low. Moreover, FDA's more-cautious review process and demand for a greater number of complex clinical trials are increasing the total time period required to market a new molecule. In addition, compliance with 21 *CFR* Part 11 and the new Health Insurance Portability and Accountability Act (HIPAA) is affecting the clinical trial process. In 1996, FDA approved 53 NCEs (new chemical entities); this figure dropped to 27 in 2000 and is declined to 18 in 2003 (Jain 2004). In the next five years, at least 20 blockbuster products with combined sales of nearly US $40 billion will lose patent protection. Hence the emergence of repatentability, achieved by the introduction of controlled-release formulations of existing immediate release products, as an attractive financial option for pharmaceutical companies, in addition to seeking new therapeutic indications for these "new" products. The recent market introduction of two products, Augmentin XR (Glaxo SmithKline) and Cipro XR (Bayer), are harbingers of this option becoming a trend. Controlled release formulations can also revive some promising compounds, which have

been abandoned due to formulation problems. These compounds will get second life and again will enjoy the marketing advantages (Shah 2004).

Most of the blockbuster formulations of Glaxo SmithKline (GSK) are controlled release formulations (Jain 2004). Cardiazem LA is a once daily formulation of dilitiazem HCl; it targets the sales of US $90-120 million by 2004 (Jain 2004). In this case, a well exploited molecule dilitiazem HCl got life extension by controlled release formulations. The controlled-release drug delivery market is expanding rapidly, paralleled with an active and aggressive research in the field. There has been a significant increase in approvals of novel drug delivery systems (NDDS) in the last few years, and this is expected to continue at an impressive rate in the near future (Verma et al. 2004).

1.2.3. Types of oral controlled release drug delivery systems

Oral controlled release delivery system is a drug-containing dosage form that releases the drug continuously in a predetermined pattern for a fixed period of time. Various devices have been studied and built up in order to control the release of the drug throughout the GIT through which the pH varies from 1 to 8 (Vergnaud 1993, Higuchi 1973, Theeuwes 1983, Chien 1987, Jerzewski and Chien 1991, Chien 1992). They can be divided into five categories as (Jantzen and Robinson 1996);

1) Dissolution controlled drug delivery Systems
2) Diffusional controlled drug delivery Systems
3) Bioerodible and combination diffusion and dissolution controlled drug delivery systems
4) Osmotically controlled drug delivery systems
5) Ion exchange controlled drug delivery systems

1.2.3.1. Matrix based controlled release drug delivery systems

Matrix technologies have often proven popular among the oral controlled drug delivery technologies because of their simplicity, ease in manufacturing, high level of reproducibility, stability of the raw materials and dosage form, and ease of scale-up and process validation. Technological advancements in the area of matrix formulation have made controlled-release product development much easier than before, and improved

upon the feasibility of delivering a wide variety of drugs with different physicochemical and biopharmaceutical properties. This is reflected by the large number of patents filed each year and by the commercial success of a number of novel drug delivery systems based on matrix technologies.

Matrix-based delivery technologies have steadily matured from delivering drugs by first-order or square-root-of-time release kinetics to much more complex and customized release patterns. In order to achieve linear or zero-order release, various strategies that seek to manipulate tablet geometry, polymer variables, and formulation aspects have been applied. Various drug, polymer, and formulation-related factors, which influence the in situ formation of a polymeric gel layer/drug depletion zone and its characteristics as a function of time, determine the drug release from matrix systems (Verma et al. 2004). In matrix devices, the drug is homogeneously dispersed in either a hydrophobic or hydrophilic polymer matrix. The release rate from matrix systems remains unaffected by thin spots, pinholes, and other similar defects, which can be a serious problem with reservoir systems (Soppimath et al. 2001, Baker 1987). These advantages, along with the low fabrication cost, outweigh the less desirable feature of declining release rates with time, which is a characteristic of matrix systems. Such devices can be conveniently prepared by using a simple polymer fabrication technique involving a physical blending of the active agent with matrix formers, followed by compaction, extrusion, or solvent casting.

1.2.3.2. Polymers used in the development of matrix based controlled release formulations

Several hydrophilic and hydrophobic polymers have been reported as carriers/ release retardant materials in the development of oral controlled release formulations of drugs. Selection of a suitable matrix material depends on the dose size, desired release rate, and the physiochemical properties of the drug of interest (Verma et al. 2004). Hydrophilic polymers have been paid considerable attention in the formulation of controlled release formulations for various drugs. Hydroxypropyl cellulose (Ranga Rao et al. 1988), carbopols (Khan and Jhu 1999), polyvinyl alcohol (Korsmeyer et al. 1983), PHEMA (polyhydroxyethylmethyl acrylate) methyl methacrylate (Zhang et al. 1990), vinyl acetate

(Zhang et al. 1988), ethylene oxide (Zhang et al. 1990), polyethylene oxide (PEO) (Dimitrov and Lambov 1999), methylcellulose and sodiumcarboxy methylcellulose (Ranga Rao et al. 1988) are some of the hydrophilic polymers, which have been extensively used in the formulation of controlled release systems. Ethyl cellulose (Sajeev and Saha 2001) is one of the most widely used hydrophobic polymers in the formulation of controlled release systems.

Hydroxypropyl methylcellulose (HPMC)

HPMC, a semi synthetic derivative of cellulose, has its popularity for the formulation of controlled release dosage forms as a swellable and hydrophilic polymer (Velasco et al. 1999. Varghas and Ghaly 1999, Maggi et al. 1999, Ford et al. 1985). From a commercial point of view, HPMC is the most prominent carrier material in pharmaceutical applications. Its nontoxic property, ease of handling, ease of compression, ability to accommodate a large percent of drug, negligible influence of the processing variables on drug release rates and relatively simple tablet manufacturing technology make it an excellent carrier material (Alderman 1984, Skoug et al. 1993, Lee et al. 1999). One of its most important characteristics is the high swellability, which has a significant effect on the release kinetics of a drug. Upon contact with a dissolution medium, water or biological fluid diffuses in to the tablet, resulting in polymer chain relaxation with volume expansion. Then, the drug diffuses out of the device (Siepmann et al. 1999). The release of drugs from the formulations can be governed by the diffusion through the hydrogel and by its subsequent erosion. Various formulation factors influence the drug release form HPMC matrices viz: polymer viscosity, polymer particle size, drug/polymer ratio, drug solubility, drug particle size, drug loading, compression force, tablet shape, formulation excipients, coatings, and processing techniques, as well as the dissolution medium (Velasco et al. 1999, Ranga Rao et al. 1988). The release kinetics of a practically water insoluble drug within two types of HPMC matrices has been studied and observed non-Fickian release behavior (Eyjolfsson 1999). However, for slightly water-soluble drugs, variation of HPMC content and viscosity grade found to produce a wide range of drug release rates (Maggi et al. 1999). Influence of drug:HPMC ratio, drug particle size, polymer particle size and compression force on the release have been studied (Velasco et

al. 1999). Effects of some formulation variables on the release of promethazine hydrochloride have been investigated (Ford et al. 1985). It has been observed that, increased compaction force increased the apparent density of HPMC based tablets, but the release characteristics were not markedly affected (Huber and Christenson 1968, Ebube et al. 1997, Mitchell et al. 1993).

Carbopol

Carbopol polymers (carbomers) are synthetic, high molecular weight acrylic acid polymers cross-linked with polyalkenyl ethers or divinyl glycol. These polymers readily hydrate, absorb water, and swell quickly (Khan and Zhu 1999) up to 1000 times their volume to form a gel when exposed to pH environment above 4 to 6. 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 (Huang and Schwartz 1995). Among these, Carbopol 934P and 971P are the most widely used pharmaceutical grade polymers for oral use (Durrani et al. 1994). Since their introduction in 1957 a number of controlled release tablet formulations have been patented (Goodrich 1987). In one report, Carbopol based matrix tablets showed to follow zero order release mechanism in most of the cases studied (Zhang and Schwartz 1988). Marcos et al. studied the release profile of atenolol from carbomer matrix tablets. They found that release profiles followed Higuchi's square root kinetics model and compression force had no effect on the drug release (Marcos et al. 1991). Researchers reported that increasing the amount of Carbopol 934P in the matrix tablet formulation resulted in a reduction in the drug release rate and linearization of the release profiles with shift in the release mechanism (Khan and Zhu 1999). Several other investigators also studied the release kinetics of drugs from Carbopol matrices. They found that the Carbopol matrices exhibited zero order release profiles at several concentrations studied (Huang and Schwartz 1995, Durrani et al. 1994).

Hydroxypropyl cellulose (HPC)

Hydroxypropyl cellulose (HPC) is non-ionic, water-soluble cellulose ether, formed by reaction of cellulose with propylene oxide. Low substituted HPCs (L-HPC) are normally

used as excipients for good binding and disintegrating properties (Kawashima et al. 1992). Nagami and Nada first reported that the L-HPC could be used as a matrix base for the formulation of controlled release tablets (Nakagami and Nada 1988). Study has been done on the effect of particle size of L-HPC on drug release rate and tensile strength of the directly compressed tablets (Kawashima et al. 1989). The effect L-HPC particle size and loading on the release rate was evaluated and was observed that drug release rate was strongly influenced by the particle size of the L-HPC, which determined their swelling ability and, drug release rate was also dependent upon the percent loading of the polymer (Kawashima et al. 1992). The effect of HPC viscosity grade on the release of theophylline was studied and it was observed that the release of theophylline decreased as the HPC viscosity grade increased (Nakano et al. 1983). The release of acetaminophen and pseudoephedrine from HPMC and HPC matrices has been evaluated. The effect of HPMC:HPC ratio, polymer loading, pH of the dissolution media, and compression force on release from the formulations containing both acetaminophen and pseudoephedrine was found to be slower than those containing only acetaminophen (Ebube and Jones 2003).

Eudragits

Eudragits are polyacrylic acid polymers that have been widely used in the development of oral controlled release formulations. Eudragit L and S grades are anionic polymers based on the combination of methacrylic acid and methyacrylic acid esters, which are useful in the development of pH dependent systems to achieve linear release profiles or to balance pH dependent drug solubility. There are several studies reported in the literature that substantiate the use of Eudragit polymers in the development of controlled release matrix tablets (Al-Taani and Tashtoush 2003, Reddy et al. 2003, Gohel et al. 2003, Rao et al. 2003). Eudragits provide good mechanical strength to the tablets and control the diffusion of embedded active ingredients through pores and channels. Release of the active ingredients from matrix structures often occurs proportionally to square root of time, i.e. an initial steep rise is followed by a gentler slope. In any case, it is the type and quantity of polymethacrylate used which dictates the release pattern of the final dosage form.

1.2.3.3. Release kinetics and release mechanism of drugs from matrix formulations

Drug diffusion and polymer relaxation/dissolution are the basic drug transport mechanisms from a matrix system. Fick's first and second laws express diffusion of a solute across a particular medium. These laws form the basis for several mathematical models proposed for drug diffusion from matrix systems. Higuchi related the release rate of a drug dispersed in ointment bases to the pertinent physical constants (Higuchi 1963). This model was modified to correlate factors governing the rate of drug release from homogenous matrices. Assuming that the matrix does not significantly dissolve and that the drug is uniformly distributed in it, the release rate dependent on the square root of time was derived for planner and spherical matrices. Experimental data demonstrating the validity of the Higuchi equation are reported (Ford et al. 1985, Ford et al. 1987, Ford et al.1991, Hogan 1989).

Several kinetic models are available for the analysis of in vitro release data like;

a) Zero order model,

$$M_r = M_0 - K_0 . t$$

$$M_t = K_0 . t$$

Where, M_0 is the initial amount, M_r is the amount remaining to be released at time 't', M_t ($M_0 - M_r$) is the amount of the drug released at time t, and K_0 is the zero order release rate constant.

b) First order model,

$$\text{Log } M_r = \text{Log } M_o - (K_1 . t)/2.303$$

Where, M_r is amount of the drug remaining to be released at time t, M_o is the initial amount of drug at zero time, and K_1 is first order release rate constant.

c) Higuchi's square root kinetics model,

$$M_t = K_H . t^{1/2}.$$

Where, M_t is the amount of the drug released at time t, and K_H is release rate constant representative of the square root kinetics.

d) Korsmeyer-Peppas model (Korsmeyer et al. 1983, Ritger and Peppas 1987),

$$M_t / M_\infty = K . t^{n}$$

Where, M_t / M_∞ is the fraction of the drug released up to time t, K is a constant incorporating structural and geometric characteristics of the release system, and, n is the diffusional exponent indicative of the release mechanism. The values of n are 0.45 and 0.89 for Fickian and Case-II transport respectively. When the n value > 0.45 and < 0.89, the release is said to be non-Fickian. When the n value is greater than that of Case--II transport (0.89), the release is said to be super Case-II transport. This equation has been used to analyze drug release profiles of several systems. Two competing release mechanisms (Fickian diffusional release and Case II relaxational release) are the limits of this phenomenon. Fickian diffusional release occurs by molecular diffusion of the drug because of the chemical potential gradient. Case II relaxational release is the drug transport mechanism associated with stresses and state transition in hydrophilic glassy polymers. These two mechanisms controlling the drug release are considered additive and can be resolved to get the contribution of either in ultimate drug release (Peppas and Sahlin 1989).

A thorough understanding of drug release mechanism and kinetics gives a fairly good idea about the variables that can be manipulated in achieving a desired release profile. An extensive review by Siepmann and Peppas on the mathematical modeling of drug release from HPMC matrices deserves a special mention (Siepmann and Peppas 2001). Narasimhan and Peppas (Narasimhan and Peppas 1996a, Narasimhan and Peppas 1996b, Narasimhan and Peppas 1997) developed mathematical models describing polymer dissolution based on the theory of macromolecular disentanglement & chain reptation. They showed that the dissolution could be either disentanglement or diffusion controlled and also can be a combination of both depending on the polymer molecular weight and the thickness of the diffusion boundary layer.

1.3. Objective of the present research endeavor

For long-term therapy like tuberculosis, the basic goal of therapy is to achieve and maintain a steady-state blood or tissue drug level, which is therapeutically effective and nontoxic for an extended period of time. This goal can be achieved ideally by delivering the drugs as controlled release formulations. These controlled release (C.R.) formulations not only reduce the toxic or adverse reactions of the antitubercular drugs but also can

enhance the bioavailability of the drugs at their existing dose levels and finally can lead to an improved patient compliance. The objective of the present research endeavor was to design the C.R. formulations of rifampicin and isoniazid, first line anti-tubercular drugs widely used in the treatment of the mycobacterium tuberculosis infection. The present work aimed at the design of C.R. formulations of rifampicin and isoniazid, alone and in combination, using different hydrophilic and hydrophobic polymers and their combinations. The designed formulations are to be studied for their in vitro characteristics like, physical characters and in vitro release in suitable release media.

As analysis is an essential component of the formulation development research, it has been planned to develop and validate analytical methods for the estimation of rifampicin and isoniazid, alone and in their combination to support bulk drug analysis, drug content estimation of designed formulations, analysis of in vitro release samples and analysis of stability study samples. Preformulation studies are essential for development of formulations and designed accordingly.

The C.R. formulations of rifampicin and isoniazid (alone and in combination) have been formulated by polymer matrix embedded technology. Polymers used for these studies are HPMC, Carbopol 934P, Eudragit L100-55, ethyl cellulose (EC) and HPC as they are already reported for other drugs and also are being used for commercial purpose. The effect of following parameters on the in vitro drug release characteristics from the matrix embedded tablets were studied and analyzed:

- Polymer type, individual or combination
- Polymer ratio
- Polymer viscosity
- Compression force
- Method of granulation
- Dissolution methodology
- Hydrodynamic conditions in release studies
- Addition of extragranular rifampicin as a free drug portion
- Change in the release media
- Change in the formulation/processing technology
- Change in the volume of release media

- Bilayer tablets of separate layer for separate drug

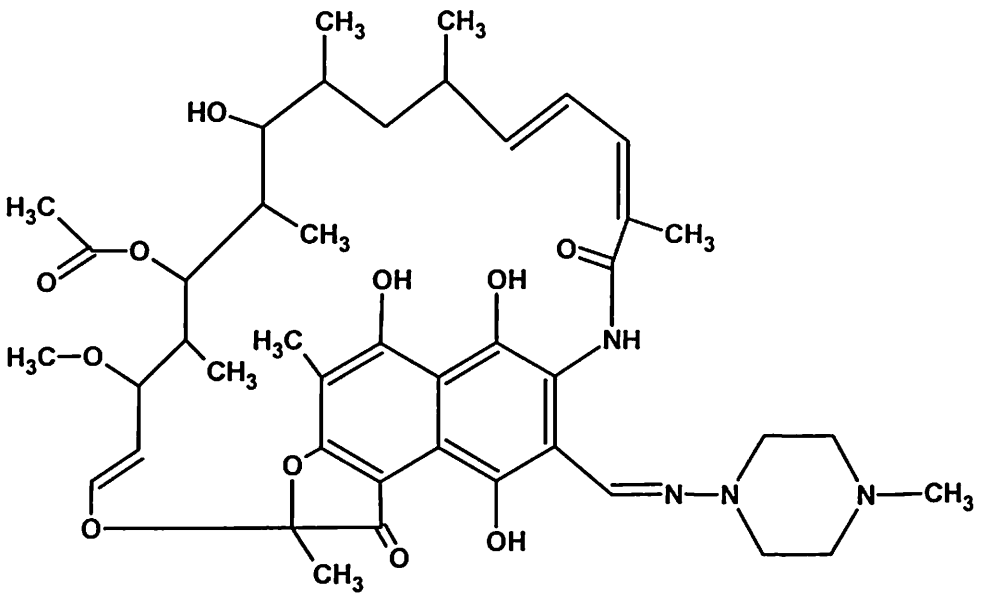
Finally these parameters were optimized to get the formulations with variety of release rates and duration of release for rifampicin and isoniazid (alone and in combination). The release profiles were analyzed by various release kinetics models like, zero order release model, first order release model, Higuchi's square root kinetics model and Korsmeyer-Peppas model. Release profiles were further analyzed for f_2 (similarity) factor values to assess the similarity or difference in the release profiles.

The selected formulation batches were tested for batch reproducibility studies in terms of physical properties like, drug content, weigh variation, friability and hardness. The release profiles of the formulations from the reproduced batches were compared with their original formulation batches with f_2 factor values. Finally, the selected formulations were undertaken for stability studies (according to ICH guidelines) at different storage conditions. The effect of storage conditions on the physical properties of the tablets and in vitro release profiles of rifampicin and isoniazid from their respective C.R. formulations have been evaluated.

Chapter 2

Drug Profiles

2.1. Rifampicin



Rifampicin is a synthetic derivative of a natural antibiotic rifamycin B produced by streptomyces mediterranei and belongs to the class of naphthalenic rifamycins (Maggi et al. 1965, Sensi 1983). It was jointly developed by Lepitit and Ciba Geigy in 1965 and introduced in 1968.

2.1.1. Chemistry

Rifampicin (rifampin, rifamycin, rifamycin AMP) is chemically 3-{[(4-methyl-1-piperazinyl) imino] methyl}rifamycin (Maggi et al. 1965). It is brick-red crystalline powder with little odor. Its molecular formula is $C_{43}H_{58}N_4O_{12}$ and, molecular weight is 823.

2.1.2. Solubility

It is freely soluble in chloroform, soluble in ethyl acetate and methanol. It is relatively insoluble in water but solubility increases at low pH values. Solubility increased by addition of ascorbic acid (Boman et al. 1975). Rifampicin dissolves better at lower pH values at which it is strongly dissociated and hence its physicochemical properties may cause unsatisfactory absorption (Mannisto 1977).

2.1.3. Ionization constant

It exists as a zwitterion in water, with isoelectric point equal to 4.8. The acidic function of rifampicin is due to the presence of hydroxyl groups (C1, C4, and C8) and basic function is due to piperazine nitrogen group. With the presence of both acidic and basic functions it has got two pKa values 1.7 and 7.9 (Kenny and Strates 1981).

2.1.4. Optical rotation

The optical rotation of rifampicin was reported to be + 10.6 of 0.5 % solution in $CDCl_3$ (Sano and Hakusui 1970).

2.1.5. Crystal properties

Grinding causes the crystallinity of rifampicin to disappear and an amorphous form to originate.

2.1.6. Thermal analysis

Rifampicin melts with decomposition at 183-188°C. The heating curve of rifampicin showed an endotherm at 193 °C corresponding to the melting, immediately followed by an exotherm corresponding to the recrystallization of the melt, which then decomposes exothermically at about 240 °C (Pelizza et al. 1977).

2.1.7. Lipid-water partition

The partition of rifampicin in the system n-octanol/ aqeuos phosphate buffer pH 7.4 was determined to be K = 15.6 (Seydel 1970).

2.1.8. Surface activity

The surface activity of rifampicin depends on the pH of the medium. In the alkaline to neutral range rifampicin is a weak surfactant, and in the acidic range (pH 4.0) a pronounced lowering of the surface tension with concentration is observed and the micelle formation is apparent at a concentration of about 10^{-5} moles/liter (Pelizza et al. 1976).

2.1.9. Official methods of analysis

Indian pharmacopoeia (IP), 1996, and British Pharmacopoeia (BP), 1998, have reported a simple spectrophotometric method for the estimation of rifampicin. The method is as follows. Accurately weighed quantity of rifampicin of (0.100 g) dissolved in sufficient methanol and the volume made up to 100 ml. Then 2 ml of this solution was taken and volume made up to 100 ml with phosphate buffer pH 7.4. The absorbance of the resulting solution was measured at the maximum at about 475 nm using phosphate buffer pH 7.4 as a blank. Calculate the content of rifampicin taking 187 as the value of A (1%, 1 cm) at the maximum at about 475 nm. Same method has been reported for the assay of rifampicin capsule in IP 1996.

A high performance liquid chromatographic (HPLC) method has been reported in the United State pharmacopoeia (USP 24), 2000, for rifampicin, rifampicin capsules and rifampicin injection. The method recommended the use of a mobile phase of water, acetonitrile, 1.0 M phosphate buffer, 1.0 M citric acid, and 0.5 M sodium perchlorate (510:350:100:20:20) at a flow rate of 1.5 ml/min, using rifampin quinone as a internal standard. The column reported was 4.6mm × 10 cm with 5 µm particles and the detection was done by UV at 254 nm.

A different HPLC method has been reported in USP 24, for the estimation of rifampicin from rifampicin and isoniazid capsules. The method recommended the use of a mobile phase of water, phosphate buffer solution, and methanol (850:100:50) at a flow rate of 1.5 ml/min. The column reported was 4.0mm × 30 cm with 10 µm particles L1 packing and the detection was done by UV at 254 nm.

2.1.10. Pharmacology

In vitro, rifampicin is bactericidal against a wide range of organisms, including mycobacteria (Kunin et al. 1969, Arioli et al. 1967). The minimum. inhibitory concentration (MIC mg/l) against mycobacterium tuberculosis is 0.005-0.2. The mode of action is by inhibition of DNA dependent RNA polymerase, inhibiting transcription. Microbial resistance to rifampicin can develop and against acute organisms resistance

develops more readily, and, therefore it is usually given in combination with other anti-tubercular drugs.

2.1.11. Pharmacokinetics

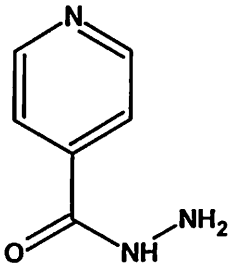
Rifampicin is most rapidly and completely absorbed from the gastrointestinal tract (Acocella 1978). The bioavailability of rifampicin is approximately 65% after oral administration (Loos et al. 1985, Nitti et al. 1977). Rifampicin may be given intravenously in patients with severe gastrointestinal abnormalities (short bowel syndrome) or in the acutely ill patient who is vomiting. Following a typical 600 mg dose peak concentrations in the region of 7-10 mg/liter are reached in 2-4 h and this is well above therapeutic levels for tuberculosis. There is considerable individual variability, and concurrent administration of p-aminosalicylic acid may delay the absorption (Boman et al. 1971). Concurrent administration of the food may delay the absorption (Verbist and Gyselen 1968, Siegler et al. 1974). Also, gastric pH is of importance and acidification of gastric juice increases the absorption and serum concentrations. Food decreases the AUC by 6% and C_{max} by 36% (Peloquin et al. 1999, Acocella 1978). Antacids have little effect on the absorption of the rifampicin (Peloquin et al. 1999). Rifampicin absorption is very sensitive to changes in the product formulation (Buniva et al. 1983, Cavenaghi 1989). There is considerable variability in the absorption of rifampicin or rifampicin containing combination products marketed globally (Ellard et al. 1986. Acocella et al. 1988, Sbarbaro et al. 1999).

Rifampicin readily diffuses in to most organs, tissues, bone and body fluids, including exudates into tuberculous lung cavities (Acocella et al. 1967). High concentrations appear in the lachrymal glands and tears. This is perhaps best exemplified by the fact that the drug may impart an orange red color to the urine, feces, saliva, sputum, tears, and sweat; the patients should be so warned (Mandell and Petri Jr 2001). The volume of distribution is approximately 1 l/kg.

The rifampicin is extensively eliminated by intestinal and hepatic metabolism mostly deacetylated, hydroxylated and formyl derivatives (Burman et al. 2001). The chief route of elimination of rifampicin is through biliary excretion of its micro biologically active desacetyl metabolite (Furesz 1970), and it is probable that this route of excretion is

saturated at quite modest doses, with the result that its elimination half life markedly increases with increasing dose sizes (Acocella et al. 1971, Furesz 1970, Furesz et al. 1967) as does the proportion of dose excreted in the urine. This first pass effect also results in peak serum/plasma concentrations increasing more than proportionally to dose size. Repeated daily administration leads to more rapid elimination of the drug as manifested by reduction in its half life (Acocella et al. 1971, Nitti et al. 1972, Immanuel et al, 1985, Acocella et al. 1985). This is generally believed to result from the autoinduction of hepatic deacetylase and resulting increased biliary excretion. The half life is reported to decrease from 3.4 h on day 1 to 2.0 h on day 14. Although kidney is not the main excretion pathway for rifampicin and its metabolites, urinary excretion increase with doses above 450 mg when the biliary excretion pathway is more saturated. With a dose of 900 mg, 25% of the rifampicin may be excreted in the urine (Acocella 1978).

2.2. Isoniazid



Isoniazid is a synthetic first line antitubercular drug widely used for the specific treatment of tuberculosis either alone for preventive therapy or in combination with other antitubercular drugs for the treatment of all active forms of the disease.

2.2.1. Chemistry

Isoniazid (INH, hydrazid, isonicotinic acid hydrazide, nydrazid) is chemically hydrazide of isonicotinic acid (Mandell and Petri Jr 2001). It is white, odorless, crystalline powder with the molecular formula $C_6H_7N_3O$. Its molecular weight is 137.14. The pH of a 1% aqueous solution 5.5-6.5.

2.2.2. Solubility

Isoniazid is having good solubility in water. Its solubility in water is 14 g in 100 ml at 25°C, and 26 g in 100 ml at 40°C.

2.2.3. Ionization constant

Isoniazid is reported to have three pKa values (basic groups), viz, 1.8, 3.5, and 10.8 (Rekker and Nauta 1964).

2.2.4. Crystal properties

Isoniazid crystals are orthorhombic in nature.

2.2.5. Thermal analysis

The melting point of isoniazid is reported to be 170-174 °C. Differential thermal analysis (D.T.A.) showed that isoniazid in the presence of zinc, copper, and iron salts and mercuric oxide gives an abnormal D.T.A. pattern. Isoniazid shows a sharp endotherm at 170 °C using DuPont thermal analyzer (Brewer 1977).

2.2.6. Partition coefficient

Partition coefficient of isoniazid has been determined in octanol/buffer (pH 7.4) system, and reported to be 0.08.

2.2.7. Official methods of analysis

IP, 1996, and BP, 1998, have reported a titrimetric method for the estimation of isoniazid and isoniazid tablets. The method is as follows. Accurately weighed quantity of isoniazid (0.25) was dissolved in sufficient quantity of water to produce 100 ml. To the 20 ml of the resulting solution was added 100 ml of water, 20 ml of hydrochloric acid, 0.2 g of potassium bromide, and 0.05 g of methyl red solution. Titrate dropwise with 0.0167 M potassium bromate, shaking continuously, until the red color disappears.

A HPLC method has been reported in the United State pharmacopoeia (USP 24), 2000, for isoniazid. The method recommended the use of a mobile phase prepared by dissolving

4.4 g of docusate sodium in 600 ml of methanol and to this was added 400 ml of water. The pH of the prepared mobile phase was then adjusted to 2.5 by adding 2 N sulfuric acid. Mobile phase was pumped at a flow rate of 1.5 ml/min. The column reported was 4.6mm × 25 cm with L1 packing and the detection was done by UV at 254 nm. Different HPLC method has been reported in USP 24 for the estimation of isoniazid from isoniazid tablets. Here, mobile phase consists of a mixture of phosphate buffer pH 6.9 (containing 0.2 mM triethanolamine) and methanol in the ratio of 95:5. Mobile phase flow rate was maintained at 1.5 ml/min. The column used was 4.6 mm × 30 cm with L1 packing and the detection was done by UV at 254 nm.

A titrimetric method has been reported in USP 24, for the estimation of isoniazid from rifampicin and isoniazid capsules. The method reported is as follows. The contents of 20 capsules were accurately weighed and mixed. Accurately weighed portion of the powder equivalent to 100 mg of isoniazid was transferred to a 250 ml separator. To this was added 20 ml of 0.1 N hydrochloric acid and the contents were shaken. The acidic solution was then extracted with six 25 ml portions chloroform, retaining any interfacial emulsion with the aqueous phase, and discarding the chloroform extracts. Acidic aqueous phase was transferred to a 100 ml volumetric flask and diluted to volume with 0.1 N HCl and mixed. 25 ml of this solution was pipetted in to a titration vessel to this 10 ml of hydrochloric acid was added and adjusted the volume to 50 ml with water. Finally this solution was titrated with 0.1 N bromine, determining the endpoint potentiometrically.

2.2.8. Pharmacology

Isoniazid is bactericidal in vitro and in vivo against actively dividing tubercle bacilli and is less active against nondividing tubercle bacilli, being only bacteriostatic. It primary action is to inhibit the synthesis of long chain mycolic acids, which are unique constituents of the mycobacterial cell walls. The minimum inhibitory concentration for M. tuberculosis is 0.025 to 0.05 mg/liter (Krishnamurthy 1975).. Isoniazid resistance is a relatively uncommon occurrence in developed countries but is an increasing problem in developing countries. Because of the risk of resistance development, this drug is usually given in combination with the other antitubercular drugs.

2.2.9. Pharmacokinetics

Isoniazid is rapidly and completely absorbed from the GIT provided there is no interference from the contact with the food or other co-administered drugs. The peak plasma concentrations of 3-7 mg/liter are achieved 1-2 h after oral adiministration of normal therapeutic doses to adults (Hurwitz and Schlozman 1974). Its bioavailability is reduced however by high carbohydrate meals and by various antacids. Food intake decreases the bioavailability of isoniazid by 12 to 43%, which may lead to treatment failure (Joshi et al. 1991, Mannisto et al. 1982, Melander et al. 1976).

Isoniazid readily diffuses in to all body fluids and cells. The drug is detectable in significant quantities in pleural and ascetic fluids (Mandell and Petri Jr 2001), concentration in the cerebrospinal fluid are similar to those in the plasma. Isoniazid distributes in total body water with a mean apparent volume of distribution (V_d) of 61 ± 11% of body weight (0.6 to 0.8 liter/kg). V_d is unrelated to acetylator status or age (Weber and Hein 1985).

Metabolism is the predominant factor affecting the elimination and pharmacokinetic profile of isoniazid. In humans N-acetylation is the most important pathway in isoniazid elimination. Numerous studies demonstrated that differences in isoniazid disposition and elimination are attributable to genetically determined differences in individual N-acetylation capacity (Weber and Hein 1985). Isoniazid undergoes appreciable presystemic (first pass) metabolism in the wall of the small intestine and liver, resulting in the concentrations in the plasma of rapid acetylators which are half those in slow acetylators after normal dose of 300 mg of the drug (Eidus and Hodgkin 1975). The data in one rapid acetylator and in one slow acetylator show that isoniazid is converted to acetylisoniazid four to five times faster in case of rapid acetylators. The half life ranges from 35 to 110 min for rapid acetylators and from 110 to 400 min for slow acetylators. It is reported that there is no measurable difference in the peak isoniazid concentration in rapid and slow acetylators after intravenous administration. This has prompted the development of different formulations to compensate for this difference, i.e. slow release matrix formulations mixed with free isoniazid to optimize blood levels in rapid acetylators (Ellard et al. 1972). As much as 95% of ingested isoniazid is excreted in the

urine within 24 h. Less than 10% of the dose is excreted in the feces. The main excretion products in the urine are N-acetylisoniazid and isonicotinic acid. The renal clearance of isoniazid plus hydrazones that decompose to isoniazid in mildly acidic urine is 43-49 ml/min. A high carbohydrate diet (300 kcal) significantly prolongs the elimination half life in slow acetylators (4.06 ± 0.23 h) compared with fasting subjects (3.25 ± 0.22 h). This effect was attributed to the formation of condensation products with various sugars (Mannisto et al. 1982).

Chapter 3

Analytical Method Development

3.1. Introduction

Analysis is an important component in the formulation development of any drug molecule. A suitable and validated method has to be available for the analysis of the drug(s) in the bulk, in drug delivery systems, from release/dissolution studies and in biological samples. If a suitable method, for specific need, is not available then it becomes essential to develop a sensitive, simple, rapid, and cost effective method for the estimation of the drug samples.

3.1.1. Analytical methods of rifampicin

Several analytical methods have been reported for the estimation of rifampicin in different study samples.

A spectrophotometric method has been reported for the estimation of rifampicin based on the reaction with chloranil (Sastry et al. 1985). In another reported method (spectrophotometric method) (Rao and Murthy 2001), a stable dissolution media of pH 7.4 phosphate buffer with 0.02% w/v of ascorbic acid has been used for the analysis of rifampicin in controlled release formulations at 475 nm. The reported method was found to be more suitable for in vitro evaluation of rifampicin controlled release formulations.

A thin-layer Chromatographic assay for the estimation of rifampicin and its degradation components in a drug excipient interaction studies was reported (Jindal et al. 1994). Several high performance liquid chromatography methods (HPLC) have been reported for the analysis of rifampicin (Calleja et al. 2004, Ratti et al. 1981, Weber et al. 1983, Woo et al. 1987).

On a detailed survey of the available literature, none of the methods, except the one reported by Rao and Murthy (Rao and Murthy 2001), found to be suitable for routine analysis of rifampicin in formulations and from in vitro release samples. Methods reported especially are not useful for the analysis of drug samples from in vitro release study of controlled release formulations. Reported colorimetric method (Sastry et al. 1985) seems to be tedious and does not assure the stability of the drug during the analysis. Thin layer chromatographic method found to be unsuitable for routine analysis of in vitro release samples as the linearity range reported is in nanograms and also the quantification method is complex. HPLC methods although seem to be sensitive and

precise but the cost and time factors do not allow these methods to be adopted for the routine analysis of the samples. In general, the method of analysis should be cost effective, simple, less time consuming besides being sensitive, accurate, precise and stability indicating. In such cases, simple spectrophotometric methods found to be very suitable for the analysis of drug samples for routine drug content analysis and analysis of in vitro release samples. Such methods are not only rapid to perform but are practical in many laboratories with relatively modest laboratory facilities. The method reported by Rao and Murthy (Rao and Murthy 2001) seems to fulfill the above mentioned requirements. This method has been reproduced in our laboratory conditions and validated. The validated method has been used for routine analysis of assay of formulations, in vitro release samples, stability study samples and for the other studies.

3.1.2. Analytical methods of isoniazid

A large number of analytical methods including colorimetric, fluorimeric, microbiologic, chromatographic and radioimmunologic methods have been reported for measuring the concentration of isoniazid in plasma, urine, and in vitro samples and are summarized as follows.

A number of authors have formed hydrazones of isoniazid with various aldehydes and ketones and used the highly colored products to determine the drug (Brewer 1977). A rapid and sensitive spectrophotometric method for the estimation isoniazid in pure and pharmaceutical formulations has been reported (Gowda et al. 2002). Nagaraja et al reported a simple, rapid and sensitive spectrophotometric method for the determination of isoniazid and ritodrine hydrochloride in pure form as well as dosage forms (Nagaraja et al. 2002). Kinetic determination of isoniazid in pure and pharmaceutical formulations was carried out by Kulkarni et al (Kulkarni et al. 2004).

HPLC methods were preferred over other methods and permitted the simultaneous analysis of isoniazid and its hydrazine metabolites in plasma and urine (Sassen et al. 1985). A HPLC method for the determination of isoniazid, acetylisoniazid, acetylhydrazine and diacetylhydrazine in plasma and urine was developed and reported (Sassen et al. 1985). Also a HPLC analysis was reported for isoniazid in its tablet and injectable dosage forms (Bailey and Abdou 1977).

Of the various methods reported for the estimation of isoniazid, FIA-spectrofluorometry, polarography, adsorptive stripping voltametry, and selective adsorption are of high cost (Kulkarni et al. 2004). All other spectrophotometric and colorimetric methods reported for the analysis of isoniazid have many drawbacks; some of these are time bound (Manna et al. 2000), involve tedious extraction procedure (Gowda et al. 2002), need cooling for a long time (Devani et al. 1985) or are less sensitive and involve complex reactions (Nagaraja et al. 2002). HPLC methods reported as usual suffer from the disadvantages of cost, time, and the requirement of sophisticated lab facilities.

Thus, to overcome the aforementioned disadvantages and to have a simple and cost effective method that could be used for the routine analysis of pure drug samples, in vitro release samples, assay of formulations, stability samples and for other purposes, the author has used an in house developed and validated UV method for the estimation of isoniazid. The method was developed using 5.0 pH phosphate buffer and the drug was estimated at the λ_{max} 262 nm. The stability indicating capability of the developed method was evaluated for a period of 36 h and, the absorbance value at 262 nm was found to be statistically same (less % CV values) for the time period studied. The developed method was validated according to standard guidelines (ICH guidelines 1996, USP 2000b) and the developed method was used to estimate the drug content in three commercially available tablet formulations of isoniazid. The developed method was also used in the analysis of isoniazid from the formulated tablets, samples from in vitro release studies, and the stability samples.

3.1.3. Simultaneous estimation methods for the analysis of rifampicin and isoniazid

A colorimetric method has been reported for the estimation of rifampicin and isoniazid along with the pyrazinamide and ethambutol in FDC tablets and capsules (Ellard 1999). A least-squares method in the matrix form was described for the simultaneous determination of rifampicin and isoniazid in a mixture (Mahalanabis et al. 1989). A rapid and simple method for the simultaneous estimation of rifampicin, isoniazid and pyrazinamide in combined dosage forms by first derivative UV spectrometry has been reported (Rote and Sharma 1997). In another report (Goyal et al. 2002), a simple and rapid method has been described for the simultaneous estimation of rifampicin and

isoniazid. Three simple spectrophotometric methods for the simultaneous determination of rifampicin (RIF) and isoniazid (INH) in pharmaceutical preparations have been developed and reported (Kakde et al. 2002). Manna et al reported a spectroscopic method for the simultaneous estimation of rifampicin and isoniazid (Manna et al. 2000). Multivariate spectrophotometric calibration has been used by Goicoechea and Olivieri for the simultaneous estimation of rifampicin, isoniazid and pyrazinamide in tablets (Goicoechea and Olivieri 1999).

For the simultaneous estimation of rifampicin, isoniazid and pyrazinamide high performance thin layer chromatography (HPTLC) (Argekar et al. 1996) and HPLC methods have been reported in the literature (Calleri et al. 2002). In another report (Erram et al. 1992), a HPLC method has been described for the simultaneous estimation of rifampicin and isoniazid in single and combined formulations. A reversed phase HPLC method has been reported for the simultaneous estimation of rifampicin and its major metabolite desacetyl rifampicin in presence of isoniazid and pyrazinamide, in human plasma and urine (Panchagnula et al. 1999). A rapid HPLC method for the simultaneous estimation of isoniazid, acetyl isoniazid, rifampicin, and desacetyl rifampicin in microsamples of deproteinized plasma has been reported (El-Yazigi and Raines 1992). Recently a voltammetric assay has been reported for the analysis of rifampicin and isoniazid (alone and in combination) from the bulk samples as well as from the serum samples (Hammam et al. 2004).

As we have already discussed, liquid chromatographic methods for the routine analysis of the drug samples, especially from in vitro release studies of controlled release formulations, involving multiple time points seem to be cumbersome, costly, and time consuming. Although several UV spectrophotometric methods have been reported in the literature none of the method found to be useful for the routine analysis of in vitro release samples from combined controlled release formulations of rifampicin and isoniazid. Also the reported methods seem to be applicable only in case of drug content analysis from the conventional combination tablets (like FDC). Reported UV methods did not guarantee the analytical stability of the drugs during analysis, which is a major drawback.

Hence, the present investigation aimed at the development of a simple, economic, sensitive and accurate analytical method for the simultaneous estimation of rifampicin and isoniazid from combination formulations and especially from the in vitro release samples of controlled release formulations. The investigation also considered the fact that the developed method should be stability indicating for the purpose of the project. The method should be such that the estimation of both the drugs, at their wavelength of estimation, should not be affected by the degradation. Hence, such method could be used for the analysis of routine in vitro release samples for the intended period of time irrespective of the pH of the media in which release studies proposed to be performed. Considering all these factors the method development has been tried in various solvent systems such as, water, 0.4% w/v of sodium lauryl sulphate in water (Jindal et al. 1994), methanol, acetonitrile, 0.1 N HCl, simulated gastric fluid without pepsin (SGF), 0.01 N HCl, phosphate buffers (pH 5.0 – 7.4), simulated intestinal fluid without pancreatin (SIFsp), all the previously mentioned media with different anti-oxidants in different ratio (like, sodium metabisulfite, ascorbic acid), and combination of different media in different proportions. Finally, it was decided to use SIFsp. The selection of the method was based on sensitivity, interference, ease of preparation, need for pH adjustment, tolerance for pH variation, suitability for drug content estimation and stability, analysis time and cost factor. First derivative UV spectroscopic method was developed for the estimation of isoniazid at 254.5 nm, where rifampicin has got zero crossing point. Rifampicin was analyzed by simple UV spectroscopy at λ_{data} 522.5 nm. Respective absorbance/amplitude values found to be statistically same, up to 28 h, for both drugs at their wavelength of estimation.

SGF: 2 g of sodium chloride, 7 ml of concentrated HCl and volume made up to 1000 ml with water (USP 24).

SIFsp: 6.8 g of monobasic potassium phosphate, 77 ml of 0.2 N sodium hydroxide and 500 ml of water, pH adjusted to 6.8 ± 0.1 with 0.2 N HCl or 0.2 N sodium hydroxide and volume made up to 1000 ml with water (USP 24).

The developed method was validated according to standard guidelines (ICH guidelines 1996, USP 2000b) and the developed method was used to estimate the drug content in three commercially available tablet formulations of isoniazid. The developed method was used in the analysis of isoniazid from the formulated tablets, samples from in vitro release studies, and the stability samples.

3.2. Experimental

Materials

Rifampicin was obtained as a gift sample from Cadila Pharma Ltd, Ahmedabad. Isoniazid was obtained as a gift sample from Lupin Laboratories, Aurangabad. Analytical grade potassium dihydrogen orthophosphate was purchased from Merck, Mumbai, India. High quality pure water was prepared with Millipore water purification system (Millipore, Molsheim, France, model Elix SA 67120). Two commercially available rifampicin capsule formulations (R-cinex 450 from Lupin Lab, Pune, India, and Macox 300 from Macleods Pharmaceuticals, Mumbai, India.) were purchased from the local market. Two commercially available isoniazid tablet formulations (Combunex from Lupin Lab, Pune, India, and Myconex 600 from Cadila Pharma, Ahmedabad, India) were purchased from the local market. Two commercial formulations of rifampicin and isoniazid combinations (R-cinex from Lupin Lab, Pune, India, and Cadirifa from Cadila Pharmaceuticals, India) were purchased from the local market.

Instruments/Equipments

A UV-visible-NIR spectrophotometer (Jasco UV-visible spectrophotometer; model – V 570, Tokyo, Japan) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells with Jasco spectra manager software was used for all absorbance measurements for UV analysis.

3.2.1. In house validation of reported analytical method for rifampicin in 7.4 pH phosphate buffer containing 0.02% w/v of ascorbic acid (Rao and Murthy 2001)

Preparation of standard curve

A stock solution of rifampicin was prepared by dissolving 10 mg of drug in 5 ml of methanol and then volume was made up to 100 ml by adding phosphate buffer (pH 7.4: 6.805 g of KH_2PO_4 + 195.5 ml of 0.2 M NaOH, volume made up to 1000 ml with water) to get a final concentration of 100 µg/ml. The λ_{max} of rifampicin in the above media was determined by scanning a suitable dilution of the stock using the UV-visible spectrophotometer (absorption spectra presented in Figure 3.1). From the stock, various standard dilutions were made to obtain solutions of 5, 10, 20, 30, 40, 50, and 60µg/ml, and their respective absorbencies were recorded at the selected wavelength. The results are listed in Table 3.1 and the results of regression analysis are presented in the Table 3.2. The absorbance characteristics, accuracy, precision and other validation parameters of the proposed methods are given in Table 3.3. The stability of rifampicin solutions during analysis was also investigated by analyzing samples at different time intervals on the same day and the subsequent day by storing at 37 ± 1 °C temperature.

Method validation

Following procedures were employed to determine various validation parameters of the selected spectrophotometric method (the results are presented in Table 3.3)

Accuracy and precision: To determine accuracy and precision of the proposed method three different (6 in each case) concentrations of rifampicin standard and test solutions were analyzed as per the procedure enlisted in the preparation of the standard curve. Three concentrations were selected in a manner that they should cover the entire calibration curve range and are designated as LQC (lower quantification concentration), MQC (medium quantification concentration) and HQC (higher quantification concentration). The selected LQC, MQC and HQC were 15, 35 and 55 µg/ml respectively. To determine intra- and inter-day precision of the assay, replicate sets (6 at each concentration) of three calibration solutions (LQC, MQC and HQC) were analyzed. The percentage relative standard deviation (%RSD) for the assay results was determined.

Linearity: Six separate series of solutions of the drug 5, 10, 20, 30, 40, 50, and 60µg/ml were prepared from the stock solution and analyzed.

Specificity: Series of six solutions of 20 µg /ml were prepared from the stock solution and analyzed for specificity.

LOQ and LOD: LOQ and LOD were calculated on the basis of standard error of the estimate and slope of the regression equation based on replicate determinations. The experiments were then performed to analyze the actual concentration that can be accurately quantified or detected by proposed method.

Ruggedness and robustness: Ruggedness was determined for the developed method by varying the analyst for analyzing standard and test solution the drug (15 and 35 µg/ml) in triplicate and by varying the instrument (using Jasco UV visible spectrophotometer model–7800). Robustness was determined by varying the pH of phosphate buffer between 7.0 – 7.8.

Estimation of rifampicin from two commercial capsule formulations

Two commercially available capsule formulations from Indian market were selected randomly for estimation of rifampicin content by the proposed method. Ten capsules from each brand were taken for the analysis. The contents of these ten capsules were mixed and powdered. An aliquot weight was transferred to a series of 100 ml volumetric flasks (five in each case). Initially 5 ml of methanol was added to dissolve the drug and final volume was made using phosphate buffer (pH 7.4) containing 0.02% w/v of ascorbic acid. The resulting solution was filtered through Whatman filter paper no. 1 and suitably diluted to get final concentration within the limits of linearity for the proposed method. The drug content per capsule of different brands of rifampicin commercial capsules was calculated (on an average basis) from the absorbance value. The results are tabulated in Table 3.4.

Recovery studies

To study the interference of formulation additives and to keep an additional check on the accuracy of the developed assay method, analytical recovery experiments were performed by adding known amount of pure drug to pre-analyzed samples of commercial

dosage forms. The percent analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are listed in Table 3.5.

Results and Discussions

To develop rugged and sensitive spectrophotometric method various solvent systems were tried such as, water, methanol, acetonitrile, 0.1 N HCl, phosphate buffers (pH 5.0 – 7.4), and the combination of different media in different proportions. The final decision of using the reported method i.e., phosphate buffer pH 7.4 containing 0.02% w/v of ascorbic acid was based on sensitivity, interference, ease of preparation, need for pH adjustment, tolerance for pH variation, suitability for drug content estimation and stability, analysis time and cost factor. The maximum variation of pH of the selected media was ±0.08, thus contributing the robustness of the method. Effect of various formulation additives on the absorbance of rifampicin has been studied at the wavelength of estimation and no interference was observed from any of the excipients in the proportion studied.

Rifampicin showed the λ_{max} at about 475 nm in phosphate buffer (pH 7.4) with 0.02% w/v of ascorbic acid and the corresponding absorption spectra is shown in Figure 3.1. Though there were other peaks observed at different wavelength regions, but those are not used for estimation due to interference from the ascorbic acid and in a view of the stability aspects. The absorbance at 475 nm found be to stable for about 36 h at 37 ± 1 °C, indicating stability of the drug in the media. There was a linear relationship between the drug concentration and the absorbance at 475 nm in the concentration range of 5-60 µg/ml. The statistical analysis (ICH guidelines 1996, USP 2000b) of the data obtained for the estimation of rifampicin in pure solution indicated high level of precision for the proposed method as evidenced by the low standard deviation values. The low values of coefficient of variation further established the precision of the proposed method.

The linear regression equation obtained was Y = 0.0182.X + (-) 0.0093, where Y is the absorbance and X is the concentration (in µg/ml) of pure rifampicin solution. Linearity of the regression equation and negligible scatter of points were demonstrated from correlation coefficient (0.9999). The reported slope values without intercept on the

ordinate, at 95% confidence limits, suggested that the calibration lines of rifampicin solution in 7.4 pH phosphate buffer with 0.02% w/v of ascorbic acid did not deviate from the origin as the above values were within the confidence limits. The precision of the fit was further confirmed with standard error values of the intercept and slope. A one-way ANOVA test was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significant levels (Table 3.2)

Validation of the developed method

The method was validated according to the standard procedure (ICH Guidelines 1996, USP 2000b) and the results obtained are tabulated in Table 3.3. The linearity range was found to be 5-60 µg/ml at a λ_{max} of 475 nm. The LOD was obtained as 1.40 µg/ml and LOQ was found to be 4.23 µg/ml. In the validation table, the accuracy and precision are reported in terms of % RSD. The low values of these parameters reflected the excellent measurement accuracy and precision of the proposed method for estimation of rifampicin. The ruggedness in the estimation of rifampicin standard and test solutions in triplicate by different analyst and on different instruments was determined to be 100.50 ± 1.214%.

The method of preparing the buffer solution was found to be simple and accurate and variation of pH of the buffer between 7.2-7.6 did not affect the sensitivity of the method. The %RSD for intra- and inter-day variation was less than 3.0%, which fall well below the acceptance criteria.

Recovery studies

The method was further validated by estimation of rifampicin in pharmaceutical formulations and analysis of reference pure drug solution. The results are presented in Table 3.4 and Table 3.5. The estimated drug content with low values of standard deviation further confirmed the precision of the proposed method and therefore suggested the non-interference from the formulation matrix present in the studied formulations. The

accuracy of the results of estimation was further tested by recovery experiments. The average recovery varied from 99.89 ± 1.51 to 101.05 ± 0.76%.

3.2.2. Analytical Method Development of Isoniazid in pH 5.0 phosphate buffer

Preparation of standard curve

A stock solution of isoniazid was prepared by dissolving 10 mg of drug in 5.0 pH phosphate buffer and then making up the volume to 100 ml (pH 5.0; dissolve 6.8 g of KH_2PO_4 in 1000 ml water and adjust the pH to 5.0 with 10 M KOH solution) to get a final concentration of 100 µg/ml. The λ_{max} of isoniazid in the above media was determined by scanning a suitable dilution of the stock using the UV-visible spectrophotometer (UV spectra presented in Figure 3.2). From the stock, various standard dilutions were made to obtain solutions of 2.5, 10, 15, 20, 25, 30, and 40 µg/ml, and their respective absorbencies were recorded at the λ_{max}. The results are listed in Table 3.1 and the results of regression analysis are presented in the Table 3.2. The absorbance characteristics, accuracy, precision and other validation parameters of the proposed methods are given in Table 3.3. The stability of isoniazid solutions during analysis was also investigated by analyzing samples at different time intervals on the same day and the subsequent day by storing at 37 ± 1 °C temperature.

Method validation

Following procedures were employed to determine various validation parameters of the developed UV spectrophotometric method (the results are presented in Table 3.3)

Accuracy and precision: The method followed for the determination of accuracy and precision remains same as for the rifampicin method development. The selected LQC, MQC and HQC were 5, 20 and 35 µg/ml respectively. To determine intra- and inter-day precision of the assay, replicate sets (6 at each concentration) of three calibration solutions (LQC, MQC and HQC) were analyzed. The %RSD for the assay results was determined.

Linearity: Six separate series of solutions of the drug 2.5, 10, 15, 20, 25, 30, and 40 µg/ml were prepared from the stock solution and analyzed.

Specificity: Series of six solutions of 20 µg /ml were prepared from the stock solution and analyzed for specificity.

LOQ and LOD: LOQ and LOD were calculated as suggested under method validation of rifampicin. The experiments were then performed to analyze the actual concentration that can be accurately quantified or detected by proposed method.

Ruggedness and robustness: Ruggedness was determined for the developed method by varying the analyst for analyzing standard and test solution the drug (10 and 30 µg/ml) in triplicate and by varying the instrument by using Jasco UV spectrophotometer model–7800. Robustness was determined by varying the pH of phosphate buffer between 4.6 – 5.4.

Estimation of isoniazid from two commercial tablet formulations by the developed UV method

Two commercially available tablet formulations from Indian market were selected randomly for estimation of isoniazid content by the proposed method. Ten tablets from each brand were taken for the analysis. These ten tablets were powdered and mixed and aliquot weight was transferred to a series of 100 ml volumetric flasks (five in each case). The volume was made up to 100 ml using 5.0 pH phosphate buffer. The resulting solution was filtered through Whatman filter paper no. 1 and suitably diluted to get final concentration within the limits of linearity for the proposed method. The drug content per tablet of different brands of isoniazid commercial tablets was calculated (on an average basis) from the absorbance value. The results are tabulated in Table 3.4.

Recovery studies

Similar procedure that used for the recovery studies of rifampicin method development was used for isoniazid. The results are shown in the Table 3.5.

Results and Discussions

Method development

To develop rugged and sensitive UV spectrophotometric method various solvent systems were tried such as, water, methanol, acetonitrile, 0.1 N HCl, phosphate buffers (pH 5.0 – 7.4), and the combination of different media in different proportions. The final decision of using the phosphate buffer pH 5.0 was based on sensitivity, interference, ease of preparation, need for pH adjustment, tolerance for pH variation, suitability for drug content estimation and stability, analysis time and cost factor. The maximum variation of pH of the selected media was ±0.07, thus contributing the robustness of the method. Effect of various formulation additives on the absorbance of isoniazid has been studied at the wavelength of estimation and no interference was observed from any of the excipients in the proportion studied.

Isoniazid showed the λ_{max} at about 262 nm in phosphate buffer (pH 5.0) and the corresponding UV spectra is shown in Fig 3.2. Though there were other peaks found at other wavelengths, but those are not used for estimation in a view of sensitivity and stability aspects. Absorbance at 262 nm found to be stable for about 36 h at 37 ± 1 °C, indicating stability of the drug in the media. There was a linear relationship between the drug concentration and the absorbance at 262 nm in the concentration range of 2.5 - 40 µg/ml. The statistical analysis (ICH guidelines 1996, USP 2000b) of the data obtained for the estimation of isoniazid in pure solution indicated high level of precision for the proposed method as evidenced by the low standard deviation values. The low values of coefficient of variation further established the precision of the proposed method.

The linear regression equation obtained was Y = 0.0305.X + 0.0148, where Y is the absorbance and X is the concentration (in µg/ml) of isoniazid. Linearity of the regression equation and negligible scatter of points were demonstrated from correlation coefficient (0.9998). The reported slope values without intercept on the ordinate, at 95% confidence limits, suggested that the calibration lines of isoniazid solution in 5.0 pH phosphate buffer did not deviate from the origin as the above values were within the confidence limits. The precision of the fit was further confirmed with standard error values of the intercept and slope. A one-way ANOVA test was performed based on the values

observed for each pure drug concentration during the replicate measurement of the standard solutions. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significant levels (Table 3.2)

Validation of the developed method

The developed method was validated according to the standard procedure (ICH Guidelines 1996, USP 2000b) and the results obtained are tabulated in Table 3.3. The linearity range was found to be 2.5-40 μg/ml at a λ_{max} of 262 nm. The LOD was obtained as 0.36 μg/ml and LOQ was found to be 1.10 μg/ml. In the validation table, the accuracy and precision are reported in terms of % RSD. The low values of these parameters reflected the excellent measurement accuracy and precision of the proposed method for estimation of isoniazid. The ruggedness in the estimation of isoniazid standard and test solutions in triplicate by different analyst and on different instruments was determined to be 99.86 ± 1.416%.

The method of preparing the buffer solution was found to be simple and accurate and variation of pH of the buffer between 4.6-5.4 did not affect the sensitivity of the method. The %RSD for intra- and inter-day variation was less than 3.0%, which fall well below the acceptance criteria.

Recovery studies

The method was further validated by estimation of isoniazid in pharmaceutical formulations and analysis of reference pure drug solution. For this study isoniazid and ethambutol combination tablets were obtained from the local market, as it was not possible to get the isoniazid alone formulations. It was observed that ethambutol didn't have any UV absorbance in the regions of isoniazid estimation, so the method could be used for the estimation of isoniazid in presence ethambutol. The results are presented in Table 3.4 and Table 3.5. The estimated drug content with low values of standard deviation further confirmed the precision of the proposed method and therefore suggested the non-interference from the formulation matrix present in the studied formulations. The accuracy of the results of estimation was further tested by recovery experiments. The average recovery varied from 99.08 ± 0.21 to 100.43 ± 2.27%.

3.2.3. Simultaneous Estimation Method for Rifampicin and Isoniazid in USP Simulated Intestinal Fluid without Pancreatin (SIFsp)

A stepwise experimental approach was followed for the development of simultaneous estimation method.

First, simple scanning of rifampicin and isoniazid was carried out in different media as enlisted in the analytical method development of individual drugs. Finally it was decided to use SIFsp, pH 6.8 as an estimation medium. Then it was decided to follow simple UV estimation of rifampicin at 522.5 nm, as isoniazid didn't interfere with the analysis of rifampicin at this wavelength (Figure 3.3). But, rifampicin found to interfere with the analysis of isoniazid at entire wavelength region of isoniazid absorption (Figure 3.4). Thus, a derivative UV spectroscopic method was tried for the estimation of isoniazid in presence of rifampicin. In this regard selection of the appropriate derivative order based on ideal point's selection was done to generate a derivative spectrum by Savitzky-Golay algorithm. First derivative method (D1) was preferred over second and third to minimize the complexity of the analysis. By first derivative rifampicin found to have a zero crossing point (ZCP) at 254.5 nm where isoniazid could be estimated without any interference (Figure 3.5).

Preparation of standard curves

The stock solutions of rifampicin and isoniazid were prepared by dissolving 10 mg of each drug separately in sufficient quantity of SIFsp (in case of rifampicin initially few ml of methanol was added to dissolve the drug) and then volume was made up to 100 ml. Appropriate amounts of stock solutions were transferred separately into 10 ml volumetric flasks and volume was made up with SIFsp to get a series of solutions containing 5, 10, 15, 20, 30, and 50 µg/ml of isoniazid and 2, 5, 10, 20, 30, and 50 µg/ml of rifampicin. Two series of 10 ml mixtures of rifampicin and isoniazid were also prepared from the stock solutions. The first series consists of constant concentration of rifampicin (20 µg/ml) and varying concentrations of isoniazid (5-50 µg/ml). Second series consists of constant concentration of isoniazid (20 µg/ml) and varying concentrations of rifampicin (2-50 µg/ml). The spectrum scanning of standard and sample solutions were carried out

against the blank (SIFsp) from 200 to 550 nm. Rifampicin measurement was done by measuring the corresponding absorbance values at 522.5 nm by simple spectroscopy. The first derivative spectrum (D1) for each solution was recorded by Savitzky-Golay parameter of Δ = 15 points and dervatized spectra were smoothened at 25 points. Ordinate minima and maxima were adjusted to the magnitude of derivative values. The isoniazid solutions were measured at 254.5 nm where rifampicin showed ZCP (Figure 3.6).

The results of the analysis are listed in Table 3.6 and 3.7 for rifampicin and isoniazid estimation respectively. The results of regression analysis are presented in the Table 3.8 and Table 3.9 for rifampicin and isoniazid estimation respectively. The validation parameters of the proposed methods are given in Table 3.10. The stability of rifampicin and isoniazid solutions during analysis was also investigated by analyzing samples at different time intervals on the same day and the subsequent day by storing at 37 ± 1 °C temperature.

Method validation

Following procedures were employed to determine various validation parameters of the developed UV spectrophotometric method (the results are presented in Table 3.10)

Accuracy and precision: The method followed for the determination of accuracy and precision remains same as for the rifampicin and isoniazid method development. The selected LQC, MQC and HQC were 2.5, 25 and 45 µg/ml respectively for rifampicin and 7.5, 25 and 45 µg/ml respectively for isoniazid. To determine intra- and inter-day precision of the assay, replicate sets (6 at each concentration) of three calibration solutions (LQC, MQC and HQC) of both rifampicin and isoniazid were analyzed. The %RSD for the assay results was determined.

Linearity: Six separate series of solutions of the rifampicin 2, 5, 10, 20, 30, and 50 µg/ml, and isoniazid 5, 10, 15, 20, 30, and 50 µg/ml were prepared from their respective stock solutions and analyzed.

Specificity: Series of six solutions of 20 µg /ml were prepared for rifampicin and isoniazid from their respective stock solutions and analyzed for specificity.

LOQ and LOD: LOQ and LOD were calculated on the basis of standard error of the estimate and slope of the regression equation based on replicate determinations. The experiments were then performed to analyze the actual concentration that can be accurately quantified or detected by proposed method.

Ruggedness and robustness: Ruggedness was determined for the developed method by varying the analyst for analyzing standard and test solution of the drug (10 and 30 µg/ml) in triplicate (for both the drugs) and by varying the instrument by using Jasco UV spectrophotometer model–7800. Robustness was determined by varying the pH of phosphate buffer between 6.4 – 6.8.

Estimation of rifampicin and isoniazid simultaneously from two commercial combination tablet formulations by the developed UV method

Two commercially available combination (rifampicin and isoniazid) tablet formulations from Indian market were selected randomly for estimation of two drugs content per tablet by the proposed method. Ten tablets from each brand were taken for the analysis. These ten tablets were powdered and mixed and aliquot weight was transferred to a series of 100 ml volumetric flasks (five in each case). The volume was made up to 100 ml using 6.8 pH SIFsp medium. The resulting solution was filtered through Whatman filter paper no. 1 and suitably diluted to get final concentration within the limits of linearity (for both rifampicin and isoniazid) for the proposed method. The drug content per tablet of different brands of rifampicin and isoniazid combination tablets was calculated (on an average basis) from the absorbance value at 522.5 nm for rifampicin and at 254.5 nm for isoniazid (D1). The results are tabulated in Table 3.11.

Recovery studies

Similar procedure that used for the recovery studies of rifampicin and isoniazid method development (individually) was used. The results are shown in the Table 3.12.

Method development

The zero order spectra showed that the rifampicin could be estimated at the wavelength of 522.5 nm in presence of isoniazid due to noninterference from isoniazid at this wavelength (Figure 3.3 for the absorption spectra of isoniazid). Also the absorbance of rifampicin at 522.5 nm was least affected by its degradation up to 28 h. Thus, this method assures the analytical stability of rifampicin for the purpose of the project. There was a linear relationship between the drug concentration and the absorbance at 522.5 nm in the concentration range of 2-50 µg/ml. The statistical analysis (ICH guidelines 1996, USP 2000b) of the data obtained for the estimation of rifampicin in pure solution and in presence of isoniazid indicated high level of precision for the proposed method as evidenced by the low standard deviation values (Table 3.6). The low values of %RSD further established the precision of the proposed method.

The zero order derivative spectra of rifampicin were found to be overlapping for isoniazid, making its estimation difficult in presence of rifampicin (Figure 3.4). The traditional simultaneous equation method was tried, but the results were not satisfactory. The reported method of simultaneous estimation by UV visible spectrophotometry couldn't be adopted because they were either non reproducible in our lab conditions or they did not assure the analytical stability of the drugs throughout the estimation. Finally it was decided to solve the problem of overlapping spectra by the use of first derivative spectra of the mixtures.

There are several methods reported for the quantitative evaluation of derivative spectra like peak-peak, peak-baseline, ratio compensation and, zero crossing point (ZCP) measurements. However, the success of a proposed measurement method depends on its sum of systematic and random errors. The zero crossing is the simplest of the measurements and is more sensitive compared to other methods. Thus, it was decided to go with the zero crossing measurement method. It was observed during initial studies that the first derivative spectra of rifampicin have ideal ZCPs at 254.5 nm and 296.5 nm, in the concentration range studied, for the estimation of isoniazid. Final decision to use ZCP at 254.5 nm was based on stability of rifampicin ZCP throughout the period of study (28

h). It was also observed that at this wavelength the derivative absorbance/amplitude value (D1) of isoniazid remains constant for the period of 28 h. So, the method could be used in the estimation of isoniazid without affected by the degradation. Selection of the concentration ranges (5-50 µg/ml) for isoniazid and its mixture with rifampicin was based on a well-planned mixture interaction studies (Figure 3.7). The study was conducted by using mixtures of a constant concentration of isoniazid and varying concentration of rifampicin as shown in the Figure 3.7. It was observed from the results that the noninterference zone for varying concentrations of rifampicin was found to be up to 70 µg/ml. Thus, the proposed concentration range of rifampicin alone and in combination with isoniazid was ideal and accurate as the estimation of isoniazid was possible at the selected concentration ratio. It was observed from the statistical analysis of the data obtained for the pure isoniazid analysis and its mixture with rifampicin that, the standard deviation associated with measurements, and percent relative standard deviation values were reasonably low (Table 3.7). This has proved the precision of the ZCP method and the negligible interference of rifampicin in the measurement of isoniazid.

The linear regression equations obtained for rifampicin estimation were Y = 0.005X + 0.0074 and Y = 0.005X + 0.0091 respectively for rifampicin alone and rifampicin in presence of isoniazid (Table 3.8). Similarly, the linear regression equations obtained for isoniazid estimation were Y = 0.0001X + (-) 0.003 and Y = 0.0001X + 0.0002 respectively for isoniazid alone and isoniazid in presence of rifampicin (Table 3.9). Linearity of the regression equation and negligible scatter of points were demonstrated from correlation coefficient (see Table 3.8 and 3.9 for the r values for rifampicin and isoniazid estimation respectively). The reported slope values without intercept on the ordinate, at 95% confidence limits, suggested that the calibration lines of rifampicin and isoniazid solutions in 6.8 pH SIFsp did not deviate from the origin as the above values were within the confidence limits. The precision of the fit was further confirmed with low standard error values of the intercept and slope. A one-way ANOVA test was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions for both drugs. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significant levels (Table 3.8 and 3.9)

Validation of the developed method

The developed method was validated according to the standard procedure (ICH Guidelines 1996, USP 2000b) and the results obtained are tabulated in Table (3.10). The linearity range for the estimation of rifampicin by zero order spectroscopy was found to be 2-50 µg/ml at a λ_{data} of 522.5 nm. The LOD was obtained as 0.62 µg/ml and LOQ was found to be 1.88 µg/ml. The linearity range for the estimation of isoniazid by first derivative spectroscopy was found to be (D1) 5-50 µg/ml at a λ_{data} of 254.5 nm. The LOD was obtained as 0.76 µg/ml and LOQ was found to be 2.31 µg/ml. In the validation table, the accuracy and precision are reported in terms of % RSD. The low values of these parameters reflected the excellent measurement accuracy and precision of the proposed method for estimation of rifampicin. The ruggedness in the estimation of rifampicin and isoniazid standard and test solutions in triplicate by different analyst and on different instruments were determined to be 99.63 ± 2.062% and 101.51 ± 1.543% respectively.

The regression equations for pure drug solutions and those of mixtures were similar (Table 3.8 and 3.9 for rifampicin and isoniazid respectively) including the obtained correlation coefficient values, which suggested the non-interference of one drug in estimation of other. The method of preparing the buffer solution was found to be simple and accurate and variation of pH of the buffer between 6.4-7.2 did not affect the sensitivity of the method. The %RSD for intra- and inter-day variation was less than 4.0%, which fall well below the acceptance criteria.

Recovery studies

The method was further validated by the simultaneous estimation of rifampicin and isoniazid in pharmaceutical formulations by the proposed methods of estimation and analysis of reference pure drug solutions. The results are presented in Table 3.11 and Table 3.12. The estimated drug content with low values of standard deviation further confirmed the precision of the proposed methods and therefore suggested the non-interference from the formulation matrix present in the studied formulations. The accuracy of the results of estimation was further tested by recovery experiments. The

average recovery varied from 98.86 ± 0.149 to 101.45 ± 1.740% for rifampicin and, 99.00 ± 1.075 to 100.88 ± 1.290% for isoniazid.

3.3. Conclusions

The validated spectrophotometric method (Rao and Murthy 2001) has been found suitable for the estimation of drug in bulk, from formulations and release study samples. Rifampicin was found stable for more than 36 h at 37 ± 1 °C in the used medium and thus suitable for the analysis of long term dissolution study samples of C.R. formulations. The recovery studies showed excellent recovery of the rifampicin from the formulations indicating the validity of the method and noninterference from the formulation excipients. The developed method for isoniazid has been found to be suitable for estimation of drug in bulk, from formulations and dissolution samples. Isoniazid found to be stable in the used medium for more than 36 h at 37 ± 1 °C. As the proposed method does not require any extraction or other complex processing steps, it is highly suitable for routine analysis. The method could be successfully used for estimation of drug in bulk, formulations and release study samples in presence of formulation excipients.

The developed UV spectrophotometric method for the simultaneous estimation of rifampicin (simple/zero order derivative method at 522.5 nm) and isoniazid (first order derivative spectroscopic method at 254.5 nm) in SIFsp found to be precise, accurate, rugged and reproducible. The method has been found to be suitable for the simultaneous estimation of rifampicin and isoniazid in bulk, from formulations and dissolution samples. As rifampicin found to interfere in the analysis of isoniazid in the entire range of absorption of isoniazid, first order derivative spectroscopic method has been used for isoniazid estimation. Further more, both drugs were found to be stable in the analysis media for more than 28 h at 37 ± 1 °C. The lack of any reported UV spectrophotometric method at present for the simultaneous estimation of rifampicin and isoniazid from in vitro release studies samples of C.R. formulations makes it possible to adopt the proposed method for the routine studies.

Table 3.1: Calibration curve points of the proposed methods of estimation of rifampicin and isoniazid.

Rifampicin estimation			Isoniazid estimation		
Conc. of solution (µg/ml)	Mean Absorbance values[a]	% RSD[b]	Conc. of solution (µg/ml)	Mean Absorbance values[a]	% RSD[b]
5.00	0.083 ± 0.001	1.25	2.50	0.090 ± 0.001	0.78
10.00	0.175 ± 0.002	0.93	10.00	0.316 ± 0.003	0.88
20.00	0.358 ± 0.004	1.10	15.00	0.476 ± 0.005	0.96
30.00	0.532 ± 0.003	0.60	20.00	0.626 ± 0.006	1.02
40.00	0.712 ± 0.004	0.56	25.00	0.776 ± 0.004	0.57
50.00	0.899 ± 0.008	0.94	30.00	0.937 ± 0.005	0.58
60.00	1.091 ± 0.008	0.71	40.00	1.230 ± 0.008	0.69

[a] Average of six determinations with standard deviation., [b] Percent relative standard deviation

Table 3.2. Results of least square regression analysis of the data for the proposed estimation methods.

Statistical Parameter	Rifampicin Estimation Method	Isoniazid Estimation Method
Regression equation[a]	Y = 0.0182.X + (-) 0.0093	Y = 0.0305.X + 0.0148
Correlation coefficient (r)	0.9999	0.9998
Slope (SE[b])	0.0182 (4.78×10^{-5})	0.0305 (6.20×10^{-5})
95 % confidence limits of slope	1.81 to 1.83 $\times 10^{-2}$	3.04 to 3.06 $\times 10^{-2}$
Intercept (SE[b])	0.0100 (1.72×10^{-3})	0.0148 (1.45×10^{-5})
95 % confidence limits of intercept	-1.34 to -6.69 $\times 10^{-2}$	1.20 to 1.77 $\times 10^{-2}$
Standard deviation of response ($S_{y.x}$)	1.02 $\times 10^{-2}$	8.13 $\times 10^{-3}$
Mean residual sum of the squares	4.24×10^{-5}	4.25×10^{-5}
Calculated *F*-value (Critical *F*-value)[c]	4.176 $\times 10^{-4}$ (2.477)	1.764 $\times 10^{-5}$ (2.477)

[a] Based on six calibration values., [b] Standard error of mean., [c] Theoretical value of F(5,36) based on one-way ANOVA test at P = 0.05 level of significance; Each value is result of six separate determinations. Y = Absorbance, X = Concentration of the drug in µg/ml.

Table 3.3. Validation parameters for the estimation of rifampicin and isoniazid by the proposed methods.

Analytical Parameter	Results	
	Rifampicin estimation method	Isoniazid estimation method
Accuracy (%)	101.06 ± 0.34	100.11 ± 0.32
Precision (%RSD)	0.68	0.54
Specificity	A 20 µg/ml solution of rifampicin in potassium phosphate buffer pH 7.4, at UV detection λ of 475 nm will show an absorbance value of 0.3482 ± 0.0039	A 20 µg/ml solution of isoniazid in potassium phosphate buffer pH 5.0, at UV detection λ of 262 nm will show an absorbance value of 0.6271 ± 0.0064
Linearity (µg/ml)	5 to 60	2.5 to 40
Limit of Detection (LOD) (µg/ml)	1.40	0.36
Limit of Quantification (LOQ) (µg/ml)	4.23	1.10
Ruggedness (mean % recovery ± SD)	100.50 ± 1.21	99.86 ± 1.42

Table 3.4. Results of assay of rifampicin and isoniazid from their commercial formulations by the proposed methods.

Rifampicin formulations		
Formulation	Assay (mg)[a]	Assay (%)[a]
R-cin - 450 mg	455.25 ± 6.56	101.17 ± 1.46
Macox - 300 mg	305.82 ± 2.62	101.94 ± 0.87
Isoniazid formulations		
Formulation	Assay (mg)[a]	Assay (%)[a]
Combunex - 300 mg	302.76 ± 3.70	100.92 ± 0.82
Myconex - 300 mg	298.19 ± 1.65	99.39 ± 0.55

[a] Mean of six triplicate determinations with standard deviation.

Table 3.5. Results of recovery studies from standard addition method (spiking studies).

Conc. of drug in formulations [a] (µg/ml)	Conc. of pure drug added (µg/ml)	Total con. of drug found [a] (µg/ml)	% Analytical recovery [a] (± SD)
Rifampicin estimation method			
20.24	5	25.23	99.89 ± 1.51
20.24	25	45.31	100.31 ± 0.66
20.24	40	60.65	101.05 ± 0.76
Isoniazid estimation method			
10.10	5	15.12	100.43 ± 2.27
10.10	15	24.87	99.08 ± 0.21
10.10	30	40.18	100.26 ± 0.90

[a] Mean of six determinations.

Table 3.6: Calibration curve points of the proposed method for the estimation of rifampicin by zero order derivative spectroscopic method at 522.5 nm.

Rifampicin Conc. (µg/ml)	Isoniazid Conc. (µg/ml)	Zero order Derivative Absorbance values[a]	% RSD[b]
2	0	0.0173 ± 0.0001	0.56
5	0	0.0321 ± 0.0007	2.14
10	0	0.0567 ± 0.0002	0.39
20	0	0.1073 ± 0.0006	0.60
30	0	0.1571 ± 0.0015	0.95
50	0	0.2554 ± 0.0023	0.92
2	20	0.0189 ± 0.0001	0.56
5	20	0.0337 ± 0.0005	1.44
10	20	0.0582 ± 0.0002	0.38
20	20	0.1090 ± 0.0006	0.59
30	20	0.1591 ± 0.0015	0.94
50	20	0.2562 ± 0.0024	0.92

[a] Average of six determinations with standard deviation., [b] Percent relative standard deviation

Table 3.7: Calibration curve points of the proposed method for the estimation of isoniazid by first order derivative spectroscopic method at 254.5 nm.

Isoniazid Conc. (µg/ml)	Rifampicin Conc. (µg/ml)	First order Derivative Absorbance values[a]	% RSD[b]
5	0	-0.000448 ± 0.00001	3.24
10	0	-0.001145 ± 0.00003	2.53
15	0	-0.001817 ± 0.00003	1.75
20	0	-0.002558 ± 0.00003	1.25
30	0	-0.003882 ± 0.00005	1.43
50	0	-0.006755 ± 0.00007	1.11
5	20	-0.000493 ± 0.00001	1.21
10	20	-0.001183 ± 0.00002	0.80
15	20	-0.001880 ± 0.00004	0.60
20	20	-0.002568 ± 0.00005	0.42
30	20	-0.003931 ± 0.00003	0.26
50	20	-0.006725 ± 0.00006	0.93

[a] Average of six determinations with standard deviation., [b] Percent relative standard deviation

Table 3.8. Results of least square regression analysis of the data for the proposed estimation method: Rifampicin Data.

Statistical Parameter	Rifampicin Alone	Rifampicin in presence of Isoniazid
Regression equation[a]	Y = 0.005X + 0.0074	Y = 0.005X + 0.0091
Correlation coefficient (r)	0.9999	0.9997
Slope (SE[b])	0.00496 (7.99×10^{-6})	0.00497 (7.93×10^{-6})
95 % confidence limits of slope	4.95 to 4.98 $\times 10^{-3}$	4.95 to 4.98 $\times 10^{-3}$
Intercept (SE[b])	0.00755 (2.25×10^{-4})	0.00933 (2.23×10^{-4})
95 % confidence limits of intercept	7.11 to 7.99 $\times 10^{-3}$	8.89 to 9.76 $\times 10^{-3}$
Standard deviation of response ($S_{y,x}$)	1.52×10^{-3}	1.51×10^{-3}
Mean residual sum of the squares	1.75×10^{-6}	1.74×10^{-6}
Calculated *F*-value (critical *F*-value)[c]	2.116×10^{-4} (2.477)	2.150×10^{-4} (2.477)

[a] Based on six calibration values., [b] Standard error of mean., [c] Theoretical value of F(5.36) based on one-way ANOVA test at P = 0.05 level of significance; Each value is result of six separate determinations. Y = Absorbance, X = Concentration of the drug in µg/ml.

Table 3.9. Results of least square regression analysis of the data for the proposed estimation method: Isoniazid Data.

Statistical Parameter	Isoniazid Alone	Isoniazid in presence of Rifampicin
Regression equation[a]	Y = 0.0001 X + (-) 0.003	Y = 0.0001X + 0.0002
Correlation coefficient (r)	0.9998	0.9999
Slope (SE[b])	1.40×10^{-4} (3.01×10^{-7})	1.38×10^{-4} (1.77×10^{-7})
95 % confidence limits of slope	1.39 to 1.41 $\times 10^{-4}$	1.38 to 1.39 $\times 10^{-4}$
Intercept (SE[b])	-2.71×10^{-4} (8.63×10^{-6})	-1.98×10^{-4} (5.08×10^{-6})
95 % confidence limits of intercept	-2.87 to -2.54 $\times 10^{-4}$	-2.08 to -1.88 $\times 10^{-4}$
Standard deviation of response ($S_{y,x}$)	5.14×10^{-5}	3.03×10^{-5}
Mean residual sum of the squares	2.61×10^{-9}	9.04×10^{-10}
Calculated *F*-value (critical *F*-value)[c]	2.596×10^{-4} (2.477)	1.268×10^{-4} (2.477)

[a] Based on six calibration values., [b] Standard error of mean., [c] Theoretical value of F(5,36) based on one-way ANOVA test at P = 0.05 level of significance; Each value is result of six separate determinations. Y = Absorbance, X = Concentration of the drug in µg/ml.

Table 3.10. Validation parameters for the estimation of rifampicin and isoniazid by the proposed method.

Analytical Parameter	Results	
	Rifampicin estimation	Isoniazid estimation
Accuracy (%)	100.25 ± 0.78	99.33 ± 0.89
Precision (%RSD)	0.61	0.91
Specificity	A 20 µg/ml solution of rifampicin in potassium phosphate buffer pH 6.8, at UV detection λ of 522.5 nm will show an absorbance value of 0.1048 ± 0.0006	A 20 µg/ml solution of isoniazid in potassium phosphate buffer pH 6.8, at UV detection λ of 254.5 nm will show an absorbance value (D1) of 0.6271 ± 0.0064
Linearity (µg/ml)	2 to 50	5 to 50
Limit of Detection (LOD) (µg/ml)	0.62	0.76
Limit of Quantification (LOQ) (µg/ml)	1.88	2.31
Ruggedness (mean % recovery ± SD)	99.63 ± 2.06	101.51 ± 1.54

Table 3.11. Results of assay of rifampicin and isoniazid from commercial combination formulations by the proposed method.

Rifampicin assay results		
Formulation[a]	Assay (mg)[b]	Assay (%)[b]
Cadirifa - 450 mg	448.88 ± 6.43	99.75 ± 1.43
R-cinex - 450 mg	454.23 ± 1.44	100.94 ± 0.48
Isoniazid assay results		
Formulation	Assay (mg)[a]	Assay (%)[a]
Cadirifa - 300 mg	303.99 ± 2.42	101.33 ± 0.54
R-cinex - 300 mg	298.91 ± 1.01	99.63 ± 0.34

Table 3.12. Results of recovery studies from standard addition method (spiking studies).

Conc. of drug in formulations [a] (µg/ml)	Conc. of pure drug added (µg/ml)	Total con. of drug found [a] (µg/ml)	% Analytical recovery [a] (± SD)
Rifampicin estimation method			
10.08	5	15.16	101.45 ± 1.74
10.08	20	30.27	100.95 ± 1.13
10.08	40	49.51	98.86 ± 0.15
Isoniazid estimation method			
10.14	5	14.99	99.00 ± 1.07
10.14	20	30.07	99.62 ± 0.82
10.14	40	50.58	100.88 ± 1.29

[a] Mean of six determinations.

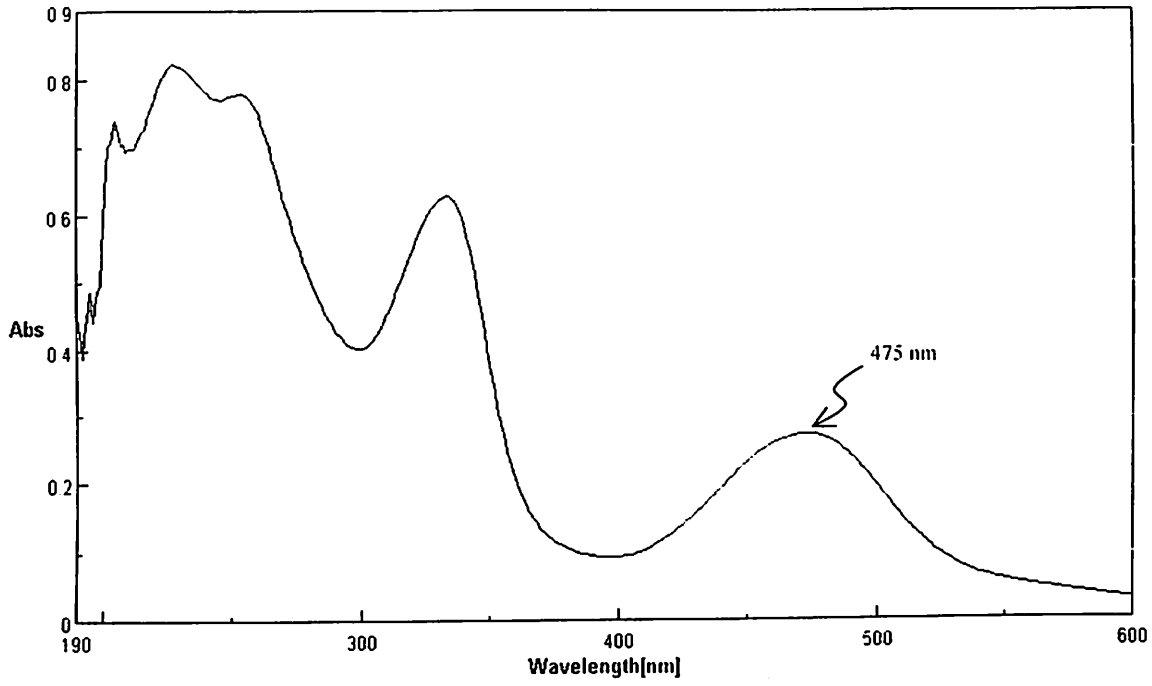


Figure 3.1. Absorption spectra of rifampicin in pH 7.4 phosphate buffer with 0.02% w/v of ascorbic acid.

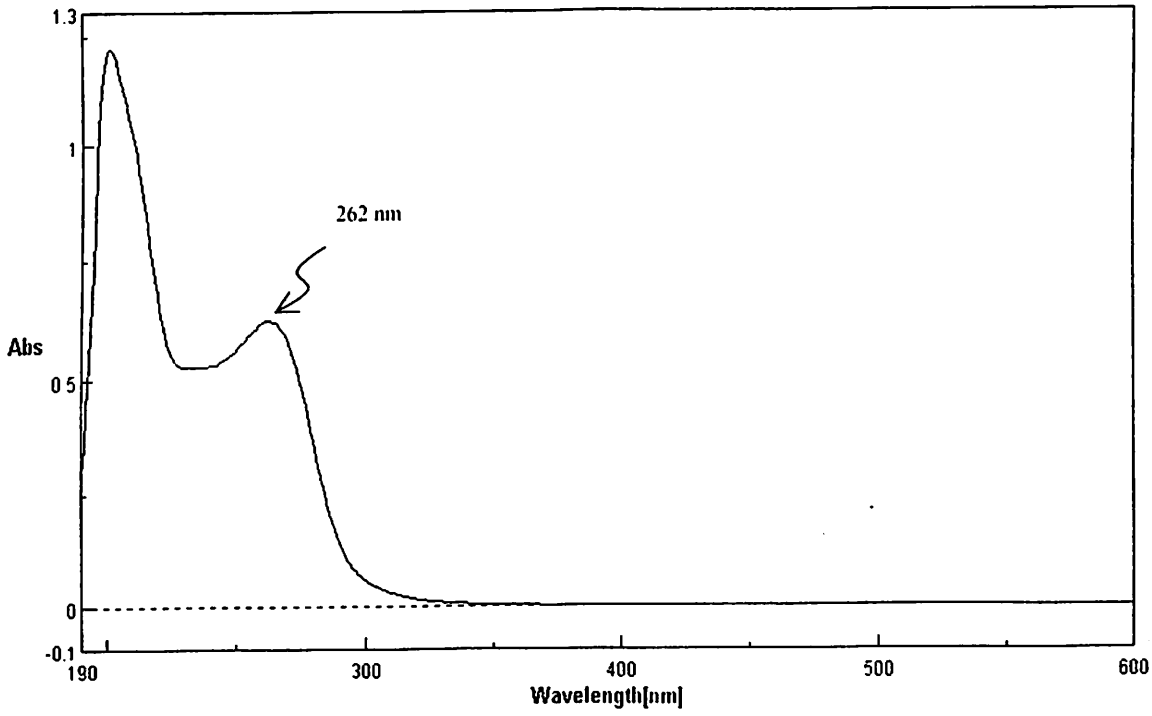


Figure 3.2. Absorption spectra of isoniazid in pH 5.0 phosphate buffer.

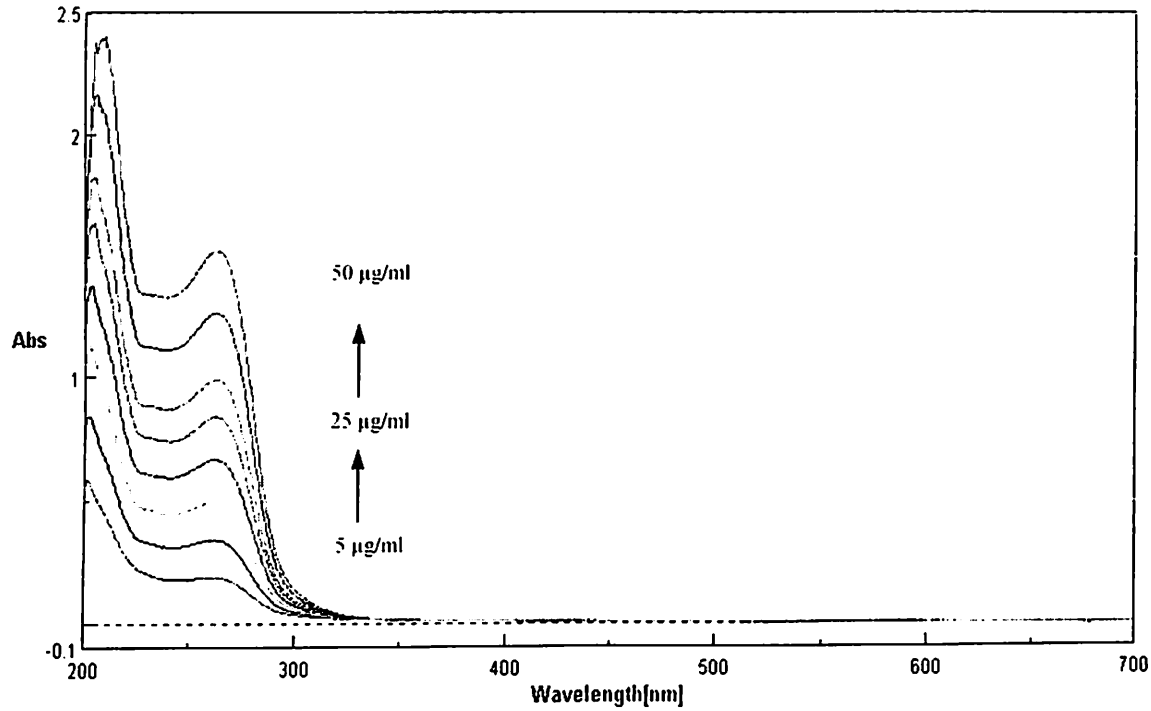


Figure 3.3. Absorption spectra of different concentrations (5, 10, 15, 20, 25, 30, 40 and 50 µg/ml) of isoniazid in SIFsp pH 6.8.

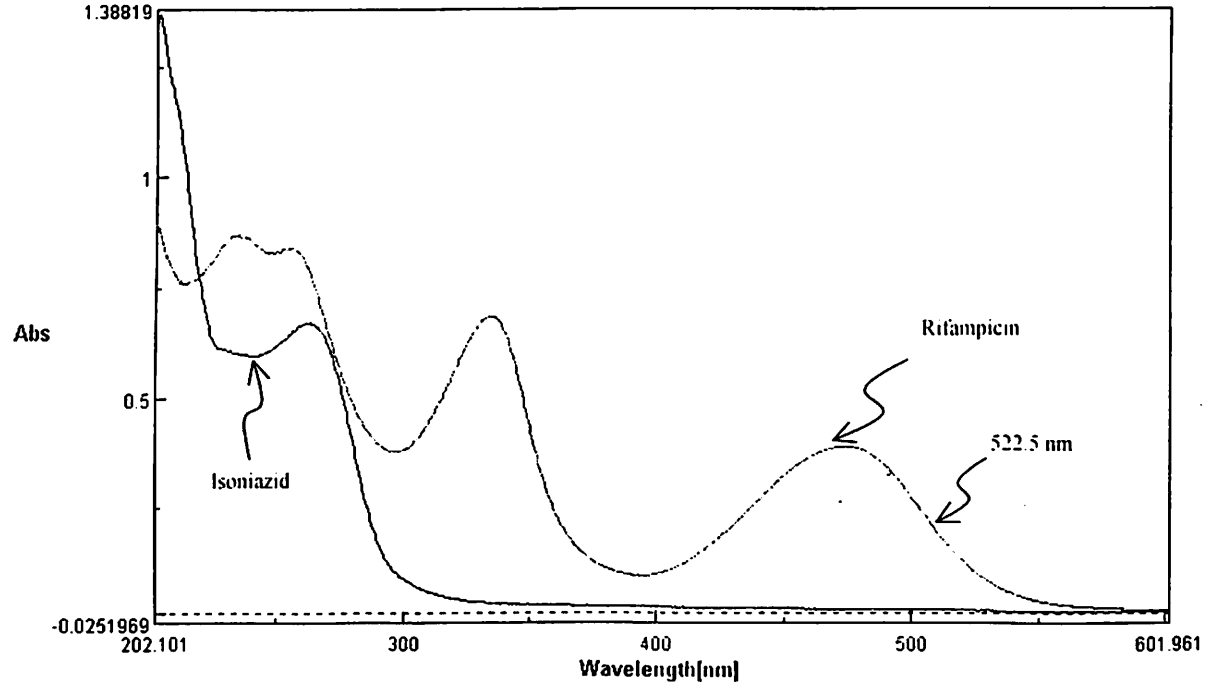


Figure 3.4. Zero order absorption spectra of isoniazid in presence of rifampicin in SIFsp pH 6.8.

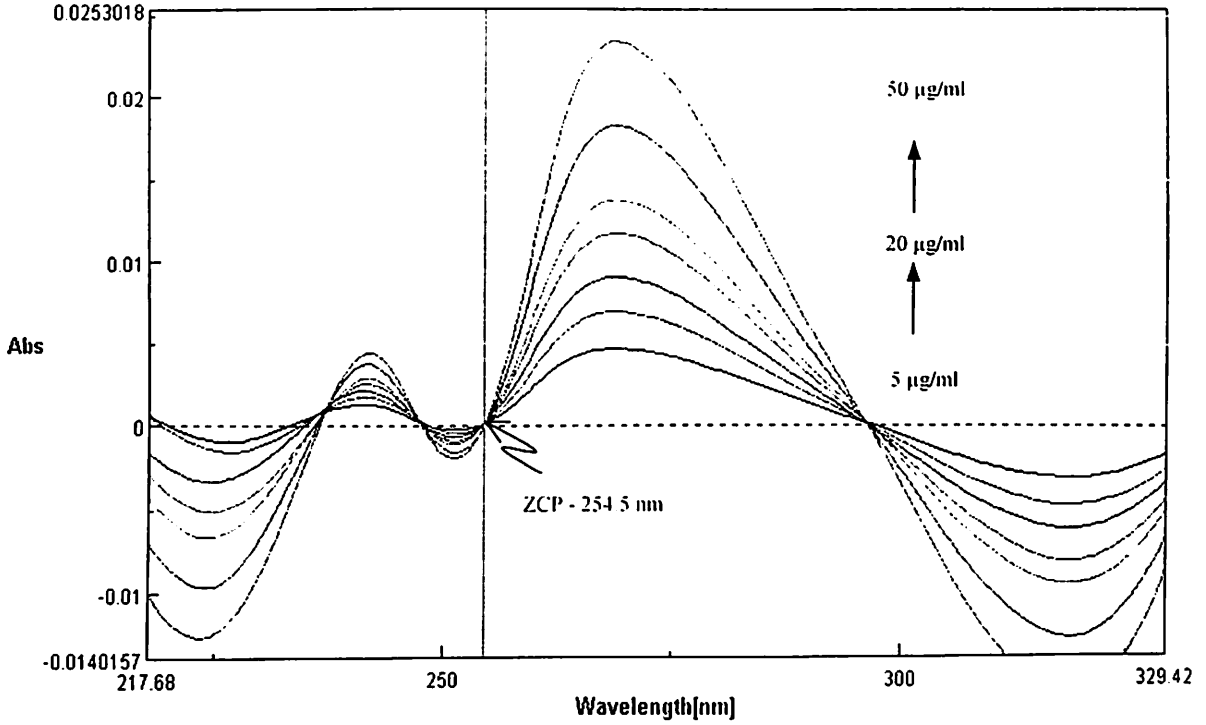


Figure 3.5. First derivative absorption spectra of different concentrations (5, 10, 15, 20, 30, 40 and 50 µg/ml) of rifampicin in SIFsp pH 6.8 in the absence of isoniazid. Zero crossing point (ZCP) at 254.5 nm.

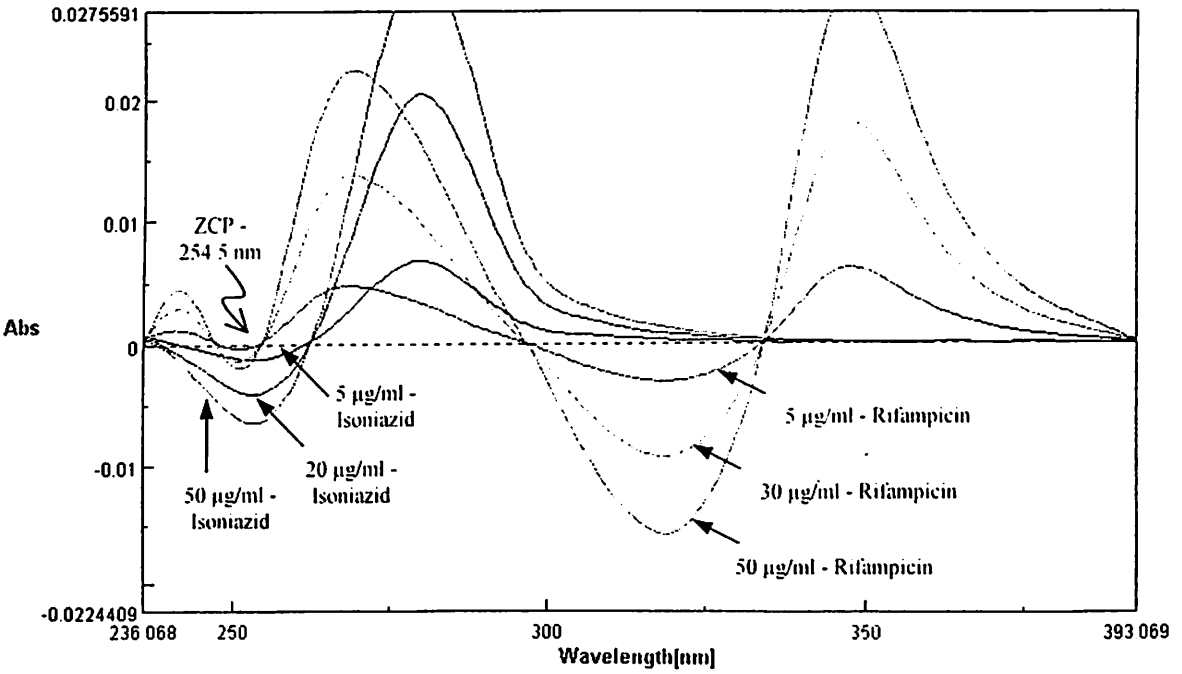


Figure 3.6. First derivative absorption spectra of different concentrations of isoniazid in SIFsp pH 6.8 in presence of different concentrations of rifampicin. Isoniazid estimation at 254.5 nm (ZCP of rifampicin).

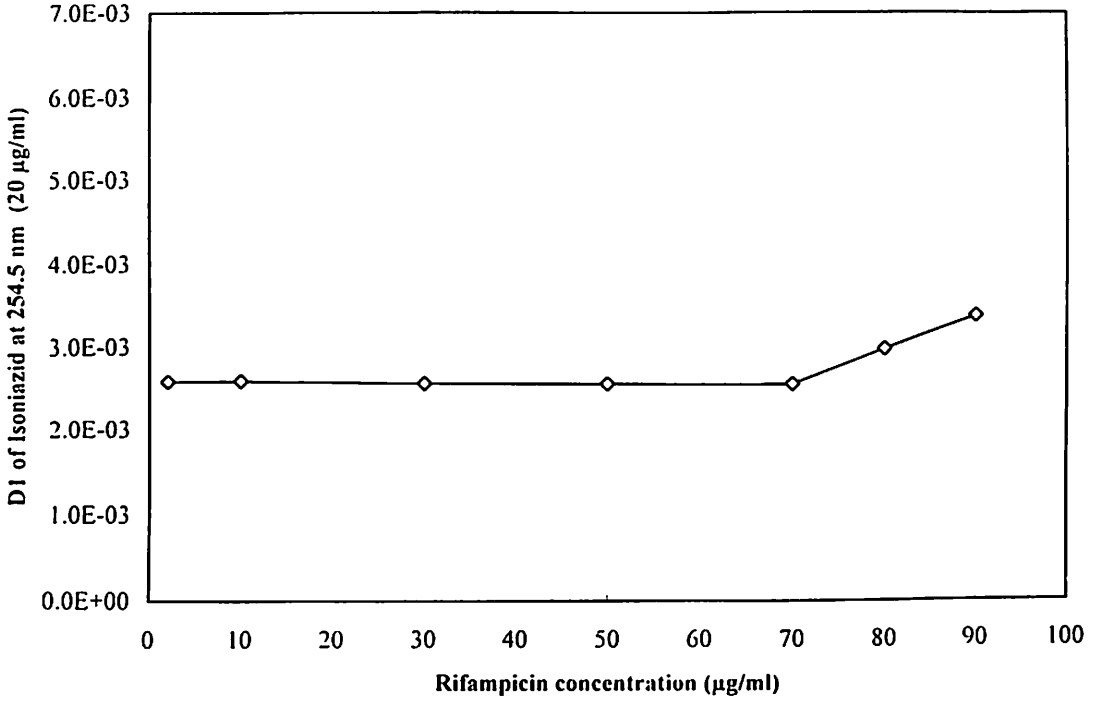


Figure 3.7. First derivative interaction graph for isoniazid (20 µg/ml) in presence of rifampicin in SIFsp pH 6.8.

Chapter 4

Preformulation Studies

4. 1. Introduction

Preformulation studies are most important part of any drug or dosage form development process. These studies focus on those physicochemical properties of the drugs that could affect drug's performance, stability and development of a suitable dosage form. To develop an effective and stable drug delivery system, it will be essential to carry out various preformulation studies including solubility, stability studies in solid state (in presence and absence of formulation additives) and in solution form under the various storage conditions (in presence of light, temperature, oxygen and humidity), etc.

Rifampicin and isoniazid are well established and widely reported drugs with several formulations available in the market. In the present investigation, as combined controlled release formulations are intended to design, main focus was on the solid state stability studies of rifampicin and isoniazid in presence and absence of various additives/polymers at various storage conditions (ICH 1996). Present investigations also focused on the stability evaluation of the two drugs in various buffered and unbuffered solutions. Solubility studies of rifampicin were carried out in various buffered and unbuffered solutions (from pH 1.2 to 7.4). As isoniazid was reported to be a Class-I drug as per biopharmaceutical classification system (BCS), detailed solubility studies were not carried out. But, for the purpose of our project, isoniazid solubility studies were done at pH 1.2, 5.0 and 7.4 buffer medias. Dissolution methodologies were either selected or developed for both rifampicin and isoniazid formulations.

4.2. Experimental

Materials

Rifampicin and isoniazid were obtained from the same source as mentioned in chapter 3. HPMC (15 cPs) and EC were obtained as gift samples from IPCA Labs Mumbai. HPMC K100LV (100 cPs), K15M (15000 cPs), Carbopol 934P and Eudragit L100-55 were received as gift samples from Zydus Cadila Research Center, Ahmedabad. HPMC K4M (4000 cPs) was purchased from Sigma Aldrich, Germany. Hydroxypropyl cellulose (HPC) (Klucel LF) was purchased from Sigma Aldrich, Bangalore. All other chemicals and reagents used were of pharmaceutical or analytical grade and were used as received.

Instruments/Equipments

A UV-visible-NIR spectrophotometer (Jasco UV-visible spectrophotometer; model – V 570, Tokyo, Japan) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells with Jasco spectra manager software was used for all absorbance measurements for UV analysis. Jasco Infrared spectrophotometer, model-TR Report 100, was used for obtaining the IR spectrum of the drugs. Stability studies were carried out in stability chamber with temperature and humidity control (MAC model, New Delhi, India). For studies at higher temperature condition (60 °C) MAC model thermostatic oven was used. Frost-free-200-litre Godrej refrigerator was used for the studies at refrigerated condition. These instruments were equipped with thermostatic temperature control unit, digital temperature recorder and relative humidity recorder (wherever applicable). All pH measurements were performed using Elico pH meter equipped with combination glass electrode filled with potassium chloride gel and auto temperature adjustments electrode.

Analytical method

In all analysis an aliquot volume of the sample, collected at different time interval, was transferred to a series of 10 ml volumetric flasks (three in each case). The final volume was made using phosphate buffer (pH 7.4) with 0.02% w/v of ascorbic acid in case of rifampicin and phosphate buffer (pH 5.0) in case of isoniazid. The resulting solutions were filtered through Whatman filter paper no. 1 and suitably diluted to get final concentration within the limits of linearity of the proposed method of estimation for individual drug.

4.2.1. Characterization of bulk drugs

The bulk drugs were characterized by various official tests of identification as per Indian Pharmacopoeia (IP), 1996 and British Pharmacopoeia (BP), 1998. Assay/percent purity analysis was carried out according to IP 1996 by the methods mentioned in the chapter 3. The IR spectra obtained were compared with that of the standard for both drugs.

4.2.2. Stability studies in solid admixture

Physical mixture of rifampicin or isoniazid (#100 mesh passed) and different formulation excipients (except talc and magnesium stearate) (#100 mesh passed) was prepared in the ratio 1:5. The excipients that were used for the study include: HPMC 15 cPs, HPMC K100LV, HPMC K4M, HPMC K15M, HPMC K100M, EC, Carbopol 934P, HPC, and Eudragit L100-55. Talc and magnesium stearate were taken in the ratio of 1:1 with respect to the drug. Drug and excipients were thoroughly blended and the mixture was passed through #100 mesh to ensure uniform blending. Drug alone (used as control) or in combination with excipients were filled and sealed in 2 ml amber colored, neutral glass ampoules and kept at different temperature conditions. For studies at 40 °C and 75% relative humidity (RH) the mixture were kept in open vials.

The prepared samples were stored at ambient as well as accelerated storage conditions. The storage conditions used for the studies were controlled room temperature (CRT: 25 ± 2 °C and 60 ± 5% RH), 40 ± 2 °C, 60 ± 2 °C, and 40 ± 2 °C/75 ± 5% RH and refrigerated condition (FT: 5 ± 2 °C). The samples in triplicate were withdrawn at predetermined time intervals (0, 30, 60, 90 days) and analyzed after suitable dilution for drug content. The observed degradation rate constants and $t_{90\%}$ (time for 90% of the drug to remain) values at different storage conditions are listed in Table 4.1 for rifampicin and in Table 4.2 for isoniazid. In an effort to establish the effect of humidity on the stability of drug in presence of excipients, the degradation constant (K_{deg}) obtained at 40 °C were compared with that obtained at 40 °C / 75% RH and the results are presented in Table 4.1 for rifampicin and in Table 4.2 for isoniazid.

4.2.3. Stability studies in solution form

Stability of rifampicin or isoniazid at various unbuffered pH solutions was studied at a concentration of 20 µg/ml. Rifampicin or isoniazid was dissolved in various unbuffered pH solutions ranging from 1.2, 2.0, 5.0, 6.0, 6.8 and 7.4 to get the concentration of 20 µg/ml. The pH was adjusted by adding varying proportion 0.1 N NaOH and 0.1 N HCl in distilled water. To study the stability of rifampicin in various buffered pH solutions, 20 µg/ml solution of the drug was prepared in pH 1.2 (SGF) (to the 7 ml of concentrated

HCl, 2 g of NaCl was added, volume made up to 1000 ml), 2.0 (Hydrochloric acid buffer: to the 50 ml of 0.2 M potassium chloride 13 ml of 0.2 M HCl was added, volume made up to 200 ml), and in phosphate buffers pH 5.0 (dissolve 6.8 g of KH_2PO_4 in 1000 ml water and adjust the pH to 5.0 with 10 M KOH solution), 6.0 (to the 50 ml of 0.2 M KH_2PO_4, 5.6 ml of 0.2 M NaOH was added, volume made up to 200 ml), 6.8 (to the 50 ml of 0.2 M KH_2PO_4, 22.4 ml of 0.2 M NaOH was added, volume made up to 200 ml) and 7.4 buffers (to the 50 ml of 0.2 M KH_2PO_4, 39.1 ml of 0.2 M NaOH was added, volume made up to 200 ml).

The prepared samples were stored at 37 ± 2 °C in 100 ml of volumetric flasks on a water bath shaker. The samples in triplicate were withdrawn at predetermined time intervals (0, 1, 2, 4, 6, 8, 12, 18, and 24 h) and analyzed after suitable dilution for rifampicin or isoniazid content. The concentration of rifampicin remaining (µg/ml) in solution at different time periods and the observed first order degradation rate constants [$K_{deg} \times 10^2$ (h^{-1})] are listed in Table 4.3 and Table 4.4 for unbuffered and buffered solutions. Similarly, the concentration of isoniazid remaining (µg/ml) in solution at different time intervals and the observed first order degradation rate constants [$K_{deg} \times 10^3$ (h^{-1})] are listed in Table 4.5 and Table 4.6 for unbuffered and buffered solutions.

4.2.4. Solubility studies

Solubility studies for rifampicin (mg/ml) were done by using the saturation shake-flask method over the pH range of 1.2 - 7.4 at 37 ± 1 °C. Selection of the pH conditions were in relevance to the in vivo physiological pH conditions (USFDA 2000). These solubility experiments were typically conducted over 24 h in different unbuffered solutions at pH values of 1.2, 2.0, 5.0, 6.0, 6.8, and 7.4. The pH was adjusted using varying proportion 0.1N NaOH and 0.1N HCl and the ionic strength were adjusted to 0.2% using sodium chloride. Solubility studies of rifampicin were also carried out in different pH buffer solutions in pH 1.2 (SGF), 2.0 (HCl buffer), and in phosphate buffers pH 5.0, 6.0, 6.8 and 7.4 with ionic strength adjusted to 0.2 using sodium chloride. In all cases, samples were withdrawn in triplicate and analyzed. The solubility values for rifampicin (µg/ml) in different media are presented in Table 4.7.

Extensive solubility studies for isoniazid were not carried out as isoniazid reported to be a Class-I drug according to biopharmaceutical classification system (BCS) (Lindenberg et al. 2004). Drugs, which belong to BCS Class-I category, are reported to have higher solubility throughout the entire gastrointestinal pH conditions (pH 1-7.5) (USFDA 2000). However, for the purpose of this project, solubility studies for isoniazid were carried out only at three pH buffers, in SGF (pH 1.2), pH 5.0 phosphate buffer and in pH 7.4 phosphate buffer with ionic strength adjusted to 0.2 using sodium chloride. The samples were withdrawn in triplicate and analyzed. The solubility values for isoniazid (μg/ml) in different media are reported in Table 4.8.

4.2.5. Selection and/or Development of Dissolution Methodology for In vitro Release Studies of C.R. Tablets

One of the most important aspects of the development of a drug product is to find a suitable in vitro characterization method for potential formulations that reflects their in vivo performance (Dressman et al. 1998). Dissolution testing is required to be done to demonstrate the performance of all solid oral dosage forms in which absorption of the drug is necessary for the product to exert a therapeutic effect. The challenge for scientists working in a research and development environment is to develop a procedure that can not only guide the formulation development process but can also be used as a regulatory test to detect manufacturing deviations and to ensure product quality consistency over the product's shelf life (Lagace et al. 2004). Of late, in vitro dissolution testing has become one of the important requirements for the development, registration and quality control aspects of solid oral controlled release dosage forms. Apart from providing very useful information regarding the in vitro drug release, they are also useful for the purposes of drug release modeling, performance evaluation of formulations and manufacturing processes through in vitro-in vivo correlations and, for monitoring drug product stability and durability as a function of time (Sood and Panchagnula 1999).

The following critical parameters were considered in the selection/development of dissolution methodology for the release studies of particular set of controlled release formulations;

- Solubility of the drug/s in the selected media

- Better in vitro in vivo correlations
- The method should be sensitive enough to detect any small changes in the formulation composition and process of manufacturing
- Analytical stability of the drug/s in the selected media for a period of study

4.3. Results and Discussions

4.3.1. Characterization of bulk drugs

The supplied drugs, rifampicin and isoniazid passed the various tests of identification and analysis as per the IP 1996 and BP 1998. The IR spectra of both the drugs found comparable with respect to their individual standard. The formulation additives and chemical or reagents, in the concentrations used, did not affect the stability and ultraviolet absorbance of the drugs at their selected wavelengths of estimation (475 nm for rifampicin and 262 nm for isoniazid).

4.3.2. Stability studies in solid admixture

The first order degradation rate constants (K_{deg}) for rifampicin alone and in presence of the different excipients, are reported in Table 4.1. The K_{deg} values of rifampicin were found to be 1.02×10^{-3} $month^{-1}$, 2.43×10^{-3} $month^{-1}$, 5.44×10^{-3} $month^{-1}$, 7.90×10^{-3} $month^{-1}$ and 10.35×10^{-3} $month^{-1}$ respectively at FT, CRT, 40 ± 2 °C, 60 ± 2 °C and 40 ± 2 °C/75 ± 5% RH conditions. The $t_{90\%}$ values in the absence of any of the excipient was found to be 103.3, 43.4, 19.4, 13.3 and 10.20 months respectively at FT, CRT, 40 ± 2 °C, 60 ± 2 °C and 40 ± 2 °C/75 ± 5% RH conditions. The K_{deg} values for rifampicin in combination with various excipients ranged from 1.14×10^{-3} $month^{-1}$ to 2.11×10^{-3} at FT, 3.33×10^{-3} $month^{-1}$ to 6.17×10^{-3} at CRT, 5.81×10^{-3} $month^{-1}$ to 11.48×10^{-3} at 40 ± 2 °C, 7.38×10^{-3} $month^{-1}$ to 13.36×10^{-3} at 60 ± 2 °C and 8.40×10^{-3} $month^{-1}$ to 19.38×10^{-3} at 40 ± 2 °C/75 ± 5% RH. The $t_{90\%}$ values for rifampicin in combination with various excipients ranged from 49.9 to 92.4 months at FT, 17.1 to 31.6 months at CRT, 9.2 to 18.1 months at 40 ± 2 °C, 7.9 to 14.3 months at 60 ± 2 °C and 5.4 to 12.5 months at 40 ± 2 °C/75 ± 5% RH.

None of the excipients found to have severe deleterious effect on the rifampicin. Among the HPMC polymers, 15 cPs and K4M found to have least effects on the stability of rifampicin at CRT. EC and HPMC 15 cPs were having least deleterious effect on rifampicin stability among the polymers studied. The rifampicin was found to be most stable in presence of talc and magnesium stearate widely used for manufacturing the tablets.

The K_{deg} values for isoniazid alone and in presence of the different excipients are presented in Table 4.2. The K_{deg} for isoniazid alone was found to be 0.88×10^{-3} $month^{-1}$, 2.89×10^{-3} $month^{-1}$, 4.29×10^{-3} $month^{-1}$, 5.73×10^{-3} $month^{-1}$and 8.86×10^{-3} $month^{-1}$ respectively at FT, CRT, 40 ± 2 °C, 60 ± 2 °C and 40 ± 2 °C/75 ± 5% RH conditions. The $t_{90\%}$ values of isoniazid were found to be 119.7, 36.5, 24.6, 18.4 and 11.9 months respectively at FT, CRT, 40 ± 2 °C, 60 ± 2 °C and 40 ± 2 °C/75 ± 5% RH conditions. The K_{deg} values for isoniazid in combination with various excipients ranged from 1.03×10^{-3} $month^{-1}$ to 2.11×10^{-3} at FT, 3.51×10^{-3} $month^{-1}$ to 6.73×10^{-3} at CRT, 5.23×10^{-3} $month^{-1}$ to 10.11×10^{-3} at 40 ± 2 °C, 5.87×10^{-3} $month^{-1}$ to 10.23×10^{-3} at 60 ± 2 °C and 7.42×10^{-3} $month^{-1}$ to 12.24×10^{-3} at 40 ± 2 °C/75 ± 5% RH. The $t_{90\%}$ values for isoniazid in presence of various excipients ranged from 49.9 to 102.3 months at FT, 15.7 to 30.0 months at CRT, 10.4 to 20.1 months at 40 ± 2 °C, 10.3 to 18.0 months at 60 ± 2 °C and 8.6 to 14.2 months at 40 ± 2 °C/75 ± 5% RH.

None of the excipients found to have severe deleterious effect on the stability of the isoniazid. Among the HPMC polymers, K4M and K100LV found to have least effect on the stability of isoniazid at CRT. EC and HPMC K4M have been found to have least effect on isoniazid stability among the polymers studied. Similarly talc and magnesium stearate also found to have least effect on the isoniazid stability among all the excipients studied.

Increase in the temperature increased the degradation process and rate constant as expected. The K_{deg} values were highest at 60 °C and lowest at FT for any drug with or without any excipient. The K_{deg} values were much higher and $t_{90\%}$ values were much lower at 40 ± 2 °C/75 ± 5% RH compared to at 40 ± 2 °C in all the cases studied indicating effect of humidity on the stability of the drug. Further, K_{deg} values were found to be higher at 40 ± 2 °C/75 ± 5% RH compared to even at 60 ± 2 °C. Thus, it can be said

that humidity was the one of the important parameter that affect the stability of both rifampicin and isoniazid. Increased K_{deg} values and decrease in $t_{90\%}$ values in presence of different hydrophilic polymers might be due to their nature to absorb moisture causing increased degradation of the drugs. EC being a hydrophobic polymer shown comparatively less deleterious effect on the stability of both rifampicin and isoniazid. The hydrophobic excipients talc and magnesium stearate also shown least deleterious effect compared to other excipients.

4.3.3. Stability studies in solution form

Rifampicin and isoniazid found to follow first order degradation in all pH of both buffered and unbuffered solutions. The results are presented in Table 4.3 and 4.4 for rifampicin in unbuffered and buffered solutions. Similarly, Table 4.5 and 4.6 present the degradation study results for isoniazid in different unbuffered and buffered solutions. The linear profile of degradation plots (Figure. 4.1 and 4.2 for rifampicin and Figure. 4.3 and 4.4 for isoniazid) confirmed the first order degradation process for both rifampicin and isoniazid in aqueous solution.

In case of rifampicin, the degradation was significantly dependent upon the pH of the media. As can be seen from the Table 4.3 (unbuffered solutions), the degradation rate constant (K_{deg}) was maximum at pH 1.2 and decreased with increase in the pH. However, K_{deg} at pH 7.4 is slightly higher than at pH 6.8 suggesting both alkaline and acidic pH causing degradation. Whereas, in case of buffered media (Table 4.4) the profiles were found to be little different. The K_{deg} values were high at acidic and alkaline pH with maximum stability at pH 5.0. The K_{deg} values were found to be 5.02×10^{-2} h^{-1} at pH 1.2, 0.87×10^{-2} h^{-1} at pH 5.0 and 1.91×10^{-2} h^{-1} at pH 7.4. Degradation rate constant observed to be higher in buffered media than in unbuffered at highly acidic pH conditions but reverse was true at higher pH. The degradation of rifampicin at acidic pH is suggested to be due to acid hydrolysis but in alkaline pH media it was due to oxidative degradation (Shishoo et al. 1999). The degradation of rifampicin at low pH values was more than that observed at high pH values.

The degradation of isoniazid found to be slower compared to rifampicin at all pH conditions. For isoniazid also, the degradation was dependent upon the pH of the media.

As can be seen from the Table 4.5 (unbuffered solutions), the K_{deg} value was lowest at pH 1.2 (0.23×10^{-3} h^{-1}) and maximum at pH 2.0 (5.07×10^{-3} h^{-1}) with further decrease with increase of pH. However, in case of buffered media (Table 4.6) the profiles were slightly different. Here also the K_{deg} value was least at pH 1.2 (0.69×10^{-3} h^{-1}) and maximum at pH 2.0 (4.38×10^{-3} h^{-1}). Also in buffered media the K_{deg} values found to decrease above pH 2.0. Degradation rate constant found to be higher in buffered media than in unbuffered except at pH 5.0 and 6.8. Thus, it was observed that the stability was maximum at pH 1.2 and least at pH 2.0, which was in accordance with the previous reports (Devani et al. 1985, Sankar et al. 2003).

4.3.4. Solubility studies

Rifampicin, a zwitterionic molecule with two pKa values (1.7 and 7.9), shows a highly pH-dependent solubility profile, especially in the pH range that exists across the GIT (pH 1.2 to 7.4). It was observed form the solubility studies that rifampicin was more soluble at acidic pH conditions and the solubility decreased as pH increased from 1.2 to 6.0 in unbuffered solutions. After pH 6.0, increase in the pH resulted in the increase in the solubility (Table 4.7). The graphical presentation of the effect of pH on rifampicin solubility in different unbuffered solutions has been shown in the Figure 4.5. The solubility was higher at pH 1.2 (91.12 mg/ml) and lower at pH 6.0 (1.27 mg/ml). Small increment in pH from 1.2 to 2.0 resulted in the significant reduction in the solubility of the drug (91.12 to 16.89 mg/ml). At pH 1.2 the proportion of the ionized form (protonated species) was much higher than that at pH 2.0 (according to Henderssons Hasselbach equation). Similarly, the difference in the solubility values at pH 2.0 and 5.0 was higher due to the same concept of ionization. There was no significant difference in the solubility at pH 5.0, 6.0 and 6.8. Further, there was slight increase in the solubility as the pH increased from 6.8 to 7.4.

Similar trend was observed even in case of buffered pH solutions. In buffered solutions also the solubility was higher at pH 1.2 (102.46 mg/ml), but lower solubility was observed at pH 5.0 (1.23 mg/ml) (Table 4.7). There was no significant difference in the solubility at pH 5.0 and 6.0 (1.36 mg/ml). However, as the pH increased from 6.0 to 6.8 and 7.4 the solubility increase was found to be significant (2.59 and 3.18 respectively at

pH 6.8 and 7.4). The graphical presentation of the effect of pH on rifampicin solubility in different buffered solutions has been shown in the Figure 4.6. The solubility values were higher in buffered solutions than in unbuffered solutions (except at pH 5.0). This might be due to the lesser ionization capacity of the sodium hydroxide and hydrochloride ions (used for adjusting the pH in case of unbuffered solutions) compared to the potassium dihydrogen phosphate ions (used in the preparation of phosphate buffer of varying pH).

Isoniazid solubility studies are represented in Table 4.8. The limited numbers of studies, for the purpose of this project, were carried out in three different pH buffer solutions. The buffer solutions selected were SGF (pH 1.2) and phosphate buffers pH 5.0 and 7.4 (selected on the basis of physiological pH conditions). In all three media the solubility was higher, however, it was observed that there was very slight decrease in the solubility as the pH was increased. The solubility found to be 326 ± 3.96 mg/ml (in SGF), 281 ± 3.52 mg/ml (at pH 5.0) and 274 ± 4.79 mg/ml (at pH 7.4).

4.3.5. Dissolution methodology

4.3.5.1. Dissolution method for rifampicin

The USP 24 suggested 0.1 N HCl as a dissolution medium for conventional rifampicin capsules using UV visible spectrophotometric method of analysis by comparison of the sample with same of reference of reference drug. This method was found to be not suitable for analysis of samples from controlled release formulations as drug is not stable for long time in 0.1 N HCl and method of analysis is tedious for routine multiple samples. This method also seems to be cumbersome, tedious and particularly unsuitable for the in vitro release studies of C.R. formulations of rifampicin. To overcome this problem a dissolution medium of pH 7.4 phosphate buffer containing 0.02% w/v of ascorbic acid has been suggested by Rao and Murthy for the in vitro evaluation of C.R. formulations of rifampicin (Rao and Murthy 2001). It has also been reported that this dissolution medium found to give better in vitro in vivo correlations for the C.R. formulations of rifampicin (Rao et al. 2001b). In one more report it has been found that the USP rotating basket method appears to correlate best with maximum urinary excretion rate, area under excretion rate versus time curve as well as total amount of drug excreted. So, the

determination of dissolution rate of rifampicin products by the USP rotating basket method could be considered as a reliable tool for predicting the in vivo performance of the drug (Ammar and Khalil 1996). Thus, it was decided to use pH 7.4 phosphate buffer containing 0.02% w/v of ascorbic acid as a dissolution medium for rifampicin C.R. formulations. Preliminary studies have been carried out to further assess the suitability of the reported method in our laboratory conditions. Stability of the rifampicin in the dissolution medium was evaluated for a period of 36 h (with the developed analytical method). Dissolution studies of some marketed immediate release formulations of rifampicin have been carried out to assess the suitability of the method as per official pharmacopoeial limits (IP 1996 and USP 24). After the preliminary studies, a dissolution volume of 900 ml was used at a temperature of 37 ± 0.5 °C. The basket rotation was kept at 100 ± 5 rpm.

4.3.5.2. Dissolution method for isoniazid

It was already discussed that isoniazid is a Class-I drug according to BCS (Lindenberg 2004). Drugs belonging to BCS Class-I category are reported to have dose solubility ratio (D:S) < 250 ml (USFDA 2000). Also it has been reported that these drugs, when formulated in to orally administered immediate-release products, release > 85% of the labeled amount within 30 min. In such conditions, a simple one-point dissolution test might be well enough to assure the bioavailability of the drug (Amidon et al 1995).

Thus, we have decided to use pH 7.4 phosphate buffer as a dissolution medium for the in vitro release studies of isoniazid controlled release formulations. The decision for the dissolution medium selection was based upon the preliminary studies in terms of analytical stability of the drug through out the dissolution study, better discriminating ability for small changes in the formulation or process variables and, finally from the dissolution study of some marketed isoniazid formulations as per official pharmacopoeial limits (IP 1996 and USP 24). The developed method was also compared with the official methods of dissolution for the suitability and acceptability. A dissolution volume of 900 ml was used at a temperature of 37 ± 0.5 °C. The basket rotation was kept at 100 ± 5 rpm.

Further to study the effect of dissolution media on isoniazid release from C.R. formulations, it was decided to carry out the in vitro release studies of some selected formulations in 0.1 N HCl (pH 1.2).

4.3.5.3. Development of dissolution method for rifampicin and isoniazid combination

The solubility of the active ingredient(s) is one of the key aspects in the screening of possible dissolution media. In selecting a medium for dissolution testing of two drugs, the solubility characteristics of the individual active ingredients must be considered. Isoniazid is freely soluble in water (>200 mg/ml solubility) at wide range of pH values (pH 1.2 to 7.4). Rifampicin is a poorly soluble drug and its solubility is maximum (102.46 mg/ml) in 0.1N HCl (SGF) due to the formation of the protonated base and decreases as pH increased. The choice of dissolution medium to accommodate both active compounds is limited. According to the USP, dissolution medium may be water, a buffered aqueous solution (typically pH 4 to 8) or a dilute acid (0.001 to 0.1N HCl). Surfactants and electrolytes may also be added to aid in the solubilization of the active ingredients (USFDA 1997).

Formulations containing more than one component can interact in different ways, thereby influencing the dissolution behavior. It is a well known fact that rifampicin interacts with the isoniazid in the solution (in acidic media). The dissolution medium specified in USP 24 for rifampicin and isoniazid capsules has been reported to be unsuitable, as it has been observed that rifampicin degrades in this medium from 17% to 22% in presence of isoniazid as found by different research groups (Gharbo et al 1989, Shishoo et al 1999). A dissolution test method has been suggested for rifampicin and isoniazid fixed dose formulations (Jindal et al. 1994) consisting of 0.4% w/v of sodium lauryl sulphate (SLS) in water.

It was initially decided to use this reported media (0.4% w/v of SLS in water) for the in vitro release studies of combination C.R. tablets of rifampicin and isoniazid. But the method found to be unsuitable for the purpose of our project as the drugs found to degrade after 3 h in the dissolution media. Hence, it was decided to develop a dissolution methodology for the combined C.R. formulations of rifampicin and isoniazid.

The dissolution method development work started systematically. Screening of 0.1 N HCl, SGF (without pepsin), 0.01 N HCl, SIFsp (pH 6.8) without pancreatin, phosphate buffer pH 7.4, SIFsp or phosphate buffer pH 7.4 with different surfactants or different antioxidants at different concentrations were carried out using USP Apparatus I at 100 rpm and 37 ± 0.5 °C. The surfactants used (in their proportions) were, SLS (0.05 to 0.5% w/v), Tween 80 (0.2 to 1% v/v) and, Pluronic 127 (0.1% to 0.5% w/v). Antioxidants used were ascorbic acid (0.01 to 0.2 % w/v) and sodium metabisulfite (0.05 to 0.4% w/v) and their combinations.

Surfactants in the concentrations used didn't significantly improve the dissolution of rifampicin. The possible reason for such observation might be due to large molecular size and molecular weight of the rifampicin that hindered its entrapment in to surfactant micelles. One more probable reason for the insignificant improvement in the dissolution in presence of SLS might be due to predominant existence of rifampicin in anionic form at alkaline pH conditions (at pH 6.8 and 7.4) of dissolution media used. Thus, it might have experienced the repulsive effect from the anionically charged SLS molecules and hence micellar entrapment became negligible. One more attempt has been made in enhancing the dissolution of rifampicin by incorporating methanol (1 to 15%) in to the selected dissolution media. But, methanol as well, didn't significantly improve dissolution of rifampicin. It was observed that when methanol was incorporated in the dissolution media it didn't improve the wettability of rifampicin indicated by the floating of drug particles on the surface of the dissolution media. This might be probable reason because of which methanol failed in enhancing the dissolution of the rifampicin. The antioxidant ascorbic acid found to be useful only for preventing oxidative degradation of rifampicin and was obviously not effective in minimizing the hydrolytic loss of rifampicin in presence of isoniazid at acidic pH conditions. Further more, the ascorbic acid, even at lower concentrations used, found to interfere with the analysis of isoniazid at the selected wavelengths of isoniazid estimation. Also the interference was observed at λ_{data} 254.5 nm, where isoniazid could be estimated in presence of rifampicin (ZCP for rifampicin) by first derivative spectroscopy. Hence, the use of ascorbic acid for the purpose of present study was not justified. Sodium metabisulfite, in the concentrations

used, didn't significantly improve the stability of rifampicin against oxidation. So, this option was also not useful in the present context of research.

The dissolution studies of marketed FDC tablets containing rifampicin and isoniazid combination were undertaken with the USP type I apparatus at 100 ± 5 rpm and at 37 ± 0.5 °C in the dissolution medias selected for screening. To challenge the ability of the dissolution procedure to demonstrate sufficient discriminating power, commercial formulations from different manufacturers were evaluated in selected dissolution medias. Both rifampicin and isoniazid have good solubilities in 0.01 N HCl, 0.1 N HCl and SGF without pepsin, that were sufficient enough to maintain their sink conditions during in vitro release studies. In selecting SGF over 0.1 N HCl, we refer back to the report by Gharbo et al (Gharbo et al. 1989) which specified that, rifampicin was found to degrade 17% and 37% in 0.1 N HCl in 45 min and 2 h respectively at 37 °C where as, the degradation was only 6% and 12% respectively within same period in SGF. It has also been reported that the extent of rifampicin degradation in presence of isoniazid increased from ~ 11 % in 0.1 N HCl to ~ 34% in 0.01 N HCl within 50 min (Sankar et al. 2003). Thus, to have a comparatively more stable environment that will aid in the exact measurement of the drugs by UV analysis we have preferred SGF over 0.1 N HCl and 0.01 N HCl. It was then decided to use SGF for in vitro release studies during initial hours to simulate in vivo conditions of the stomach. As normally the anti-TB formulations (containing rifampicin and isoniazid) are suggested to be administered on an empty stomach, SGF can be an ideal choice. It has been reported that the volume of stomach fluid in the fasted state might be as little as 20-30 ml (Dressman et al. 1998). Normally patient takes the formulation with a glass of water, then the total fluid volume of stomach in fasted state comes around 200-300 ml. Thus, it was decided to use 350 ml of SGF during initial hours of release studies to approximately simulate in vivo fasted state conditions. It was decided to use SGF in initial 2 h of the study as normal gastric retention time varies from 15 to 210 min with an average of 112.5 min for a larger pellets/tablets (~ 14 mm) (Dressman et al. 1998). It was decided to use SIFsp (pH 6.8) (without pancreatin) for in vitro release studies in the later hours (after 2 h studies in SGF). This medium also found to be quite discriminating for both rifampicin and isoniazid. But it was difficult to maintain the exact sink conditions for rifampicin

dissolution in this medium. Where as, the dose solubility ratio (according to BCS classification system) for 450 mg rifampicin formulations was found to be 173.75 ml, which was well below the specified limit of 250 ml. Thus, it was finally decided to go with SIFsp as a release medium from 3rd h to 24th h along with SGF.

4.4. Conclusions

Rifampicin and isoniazid passed the various tests of identification and analysis as per the IP 1996 and BP 1998.

Degradation profiles of both the drugs were found to follow first order at all the storage conditions studied. None of the excipients found to have severe deleterious effect on the stability of the rifampicin and isoniazid. It was observed in both rifampicin and isoniazid that with the increase in the temperature the K_{deg} values increased and $t_{90\%}$ values decreased in presence of all excipients. The degradation of both rifampicin and isoniazid was enhanced at higher humidity conditions (40 ± 2 ^{o}C/75 ± 5% RH). Increased K_{deg} values in presence of different polymers compared to drugs alone might be due to the tendency of polymers to absorb moisture that caused degradation of the drugs. The hydrophobic excipients talc and magnesium stearate thus, were shown negligible effect.

The degradation of both rifampicin and isoniazid was significantly dependent upon the pH of the solution (buffered or unbuffered). The degradation of rifampicin was higher at lower pH values and the degradation decreased as the pH of the solution (buffered or unbuffered) increased. However, the stability of the isoniazid was maximum at pH 1.2 and degradation rate constant was maximum at pH 2.0. The degradations rate constants were not much differed in buffered and unbuffered media.

Rifampicin showed a highly pH-dependent solubility profile. It was more soluble at acidic pH conditions. The solubility values were higher in buffered solutions than in unbuffered solutions (except at pH 5.0). It was observed that the isoniazid was highly soluble in all three medias studied (pH 1.2. pH 5.0 and pH 7.4).

It was decided to use pH 7.4 phosphate buffer containing 0.02% w/v of ascorbic acid as a dissolution medium for in vitro release studies of rifampicin C.R. formulations. After preliminary studies pH 7.4 phosphate buffer was used as a release medium for in vitro release studies of isoniazid C.R. formulations. In case of combined C.R. formulations of

rifampicin and isoniazid, it was decided to use 350 ml of SGF for first 2 h release studies to be followed by the use of 900 ml of SIFsp (pH 6.8) from 3-24 h.

Table 4.1. Observed first order degradation rate constants for rifampicin in presence of excipients (solid admixture) at different storage conditions

Drug/ Drug + Excipient	FT (5 ± 2°C)		CRT		40 ± 2°C		60 ± 2°C		40 °C/75% RH	
	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)
Rifampicin (R)	1.02	103.3	2.43	43.4	5.44	19.4	7.90	13.3	10.35	10.2
R + HPMC 15 cPs (1:5)	1.52	69.3	3.33	31.6	9.07	11.6	9.10	11.6	11.82	8.9
R + HPMC K 100LV (1:5)	2.11	49.9	4.53	23.3	6.49	16.2	9.30	11.3	11.52	9.1
R + HPMC K 4M (1:5)	1.72	61.3	4.20	25.1	7.26	14.5	8.97	11.7	12.24	8.6
R + HPMC K 15M (1:5)	1.64	64.3	4.80	22.0	6.26	16.8	9.84	10.7	13.14	8.0
R + HPMC K 100M (1:5)	1.47	71.7	4.56	23.1	6.74	15.6	8.13	13.0	13.67	7.7
R + Carbopol 934P (1:5)	1.52	69.3	5.10	20.7	11.48	9.2	11.65	9.0	19.38	5.4
R + Ethyl cellulose (1:5)	1.58	66.7	3.75	28.1	5.81	18.1	7.38	14.3	16.0	6.6
R + Eudragit L 100 D-55 (1:5)	1.52	69.3	5.58	18.9	9.40	11.2	11.58	9.1	12.45	8.5
R + HPC (1:5)	2.09	50.4	6.17	17.1	8.54	12.3	13.36	7.9	13.78	7.6
R + Talc (1:1)	1.14	92.4	3.43	30.7	6.21	17.0	8.05	13.1	8.40	12.5
R + Magnesium stearate (1:1)	1.27	83.0	4.28	24.6	6.26	16.8	9.14	11.5	8.57	12.3

Table 4.2. Observed first order degradation rate constants for isoniazid in presence of excipients (solid admixture) at different storage conditions

Drug/ Drug + Excipient	FT (5 ± 2°C)		CRT		40 ± 2°C		60 ± 2°C		40 °C/75% RH	
	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)	$t_{90\%}$ (month)
Isoniazid (H)	0.88	119.7	2.89	36.5	4.29	24.6	5.73	18.4	8.86	11.9
H + HPMC 15 cPs (1:5)	1.52	69.3	4.61	22.9	8.93	11.8	6.36	16.6	10.44	10.1
H + HPMC K 100LV (1:5)	1.33	79.2	4.59	23.0	7.59	13.9	5.87	18.0	9.15	11.5
H + HPMC K 4M (1:5)	1.20	87.8	3.85	27.4	5.48	19.2	6.93	15.2	7.46	14.1
H + HPMC K 15M (1:5)	1.74	60.6	5.57	18.9	7.07	14.9	9.45	11.2	11.51	9.2
H + HPMC K 100M (1:5)	1.65	63.9	6.73	15.7	8.53	12.4	10.23	10.3	12.24	8.6
H + Carbopol 934P (1:5)	1.40	75.3	4.99	21.1	7.79	13.5	9.62	11.0	10.67	9.9
H + Ethyl cellulose (1:5)	1.30	81.1	4.79	22.0	5.23	20.1	6.92	15.2	9.23	11.4
H + Eudragit L 100 D-55 (1:5)	2.11	49.9	5.66	18.6	7.39	14.3	9.29	11.3	10.08	10.5
H + HPC (1:5)	1.57	67.1	5.19	20.3	10.11	10.4	6.52	16.2	9.37	11.2
H + Talc (1:1)	1.03	102.3	3.51	30.0	5.95	17.7	6.85	15.4	8.22	12.8
H + Magnesium stearate (1:1)	1.44	73.2	4.27	24.7	6.12	17.2	7.42	14.2	7.42	14.2

Table 4.3. Stability studies of Rifampicin at different pH (unbuffered solutions)

Time (h)	Concentration (µg/ml)[a] with SD					
	pH 1.2	pH 2.0	pH 5.0	pH 6.0	pH 6.8	pH 7.4
0	19.67 ± 0.53	18.22 ± 0.54	20.99 ± 0.36	20.34 ± 0.19	19.39 ± 0.30	21.11 ± 0.52
1	16.44 ± 0.44	17.12 ± 0.43	19.38 ± 0.53	19.48 ± 0.36	18.47 ± 0.10	18.74 ± 0.34
2	15.36 ± 0.50	16.30 ± 0.33	18.43 ± 0.61	18.07 ± 0.38	17.70 ± 0.38	17.99 ± 0.29
4	12.40 ± 0.49	15.46 ± 0.25	17.53 ± 0.46	17.13 ± 0.31	16.81 ± 0.22	16.53 ± 0.14
6	11.02 ± 0.37	14.51 ± 0.47	16.75 ± 0.28	16.47 ± 0.44	15.62 ± 0.36	15.79 ± 0.27
8	10.19 ± 0.28	13.10 ± 0.31	16.20 ± 0.18	15.73 ± 0.16	14.95 ± 0.25	15.17 ± 0.10
12	8.98 ± 0.63	12.03 ± 0.26	15.10 ± 0.28	14.85 ± 0.28	14.27 ± 0.18	13.90 ± 0.37
18	7.69 ± 0.43	10.67 ± 0.21	14.11 ± 0.19	13.89 ± 0.19	13.82 ± 0.30	12.56 ± 0.25
24	7.03 ± 0.33	8.22 ± 0.18	12.97 ± 0.27	13.14 ± 0.20	13.41 ± 0.33	12.24 ± 0.17
$K_{deg} \times 10^2$ (h^{-1})*	4.01	2.44	1.82	1.73	1.50	2.09

[a] Average of triplicate readings with standard deviation values.
* First order degradation rate constant.

Table 4.4. Stability studies of Rifampicin at different pH (buffered solutions)

Time (h)	Concentration (µg/ml)[a] with SD					
	SGF (pH 1.2)	HCl (pH 2.0)	PO_4 (pH 5.0)	PO_4 (pH 6.0)	PO_4 (pH 6.8)	PO_4 (pH 7.4)
0	19.39 ± 0.52	19.56 ± 0.42	21.23 ± 0.33	21.62 ± 0.38	20.30 ± 0.40	19.96 ± 0.376
1	15.40 ± 0.37	17.40 ± 0.23	20.84 ± 0.19	20.81 ± 0.41	18.04 ± 0.39	18.66 ± 0.42
2	13.89 ± 0.32	16.24 ± 0.18	20.21 ± 0.22	20.05 ± 0.20	17.48 ± 0.23	18.12 ± 0.24
4	10.97 ± 0.46	15.42 ± 0.10	19.59 ± 0.41	19.29 ± 0.53	15.72 ± 0.44	16.74 ± 0.39
6	8.79 ± 0.34	14.66 ± 0.32	19.27 ± 0.22	18.42 ± 0.33	15.38 ± 0.31	15.90 ± 0.26
8	8.13 ± 0.17	13.17 ± 0.20	18.95 ± 0.52	17.63 ± 0.35	15.12 ± 0.30	15.21 ± 0.48
12	6.90 ± 0.20	12.06 ± 0.11	18.37 ± 0.59	16.99 ± 0.19	14.65 ± 0.39	14.16 ± 0.44
18	5.90 ± 0.13	10.89 ± 0.23	17.44 ± 0.47	15.83 ± 0.23	14.21 ± 0.31	12.87 ± 0.39
24	5.34 ± 0.10	9.93 ± 0.17	17.15 ± 0.20	14.60 ± 0.12	13.94 ± 0.19	12.50 ± 0.20
$K_{deg} \times 10^2$ (h^{-1})	5.02	2.62	0.87	1.54	1.27	1.91

[a] Average of triplicate readings with standard deviation values.
* First order degradation rate constant.

Table 4.5. Stability studies of Isoniazid at different pH (unbuffered solutions)

Time (h)	Concentration (µg/ml)[a] with SD					
	pH 1.2	pH 2.0	pH 5.0	pH 6.0	pH 6.8	pH 7.4
0	20.65 ± 0.02	20.12 ± 0. 07	19.98 ± 0.07	21.91 ± 0.08	20.48 ± 0.03	19.63 ± 0.02
1	20.58 ± 0.06	18.68 ± 0.04	19.74 ± 0.06	21.73 ± 0.07	20.35 ± 0.01	19.58 ±0.03
2	20.53 ± 0.03	18.13 ± 0.03	19.46 ± 0.05	21.64 ± 0.01	20.28 ± 0.06	19.53 ± 0.04
4	20.51 ± 0.02	17.76 ± 0.06	19.36 ± 0.05	21.55 ± 0.03	20.21 ± 0.01	19.46 ± 0.05
6	20.49 ± 0.03	17.55 ± 0.04	19.28 ± 0.03	21.46 ± 0.02	20.16 ± 0.02	19.41 ± 0.03
8	20.47 ± 0.05	17.51 ± 0.01	19.23 ± 0.02	21.38 ± 0.07	20.10 ± 0.01	19.36 ± 0.02
12	20.46 ± 0.02	17.28 ± 0.05	19.15 ± 0.04	21.36 ± 0.09	20.04 ± 0.02	19.28 ± 0.02
18	20.45 ± 0.01	17.14 ± 0.04	18.86 ± 0.02	21.30 ± 0.01	19.87 ± 0.04	19.17 ± 0.06
24	20.43 ± 0.04	16.95 ± 0.02	18.82 ± 0.04	21.28 ± 0.02	19.43 ± 0.03	19.08 ± 0.04
$K_{deg} \times 10^3$ (h^{-1})	0.23	5.07	2.07	0.92	1.84	1.16

[a] Average of triplicate readings with standard deviation values.
* First order degradation rate constant.

Table 4.6. Stability studies of Isoniazid at different pH (buffered solutions)

Time (h)	Concentration (µg/ml)[a] with SD at					
	SGF (pH 1.2)	HCl (pH 2.0)	PO_4 (pH 5.0)	PO_4 (pH 6.0)	PO_4 (pH 6.8)	PO_4 (pH 7.4)
0	18.92 ± 0.01	19.34 ± 0.10	20.16 ± 0.01	19.92 ± 0.04	19.50 ± 0.02	19.84 ± 0.02
1	18.89 ± 0.01	18.78 ± 0.06	20.07 ± 0.02	19.90 ± 0.01	19.43 ± 0.02	19.78 ±0.03
2	18.86 ± 0.03	18.34 ± 0.07	19.68 ± 0.01	19.86 ± 0.01	19.38 ± 0.01	19.72 ± 0.01
4	18.82 ± 0.02	18.22 ± 0.01	19.55 ± 0.03	19.80 ± 0.02	19.31 ± 0.04	19.65 ± 0.02
6	18.78 ± 0.03	17.86 ±0.03	19.48 ± 0.01	19.75 ± 0.01	19.25 ± 0.04	19.59 ± 0.02
8	18.76 ± 0.02	17.63 ± 0.02	19.41 ± 0.02	19.62 ± 0.03	19.18 ± 0.03	19.53 ± 0.03
12	18.71 ± 0.01	17.40 ± 0.03	19.20 ± 0.03	19.52 ± 0.03	19.11 ± 0.01	19.45 ± 0.01
18	18.69 ± 0.01	17.26 ± 0.08	19.14 ± 0.04	19.36 ± 0.01	19.05 ± 0.02	19.37 ±0.02
24	18.65 ± 0.01	17.07 ± 0.04	19.07 ± 0.02	19.20 ± 0.04	18.99 ± 0.01	19.33 ± 0.02
$K_{deg} \times 10^3$ (h^{-1})	0.69	4.38	2.06	1.61	1.14	1.27

[a] Average of triplicate readings with standard deviation values.
* First order degradation rate constant.

Table 4.6. Stability studies of Isoniazid at different pH (buffered solutions)

Time (h)	Concentration (µg/ml)[a] with SD at					
	SGF (pH 1.2)	HCl (pH 2.0)	PO_4 (pH 5.0)	PO_4 (pH 6.0)	PO_4 (pH 6.8)	PO_4 (pH 7.4)
0	18.92 ± 0.01	19.34 ± 0.10	20.16 ± 0.01	19.92 ± 0.04	19.50 ± 0.02	19.84 ± 0.02
1	18.89 ± 0.01	18.78 ± 0.06	20.07 ± 0.02	19.90 ± 0.01	19.43 ± 0.02	19.78 ±0.03
2	18.86 ± 0.03	18.34 ± 0.07	19.68 ± 0.01	19.86 ± 0.01	19.38 ± 0.01	19.72 ± 0.01
4	18.82 ± 0.02	18.22 ± 0.01	19.55 ± 0.03	19.80 ± 0.02	19.31 ± 0.04	19.65 ± 0.02
6	18.78 ± 0.03	17.86 ±0.03	19.48 ± 0.01	19.75 ± 0.01	19.25 ± 0.04	19.59 ± 0.02
8	18.76 ± 0.02	17.63 ± 0.02	19.41 ± 0.02	19.62 ± 0.03	19.18 ± 0.03	19.53 ± 0.03
12	18.71 ± 0.01	17.40 ± 0.03	19.20 ± 0.03	19.52 ± 0.03	19.11 ± 0.01	19.45 ± 0.01
18	18.69 ± 0.01	17.26 ± 0.08	19.14 ± 0.04	19.36 ± 0.01	19.05 ± 0.02	19.37 ±0.02
24	18.65 ± 0.01	17.07 ± 0.04	19.07 ± 0.02	19.20 ± 0.04	18.99 ± 0.01	19.33 ± 0.02
$K_{deg} \times 10^3$ (h^{-1})	0.69	4.38	2.06	1.61	1.14	1.27

[a] Average of triplicate readings with standard deviation values.
* First order degradation rate constant.

Table 4.7. Solubility studies of rifampicin in different pH buffered and unbuffered solutions

Solubility of Rifampicin in unbuffered solutions		Solubility of Rifampicin in buffered solutions	
Media	Conc. (mg/ml)[a]	Media	Conc. (mg/ml)[a]
pH 1.2	91.12 ± 1.4	SGF (pH 1.2)	102.46 ± 2.01
pH 2.0	16.89 ± 0.56	HCl buffer (pH 2.0)	22.43 ± 0.77
pH 5.0	1.54 ± 0.20	PO_4 (pH 5.0)	1.23 ± 0.11
pH 6.0	1.27 ± 0.12	PO_4 (pH 6.0)	1.36 ± 0.16
pH 6.8	1.46 ± 0.23	PO_4 (pH 6.8)	2.59 ± 0.21
pH 7.4	1.74 ± 0.18	PO_4 (pH 7.4)	3.18 ± 0.34

[a] Average of three determinations with standard deviation values. SGF – Simulated gastric fluid without pepsin. HCl buffer – Hydrochloric acid buffer. PO_4 – Phosphate buffer.

Table 4.8. Solubility studies of isoniazid in different pH buffered solutions

Media	Conc. (mg/ml)[a]
SGF (pH 1.2)	326 ± 3.96
PO_4 (pH 5.0)	281 ± 3.52
PO_4 (pH 7.4)	274 ± 4.79

[a] Average of three determinations with standard deviation values.
SGF – Simulated gastric fluid without pepsin.
PO_4 – Phosphate buffer.

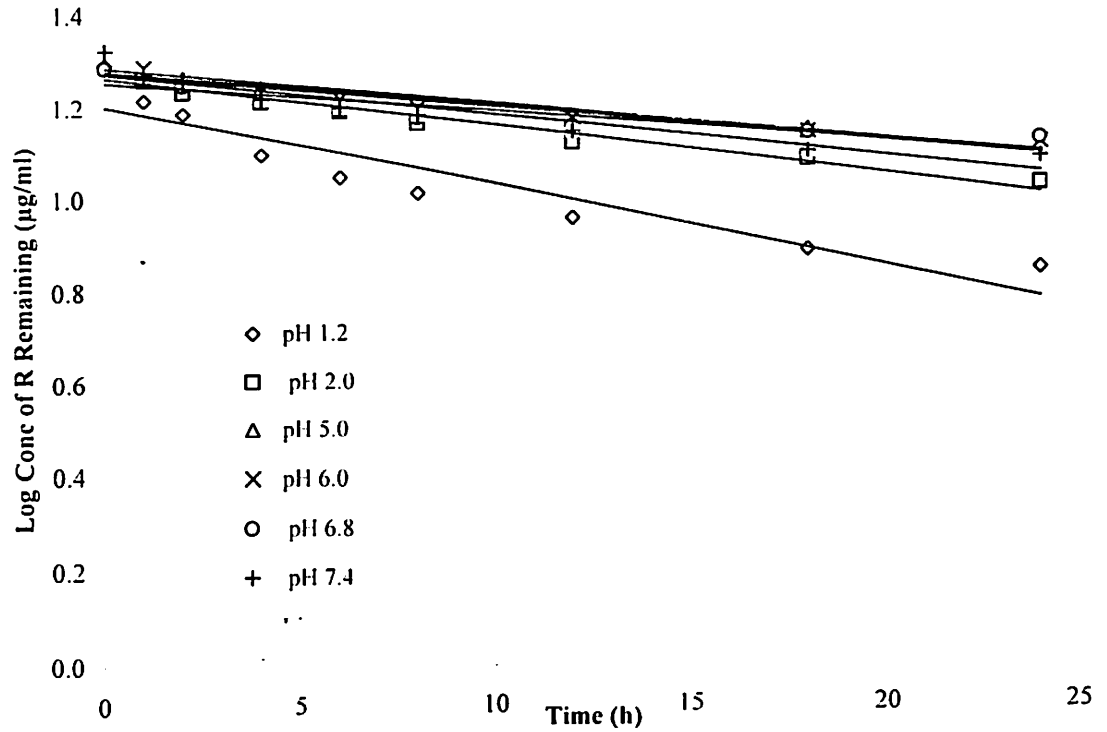


Fig. 4.1. Stability study plots of rifampicin in different pH unbuffered solutions. Each data represents average of three readings with SD less than ± 0.6% in all cases.

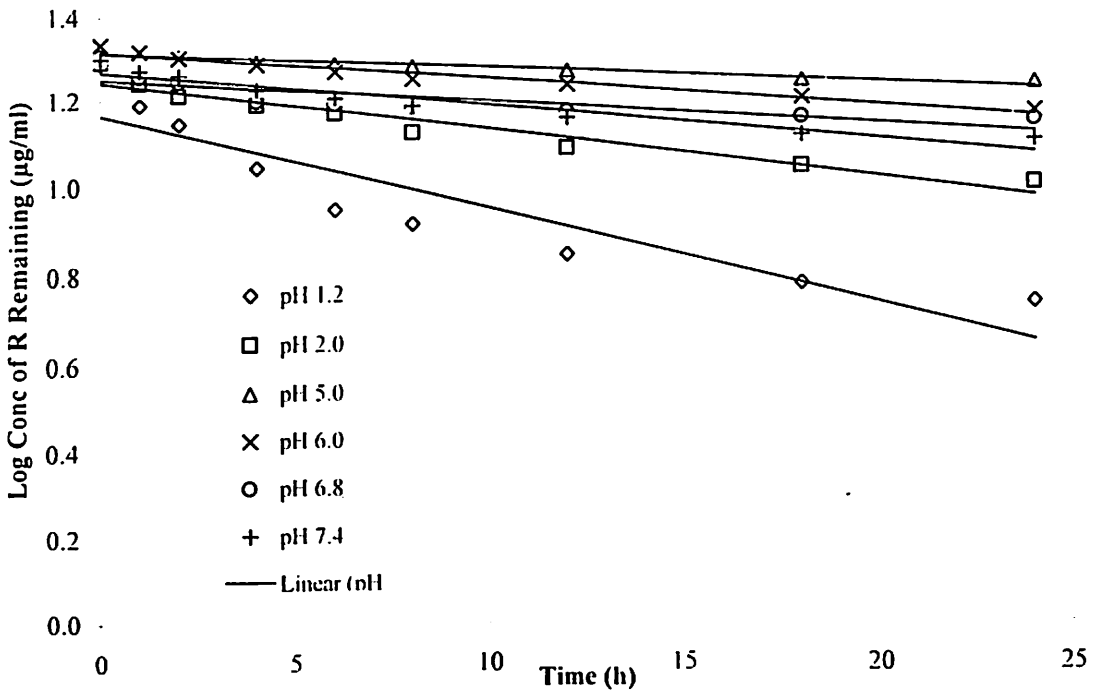


Fig. 4.2. Stability study plots of rifampicin in different pH buffered solutions. Each data represents average of three readings with SD less than ± 0.6% in all cases.

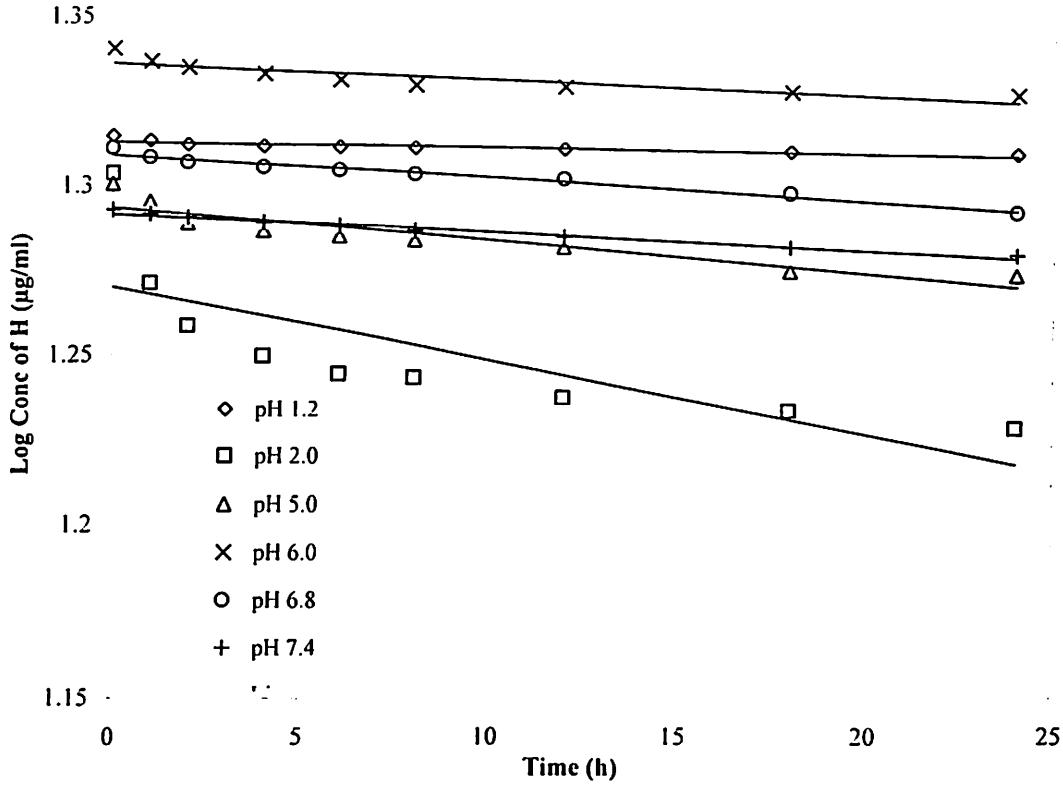


Fig. 4.3. Stability study plots of isoniazid in different pH unbuffered solutions. Each data represents average of three readings with SD less than ± 0.6% in all cases.

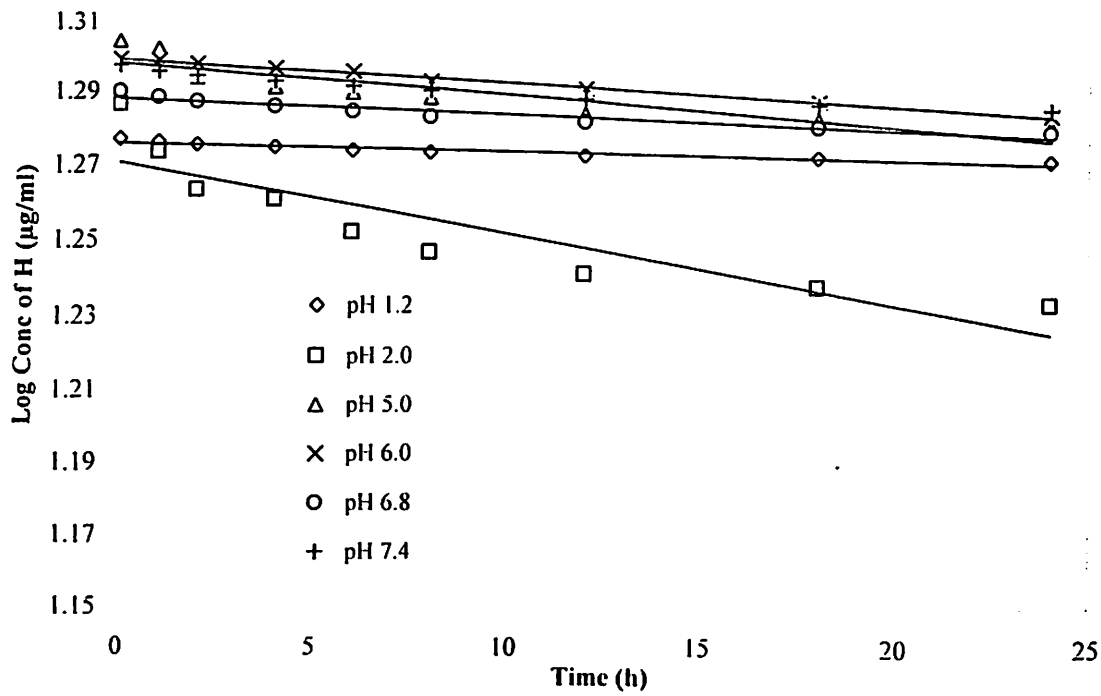


Fig. 4.4. Stability study plots of isoniazid in different pH buffered solutions. Each data represents average of three readings with SD less than ± 0.6% in all cases.

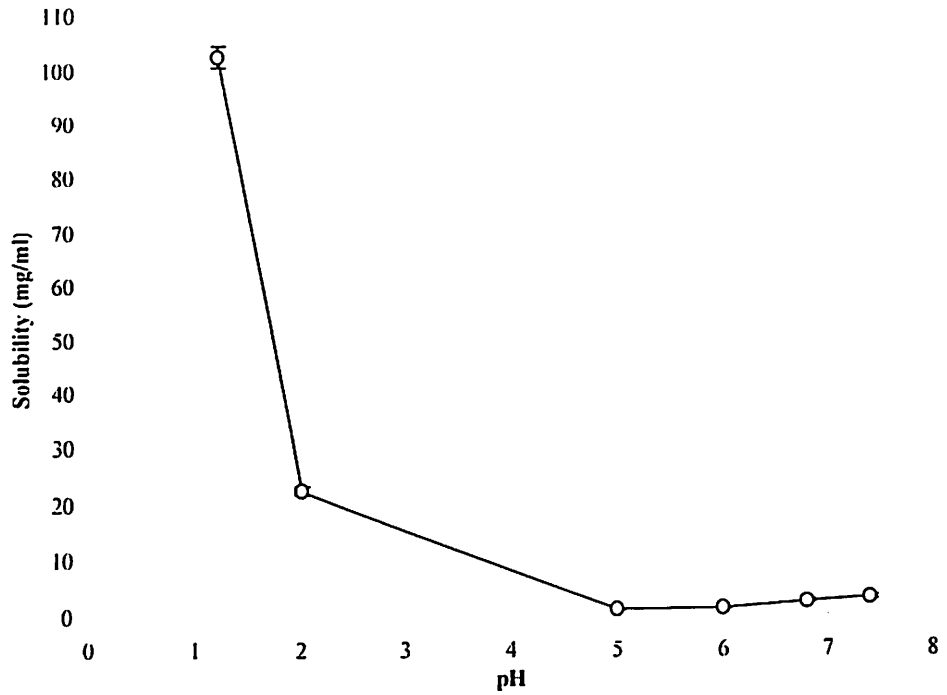


Fig. 4.5. Solubility study of rifampicin in different pH unbuffered solutions. Each data represents average of three readings with SD.

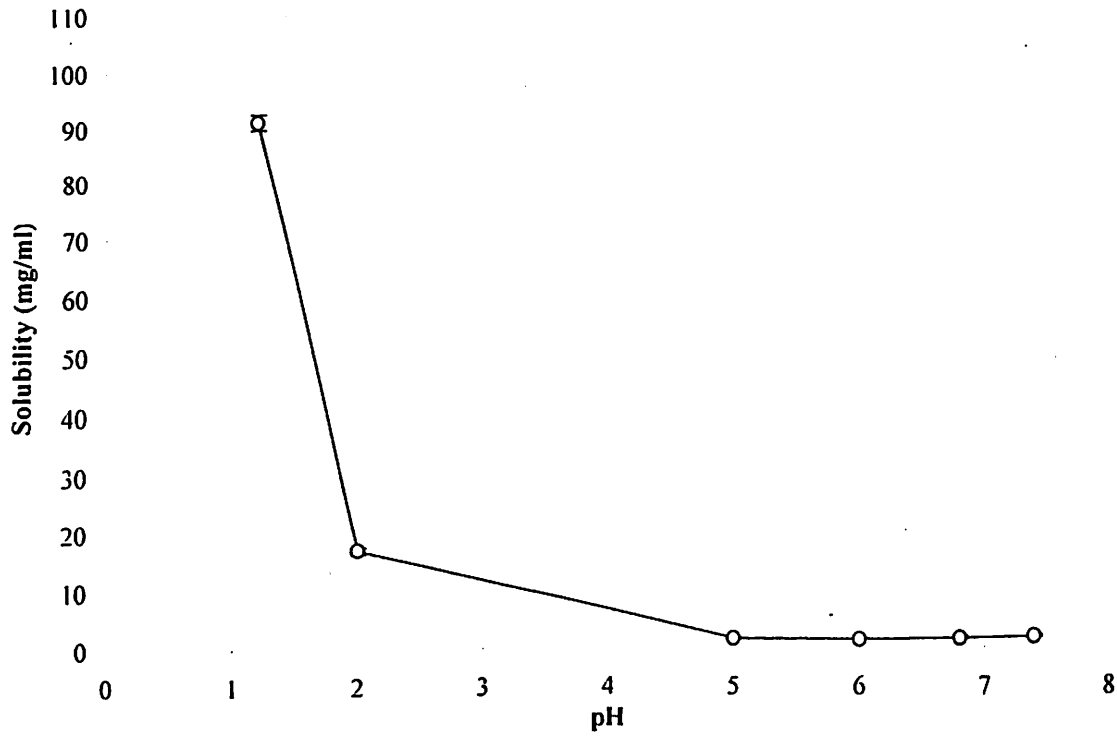


Fig. 4.6. Solubility study of rifampicin in different pH buffered solutions. Each data represents average of three readings with SD.

Chapter 5

Design and Studies of Oral Controlled Release Formulations of Rifampicin

Rifampicin is a first line anti-tubercular drug popularly used in the treatment of tuberculosis. Even though it has got excellent anti-tubercular activity it suffers from many disadvantages, which are discussed in chapter 1. Thus, there is a need for development of C.R. formulations of rifampicin. In this regard attempts have been made to design C.R. formulations of rifampicin and their evaluation has been carried out.

5.1. Experimental

Materials

Rifampicin was obtained as a gift sample from Cadila Pharma Ltd, Ahmedabad, and from Lupin Laboratories, Aurangabad. All other chemicals and polymers were obtained from the sources as mentioned in chapter 4.

Instruments/Equipments

Tablet compression was carried out on a single station tablet compression machine (Cadmach) using 13 mm standard concave (SC) punches. In vitro release were carried out using Electrolab - Tablet Dissolution Tester (USP 24, Model TDT 06P). A UV-visible-NIR spectrophotometer (Jasco UV-visible spectrophotometer; model – V 570, Tokyo, Japan) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells with Jasco spectra manager software was used for all absorbance measurements for UV analysis.

Analytical Method

Analysis of rifampicin was done by the spectrophotometric method as mentioned in chapter 3.

5.1.1. Formulation of rifampicin matrix tablets

5.1.1.1. Formulation of rifampicin tablets with HPMC polymers

Controlled release matrix tablets with HPMC 15 cPs were formulated by wet granulation method using different proportion of polymers (Table 5.1). The drug and polymer (both

passed through 60# mesh) were mixed uniformly and granulated with isopropyl alcohol (IPA), and dried in a tray drier at 60 °C. The final granules were blended with talc (0.6% w/w) and magnesium stearate (0.4% w/w) and compressed in a single station tablet compression machine using 13 mm SC punches.

Controlled release matrix tablets with HPMC K100LV, HPMC K4M and HPMC K15M were formulated by direct compression method using different proportions of polymers (Table 5.2 to 5.4). The drug and polymer (passed through 60# mesh) were mixed uniformly and were blended with talc (0.6% w/w) and magnesium stearate (0.4% w/w) and compressed in a single station tablet compression machine (Cadmach). The compression force was kept at a constant level to get the tablets of about 6-7 kg/cm^2 hardness, except for the studies on the effect of compression force on the release rate.

Three batches of tablets were prepared for each formulation with each tablet contained 450 mg of rifampicin. The formula and the physical characteristics of the formulated tablets are given in Table 5.2 (for HPMC K100LV formulations), Table 5.3 (for HPMC K4M formulations) and in Table 5.4 (for HPMC K15M formulations).

5.1.1.2. Formulation of rifampicin tablets with Carbopol 934P polymer

Controlled release matrix tablets with Carbopol 934P were formulated by direct compression method using different proportions of polymer (Table 5.5). The drug and polymer (passed through 60# mesh) were mixed uniformly and were blended with talc (0.6% w/w) and magnesium stearate (0.4% w/w) and compressed in a single station tablet compression machine (Cadmach) using 13 mm SC punches. Three batches of tablets were prepared for each formulation with each tablet contained 450 mg of rifampicin. The formula and the physical characteristics of the formulated tablets are given in Table 5.5.

5.1.1.3. Formulation of rifampicin tablets with HPC (Klucel LF) polymer

Controlled release matrix tablets with HPC (Klucel LF) were formulated by direct compression method using different proportions of polymer (Table 5.6). The drug and polymer (passed through 60# mesh) were mixed uniformly and were blended with talc (0.6% w/w) and magnesium stearate (0.4% w/w) and compressed in a single station tablet compression machine (Cadmach) using 13 mm SC punches. Three batches of tablets

were prepared for each formulation with each tablet contained 450 mg of rifampicin. The formula and the physical characteristics of the formulated tablets are given in Table 5.6.

5.1.2. Physical characterization of the tablets

Formulated tablets were subjected to different physical characterization studies (Table 5.1 to 5.6). The drug content of each batch of the formulated tablets was determined in triplicate as per the procedure given in the IP 1996 for rifampicin capsules. The weight variation was determined on 20 tablets using electronic balance (Afcoset). Tablet hardness was determined for minimum 6 tablets of each batch using Monsanto (Standard type) tablet hardness tester. Friability was determined with 20 tablets in a Cambell Electronic Friabilator for 5 min at 25 rpm.

5.1.3. In vitro release studies

Release studies were carried out in pH 7.4 phosphate buffer containing 0.02% w/v of ascorbic acid, at 37 ± 0.5 °C as selected and mentioned in chapter 4. The volume of the dissolution medium was 900 ml and the stirring speed was set at 100 rpm. At predetermined time intervals 10 ml of sample was withdrawn and replaced with fresh dissolution media. After appropriate dilutions, the samples were analyzed by spectrophotometric method. Cumulative percent of drug released was calculated, and mean of six tablets from three different batches were used in the data analysis.

The in vitro release data was analyzed by using different kinetic models as mentioned in chapter 1. The following parameters were varied and their effect on in vitro release rate, release mechanism and nature of release were studied.

5.1.3.1. Effect of polymer proportion

Tablets containing 10%, 20%, 30%, 40%, 50% and 60% HPMC (w/w of the drug) were made in case of formulations with HPMC 15 cPs. Effect of HPMC K100LV proportion were studied using 10%, 20%, 30%, 40%, and 60% (w/w of the drug). HPMC K4M ratios studied were, 10%, 20%, 30%, and 40%. (w/w of the drug). In case of HPMC K15M the proportion was varied using 5%, 10%, 20%, and 30%. (w/w of the drug). Tablets were made containing 10%, 20%, 30% and 40% (w/w of the drug) in case of

Carbopol 934P and 20%, 40%, 60% and 80% (w/w of the drug) in case of HPC (Klucel LF). The results of effect of polymer ratio on rifampicin release are shown in the Figure 5.1, 5.2, 5.3, 5.4, 5.5, and 5.6 for formulations with HPMC 15 cPs, HPMC K100LV, HPMC K4M, HPMC K15M, Carbopol 934P, and HPC (Klucel LF) respectively.

5.1.3.2. Effect of viscosity of HPMC

Three different viscosity grade HPMCs were used in the present investigation, HPMC K100LV (100 cPs), K4M (4000 cPs) and K15M (15000 cPs). The results of effect of viscosity in case of formulations containing 20% polymer (HPMC) proportion are shown in Figure 5.7.

5.1.3.3. Effect of compression force

The tablet batches containing 40% of HPMC 15 cPs, 40% of HPMC K100LV and 20% of HPMC K4M were compressed at three different hardness levels to get the tablets of about 4, 6 and 10 kg/cm^2 hardness. In case of Carbopol 934P, 20% formulation was compressed at three different hardness levels, 4, 6, and 11 kg/cm^2. In case of HPC (Klucel LF), 20% formulation was compressed at three different hardness levels, 4, 8, and 12 kg/cm^2. Results are shown in the Figure 5.8, 5.9, 5.10, 5.11 and 5.12 respectively for formulations with HPMC 15 cPs, HPMC K100LV, HPMC K4M, Carbopol 934P and HPC (Klucel LF).

5.1.3.4. Effect of method of granulation

The effect of granulation methodology was studied in tablets with 40% HPMC 15 cPs. The mixture of drug and excipient was first directly compressed and the same formula was granulated with IPA and the granules were compressed. The results are shown in the Figure 5.13.

5.1.3.5. Effect of change in the dissolution methodology

In this case formulation with HPMC 15 cPs (50%) was selected for the study. The formulations were undertaken for the in vitro release studies by two different methods,

USP type I (basket) and USP type II (paddle) method, at 100 rpm. The results are shown in the Figure 5.14.

5.1.3.6. Effect of hydrodynamic conditions (stirring speed of basket)

This study was undertaken on formulations with HPMC 15 cPs (40%) and HPMC K4M (20%). In vitro release studies were carried out at three different stirring speeds, 50, 100 and 150 rpm. The results are shown in the Figure 5.15 and 5.16 for formulations with HPMC 15 cPs and HPMC K4M respectively.

5.1.3.7. Effect of addition of free drug

In this case 15% of the total rifampicin was separated and added as a free drug (extragranularly) to the granules of remaining amount of drug with 50% HPMC 15 cPs (R4), while maintaining the drug to polymer ratio same. The results are shown in the Figure 5.17.

5.1.4. Erosion studies

Tablet erosion studies were performed using the USP type I (basket) method under the same conditions described in the dissolution studies. Individual tablets were removed at different time intervals during the dissolution studies and carefully placed on pieces of aluminum foil and dried at 60°C under reduced pressure to a constant weight as determined with an analytical balance. The percentage of tablet eroded was calculated from the weight loss of the tablets for each time interval. The procedure was repeated for three tablets for each batch and the mean was used in the data analysis.

The erosion study results of tablets with HPMC 15 cPs only were presented (Figure 5.18).

5.1.5. Batch Reproducibility

Three batches of each formulation were prepared and their quality and respective release characters were evaluated under the same conditions as prescribed in previous sections. In vitro release data pertaining to reproducibility studies were compared by f_2 factor

(similarity factor) values. The f_2 factor values for the selected formulation batches were calculated.

5.1.6. Stability Studies

The two best formulations from each polymer, one with lower polymer proportion and another with higher, were selected for stability studies. The selected formulations were sealed in airtight cellophane packets and stored at ambient as well as accelerated storage conditions as per ICH guidelines (ICH 1996). The storage conditions used for the studies were controlled room temperature (CRT: 25 ± 2 °C and 60 ± 5% RH), 40 ± 2 °C, and 40 ± 2 °C/75 ± 5% RH. The samples in triplicate were withdrawn from each batch at predetermined time intervals (0, 0.5, 1, 3 and 6 months) and analyzed for physical characters and in vitro release behavior. The physical characters such as appearance, hardness and friability were evaluated as per the specifications enlisted in previous sections and compared with the initial values. The drug content in triplicate was determined for each formulation by the method described in chapter 3 after suitable dilutions. The observed degradation rate constants (K_{deg}) and $t_{90\%}$ (time for 90% of the drug to remain) at different storage conditions are listed in Table 5.8. In an effort to study the effect of humidity on the stability characteristics of drug in tablet formulations the K_{deg} and $t_{90\%}$ obtained at 40°C were compared with that obtained at 40°C / 75% RH and the result are presented in Table 5.8. The in vitro release profiles were studied as per the specifications enlisted in previous sections and compared with their respective initial release profiles. The in vitro release profiles of the formulations stored at CRT for 6 months were compared with their initial release profiles (0 time samples at CRT) with f_2 factor values.

5.2. Results and Discussions

5.2.1. Formulation of rifampicin matrix tablets

It has been reported that for a drug substance that is adjudged to be physically or chemically unstable when exposed to moisture, a direct compression or nonaqueous

granulation method is to be recommended for the preparation of the tablet/capsule formulations (Wadke and Jackobson 1980). From the preformulation studies (Chapter 4) it was observed that the rifampicin degradation enhanced in presence of moisture or high humidity. Thus, it was decided to use either direct compression method or nonaqueous granulation with IPA for the formulation of matrix tablets of rifampicin.

In case of formulations with HPMC 15 cPs the direct compression method did not produce good tablets. The in vitro release studies of these formulations resulted in the immediate disintegration of the tablets and quicker release of the drug within a short period of time. Thus, the formulations were made with nonaqueous wet granulation method using IPA. These formulations found to have good physical characters (Table 5.1) and found to control the drug release when in vitro release studies were carried out.

With higher viscosity HPMC (K100LV, K4M and K15M), Carbopol 934P and HPC (Klucel LF) polymers, direct compression found to give better quality tablets. In these cases, wet granulation with IPA resulted in the nonreproducible release profiles. Thus, in all these cases (with HPMC K100LV, K4M, K15M, Carbopol 934P and HPC) it was decided to use direct compression method for the preparation of matrix tablet formulations.

As the drug dose itself was high no excipients (such as microcrystalline cellulose, lactose, dicalcium phosphate, etc) other than controlled release polymers have been incorporated to increase the bulk further or to improve the granule properties. The use of polymer levels also restricted to minimum so as to make the formulations practically useful for the purpose of administration in terms of tablet height and thickness.

5.2.2. Physical characterization of the tablets

Physical appearance, tablet hardness, friability, weight variation, and drug content uniformity of all formulations were found to be satisfactory as can be observed from the data in Table 5.1, 5.2, 5.3, 5.4, 5.5 and 5.6 for formulations with HPMC 15 cPs, HPMC K100LV, HPMC K4M, HPMC K15M, Carbopol 934P and HPC respectively. Hardness was found to be 6-7 kg/cm^2 in all HPMC formulations and, in case of Carbopol 934P and HPC formulations the hardness ranged from 7-8 kg/cm^2 (except for the studies involving the effect of compression force on release). The friability was less than 0.9% (w/w) in all

cases. The formulated tablets showed very low weight variation, high degree of content uniformity, indicating that the method of preparation of formulation is an acceptable method for preparing good quality matrix tablets of rifampicin.

5.2.3. In vitro release studies

5.2.3.1. Effect of polymer and its proportion

HPMC formulations

It is well known fact that the drug release form the HPMC matrices can be either disentanglement or diffusion controlled depending on the viscosity of the polymer and the thickness of the diffusion boundary layer (Narasimhan and Peppas 1997). Rifampicin being poorly water soluble drug, its release character and release mechanism from HPMC matrix formulations was significantly affected by the viscosity grade and ratio of the HPMC polymer used. These findings have been depicted in the Figures 5.1-5.4, which depict the influence of drug:HPMC ratio on the release rates and release characteristics of rifampicin from HPMC matrix tablets of four different viscosity grades [15 cPs, K100LV (100 cPs), K4M (4000 cPs) and K15M (15000 cPs)].

Plots of percent cumulative drug released vs. time for HPMC 15 cPs matrix tablet formulations, R1, R2, R3, R4 and R5 (20, 30, 40, 50 and 60%), are shown in the Figure 5.1. The release was significantly dependent on the proportion of the polymer used. As the polymer level was increased from 20% to 40% the release rate decreased. Matrices containing HPMC below 20% did not sustain the drug release for long time and they disintegrated and quickly released the drug within 2 h, hence their release profiles are not given. The release was extended up to 8 h and 10 h respectively in case of formulations containing 20% (R1) and 30% (R2) HPMC respectively, indicating insufficient polymer proportion to extend the release for long period. Release kinetics studies indicated that the nature of release was observed to be first order in case of both R1 and R2 formulations as the plot of log percent remaining to be released vs. time produced very high 'r' values (Table 5.7). The initial release was faster and the release rate decreased with time. The release was extended up to 16 h when HPMC was increased to 40% (R3)

and found to be zero order at least up to 10-12 h beyond which the release rate was decreased. The most interesting observation was that, there was no significant difference between the release profiles of R3 (40%), R4 (50%) and R5 (60%) formulations as further established by the f_2 (similarity) factor values ($f_2 \geq 85$). Similar results were also reported by the other research group (Varghas and Ghaly1999), when they studied the effect of HPMC level on theophylline release. The reasons for such observations need to be explained, but we can say that, with the use of this lower viscosity grade (15 cPs) HPMC it was possible to control the release only up to 16 h at the applied hardness levels. These findings were again supported by the erosion study results. The nature of release was found to be zero order in case of both R3, R4 and R5 formulations as the plot of percent drug released vs. time was found to be linear with good 'r' values (see Table 5.7). Initial release was less (below 10% in about 1 h) in case of R3, R4 and R5 necessitating the need for the incorporation of a loading dose to achieve the desired drug levels in the initial hours.

Plots of percent cumulative drug released vs. time for HPMC K100LV matrix tablet formulations are shown in the Figure 5.2. The release was significantly dependent on the ratio of the polymer used, as the polymer level was increased from 10% to 60% [10% (R6), 20% (R7), 30% (R8), 40% (R9) and 60% (R10)] the release decreased. Tablets containing HPMC below 10% did not sustain the drug release for long time and they disintegrated and quickly released the drug, hence their release profiles are not given. The release was extended up to 4 h, 12 h and 16 h respectively in case of R6, R7 and R8 formulations containing 10%, 20% and 30% polymer respectively. When the polymer ratio was increased to 40% (R9) the release was extended up to 24 h with ~ 92% release at the end of 24 h. Further increase in the polymer proportion to 60% (R10) resulted in the extension of the release beyond 24 h with ~ 69% release at the end of 24 h. The nature of release was found to be zero order in case of all the formulations as indicated by very high 'r' values (Table 5.7). At 40% and 60% polymer levels the tablet matrices were behaving like a perfect zero order release system with 'r' values of 0.999 and 0.998 respectively. Since the drug release was less in the initial hours (especially at higher polymer levels), a separate loading dose would be required to attain the desired drug levels in the initial hour. Also, the high dose requirement of rifampicin would result in

the use of lower proportion of the polymer in the tablet. The above-mentioned drawbacks have been overcome by using higher viscosity grade HPMC polymers, which will be discussed further.

Plots of percent cumulative drug released vs. time for formulations with HPMC K4M and K15M matrix are shown in the Figure 5.3 and 5.4 respectively. Similar trend of decreased release with the increasing polymer ratio was observed in both K4M and K15M formulations. In case of formulations with K4M (Figure 5.3), the drug release was extended up to 6 h at 10% polymer proportion (R11). Even at only 20% polymer level (R12) ~ 83% of drug was released at the end of 24 h. When the polymer ratio was increased to 30% (R13) and 40% (R14) only about 60% and 35% of drug was released respectively within 24 h. Significant amount of drug was released in the initial hours indicating that there was no need for the incorporation of the loading dose. One more formulation was prepared containing 10% HPMC K4M and 30% HPMC K100LV (R15) combinations to know the effect of combination of higher and lower viscosity grade HPMC polymers on rifampicin release. As can be seen from the Figure 5.3, a complete drug release was there (~ 98%) within 24 h, which was absent when HPMC K4M alone was used (from 20-40%). Thus, a suitable combination of lower and higher viscosity grades of HPMC polymers (in suitable proportions) could give the combined advantages of both extended release and complete drug release. In case of HPMC K15M formulations the drug release was extended up to 4 h with 5% polymer ratio (R16). Only about 10% of the polymer (R17) was sufficient to control the release up to 24 hours (Figure 5.4), at the end of 24 h ~ 88% drug was released. Also, there was good initial release indicating that no necessary of loading dose incorporation. When the HPMC K15M amount was increased to 20% and 30% only about 56% and 37% drug was released respectively within 24 h. The reason for initial higher release and decrease in the rate of release with time in case of high viscosity grade polymers can be explained as follows. Initially, drug close to matrix surface might be released before the surrounding polymer reached the polymer disentanglement concentration (the concentration of the polymer in a fully hydrated state at which there are no polymer-polymer linkages), because the diffusion coefficient for drug is higher than the polymer. Higher the polymer viscosity/molecular weight longer will be the time taken to form a gel layer. This is

confirmed by the faster drug release in the initial hours of the dissolution study. But, in case of lower viscosity grade polymer, the gel layer could form immediately as polymer disentanglement starts immediately once the matrix comes in the contact with the dissolution medium. Thus in case of K100LV matrices there was less drug release in the initial hours. The release rate decreased with time and the polymer matrices exhibited first order release profiles as can be seen from the 'r' values for first order release plots (Table 5.7). Rifampicin release from K4M and K15M matrix tablets was found to show better fitting in Higuchi's square root kinetics model though also showing good correlation in first order fitting (Table 5.7).

In general, increase in the polymer ratio irrespective of the viscosity grade resulted in the decrease in the drug release rates. The reason for such observation may be explained as follows. An increase in the polymer concentration causes increase in the viscosity of the gel as well as the formation of a gel layer with a longer diffusional path. This could cause a decrease in the effective diffusion of the drug and therefore a reduction in the drug release rate. All the polymers used produced similar effect.

The release mechanism and kinetics of the release profile was further analyzed by Korsmeyer-Peppas model ($M_t / M_\infty = K. t^n$) up to 60% release. The values of K, n, $t_{50\%}$ (time for 50% of drug release) and r (correlation coefficient) obtained for various formulations are listed in Table 5.7. The n values for the HPMC 15 cPs formulations found to be about 1, except in 30% (R2) formulation (n = 1.28), indicating that the release mechanism was super Case-II. Such mechanism of release mainly involves swelling and erosion of the matrix structure due to relaxation of the polymer chains. The n values for HPMC 15 cPs formulations thus found to produce zero order release profiles. The release rate was fastest from the formulations containing 20% HPMC (R1) with a K value of 0.297 $h^{-1.03}$ and $t_{50\%}$ 1.68 h. The release rate was slowest in case of formulations containing 40% HPMC (R3) with a K value of 0.075 $h^{-1.0}$ and $t_{50\%}$ of 6.70 h. The release rates of R3, R4 and R5 were almost similar, and, there was no significant difference between $t_{50\%}$ values (6.70, 6.20 and 6.03 h) and K values (0.075 $h^{-1.0}$, 0.080 $h^{-0.96}$ and 0.083 $h^{-0.95}$) for these three formulations. These results again proved that the optimum percentage of polymer, in this case, found to be 40% beyond which there was no significant change observed in the release profile and the release kinetics parameters. The

zero order release observed could be due to synchronization of the swelling and erosion fronts. In the case of a low solubility drug like rifampicin there were due chances that the drug particles remain in the vicinity of the swelling front. Then, they might be pushed towards the gel layer by a spring-like action of the macromolecular chains as these pass from the glassy to the rubbery (gel-like) state. This presence of insoluble drug particles reduces the gel layer strength and enhances the erosive tendency of the matrix. Thus, a constant balance was always maintained between the two fronts, swelling and erosion, that resulted in the zero order release.

The n values for different formulations with HPMC K100LV found to be 0.65 for R7 but about 1 for R8, R9 and R10. Increase in the ratio of the polymer from 20% (R7) to higher resulted in the change of release character from anomalous non-Fickian release towards a zero order. This may be due to a reduction in the regions of low microviscosity and closing the micropores in the swollen tablets, which ultimately restricted the drug diffusion through the swollen layer. Similar types of results were observed by several other investigators in their study of impact of different polymer ratios on the release kinetics (Capan et al. 1991, Seng et al. 1985). Among these formulations release rate was fastest from the formulation containing 20% polymer (R7) with a K value of 0.190 $h^{-0.65}$ and $t_{50\%}$ 4.61 h. The release rate was slowest in case of formulations containing 60% polymer (R10) with a K value of 0.027 $h^{-1.01}$ and $t_{50\%}$ 17.60 h. In case of HPMC K4M and K15M formulations the n values ranged from 0.48 to 0.55 (Table 5.7) except for 30% K15M formulation (n value 0.76) indicating that the release mechanism was anomalous non-Fickian diffusion and very close to Fickian diffusion. These n values again confirmed that the release profiles followed Higuchi's square root kinetics model ('r' values of 0.969-0.998, Table 5.7) in case of higher viscosity grade HPMC formulations. Among K4M formulations release was fastest from 10% (R11) formulations with a K value of 0.38 $h^{-0.54}$ and $t_{50\%}$ 1.64 h. The release rate was slowest in case of formulations containing 40% polymer (R14) with a K value of 0.062 $h^{-0.52}$ and $t_{50\%}$ as high as 54.13 h. The n, K, and $t_{50\%}$ values from R15 formulation (containing 10% K4M and 30% K100LV combination) were found to be 0.46, 0.241 $h^{-0.54}$ and 4.83 h respectively. The optimized values for K and $t_{50\%}$ (increased K value along with increase in the $t_{50\%}$) could be obtained in case of R15 formulations that were desirable for attaining extended drug

release levels along with the complete drug release. In case of HPMC K15M, release was fastest from 10% (R17) formulations with a K value of 0.20 $h^{-0.51}$ and $t_{50\%}$ 5.75 h. The release rate was slowest in case of 30% formulations (R19) with a K value of 0.034 $h^{-0.76}$ and $t_{50\%}$ as high as 33.18 h.

Carbopol 934P formulations

Plot of percent cumulative drug released vs. time for Carbopol 934P formulations R20, R21, R22 and R23 (10, 20, 30 and 40%), are shown in the Figure 5.5. It can be observed from the graph that increase in the polymer ratio resulted in the decrease in the release. As the polymer ratio increased from 10% to 40% the release rate decreased. Matrices containing Carbopol 934P below 10% did not sustain the drug release for long time and they disintegrated with quick release of the drug within short period of time. There was also high variability in the release profiles between and within the batch in case of matrices containing Carbopol 934P below 10%, hence their release profiles are not given. The release was extended up to 12 h and 16 h respectively for 10% (R20) and 20% (R21) Carbopol 934P formulations. When the polymer proportion increased to 30% (R22) and 40% (R23), only ~ 74% and ~ 43% drug was released respectively within at the end of 24 h. Release kinetics studies indicated that the nature of release was observed to be zero order in case of all the formulations (R20 to R23) (Table 5.7 and Figure 5.5). In all the cases, initial drug release rate was slightly lower (for up to 5 h) followed by increase in the release. In other words, a bimodal drug release observed. Similar findings were reported by other research group in their study on the release of insoluble (sparingly soluble) drug from hydrophilic (swellable) matrices (Bettini et al. 2001). The reason for such observation might be explained as follows. Carbopol polymers have been known for their excellent swelling properties. These polymers readily hydrate, absorb water, and swell quickly (Khan and Zhu 1999) up to 1000 times their volume to form a gel when exposed to pH environment above 4 to 6. They also have very less solubility in water (Huang and Schwartz 1995). Thus, when Carbopol 934P polymeric tablet matrix came in contact with the release media a stable hydrated gel layer was formed quickly (noticed visually during the release studies) that prevented the initial release of the drug by diffusion through the pores (present in the matrix structure). This also caused poor

infiltration of the release media in to the tablet and hence there was some lag period for the complete hydration of the tablet. Once the tablet matrix completely hydrated then the hydrated matrix gradually started eroding. The drug release thus increased in the later hours (after 5 h to 6 h) by increased diffusion through the hydrated gel layer and/or erosion. The lower release during initial hours necessitated the need for the incorporation of a loading dose to achieve the desired drug levels in the initial hours.

The release mechanism and kinetics of the release profiles were analyzed by Korsmeyer-Peppas model ($M_t / M_\infty = K. t^n$) up to 60% release. The values of K, n, $t_{50\%}$ (time for 50% of drug release) and r (correlation coefficient) obtained for various formulations are listed in Table 5.7. The n values for the formulations ranged from 1.33 to 1.51 indicating that the release mechanism was super Case-II. The release rate was fastest from the formulations containing 10% polymer (R20) with a K value of 0.027 $h^{-1.33}$ and $t_{50\%}$ of 8.85 h. The release rate was slowest in case of formulations with 40% polymer (R23) with a K value of 0.010 $h^{-1.34}$ and $t_{50\%}$ of 19.38 h. There were significant differences in the release profiles with the small increase in the polymer proportions (10% increment in each case). In this case also (like high viscosity HPMC formulations) less amount of polymer was sufficient to control the release for longer periods. The reason for swelling/relaxation controlled release of rifampicin from the Carbopol 934P matrix tablets can be explained as follows. Once the polymer came in contact with the release media the polymer swelling commenced. Microscopically, there was a relaxation response of the polymer chains due to the stresses introduced by the release media (permeated in to the tablet) that resulted in the increase in the molecular volume of the hydrated polymer. This reduced the free volume available for the diffusion due to the presence of micropores. Thus, the drug release was mainly swelling/relaxation and erosion controlled.

HPC (Klucel LF) formulations

There have been several reports which proved that the drug release from HPC matrix tablets significantly influenced by the particle size of the L-HPC that determines their swelling ability (Kawashima et al. 1992), percent loading of the polymer (Kawashima et

al. 1992), HPC viscosity grade (Nakano et al. 1983), pH of the dissolution media (Ebube and Jones 2003), and compression force (Ebube and Jones 2003).

In Figure 5.6 is presented the plots of percent cumulative released vs. time of rifampicin from formulations with 20% (R24), 40% (R25), 60% (R26) and 80% (R27) HPC (Klucel LF). The release was significantly dependent on the ratio of the polymer. There was a decrease in the release as the polymer level was increased from 20% to 80%. Tablets containing HPC below 20% immediately disintegrated and released the drug quickly, hence their release profiles are not given. The release was extended beyond 24 h even at 20% polymer proportion (R24). At the end of 24 h release studies only about 87%, 60%, 38% and 28% drug was released respectively from formulations with R24, R25, R26 and R27. Release kinetics studies indicated that the nature of release was observed to be zero order in case of all the formulations (except some higher release during initial hours) (Figure 5.6). Since the drug release was less in the initial hours (especially at higher polymer levels), a separate loading dose would be required to attain the desired drug levels in the initial hour. The reasons for the observed zero order release profiles at all polymer ratios studied might be explained as follows. It was observed during release studies that the polymer swelling was very negligible. However, there was a continuous erosion of the tablet matrix with time. The presence of poorly soluble drug particles (rifampicin) further reduced the gel layer strength by reducing the swellability of the matrix and enhanced the erosive tendency of the tablet matrix. Thus, a constant balance was always maintained between the swelling front and erosion front that resulted in the zero order release. The HPC polymer used in the study has got fine particle sizes and due to the large effective (specific) surface area of the fine particles, the hydrogen bonding interaction would have been very extensive and this might have greatly reduced the swelling tendency of the tablet matrix. Also the viscosity of the HPC used in the present investigation (Klucel LF) was low (75-150 mPas of 5% aqueous solution) that didn't allowed the formation of gel layer that could remain stable for sufficient period of time to act as a retarding membrane for the diffusion of the drug. All these factors contributed towards zero order release profiles.

The release mechanism and kinetics of the release profiles were analyzed by Korsmeyer-Peppas model ($M_t / M_\infty = K. t^n$) up to 60% release. The values of K, n, $t_{50\%}$ (time for 50%

of drug release) and r (correlation coefficient) obtained for various formulations are listed in Table 5.7. The n values for the formulations ranged from 0.65 to 0.76 indicating that the release mechanism was anomalous non-Fickian diffusion. Such mechanism of release indicates the combined contribution of diffusion and erosion towards the release. As the polymer ratio increased the release mechanism shifted from relaxation/erosion based release to diffusion/relaxation based release with decrease in the n values (0.76, 0.70, 0.68 and 0.64 at 20%, 40%, 60% and 80% polymer proportions respectively). This might be due the fact that as the polymer ratio increased the matrix became more resistant to erosion (because of the increased strength) and hence the release proceeded predominantly by diffusion through the pores present in the matrix structure. The release rate was fastest from the formulations containing 20% polymer (R24) with a K value of 0.096 $h^{-0.76}$ and $t_{50\%}$ 8.92 h. The release rate was slowest in case of 80% polymer formulations (R27) with a K value of 0.035 $h^{-0.64}$ and $t_{50\%}$ of 57.48 h.

5.2.3.2. Effect of HPMC viscosity

The effect of polymer viscosity grade on the release was studied at 20% polymer ratio. Three formulations containing constant proportion of HPMC (but different viscosity grades) were evaluated for in vitro release behavior. Plots of percent cumulative release vs. time for HPMC K100LV, K4M and K15M were shown in Figure 5.7. It was observed that at a constant polymer ratio the rate of drug release decreased with the increasing polymer viscosity or polymer molecular weight. This observation is in agreement with the other published works (Eyjolfsson 1999, Gao et al. 1996, Kim and Fassihi 1997). As shown in the Figure 5.6, in case of the lower viscosity HPMC K100LV (100 cPs) the release was extended only up to 12 h, but at the same levels HPMC K4M (4000 cPs) and HPMC K15M (15,000 cPs) could extend the release beyond 24 h. At the end of the 24 h only 83% and 56% of the drug was released from formulations with HPMC K4M and HPMC K15M. Initially (up to 5 h) there was no significant difference between the release profiles of rifampicin from K 100LV and K4M formulations. Infact, there was little lower release in case of K100LV formulations than K4M during initial hours. This might be due to the differences in their polymer disentanglement concentrations, which resulted in the quicker formation of gel layer in case of K100LV formulations compared to K4M

formulations. In the later hours, once the gel layer has been formed, it was the thickness or strength of the gel layer that caused the differences in the release profiles. Thus, in the later hours (after 5 h) the drug release rate was higher in K100LV formulations with complete release within 12 h as compared to K4M where the drug release was extended beyond 24 h. But, the difference in the release profiles between K4M and K15M formulations were very much evident from the first hour onwards (Figure 5.7).

Conceptually, the polymer viscosity effects can be explained from the perspective of polymer chain disentanglement. At the same polymer concentration, a polymer of higher viscosity induces greater chain entanglement than a polymer of low viscosity. Therefore, it is harder for longer chains to dissolve, because of high energy required for pulling them of the matrix. The result is slower dissolution rates for matrices made of higher viscosity grade polymer. In general, the fractional polymer release decreases with increasing viscosity/molecular weight as a result of increased chain entanglement. The drug release also decreases with increasing polymer viscosity as a result of decreased polymer dissolution. The above agreement suggests that, the polymer disentanglement concentration and diffusion coefficient of the polymer are primary determinants of the viscosity grade/molecular weight dependence of the drug release.

5.2.3.3. Effect of compression force on release

In general term increase in the compression force result in increase in the apparent density and thus reduction in matrix porosity. The exact nature of the pressure-density relationship is highly material dependent and also depends on experimental factors such as compression speed and size and shape of the tooling (York 1979, Nokhodchi et al. 1996). However, as the porosity of the hydrated matrix is independent of the initial porosity, the compression force seems to have little influence on the drug release once the hydrated layer forms. However, in case of nondisintegrating type of matrix tablets certain minimum level of hardness would be required to prevent the immediate disintegration of the tablet and burst release of the incorporated drug which would otherwise result in failure of the controlled drug release.

HPMC formulations

The effect of compression force reported to be more pronounced in case of lower viscosity grade HPMC polymers because they could deform most readily to fill interparticulate voids than the higher viscosity grade HPMC polymers. The lower viscosity grade HPMC polymers have an increased tendency to under go plastic deformation during compression, thereby showing more dependence on compression speed and compression force. Results were in good agreement with the above hypothesis. The effect of compression force was studied in three tablet batches containing 40% of HPMC 15 cPs (R3), 40% of HPMC K100LV (R9) and 20% of HPMC K4M (R12) at three different hardness levels of about 4, 6 and 10 kg/cm^2. In case of formulations with HPMC 15 cPs (Figure 5.8) the effect of compression force was very much pronounced. The drug release was extended only up to 3 h at lower applied compression force (~ 4 kg/cm^2 hardness). The drug release was extended up to 16 h and 20 h respectively when the compression force was increased to get the hardness of ~ 6 and 10 kg/ cm^2. The nature of the release profile was found to be zero order up to 20 h in case of formulations compressed at 10 kg/ cm^2 (r = 0.992). Similar results were reported by other research groups (Kim and Fassihi 1997, Nokhodchi et al. 1996). At the lower hardness levels (6-7 kg/ cm^2) there was a greater level of porosity (void spaces within the matrix), which allowed a greater liquid penetration in to the matrix, thus enhancing the polymer erosion and drug dissolution. But at the higher hardness levels (10-11 kg/ cm^2), there was an increased entanglement and increased rigidity of the matrix structure resulting in the reduced porosity of the matrix. The reduced porosity further resulted in the reduced surface area available for dissolution and the reduced wettability and water ingress leading to reduced polymer dissolution/erosion, which ultimately resulted in the reduction in the release rate of the drug.

Figure 5.9 and 5.10 depict the effect of compression force on the rifampicin release profiles from formulations with HPMC K100LV and K4M respectively. It can be seen from the Figure 5.9 that, at lower compression force (hardness 4 kg/cm^2) the tablet got immediately disintegrated and resulted in the higher initial release, and the complete drug release was occurred within 6 h in case of formulations with HPMC K100LV. This might be due insufficient tablet strength and there was a greater level of porosity (void spaces

within the matrix), which allowed a greater liquid penetration in to the matrix, thus enhancing the polymer erosion and drug dissolution. However, there was no significant difference between the release profiles of the formulations compressed at 6 and 10 kg/cm^2 hardness levels, even though there was a slight decrease in the release rate of the formulations with 10 kg/cm^2 hardness observed. In case of formulations with HPMC K4M (Figure 5.10), at lower compression force (4 kg/cm^2 hardness) the formulation showed an initial burst release due to a partial initial disintegration. Similar types of observations were reported by other research group (Kabanda et al. 1994). There were no significant differences in the release profiles of the tablet compressed at 6 ad 10 kg/cm^2 hardness levels. Thus, at the low applied compression force the lag time in the drug release was lower, may be because of the gel layer is formed after drug release has commenced. From our studies it was observed that the minimum hardness level of 6 kg/cm^2 was required in case of both K100LV and K4M HPMC formulations. Below 6 kg/cm^2 hardness, there was a chance of burst effect in case of higher viscosity grade (K4M) formulations and reduction in the extension of the release. Where as, in case of formulations with lower viscosity HPMC (K100LV), there was immediate disintegration of the tablets with complete drug release within 6 h. It was also observed that once the hardness of 6 kg/cm^2 was achieved, further increase in the compression force did not result in the significant changes in the release profiles and release character of the drug. Thus, in the present investigation the compression force that was applied to get the hardness of 6 kg/cm^2 was found to be optimum to get the desired extended release profiles.

There was a distinct difference in the release mechanism due to variation in the compression force in case of formulations with HPMC 15 cPs. The release mechanism was shifted from predominantly swelling/erosion controlled (at 6 kg/cm^2 hardness) to diffusion/polymer relaxation controlled due to increase in the compression force (at 10 kg/cm^2). The release mechanism was shifted to anomalous non-Fickian diffusion with a K value of 0.098 $h^{-0.77}$. The observed variation in the release mechanism may be due to enhanced entanglement and rigidity of the matrix at the increased compression force, which minimized the erosion of the matrix. The drug release was due to combination of both diffusion and polymer relaxation as indicated by the n value (0.77). There were no

significant differences in the release kinetics parameters for the HPMC K100LV formulations with 6 and 10 kg/cm^2 hardness (K = 0.037 h$^{-0.98}$, n = 0.98 and $t_{50\%}$ = 14.2 h at 10 kg/cm^2). In case of HPMC K4M formulations the release kinetics parameters were different for the formulations with 4 kg/cm^2 hardness than that for the formulations with 6 kg/cm^2 hardness (K = 0.352 h$^{-0.44}$, n = 0.44 and $t_{50\%}$ = 2.21 h). Where as, there was no significant difference in the release kinetics parameters between the formulations with 6 and 10 kg/cm^2 hardness (K = 0.206 h$^{-0.55}$, n = 0.55 and $t_{50\%}$ = 5.79 h). Thus, the compression force found to have insignificant influence on the release mechanism and release kinetics of the rifampicin from high viscosity HPMC (K100LV and K4M) formulations.

Carbopol 934P formulations

The effect of compression force was studied in case of 20% polymer formulations (R21). Compression force was varied to get three different hardness levels, 4, 6 and 11 kg/cm^2. The results are shown in the Figure 5.11. At lower compression force (hardness 4 kg/cm^2) the release rate was higher and the complete drug release was occurred within 10 h compared to 16 h in case of formulations with 7 kg/cm^2 hardness. This might be due the greater level of porosity (void spaces within the matrix) at low applied compression force, which allowed a greater liquid penetration in to the matrix and higher initial release of the drug through the pores present in the matrix. This might have also created more pores or pathways through the matrix structure as high amount of the drug released initially enhancing the diffusion of the remaining drug particles. The f_2 value of 34.51 between the release profiles of the formulations with 4 and 6 kg/cm^2 hardness further proved the significant difference between the release profiles. However, there was no significant difference between the release profiles of the formulations with 6 and 11 kg/cm^2 hardness, even though there was a slight decrease in the release rate of the formulations with 11 kg/cm^2 hardness (f_2 value of 85.66). Thus, at the low applied compression force (6 kg/cm^2 hardness) the lag time in the drug release was lower, may be because of the gel layer was formed after drug release has commenced.

There was no significant change in the release mechanism at different hardness levels (n value ranged from 1.10 to 1.44). Also the K values (0.019 h$^{-1.44}$and 0.018 h$^{-1.43}$

respectively) $t_{50\%}$ (9.91 h and 10.03 h) remained significantly unchanged at 6 kg/cm^2 and 11 kg/cm^2 hardness levels respectively. However, the K value increased (0.054 $h^{-1.10}$) and $t_{50\%}$ decreased (7.58 h) at 4 kg/cm^2 hardness compared to the formulations compressed at 6 kg/cm^2 (0.019 $h^{-1.44}$ and 9.91 h respectively).

HPC (Klucel LF) formulations

Effect of compression force on release was studied in the 20% formulation batch (R24). Compression force was varied to get three different hardness levels, 4, 8, and 12 kg/cm^2 (Figure 5.12). As can be seen form the Figure 5.12, there was a significant difference in the release profiles among the formulations compressed at different compression force levels. The release was extended only up to 8 h in case of formulations with 4 kg/cm^2 hardness. Where as, in case of formulations with 8 kg/cm^2 and 12 kg/cm^2 hardness, ~ 87% and ~ 70% drug was released respectively at the end of 24 h release studies. This might be due to the fact that low viscosity HPC (Klucel LF) polymers were very much sensitive to the effect of particle size and compression force. At increased compression force probably there might have been increased brittle fracture of the material that resulted in the particle size reduction. Because of the increased effective surface area (due to decrease in the particle size) there might be increased interparticulate friction and cohesive force (hydrogen bonding) that lead to the formation of a very strong compact mass at increased hardness levels. This compact matrix (that contained less interparticulate voids and pores) provided the higher resistance for the drug diffusion and matrix erosion, which ultimately reduced the drug release rate. The f_2 factor value found to be 24.74 between the release profiles of the formulations with 4 and 8 kg/cm^2 hardness respectively indicating the significant difference between the release profiles. The f_2 factor value found to be 44.23 between the release profiles of the formulations with 8 and 12 kg/cm^2 hardness respectively indicating the significant difference between the release profiles.

There was significant difference in the release mechanism and release kinetics parameters among the formulations compressed at different compression forces. At lower compression force (4 kg/cm^2 hardness) drug release was predominantly by diffusion (n = 0.54), at 8 kg/cm^2 the release was by the combination of diffusion and erosion (by

anomalous non-Fickian diffusion) (n = 0.76) and at higher compression force (12 kg/cm^2 hardness) the drug was released predominantly by relaxation/erosion mechanism (n = 0.83). The change in the release mechanism might be due to the compactness of the matrix structure and initial porosity of the matrix. At low applied force the tablet matrix might contained more interparticulate voids and pores that enhanced the diffusion of the drug molecules. As the compression force increased the matrix became more compact (decreased void spaces and reduced porosity of the matrix) and thus drug release was mainly by erosion. The K values were significantly different at different compression forces (0.26 $h^{-0.54}$, 0.096 $h^{-0.76}$ and 0.078 $h^{-0.83}$ respectively at 4, 8 and 12 kg/cm^2 hardness respectively). There was a significant difference between the $t_{50\%}$ values as well (3.35, 8.92 and 9.73 h) at 4, 8 and 12 kg/cm^2 hardness respectively.

5.2.3.4. Effect of method of granulation

Although preliminary studies were carried out in selecting the method of granulation for a particular formulation batch, the effect of granulation methodology in case of formulation with 40% HPMC 15 cPs (R3) was evaluated. The results are shown in the Figure 5.13. It can be observed from the release profile that the drug release was faster in case of formulations made by direct compression compared to those formulated with IPA granulation. The drug release was extended up to 8 h in case of formulations made by direct compression (DC) compared to 16 h in case of IPA granulated formulations. Especially the drug release was very much higher during initial hours in case of DC formulations where about 34% of the drug was released within 1 h compared to just about 4% in case of IPA granulated matrices. The observed variation in the drug release with the change in the method of granulation might be explained as follows. In case of IPA granulated matrices the drug either has to diffuse only through the hydrated gel layer or has to be released as the matrix gets eroded with time because the drug exist within the granules along with the polymer and rarely any amount of the drug was present in between the granules (intergranularly) as a free drug immediately available for diffusion or release. But in DC formulations the drug was present in a physical mixture along with polymer as a uniform dispersion. Thus, the drug was free to diffuse through the pores present in the matrix structure before the gel layer has been formed. This resulted in the

increased drug release during initial hours by diffusion through the pores present in the matrix also because of the availability of the free drug on the unhydrated matrix surface. The method of granulation also profoundly affected the release mechanism and kinetics of rifampicin release from the HPMC 15 cPs matrix tablets. The release mechanism was found to be predominantly a non-Fickian diffusion with n value of 0.57 in case of DC tablets, where as with IPA granulated formulations the n value observed to be 1.00. This was due to the predominance of diffusional release in DC tablets through the less tortuous (unswelled and more porous) matrices compared to erosional release in case of IPA granulated tablets. The K value increased in DC tablets (0.336 $h^{-0.57}$) with reduction in the $t_{50\%}$ to 2 h.

5.2.3.5. Effect of change in the dissolution method (type)

For this study, formulation with 50% HPMC 15 cPs (R4) was selected. The in vitro release studies have been carried out by two different methods, USP type I (basket) and USP type II (paddle) method, at 100 rpm. The results are shown in the Figure 5.14. When the method was changed from basket to paddle, it was observed that the release rate got enhanced and there was a reduction in the extension of the release. In case of basket method the release was extended up to 16 h where as, with paddle method the release was extended up to 12 h. The difference in the release rates from the formulations between basket and paddle method was further analyzed by f_2 factor value. The f_2 factor value was found to be 34.61 indicating the significant difference. The observed difference in the release rates might be due to the increased hydrodynamic stress because of greater turbulence in case of paddle method compared to basket at a given speed of rotation (dissolution rpm). This might be the probable reason for different 'rpm' (rotations per minute) specifications for basket and paddle dissolution methods prescribed by US FDA and US Pharmacopoeia. According to these guidelines for the in vitro release studies of controlled release (extended release) dosage forms the rpm mentioned is 50-75 in case of paddle method and 100 in case of basket method. As per these guidelines it can be assumed that equal amount of the drug would be released at lower rpm speed for paddle method (50-75 rpm) compared to higher rpm levels for basket method (100 rpm). Thus, the results of the present investigations were in accordance with the guidelines.

There was no change in the release mechanism between the release profiles of basket and paddle methods. In both the cases the mechanism of release was found to be super Case-II (n = 0.96 for basket and 1.12 for paddle method respectively). Even the K values remained almost same (0.080 for basket and 0.078 for paddle method respectively). But the $t_{50\%}$ values were slightly different in both cases; it was 6.20 h for basket method and 5.34 for paddle method. Thus, the $t_{50\%}$ values again proved that the release was extended for a longer period in case of basket method compared to paddle method.

5.2.3.6. Effect of hydrodynamic conditions (stirring speed of basket)

This study was undertaken on formulation with 40% HPMC 15 cPs (R3) and 20% HPMC K4M (R12) formulations. In vitro release studies were carried out at three different stirring speeds, 50, 100 and 150 rpm. The results are shown in the Figure 5.15 for formulations HPMC 15 cPs. As the rpm (speed of rotation of basket) increased from 50 to 150, the release rate increased. At 50 rpm, ~ 86% drug was released after 16 h release studies compared to ~ 100% at 100 rpm within same time period. Where as, at 150 rpm complete drug release was there (~ 98%) within 10 h. The observed variation in the release with change in the rpm might be due to the difference in the hydrodynamic stress around the surface of the tablet undergoing dissolution. At lower rpm (50), there was a slow fluid (release media) motion and formation of stable stagnant layer surrounding the tablet. This prevented the quick entry of the release medium in to the tablet and also the release of the drug out of the tablet. But, as the rpm was increased (to 100 and 150), there was a greater fluid flow that resulted in the increased attrition of the tablet matrix at the swelling/dissolution front. This phenomenon caused the increased erosion of the matrix and decrease in the stagnant diffusion layer thickness that ultimately resulted in the increased drug release. The increase in the drug release with increase in the rpm again confirmed that the mechanism of release in case of HPMC 15 cPs formulations was erosion dependent as erosion based drug delivery systems are more susceptible to the effect of hydrodynamic conditions (Kim and Fassihi 1997). The f_2 (similarity factor) value was 46.52 between 50 and 100 rpm in case of 15 cPs formulations, and it was 43.18 between 100 and 150 rpm. These f_2 values indicated the significant difference between the release rates at different speed of rotations of basket (rpm). There was no significant

change in the release mechanism (n value ranged between 0.93-1.00). However, K value decreased at 50 rpm to 0.057 $h^{-1.02}$ and increased at 150 rpm to 0.131 $h^{-0.92}$ compared to that at 100 rpm (0.075 $h^{-1.00}$). Similarly there was increase in the $t_{50\%}$ to 8.34 h at 50 rpm and decrease at 150 rpm to 4.67 h compared to that at 100 rpm (6.70 h). These differences in the K and $t_{50\%}$ values further demonstrated the difference between the rate and extent of release.

The results of effect of hydrodynamic conditions on release for formulations with HPMC K4M are shown in the Figure 5.16. No significant changes in the release profiles were observed with variations in the basket rpm. However, there was slight increase in the drug release at higher rpm and decrease in the release at lower rpm. This might be due to the fact that the diffusional controlled drug delivery systems, where the drug release follows square root kinetics model, are less sensitive to the effect of hydrodynamic conditions or stirring rates (rpm) (Lapidus and Lordi 1968). Thus, we could say that these formulations were more robust to the effects of hydrodynamic conditions (in terms of basket rpm), which is one of the important desired characters for an ideal oral controlled release drug delivery system. The release profiles were further analyzed by f_2 factor values. The f_2 values were found to be 67.00 between 50 and 100 rpm and, 57.31 between 100 and 150 rpm. These f_2 values for K4M formulations further indicated that the release rates were not significantly different at different speed of rotations of basket (rpm). There were no significant changes observed in the release mechanism (0.48-0.50) and K values (0.253 $h^{-0.48}$, 0.222 $h^{-0.48}$ and 0.260 $h^{-0.50}$ respectively for 50, 100 and 150 rpm), but the $t_{50\%}$ values were slightly different (5.84 h, 5.36 h and 4.14 h respectively at 50, 100 and 150 rpm).

5.2.3.7. Effect of addition of free drug

This study was done in case of formulation with 50% HPMC 15 cPs (R4). Here 85% of the drug was granulated along with the polymer and rest of the drug (15%) was added along with talc and magnesium stearate extragranularly while maintaining the drug to polymer ratio same. The results are shown in the Figure 5.17. As can be seen from the release profile, there was increase in the release in case of formulation containing 15% free drug with release extended up to 12 h compared to the 16 h in case formulation

containing no (0%) free drug. The difference in the release was notable starting from the first hour of release studies. During initial hours of release the free drug might have been immediately available for the release as it was present out side the granules and as tablet came in contact with the release medium free drug (present on the surface and in between the granules) immediately got exposed to the medium and released before the formation of hydrated gel layer. This initial drug release caused formation of more pores or channels (pathways) through which the remaining drug particles (present in side the granules) diffused/released at a faster rate. Thus, overall drug release was increased in case of formulations containing free drug. The release mechanism however, remained unchanged (Super Case-II release with n value of 0.97). But the K value increased to 0.123 $h^{-0.97}$ compared to 0.080 $h^{-0.96}$ in the absence of free drug, and the $t_{50\%}$ value decreased to 4.26 h compared to 6.20 h in the absence of the free drug. Release profiles were further analyzed for f_2 factor value. The f_2 factor value observed to be 40.10 indicating the significant difference between the release profiles. The increase in the K value, decrease in the $t_{50\%}$ value and f_2 factor value all together supported the fact that inclusion of free drug (15% extragranularly) increased the drug release rate. Thus, it could be used as an alternative tool for achieving initial higher release to avoid the incorporation of loading dose in case of formulations with low viscosity HPMC (15 cPs).

5.2.4. Erosion studies

Erosion studies with tablets containing 40%, 50% and 60% HPMC 15 cPs (R3, R4 and R5) revealed that there was no significant difference in their erosion rates as indicated by the f_2 factor (similarity factor) values ($f_2 \geq 85$). The results (Figure 5.18) showed that the matrix erosion follows a linear profile with the time in all three cases. The linear matrix erosion profiles correspond with the linear release profiles of the drug in each case, which again supported the fact that the release was significantly dependent upon the erosion mechanism. Matrix erosion might occurred because of the stress developed due to hydration and swelling of the HPMC polymer could not be compensated, as rifampicin was a poorly soluble drug. Due to faster erosion rates in R3, R4 and R5 formulations, the diffusion pathlength for the drug might be constant and perhaps due to this reason the release rate was found to be nearly zero order.

The reason for the erosion based drug release in case of low viscosity HPMC formulations containing poorly soluble drug rifampicin could be explained as follows. As the drug solubility was low in the selected release media, the release rate slowed down. Then, there was an increased probability for the drug particles to be transported within the gel. This particle translocation reduced the stability of the gel layer that lead to the reduction in the thickness of the diffusive pathway. Since the presence of solid particles also affects the entanglement of polymer chains, thus increasing the fragility of the gel, erosion was favored. The higher viscosity/molecular weight HPMC polymers were resistant to erosion as the gel layer formed was rigid enough and polymer entanglement was stronger that reduced the tendency to erosion.

5.2.5. Batch Reproducibility

Three batches of each formulation were prepared and their quality and respective release characters were evaluated under the same conditions as prescribed in previous sections. The physical properties of the tables from all three batches were evaluated in the same manner as for the original batch formulations. The tablets showed low standard deviation values for the drug content, friability, weight variation and hardness from three different batches prepared separately (data not shown). These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility and absence of significant variations between batch-to-batch. In vitro release data pertaining to reproducibility studies were compared by f_2 metric (similarity factor) values. No significant difference was observed in the release profiles of the formulations between different batches as indicated by the low standard deviation values of the percent cumulative release data at different time points obtained from the replicate release studies of the samples and from high similarity factor values. The detailed release profiles (percent cumulative release vs. time data points) of the formulations from different batches have not shown or given. Instead, the f_2 factor values for the selected formulation batches have been calculated and given as follows. The f_2 values varied from 69.72 to 87.39 for release profiles of R2, R5, R7, R10, R12, R14, R17, R19, R20, R22, R24 and R26 formulations between original and reproduced batches (calculated from the average percent release at each time point with standard deviation values less than 3%). Thus, the

reproducible physical properties in terms low standard deviation for each parameter from different batches and significantly similar release profiles of rifampicin from all batches (proved by f_2 factor values) indicated that the formulation methodology employed (IPA granulation in case of formulations with HPMC 15 cPs and direct compression with other polymer formulations) found to be suitable for manufacturing the good quality C.R. matrix tablets of rifampicin.

5.2.6. Stability Studies

The two best formulations from each batch of formulations, one with lower polymer proportion and another with higher, were selected for stability studies (Table 5.8). The drug content in triplicate was determined for each formulation by the spectrophotometric method described in chapter 3 and the observed degradation rate constants at different storage conditions and corresponding $t_{90\%}$ values are listed in Table 5.8. The rifampicin in matrix embedded tablets (in case of all polymer formulations) found to follow first order degradation as the plots of log percent drug content remaining vs. time found to be linear (with "r" value more than 0.956 in all cases and individual plots not given). The K_{deg} values for rifampicin in various formulations ranged from 4.94×10^{-3} $month^{-1}$ to 6.78×10^{-3} at CRT, 6.31×10^{-3} $month^{-1}$ to 7.86×10^{-3} $month^{-1}$ at 40 ± 2 °C and 8.25×10^{-3} $month^{-1}$ to 12.04×10^{-3} $month^{-1}$ at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 5.8). In case of all matrix tablets the degradation rate constant increased with increase in the polymer proportion. The $t_{90\%}$ values for rifampicin in various formulations ranged from 15.55 to 22.11 months at CRT, from 13.41 to 16.71 months at 40 ± 2 °C and from 8.75 to 12.77 months at 40 ± 2 °C/75 ± 5% RH (Table 5.8). Rifampicin found to be more stable at CRT and less stable at 40 ± 2 °C/75 ± 5% RH in all formulations. Among the HPMC polymers, rifampicin was comparatively more stable in HPMC K4M formulations as the degradation rate constant values found to be comparatively low and $t_{90\%}$ found be comparatively high at all storage conditions (Table 5.8). Rifampicin found to be comparatively less stable in HPMC 15 cPs formulations (among HPMC formulations) (Table 5.8). Among the HPC and Carbopol formulations, rifampicin found to be more stable in HPC formulations (Table 5.8). Among all the

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The two best formulations from each batch of formulations, one with lower polymer proportion and another with higher, were selected for stability studies (Table 5.8). The drug content in triplicate was determined for each formulation by the spectrophotometric method described in chapter 3 and the observed degradation rate constants at different storage conditions and corresponding $t_{90\%}$ values are listed in Table 5.8. The rifampicin in matrix embedded tablets (in case of all polymer formulations) found to follow first order degradation as the plots of log percent drug content remaining vs. time found to be linear (with "r" value more than 0.956 in all cases and individual plots not given). The K_{deg} values for rifampicin in various formulations ranged from 4.94×10^{-3} $month^{-1}$ to 6.78×10^{-3} at CRT, 6.31×10^{-3} $month^{-1}$ to 7.86×10^{-3} $month^{-1}$ at 40 ± 2 °C and 8.25×10^{-3} $month^{-1}$ to 12.04×10^{-3} $month^{-1}$ at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 5.8). In case of all matrix tablets the degradation rate constant increased with increase in the polymer proportion. The $t_{90\%}$ values for rifampicin in various formulations ranged from 15.55 to 22.11 months at CRT, from 13.41 to 16.71 months at 40 ± 2 °C and from 8.75 to 12.77 months at 40 ± 2 °C/75 ± 5% RH (Table 5.8). Rifampicin found to be more stable at CRT and less stable at 40 ± 2 °C/75 ± 5% RH in all formulations. Among the HPMC polymers, rifampicin was comparatively more stable in HPMC K4M formulations as the degradation rate constant values found to be comparatively low and $t_{90\%}$ found be comparatively high at all storage conditions (Table 5.8). Rifampicin found to be comparatively less stable in HPMC 15 cPs formulations (among HPMC formulations) (Table 5.8). Among the HPC and Carbopol formulations, rifampicin found to be more stable in HPC formulations (Table 5.8). Among all the

polymer formulations, rifampicin found to be comparatively more stable in HPMC K4M and HPC polymer formulations.

It was observed that with the raise in the temperature (from 25± 2 °C to 40 ± 2 °C) the K_{deg} values increased and $t_{90\%}$ values decreased in case of all polymer formulations (in all polymer ratios). This might be due to the increased frequency of collisions between the reacting drug molecules at higher temperature conditions (according to Arrhenius theory). To establish the effect of humidity on the stability characteristics of the rifampicin in polymer formulations the degradation studies were carried out at 40 ± 2 °C and 40 ± 2 °C/75 ± 5% RH and, the results were compared (Table 5.8). The K_{deg} values were much higher at 40 ± 2 °C/75 ± 5% RH compared to 40 ± 2 °C in all the cases studied (Table 5.8). Thus, from our studies, it was observed that the humidity was the one of the most important parameter that affected the stability of rifampicin in all polymer formulations. It was also observed that the deleterious effect of humidity on the stability of the rifampicin was more pronounced than the temperature (as can be observed from the K_{deg} values and $t_{90\%}$ values at CRT, 40 ± 2 °C and 40 ± 2 °C/75 ± 5% RH in case of all formulation batches). Thus, the applicability of the Arrhenius relationship for the estimation of room temperature stability from extrapolated high temperature data found to be inappropriate (not justified) as the degradation was not solely dependent upon the temperature and other mechanism (in this case humidity) found to have significant contribution in the degradation of rifampicin (USFDA 2003, Lachman et al. 1991). The increased K_{deg} values found at higher humidity condition again supported that the avoidance of aqueous granulation technology (use of either IPA granulation or direct compression) in the manufacturing of rifampicin matrix tablets was significantly beneficial in obtaining the stable C.R. matrix tablets of rifampicin.

The in vitro release profiles were studied as per the specifications enlisted in previous sections and compared with their respective initial release profiles. The in vitro release profiles of the formulations stored at CRT for 6 months were compared with their initial release profiles (0 time samples at CRT) with f_2 factor values. The f_2 factor values in all cases found to be more than 58 indicating that the rifampicin release profiles were significantly similar for zero time samples and 6 months samples (stored at CRT). Thus, the in vitro release characteristics were not significantly affected by the stability studies

(storage at CRT) for about 6 months showing that the formulations were stable in terms of release characteristics.

5.3. Conclusions

The designed matrix tablets of rifampicin showed good physical properties indicating that the method of preparation of formulation is suitable and acceptable method for preparing good quality and reproducible matrix tablets of rifampicin. The tablet manufacturing method was relatively simple and can be easily adopted in conventional tablet manufacturing units in industries on a commercial scale.

Increase in the polymer ratio, irrespective of the polymer type and viscosity grade, decreased the rifampicin release except in case of formulations with HPMC 15 cPs where there was no significant difference in the release profiles when the polymer proportion increased beyond 40% (w/w of the rifampicin). Increase in the polymer viscosity decreased the rifampicin release rate. The effect of compression force was more pronounced in case of formulations with lower viscosity HPMC (15 cPs) and in HPC (Klucel LF). The rifampicin release was faster from the directly compressed tablets than from the IPA granulated formulations. The rifampicin release was higher in USP type II (paddle) method compared to USP type I (basket method). Increased stirring speed in the release media resulted in the increased rifampicin release from HPMC 15 cPs formulations (that exhibit erosional release of rifampicin). Addition of 15% free drug (rifampicin) resulted in the increased initial release.

The rifampicin release found to follow super Case-II release mechanism (erosion dependent) in case of formulations with low viscosity HPMC (15 cPs) and in Carbopol 934P. The mechanism of rifampicin release in case of medium viscosity HPMC (K100LV) formulations found to be dependent on the polymer proportion. In case of formulations with high viscosity HPMC (K4M and K15M) and in HPC (Klucel LF), the mechanism of rifampicin release found to be predominantly by non-Fickian diffusion at all polymer ratios.

In the present study a series of formulations was developed with different release rates and duration. The duration of rifampicin release was extended from 4 h to beyond 24 h by varying the polymer type, polymer ratio, polymer viscosity and processing techniques.

The nature of the rifampicin release followed zero order, first order, and Higuchi's square root kinetics depending on polymer type and their viscosity. The low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility. The formulations were found to be stable as observed from the stability studies. Avoidance of aqueous granulation technology (use of either IPA granulation or direct compression) in the manufacturing of rifampicin matrix tablets was found to be significantly beneficial in obtaining the stable matrix tablets of rifampicin.

These systems could be the alternate treatment regimens for the TB control with improved patient compliance as well as lower probability of incidence of toxic side effects. These systems can reduce the pH dependent degradation and bioavailability problems associated with rifampicin and hence can lead to the better bioavailability of the drug at the existing dose levels.

Table 5.1. Formula and physical properties of rifampicin matrix tablets prepared with HPMC 15 cPs.

Formulations	R 1	R 2	R 3	R 4	R 5
Components *					
Drug (mg)	450	450	450	450	450
HPMC[a] (%)	20	30	40	50	60
Physical Properties					
Drug Content (% Label Claim)[b]	101.4 ± 1.3	100.8 ± 1.4	100.9 ± 0.6	101.2 ± 0.9	99.2 ± 1.5
Weight variation (%)[c]	± 1.8	± 2.1	± 1.9	± 2.4	± 2.2
Hardness (kg/cm^2)[d]	6.5 ± 0.6	6.2 ± 0.4	6.7 ± 0.4	6.4 ± 0.6	6.8 ± 0.3
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 5.2. Formula and physical properties of rifampicin matrix tablets prepared with HPMC K100LV.

Formulations	R 6	R 7	R 8	R 9	R 10
Components *					
Drug (mg)	450	450	450	450	450
HPMC[a] (%)	10	20	30	40	60
Physical Properties					
Drug Content (% Label Claim)[b]	102.1 ± 0.7	100.2 ± 1.3	99.6 ± 0.9	100.7 ± 1.4	98.9 ± 1.2
Weight variation (%)[c]	± 2.8	± 1.6	± 2.4	± 2.7	± 1.8
Hardness (kg/cm^2)[d]	6.6 ± 0.7	6.4 ± 0.6	6.8 ± 0.5	6.9 ± 0.8	6.5 ± 0.5
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 5.3. Formula and physical properties of rifampicin matrix tablets prepared with HPMC K4M.

Formulations	R 11	R 12	R 13	R 14	R 15†
Components *					
Drug (mg)	450	450	450	450	450
HPMC[a] (%)	10	20	30	40	10
Physical Properties					
Drug Content (% Label Claim)[b]	101.1 ± 0.9	102.4 ± 1.6	99.4 ± 1.4	101.3 ± 1.2	99.9 ± 1.3
Weight variation (%)[c]	± 1.9	± 2.5	± 2.2	± 1.9	± 2.3
Hardness (kg/cm^2)[d]	6.8 ± 0.4	6.6 ± 0.8	6.5 ± 0.9	6.3 ± 0.6	6.7 ± 0.7
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

† Also contains 30% HPMC K100LV. * Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 5.4. Formula and physical properties of rifampicin matrix tablets prepared with HPMC K15M.

Formulations	R 16	R 17	R 18	R 19
Components *				
Drug (mg)	450	450	450	450
HPMC[a] (%)	5	10	20	30
Physical Properties				
Drug Content (% Label Claim)[b]	101.1 ± 1.2	99.6 ± 1.5	99.8 ± 1.4	102.3 ± 1.0
Weight variation (%)[c]	± 1.9	± 2.3	± 1.8	± 2.2
Hardness (kg/cm^2)[d]	6.4 ± 0.7	6.8 ± 0.8	6.6 ± 0.6	6.5 ± 0.6
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 5.5. Formula and physical properties of rifampicin matrix tablets prepared with Carbopol 934P.

Formulations	R 20	R 21	R 22	R 23
Components *				
Drug (mg)	450	450	450	450
Carbopol[a] (%)	10	20	30	40
Physical Properties				
Drug Content (% Label Claim)[b]	102.2 ± 1.4	100.8 ± 0.7	99.4 ± 1.6	101.9 ± 1.3
Weight variation (%)[c]	± 2.1	± 1.5	± 1.9	± 2.5
Hardness (kg/cm^2)[d]	7.3 ± 0.5	7.6 ± 0.7	7.7 ± 0.8	7.5 ± 0.8
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 5.6. Formula and physical properties of rifampicin matrix tablets prepared with HPC (Klucel LF).

Formulations	R 24	R 25	R 26	R 27
Components *				
Drug (mg)	450	450	450	450
HPC[a] (%)	20	40	60	80
Physical Properties				
Drug Content (% Label Claim)[b]	101.9 ± 1.3	102.4 ± 1.6	100.3 ± 1.7	99.2 ± 1.2
Weight variation (%)[c]	± 2.4	± 1.9	± 2.3	± 2.7
Hardness (kg/cm^2)[d]	7.4 ± 0.6	7.8 ± 0.9	7.5 ± 0.7	7.9 ± 0.5
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 5.7. Release kinetics parameters for rifampicin C.R. formulations.

Formulations	Peppas model parameters				r^d for Zero Order	r^d for First Order	r^d for Higuchi's Kinetics
	n^a	K^b (h^{-n})	$t_{50\%}{}^c$ (h)	r^d			
R1	1.03	0.297	1.68	0.999	0.812	0.998	0.944
R2	1.28	0.172	2.91	0.985	0.820	0.993	0.926
R3	1.00	0.075	6.70	0.994	0.974	0.700	0.920
R4	0.96	0.080	6.20	0.997	0.972	0.875	0.921
R5	0.95	0.083	6.03	0.996	0.976	0.856	0.924
R7	0.65	0.190	4.61	0.994	0.974	0.971	0.971
R8	0.93	0.063	9.28	0.998	0.998	0.812	0.891
R9	0.98	0.039	13.41	0.999	0.999	0.895	0.902
R10	1.01	0.027	17.60	0.999	0.998	0.951	0.897
R11	0.54	0.384	1.64	0.994	0.921	0.859	0.998
R12	0.48	0.222	5.36	0.998	0.780	0.942	0.969
R13	0.54	0.103	18.17	0.997	0.931	0.982	0.994
R14	0.52	0.062	54.13	0.995	0.943	0.969	0.992
R15	0.46	0.241	4.83	0.974	0.846	0.973	0.987
R17	0.51	0.200	5.75	0.980	0.822	0.932	0.981
R18	0.55	0.096	20.39	0.997	0.929	0.954	0.996
R19	0.76	0.034	33.18	0.997	0.970	0.989	0.974
R20	1.33	0.027	8.85	0.959	0.950	0.652	0.762
R21	1.44	0.019	9.91	0.961	0.958	0.824	0.802
R22	1.51	0.012	11.46	0.986	0.915	0.952	0.875
R23	1.34	0.010	19.38	0.969	0.953	0.975	0.921
R24	0.76	0.096	8.92	0.987	0.935	0.973	0.972
R25	0.70	0.071	16.26	0.988	0.934	0.981	0.976
R26	0.68	0.050	29.46	0.984	0.908	0.941	0.978
R27	0.64	0.035	57.48	0.986	0.961	0.983	0.969

[a] Diffusional exponent indicative of the release mechanism; [b] release rate constant; [c] time for 50 % of the drug release; [d] correlation coefficient.

Table 5.8. Stability studies of rifampicin C.R. formulations at different storage conditions.

Formulations	CRT		40 ± 2°C		40 °C/75% RH	
	$K_{deg} \times 10^3$ ($month^{-1}$)*	$t_{90\%}$ (month)	$K_{deg} \times 10^3$ ($month^{-1}$)*	$t_{90\%}$ (month)	$K_{deg} \times 10^3$ ($month^{-1}$)*	$t_{90\%}$ (month)
R2	6.28	16.78	7.41	14.23	10.97	9.61
R5	6.78	15.55	7.69	13.70	11.71	9.00
R7	5.82	18.12	6.75	15.61	9.69	10.88
R10	6.28	16.78	7.14	14.76	10.30	10.23
R12	4.77	22.11	6.31	16.71	8.25	12.77
R14	5.00	21.09	6.64	15.86	8.82	11.95
R17	5.19	20.31	6.57	16.05	9.03	11.67
R19	5.53	19.04	6.91	15.25	9.71	10.85
R20	6.14	17.15	7.34	14.35	10.75	9.80
R22	6.60	15.97	7.86	13.41	12.04	8.75
R24	4.94	21.34	6.34	16.61	8.77	12.01
R26	5.16	20.41	6.69	15.75	9.45	11.15

* First order degradation rate constant based on average of triplicate assay values at five time points with C.V. less than 4.0% in all cases.

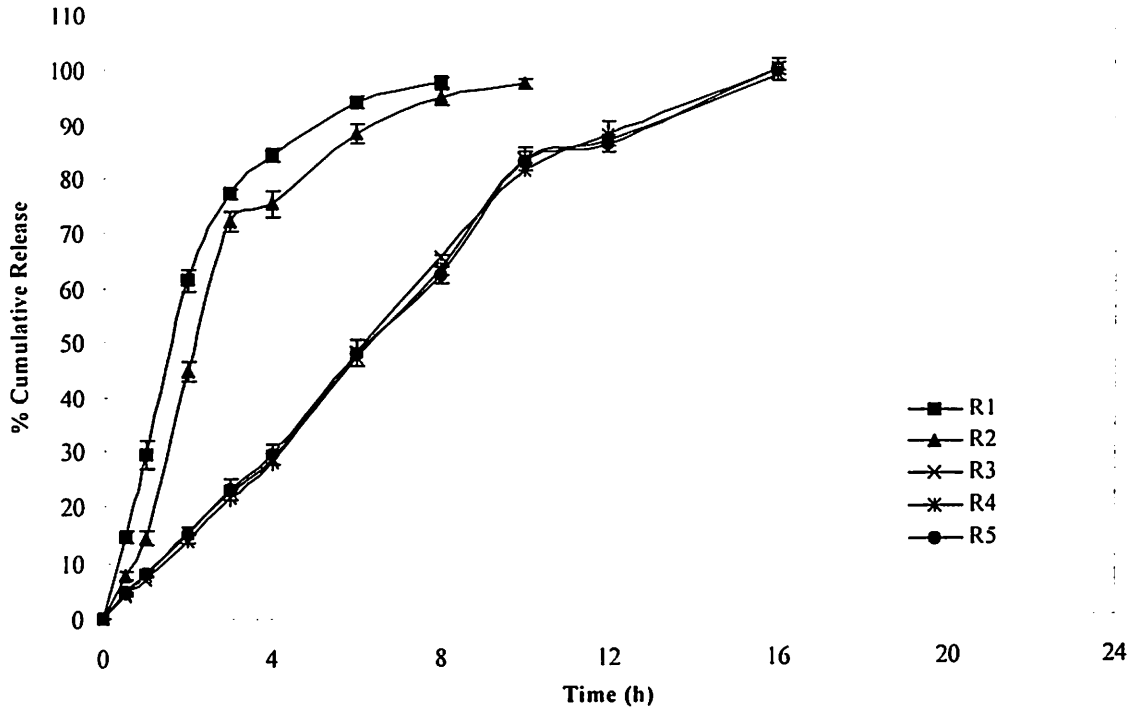

Fig 5.1. Comparative release profiles of rifampicin from HPMC 15 cPs formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

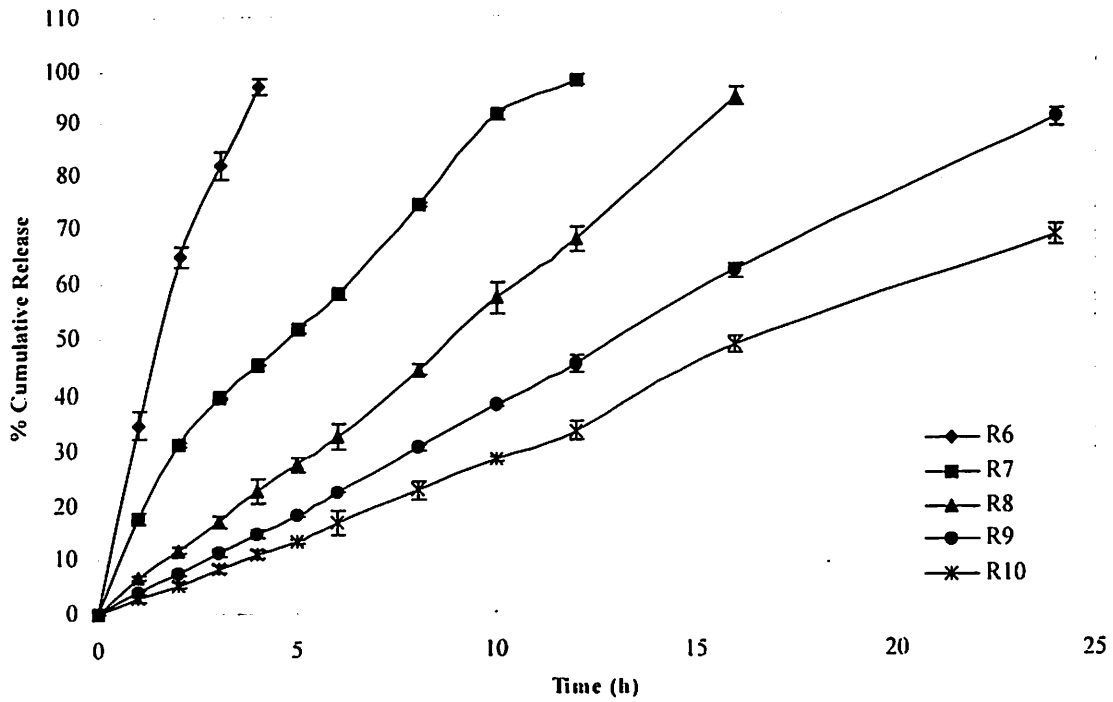

Fig 5.2. Comparative release profiles of rifampicin from HPMC K 100LV formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

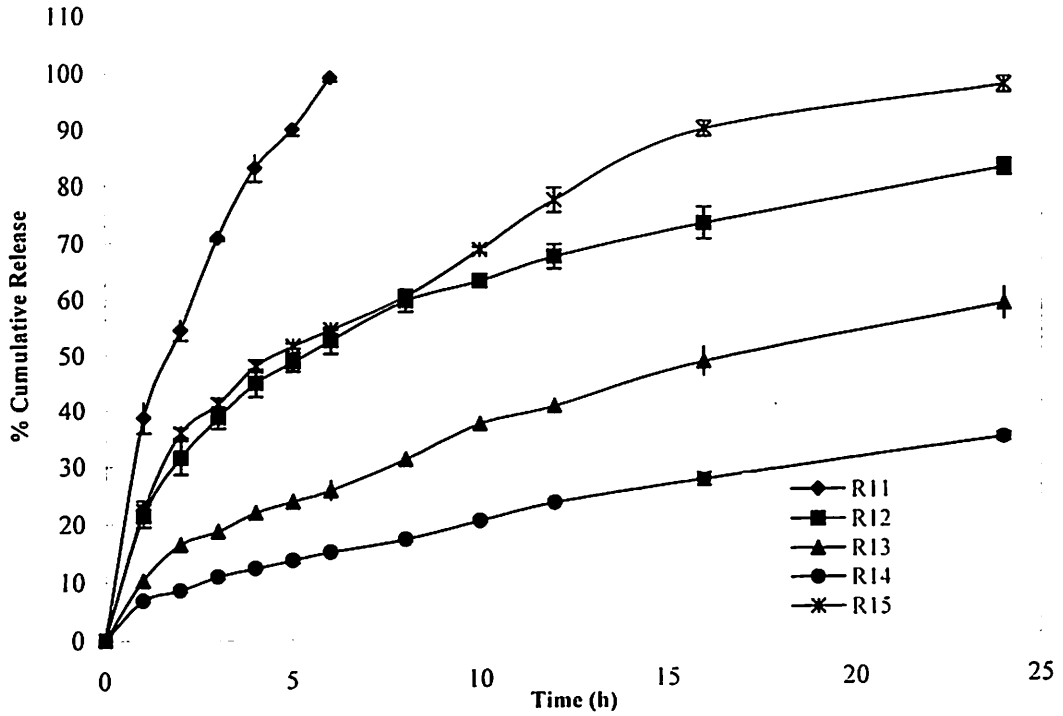

Fig 5.3. Comparative release profiles of rifampicin from HPMC K4M formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

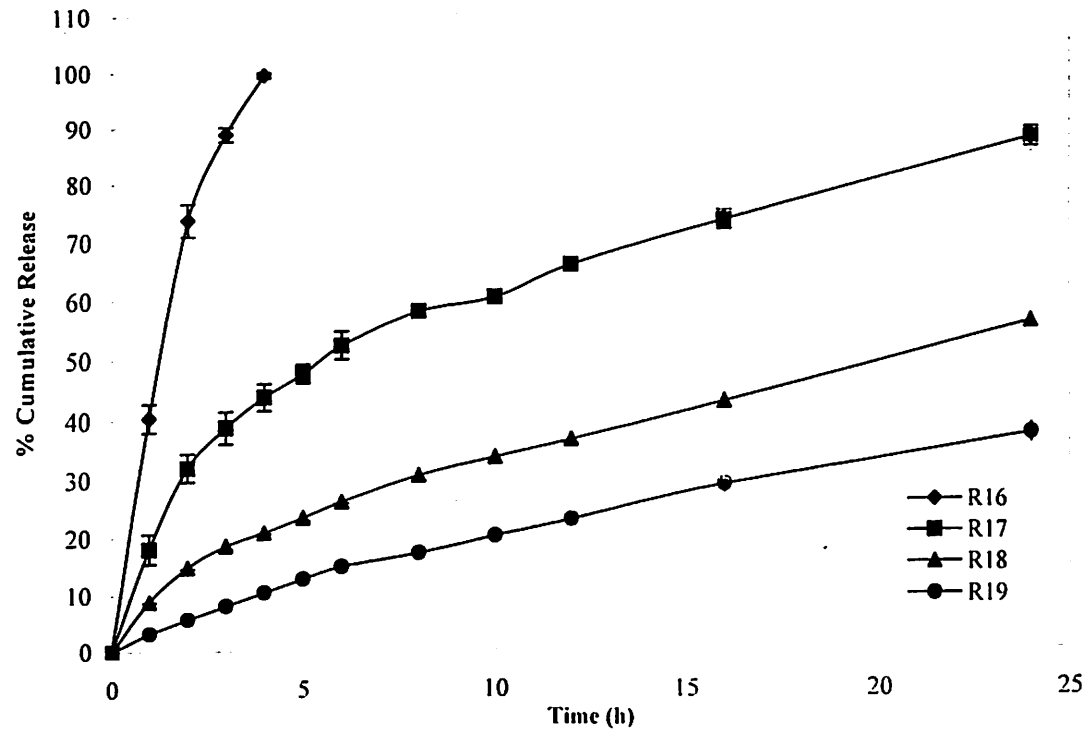

Fig 5.4. Comparative release profiles of rifampicin from HPMC K15M formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

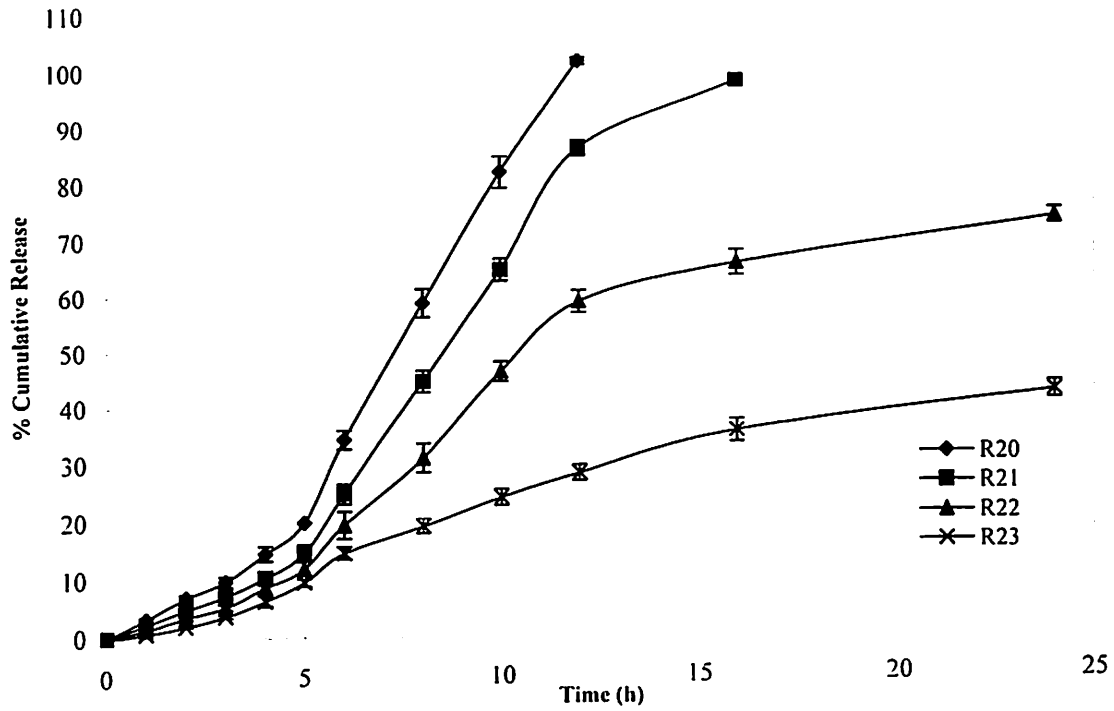

Fig 5.5. Comparative release profiles of rifampicin from Carbopol 934P formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

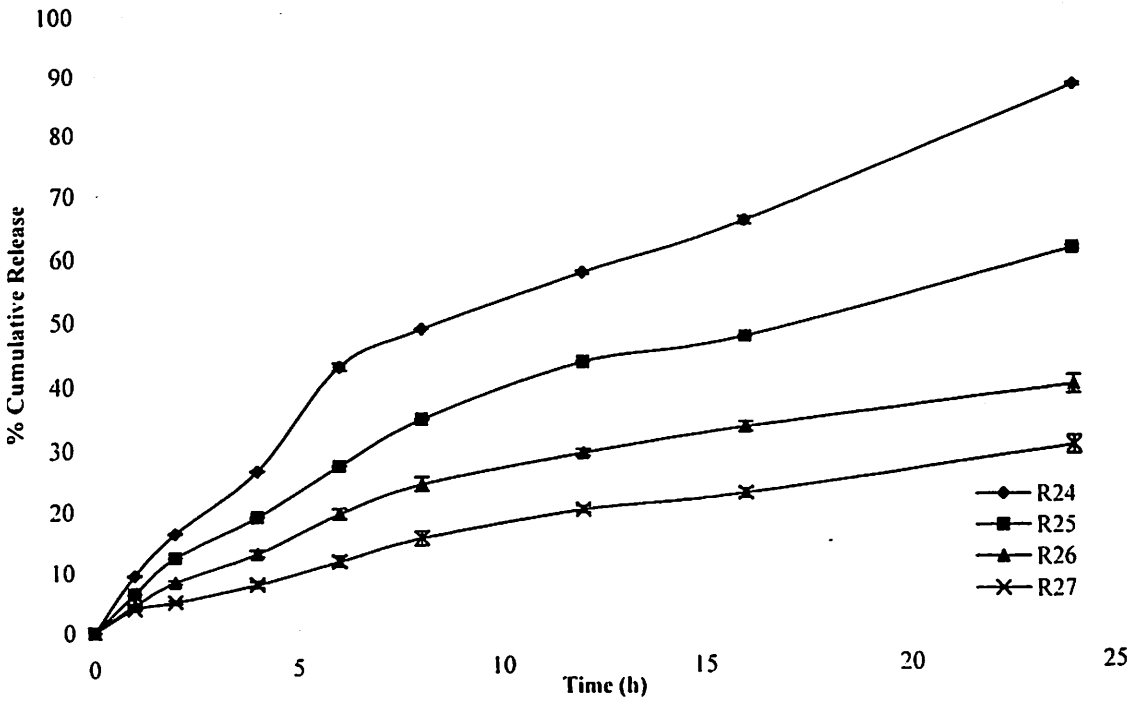

Fig 5.6. Comparative release profiles of rifampicin from HPC (Klucel LF) formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

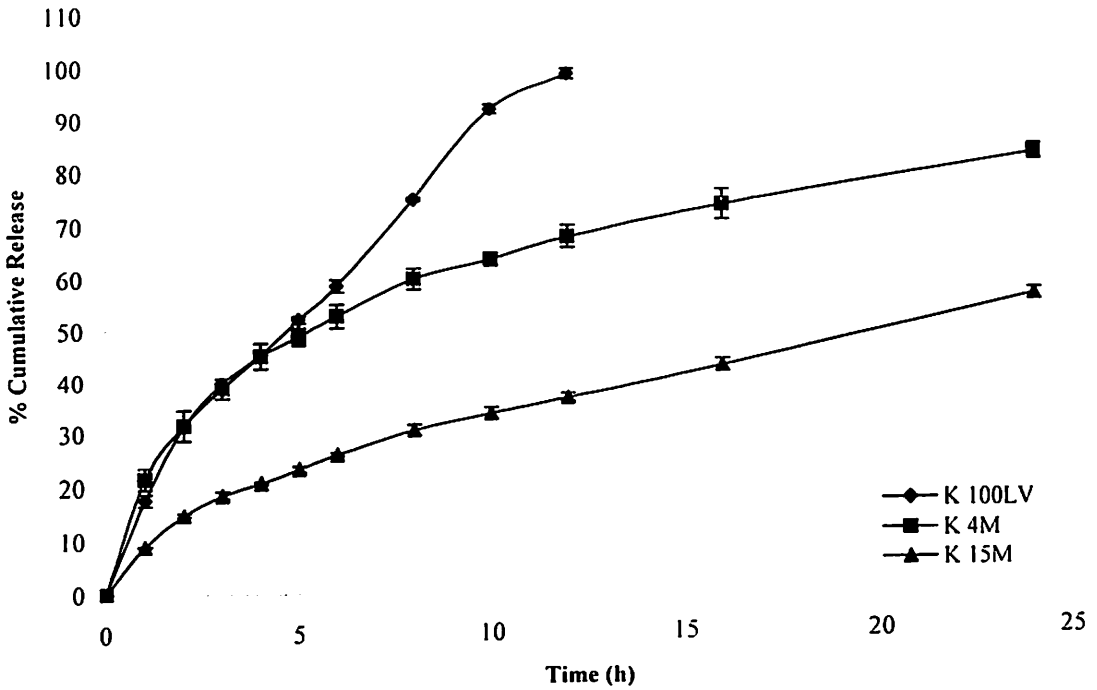

Fig 5.7. Effect of HPMC viscosity on rifampicin release profiles from 20% HPMC formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid. Each data point represents the average of six tablets from three batches with S.D.

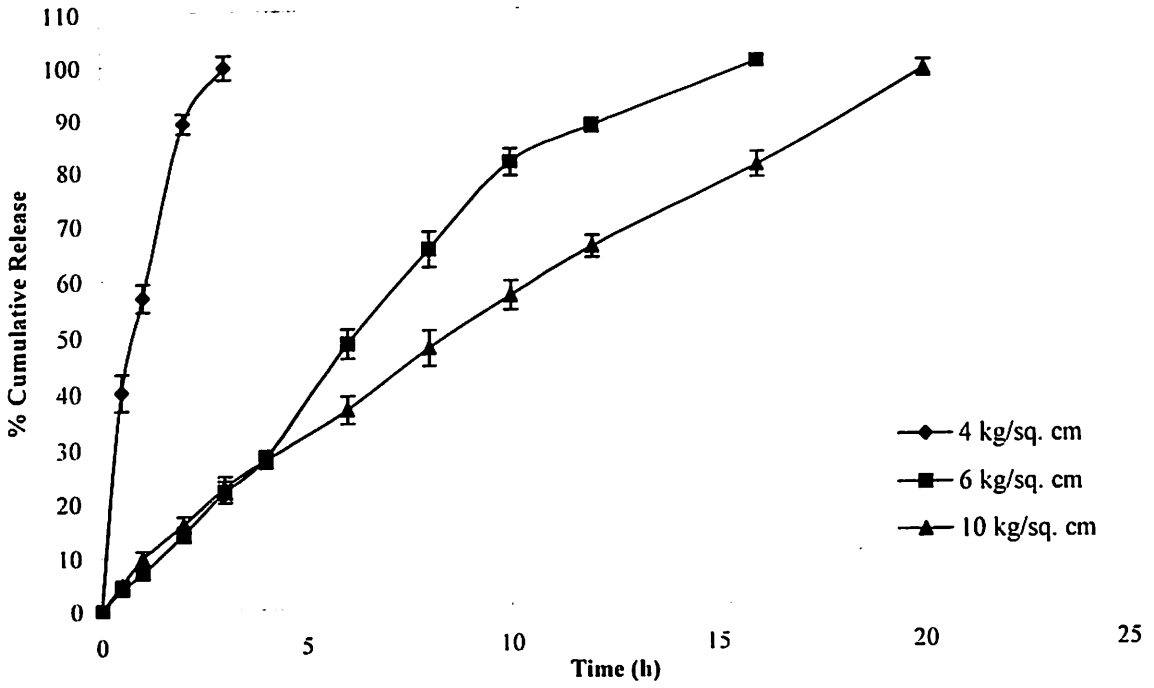

Fig 5.8. Effect of compression force on rifampicin release profiles from 40% HPMC 15 cPs formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

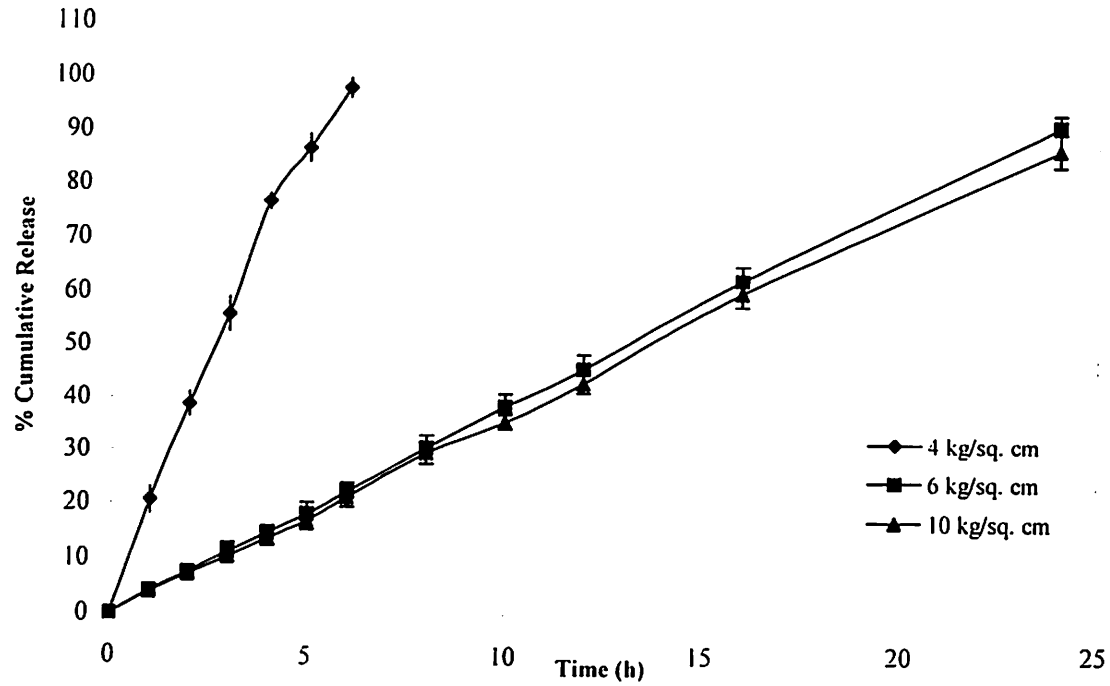

Fig 5.9. Effect of compression force on rifampicin release profiles from 40% HPMC K100LV formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

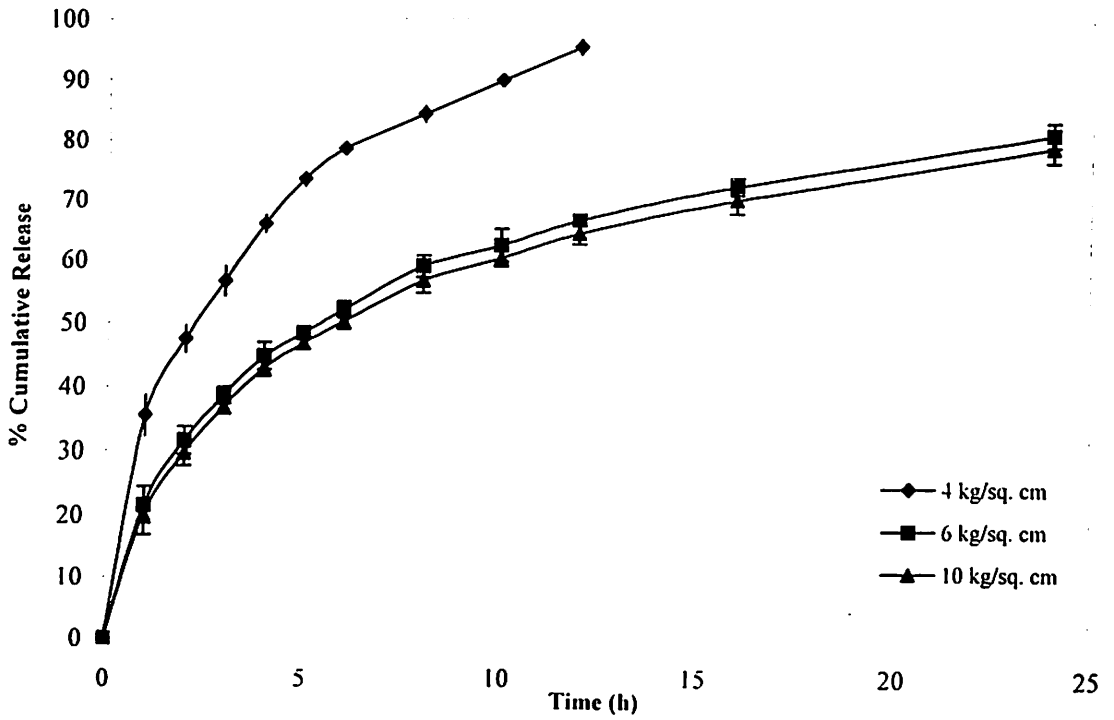

Fig 5.10. Effect of compression force on rifampicin release profiles from 20% HPMC K4M formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

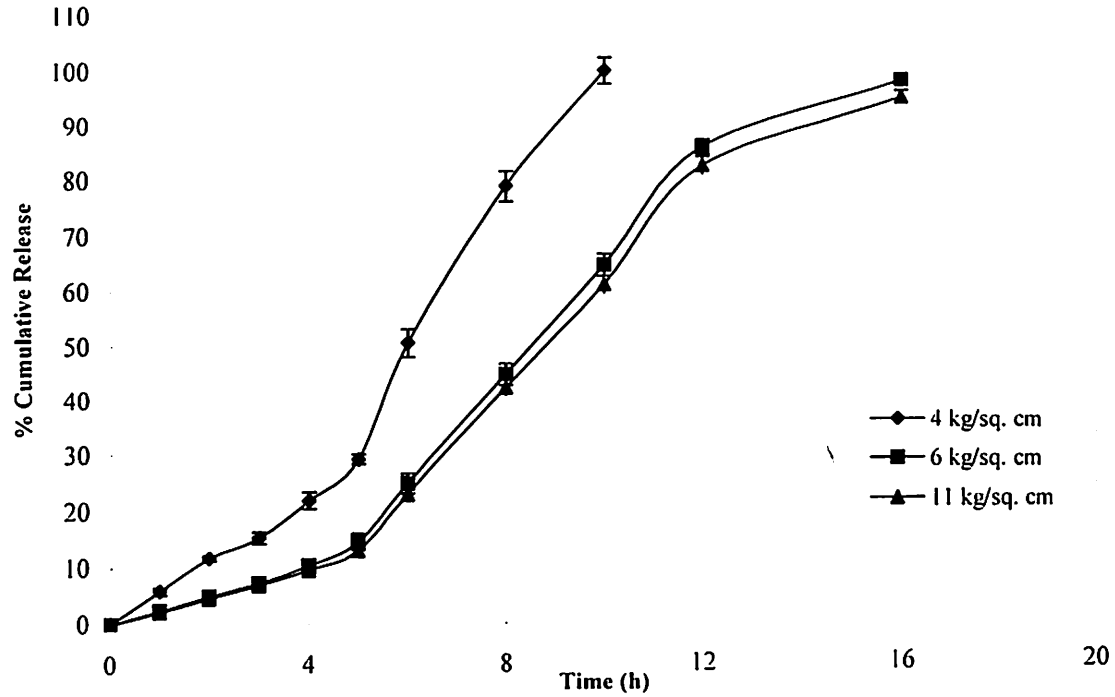

Fig 5.11. Effect of compression force on rifampicin release from 20% Carbopol 934P formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

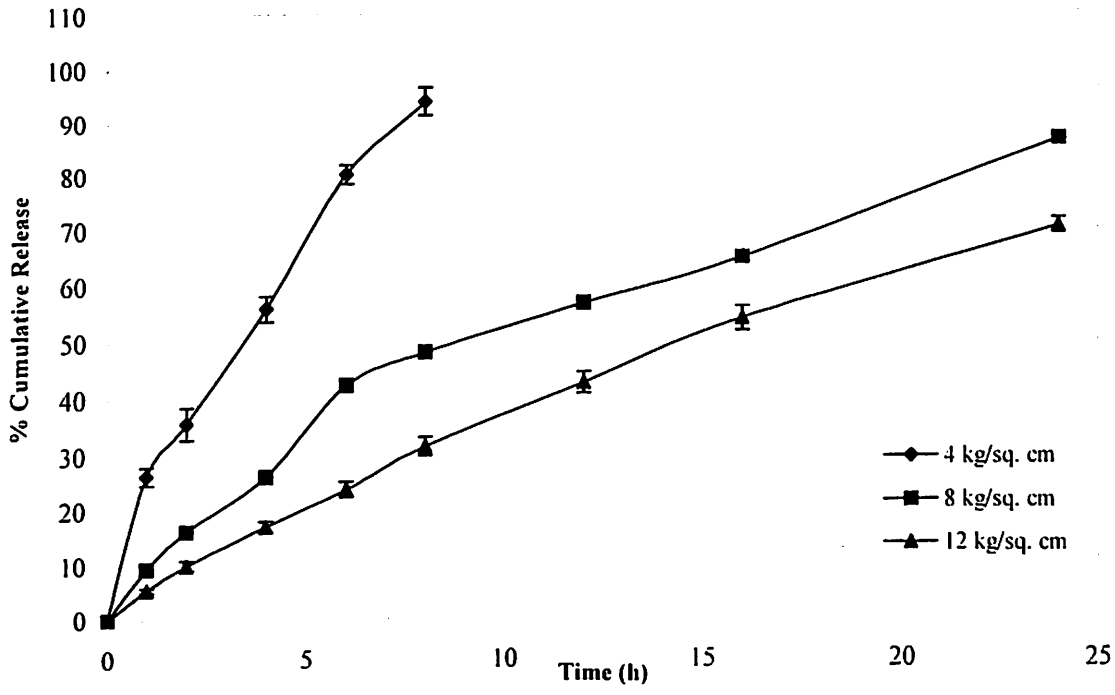

Fig 5.12. Effect of compression force on rifampicin release from 20% HPC (Kluel LF) formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

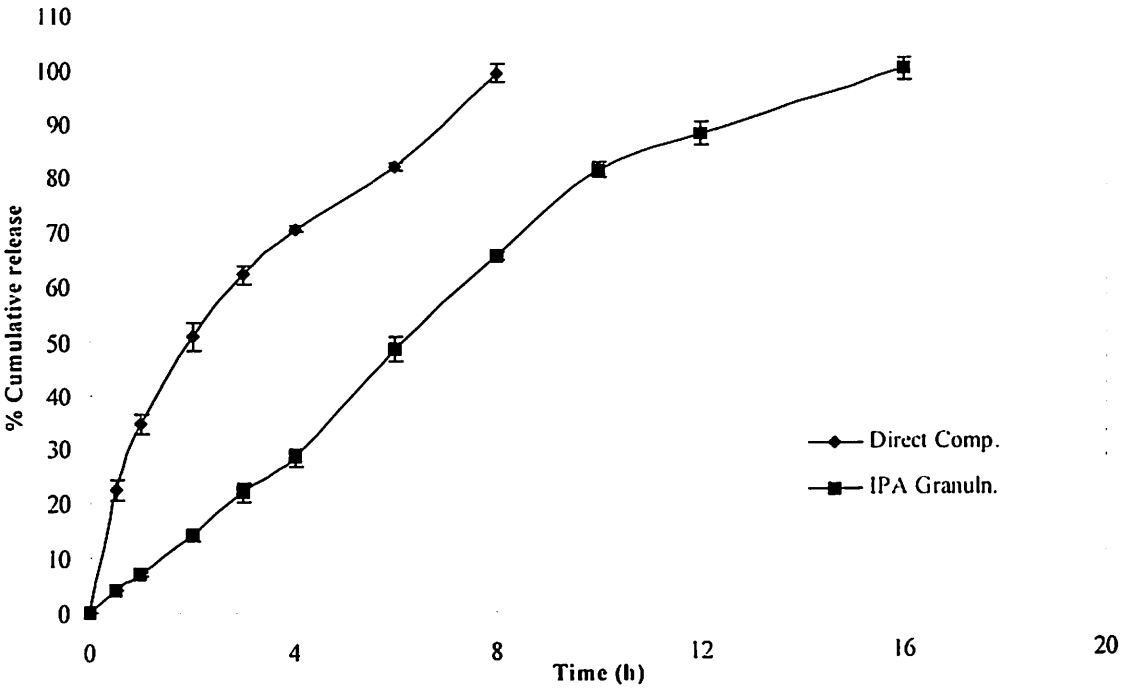

Fig 5.13. Effect of method of granulation on rifampicin release profiles from 40% HPMC 15 cPs formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

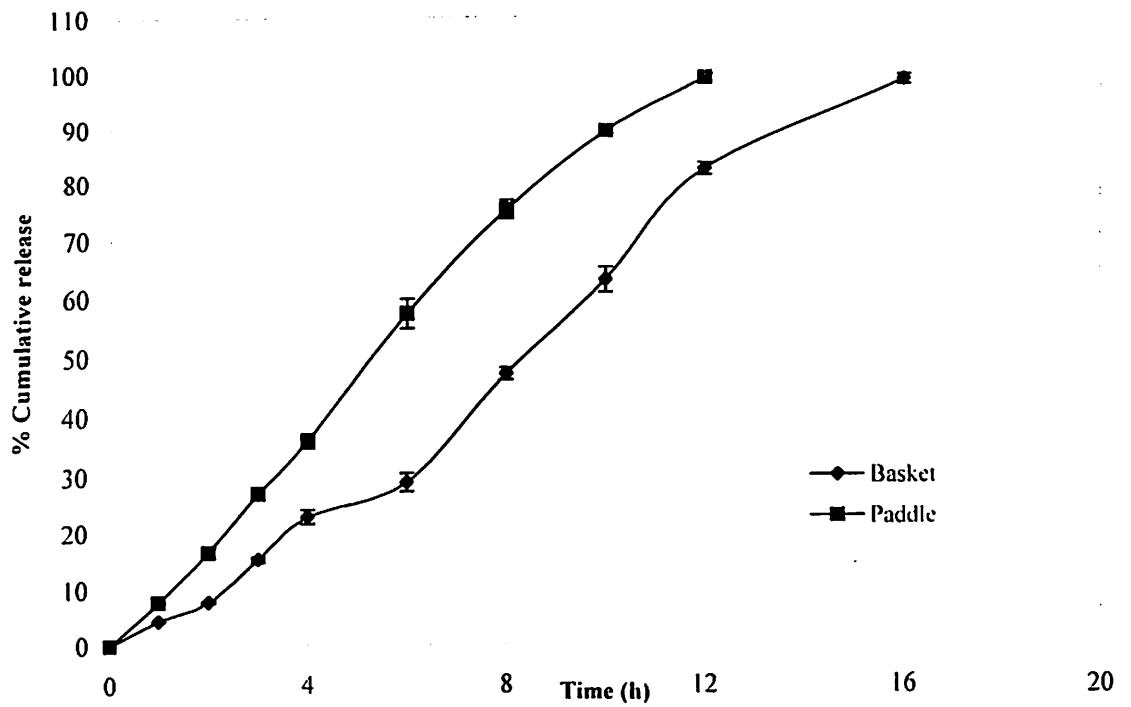

Fig 5.14. Effect of dissolution method (type) on rifampicin release profiles from 50% HPMC 15 cPs formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

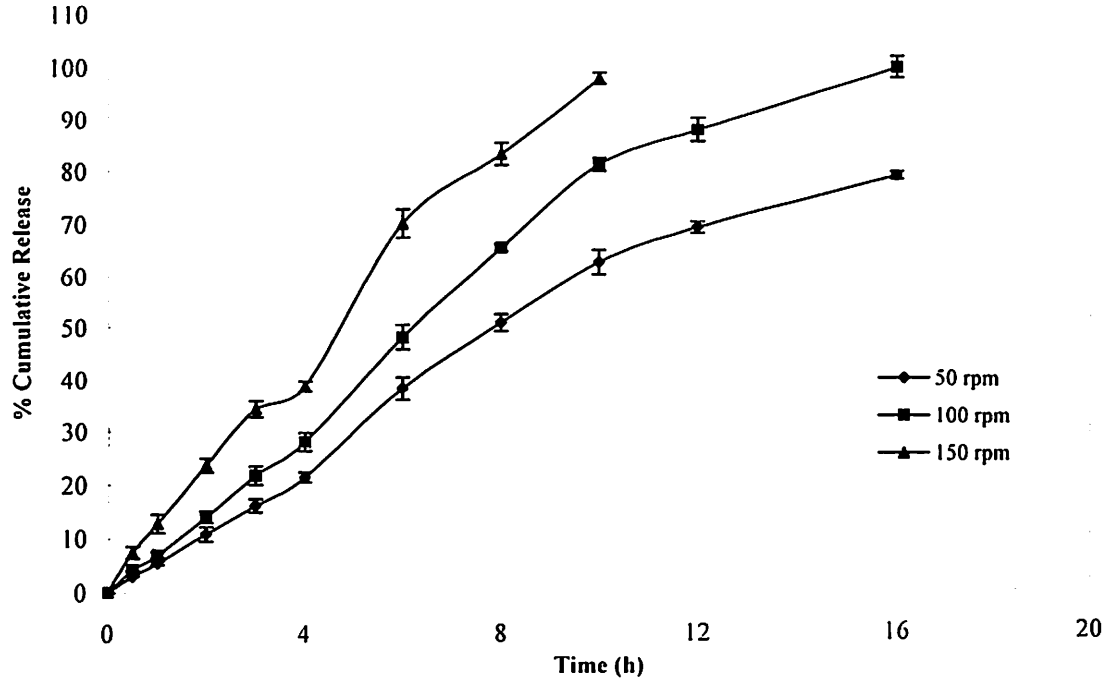

Fig 5.15. Effect of hydrodynamic conditions (stirring speed) on rifampicin release profiles from 40% HPMC 15 cPs formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

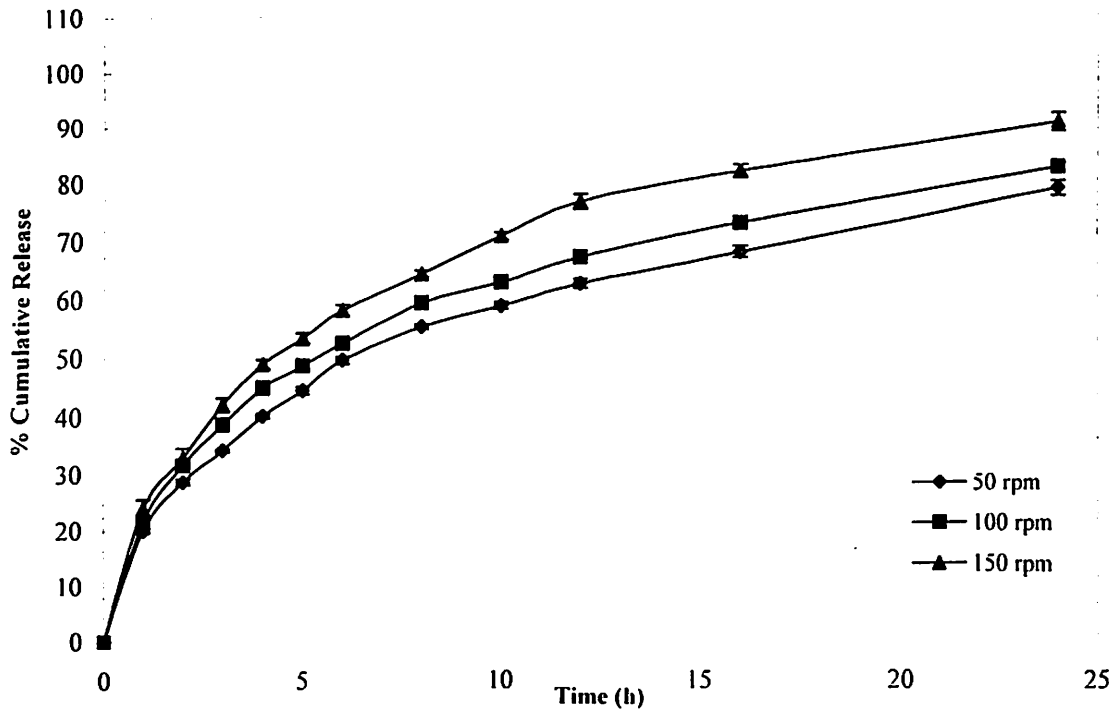

Fig 5.16. Effect of hydrodynamic conditions (stirring speed) on rifampicin release profiles from 20% HPMC K4M formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

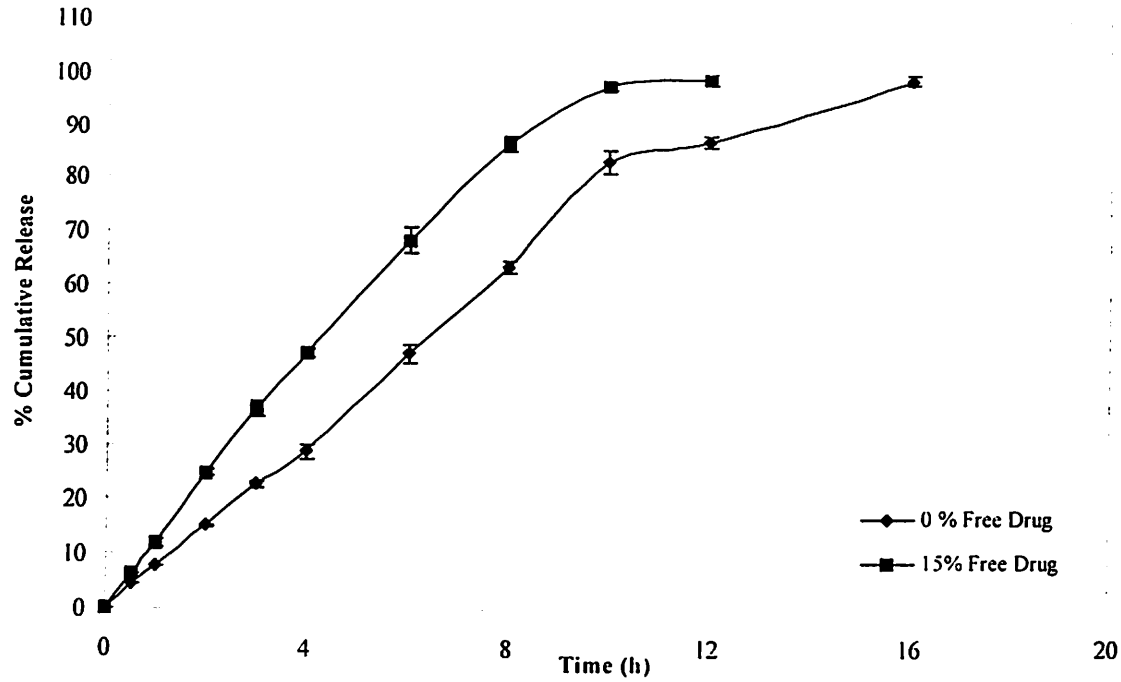

Fig 5.17. Effect of free drug addition (as extragranular rifampicin) on rifampicin release profiles from 50% HPMC 15 cPs formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

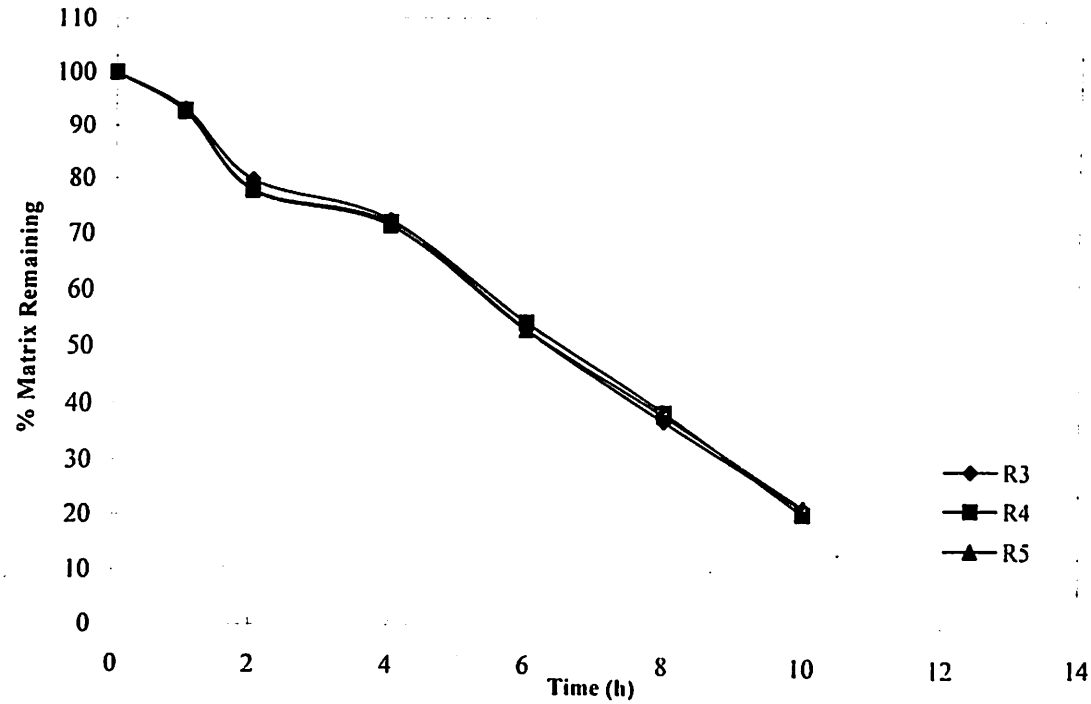

Fig 5.18. Comparative erosion profiles of rifampicin from HPMC 15 cPs formulations in pH 7.4 PO_4 with 0.02% w/v of ascorbic acid (Each data point represents the average of six tablets from three batches with S.D.).

Chapter 6

Design and Studies of Oral Controlled Release Formulations of Isoniazid

The major drawbacks in the use of isoniazid for the treatment of tuberculosis are severe toxic/adverse effects associated with it and its first pass metabolism. In this regard few C.R. formulations have been developed and reported in the literature, which have been discussed in chapter 1. In the present work, isoniazid C.R. matrix tablet formulations have been designed and evaluated which will be discussed in detail in forthcoming sections.

6. 1. Experimental

Materials

Isoniazid was obtained as a gift sample from Lupin Laboratories, Aurangabad. HPMC K100M (Metolose 90SH 100000) was purchased from Sigma Aldrich, Bangalore. All other polymers, chemicals and reagents remain same as mentioned in chapter 4.

Instruments/Equipments

All the instruments/equipments used were same as mentioned in chapter 5.

Analytical Method

Analysis of isoniazid was done by the UV spectrophotometric method as mentioned in chapter 3.

6.1.1. Formulation of isoniazid matrix tablets

6.1.1.1. Formulation of isoniazid tablets with HPMC polymers

Controlled release matrix tablets were formulated using different proportions of HPMC K100LV, HPMC K4M, HPMC K15M and HPMC K100M by wet granulation method (Table 6.1 to 6.4). The drug and polymer (passed through 60# mesh) were mixed uniformly and granulated with IPA, and dried in a tray drier at 60°C. The final granules were blended with talc (1% w/w) and magnesium stearate (1% w/w) and compressed in a single station tablet compression machine (Cadmach) using 13 mm SC punches. The

compression force was kept at a constant level to get the tablets of about 6 kg/cm^2 hardness, except for the studies on the effect of compression force on the release rate.

Three batches of tablets were prepared for each formulation with each tablet contained 300 mg of isoniazid. The formula and the physical characteristics of the formulated tablets are given in Table 6.1 (for HPMC K100LV formulations), Table 6.2 (for HPMC K4M formulations), Table 6.3 (for HPMC K15M formulations) and in Table 6.4 (for HPMC K100M formulations).

6.1.1.2. Formulation of isoniazid tablets with Carbopol 934P polymer

Formulation of tablets by wet (IPA) granulation method

In this method, controlled release matrix tablets were formulated by wet granulation method using different proportion of Carbopol 934P (Table 6.5). The procedure remains same as described in case of for HPMC formulations. The formula and the physical characteristics of the formulated tablets are given in Table 6.5.

Formulation of tablets by direct compression method

In this method, controlled release matrix tablets were formulated by direct compression method using different proportions of carbopol 934P (Table 6.6). The drug and polymer (passed through 40# mesh) were mixed uniformly and were blended with talc (1% w/w) and magnesium stearate (1% w/w) and compressed in a single station tablet compression machine (Cadmach) using 13 mm SC punches. Three batches of tablets were prepared for each formulation with each tablet contained 300 mg of isoniazid. The formula and the physical characteristics of the formulated tablets are given in Table 6.6.

6.1.1.3. Formulation of isoniazid tablets with HPC (Klucel LF) polymer

Controlled release matrix tablets were formulated by wet granulation method using different proportion of HPC (Klucel LF) (Table 6.7). The procedure remains same as described in case of for HPMC formulations. The formula and the physical characteristics of the formulated tablets are given in Table 6.7.

6.1.2. Physical characterization of the tablets

Formulated tablets were subjected to different physical characterization studies (Table 6.1 to 6.7). The drug content of each batch of the formulated tablets was determined in triplicate. The weight variation was determined on 20 tablets using electronic balance (Afcoset). Tablet hardness was determined for minimum 6 tablets of each batch using Monsanto (Standard type) tablet hardness tester. Friability was determined with 20 tablets in a Cambell Electronic Friabilator for 5 min at 25 rpm.

6.1.3. In vitro release studies

Release studies were carried out in pH 7.4 phosphate buffer at 37 ± 1° C. The volume of the dissolution medium was 900 ml and the stirring speed was set at 100 rpm. At predetermined time intervals 10 ml of sample was withdrawn and replaced with fresh dissolution media. After appropriate dilutions, the samples were analyzed by in house developed UV spectrophotometric method. Cumulative percent of drug released was calculated, and mean of six tablets from three different batches were used in the data analysis.

The in vitro release data was analyzed by using various kinetic models as reported in chapter 1. The following variations in tablet formulae were done and their effect on in vitro release rate, release mechanism and nature of release were studied.

6.1.3.1. Effect of polymer proportion

Tablets were made containing 20%, 30%, 40%, 60%, and 80% (w/w of the drug) of HPMC K100LV. But, for HPMC K4M, K15M and K100M, 10%, 20%, 40%, 60% and 80% of polymers were used. Results are shown in the Figure 6.1, 6.2, 6.3 and 6.4 for formulations with HPMC K100LV, K4M, K15M and K100M respectively. Tablets were made by wet granulation method using IPA containing 15%, 30%, 45%, 60% and 80% (w/w of the drug) of Carbopol 934P and the results are shown in the Figure 6.5. In case of direct compression method using Carbopol 934P, the polymer proportions studied were 10%, 20%, 40%, 60% and 80%, the results are shown in the Figure 6.6. HPC (Klucel LF)

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was used in 20%, 40%, 60%, 80% and 100% to make tablets and release data are shown in the Figure 6.8.

6.1.3.2. Effect of HPMC viscosity

Three different viscosity grade HPMCs (60% w/w of the drug) were used in the present investigation, HPMC K100LV (100 cPs), HPMC K4M (4000 cPs), HPMC K15M (15,000 cPs) and HPMC K100M (1,00,000 cPs). The results are depicted in the Figure 6.9.

6.1.3.3. Effect of compression force

The tablet batches containing 60% of HPMC K100LV and HPMC K100M were compressed at three different hardness levels to get the tablets of about 4, 7 and 11 kg/cm^2 hardness. The results are shown in the Figure 6.10 and 6.11 for HPMC K100LV and K100M formulations respectively. Compression force was varied to get three different hardness levels, 4, 7, and 11 kg/cm^2 in case of formulations with 60% Carbopol 934P (made by IPA granulation method) and results are shown in the Figure 6.12. Compression force was varied to get three different hardness levels, 4, 7, and 11 kg/cm^2 in case of formulations with 60% Carbopol 934P (made by direct compression) and results are shown in the Figure 6.13. In case of formulations with 80% HPC, compression force was varied from 4, 7, and 11 kg/cm^2 and the results are shown in the Figure 6.14.

6.1.3.4. Effect of change in the release media

To study the effect of dissolution media, the release studies were done in 0.1 N HCl (pH 1.2) apart from the 7.4 pH phosphate buffer. The results are shown in the Figure 6.15, 6.16, 6.17, 6.18 and 6.19 for 40% HPMC K100LV, 40% HPMC K15M, 60% Carbopol 934P (IPA granulation method), 60% Carbopol 934P (direct compression method) and 100% HPC formulations respectively.

6.1.4. Batch Reproducibility

Batch reproducibility studies were carried out in a similar manner as performed for rifampicin C.R. formulations in chapter 5.

6.1.5. Stability Studies

Stability studies were carried out in a similar manner as performed for rifampicin C.R. formulations in chapter 5. The observed degradation rate constants and $t_{90\%}$ at different storage conditions are listed in Table 6.9. The f_2 factor values of in vitro release profiles for the selected formulation batches are presented in Table 6.9.

6.2. Results and Discussions

6.2.1. Formulation of isoniazid matrix tablets

It has already been discussed that for a drug substance that is sensitive (physically or chemically unstable) to moisture, a direct compression or nonaqueous granulation method should be used for the preparation of the tablet/capsule formulations (Wadke and Jackobson 1980). From the preformulation studies it was observed that the isoniazid degradation enhanced in presence of moisture (higher humidity conditions compared to dry environment) (Table 4.1). Thus, it was decided to use either direct compression method or nonaqueous granulation with IPA for the formulation of matrix tablets of isoniazid.

In case of formulations with HPMC and HPC the direct compression method was not found suitable in obtaining good quality matrix tablets. The tablets obtained found to have very low hardness (less than 2-3 kg/cm^2) values even at higher applied forces. Further increase in the compression force to get the better hardness resulted in the capping and lamination problems. Thus, the formulations were prepared with nonaqueous granulation method using IPA. These formulations (made with IPA granulation) found to have good physicochemical properties (including hardness) and found to control the drug release well when in vitro release studies were carried out. Where as in case of formulations with Carbopol 934P, good quality matrix tablets could be obtained by both direct compression method and nonaqueous granulation using IPA. Thus, the formulations were prepared by both the methods and their release profiles were compared.

Similar to rifampicin C.R. formulations, in this case also no excipients other than controlled release polymers have been incorporated in to the matrices and the use of polymer proportions also restricted.

6.2.2. Physical characterization of the tablets

Physical appearance, tablet hardness, friability, weight variation, and drug content uniformity of all formulations were found to be satisfactory as can be observed from the data in Table 6.1, 6.2, 6.3, 6.4, 6.5, 6.6 and 6.7 for formulations with HPMC K100LV, HPMC K4M, HPMC K15M, HPMC K100M, Carbopol 934P (IPA granulated tablets), Carbopol 934P (directly compressed tablets) and HPC (Klucel LF) respectively. Hardness was found to be 7-8 kg/cm^2 for formulations with HPMC and Carbopol 934P. In case of formulations with HPC, the tablets were compressed to get the hardness of 8-9 kg/cm^2. But, in case of the studies on the effect of compression force on release the hardness was varied from 4-12 kg/cm^2 in all cases. The friability was less than 0.9% (w/w) in all cases. The formulated tablets showed very low weight variation and high degree of content uniformity, indicating that the method of preparation of formulation is an acceptable method for preparing good quality matrix tablets of isoniazid.

6.2.3. In vitro release studies

6.2.3.1. Effect of polymer and its proportion

HPMC formulations

Plots of percent cumulative drug released vs. time for HPMC K100LV matrix tablet formulations, H1, H2, H3, H4 and H5 (20, 30, 40, 60 and 80%), are shown in the Figure 6.1. As the polymer ratio increased from 20% to 80% the release rate decreased. Tablets containing HPMC K100LV below 10% immediately disintegrated and quickly released the drug; hence their release profiles are not given. The release was extended up to 2 h, 4 h, 6 h, 10 h and 12 h respectively in case of H1, H2, H3, H4 and H5 formulations. The corresponding 'r' values for different release models are shown in the Table 6.8. The H2, H3, H4 and H5 formulations found to follow Higuchi's square root kinetics model as the

plots of percentage drug released vs. square root of time found to be linear ('r' values are shown in the Table 6.8). There was higher initial release at all polymer ratios. This higher initial release might be due to very high solubility of the drug in the release media. So, when the tablet matrix came in contact with the release media, increased amount of the drug (which was present on the surface) might have been released immediately. As can be seen from the Figure 6.1, about 64%, 44%, 38%, 32.5% and 29% of the drug was released within 1 h from H1, H2, H3, H4 and H5 matrices. The high amount of the drug released during initial hours made the matrix more porous (less tortuous) for the diffusion of the remaining drug molecules through the hydrated gel layer in the later hours. Best fit of data with square root kinetics and first order models confirmed the diffusional release of drug with decreasing release rates as the time increased.

Plots of percent cumulative drug released vs. time for formulations with HPMC K4M, H6, H7, H8, H9 and H10 (10, 20, 40, 60 and 80%), are shown in the Figure 6.2. Plots of percent cumulative drug released vs. time for HPMC K15M matrix tablet formulations, H11, H12, H13, H14 and H15 (10, 20, 40, 60 and 80%), are shown in the Figure 6.3. Similarly, percent cumulative release vs. time plots for HPMC K100M, H16, H17, H18, H19 and H20 (10, 20, 40, 60 and 80%) are shown in the Figure 6.4. In all these cases increase in the polymer ratio resulted in the decrease in the release. Tablets containing less than 10% of HPMC K4M, K15M and K100M did not extend the release; hence their release profiles are not given. The release was extended from 3 h to 20 h in case of formulations with HPMC K4M. In case of HPMC K15M (H11, H12, H13, H14 and H15), the release was extended from 4 h to 20 h. Similarly in case of formulations with HPMC K100M (H16, H17, H18, H19 and H20); the release was extended from 4 h to 20 h. The reason for decrease in the release with increase in the polymer proportion is that increase in polymer proportion has increased the diffusional barrier. Upon contact with the release media the drug dissolved (as it has high solubility) and due to concentration gradient diffused out of the device. As the drug to polymer ratio was comparatively higher at low polymer proportion, the inner structure of the matrix became more and more porous upon drug depletion (release). But as the polymer ratio increased then the matrix was covered with more number of polymer molecules. Thus, upon swelling a tight network of polymer chains resulted that presented a significant hindrance for drug

diffusion and drug release. The release profiles were fitted with several different kinetics models. The correlation coefficient 'r' values for different release models are shown in the Table 6.8. Formulations with HPMC K4M, K15 M and K100M also found to follow Higuchi's square root kinetics model as the plots of percentage drug released vs. square root of time found to be linear ('r' values are 0.941 to 0.995). The reason for initial higher release and decrease in the release rate of isoniazid with time can be explained as follows. At initial stage, drug on or close to matrix surface was released before the surrounding polymer reached the polymer disentanglement concentration, because the diffusion coefficients for drug molecules were higher than the polymer. And we have already discussed that the high viscosity polymers would take longer time to form a gel layer. Within this time major amount of the drug might have been released. The decreasing rate of release with time was expected in a purely diffusional release due to increasing diffusional pathlength and decreasing diffusion coefficient of the drug as the release proceeded. The increase in the diffusion pathlength with time might be due to less erosive tendency of the high viscosity HPMC formulations. The presence of soluble drug further helped in water (release media) up take by the polymer and the formation of a strong polymer network (gel layer) that remained stable for sufficient period of time to allow diffusional release of the drug. Thus, the nature of release in all HPMC formulations (K100LV, K4M, K15M and K100M) found to follow first order and square root kinetics. The higher initial release might be advantageous as it avoids the need for the incorporation of loading dose to achieve the desired drug levels in the initial hours. It has been reported that, to get best results, the controlled release formulations in case of isoniazid should contain 37% free isoniazid and 63% matrix component (Eidus and Hodgkin 1975). The free isoniazid was to achieve initial amount of release required to elicit necessary therapeutic action (as loading dose) and the remaining part (matrix component) was required as a maintenance dose for continuing/maintaining the blood levels achieved by the loading dose for sufficient duration of time. Thus, from our present studies it was observed that the formulations with HPMC could give both of these advantages in a single controlled release tablet formulation (providing the initial release required for achieving required blood levels and maintenance of that level for a required period of time).

The release mechanism and kinetics of the release profiles were analyzed by Korsmeyer-Peppas model ($M_t / M_\infty = K. t^n$) up to 60% release. The values of K, n, $t_{50\%}$ (time for 50% of drug release) and r (correlation coefficient) obtained for various formulations are listed in Table 6.8. The n values ranged from 0.59 to 0.70 in case of HPMC K100LV matrix tablets indicating that the mechanism of release was by combination of diffusion and swelling/relaxation (anomalous non-Fickian diffusion). The release rate was fastest from H2 formulations with a K value of 0.438 $h^{-0.60}$ and $t_{50\%}$ 1.25 h. The release rate was slowest in case of H5 formulations with a K value of 0.282 $h^{-0.70}$ and $t_{50\%}$ of 2.26 h. The n values ranged from 0.53 to 0.64 in case of HPMC K4M, K15M and K100M matrix tablets indicating that the mechanism of release was by combination of diffusion and swelling/relaxation (anomalous non-Fickian diffusion) with the contribution of diffusion remaining predominant. The release rate was fastest from H17 formulation with a K value of 0.359 $h^{-0.63}$ and $t_{50\%}$ 1.39 h. The release rate was slowest in case of H20 formulation with a K value of 0.256 $h^{-0.53}$ and $t_{50\%}$ of 3.43 h. Even though, the extension of release was significantly different among the formulations with different polymer ratios the K and $t_{50\%}$ values found to be not that much affected. This might be due to the fact that the K and $t_{50\%}$ values were calculated with the Korsmeyer and Peppas model (Korsmeyer et al. 1983, Ritger and Peppas 1987), which could be applied up to 60% release only. We have already discussed that the drug release was higher during initial hours irrespective of the polymer ratio or viscosity. Thus, there were not much differences in the release profiles of the formulations during initial hours (compared to the differences in the later hours of the release studies) and hence the K and $t_{50\%}$ values did not differ to the extent shown by the differences in the release extensions or differences in the release profiles during later hours. It has been also reported that the higher K value in case of the drug release from matrix embedded C.R. tablet formulations is an indication of burst release from the formulations (Levina and Rajabi-Siahboomi 2004). Thus, the burst release of highly soluble isoniazid from HPMC formulations might have resulted in the higher K values and lower $t_{50\%}$ values from HPMC matrix tablets.

Carbopol 934P formulations

Plot of percent cumulative drug released vs. time for formulations with Carbopol 934P H21, H22, H23, H24 and H25 (15, 30, 45, 60 and 80%), prepared by wet granulation using IPA, are shown in the Figure 6.5. It can be seen from the graph that increase in the polymer ratio resulted in the decrease in the release. As the polymer ratio increased from 15% to 80% the release rate decreased. Matrices containing Carbopol 934P below 15% did not sustain the drug release for long time hence their release profiles are not given. The release was extended from 4 h to 20 h as the polymer ratio increased from 15% to 80%. The release profiles were fitted with several different kinetics models and data are shown in the Table 6.8. The nature of release found to be Higuchi's square root kinetics model as the plots of percentage drug released vs. square root of time found to be linear ('r' values are shown in the Table 6.8) at all polymer proportions. During the release studies following trend was observed in all formulations. Initially when the tablet placed in a release media it started hydrating and swelling. The reason for swelling of the polymer in the release media was due to the higher pH (7.4) of the release media that caused the ionization of the carboxylic groups of the polymer. This resulted in the ionic repulsion between the polymer molecules that manifested on a macro level as swelling. More the polymer in the tablet more the matrix swelled. There was higher release during initial hours at all polymer ratios. The higher initial release might be due to the high solubility of the drug in the release media that caused the initial burst effect. Another reason might be the surface drug (drug particles present on the surface of the matrix) might have been released before the formation of hydrated gel layer (that acted as rate controlling membrane for drug diffusion), thus, there was higher initial release. The escape of these drug particles (present on the surface) through the matrix might have increased the porosity of the matrix (decreased the tortuosity) that facilitated the diffusion of the remaining drug particles. When the formation of hydrated gel layer was complete, there was a reduction in the release rate due to the gel barrier to the diffusion. As the time proceeded the release rate decreased because of the increase in the diffusion pathlength and decrease in the concentration gradient.

The release mechanism and kinetics of the release profiles were analyzed by Korsmeyer-Peppas model ($M_t / M_\infty = K. t^n$) up to 60% release. The values of K, n, $t_{50\%}$ (time for 50%

of drug release) and r (correlation coefficient) obtained for various formulations are listed in Table 6.8. The n values for the formulations ranged from 0.57 to 0.68 indicating that the release mechanism was anomalous non-Fickian diffusion. This mechanism was due to a combination of diffusion and polymer swelling/relaxation. The release rate was fastest from H22 formulations with a K value of 0.388 $h^{-0.68}$ and $t_{50\%}$ 1.46 h. The release rate was slowest in case of H25 formulations with a K value of 0.227 $h^{-0\,63}$ and $t_{50\%}$ of 3.50 h. The reason for anomalous non-Fickian diffusional release of isoniazid from the Carbopol 934P matrix tablets can be explained as follows. The drug (isoniazid) was highly soluble in the release media, thus, when the tablet came in contact with release media and media entered in to the tablet matrix the drug dissolution immediately begun (within the matrix). As the drug was having good solubility it also aided in the uptake of release media and thus swelling of the polymer. This reduced the erosive tendency of the matrix, thus, a constant and stable gel layer was maintained for the diffusion of the drug for sufficient period of time. Hence, the drug release was predominantly by the diffusion through this gel layer.

In case of formulations made by direct compression method, plot of percent cumulative drug released vs. time for H26, H27, H28, H29 and H30 (10, 20, 40, 60 and 80%), are shown in the Figure 6.6. In this case also, increase in the polymer ratio resulted in the decrease in the release. The release was extended from 6 h to 24 h as the polymer ratio was increased. Similar to wet granulated formulations, the nature of release found to be Higuchi's square root kinetics model as the plots of percentage drug released vs. square root of time found to be linear ('r' values are shown in the Table 6.8) at all polymer proportions. There were no significant differences in the nature of release profiles between the formulations made by wet granulation and by direct compression method. Thus, the reason for observed nature of the isoniazid release from directly compressed Carbopol 934P matrix tablet formulations remains same as that for the formulations made by IPA granulation method.

However, the main difference in the release profiles of isoniazid from wet granulated and directly compressed Carbopol 934P formulations was in the rate and extent of isoniazid release. The effect of method of granulation on isoniazid release from Carbopol 934P tablet formulations is shown in the Figure 6.7. The release of isoniazid was more

controlled in case of directly compressed matrices compared to IPA granulated tablets (at the same polymer ratio). Also there was significant difference in the initial release with release higher in IPA granulated formulations compared to directly compressed formulations. The release was extended up to 20 h and 16 h respectively from 60% formulations made by direct compression and IPA granulation. The release was extended up to 24 h and 20 h respectively from 80% formulations made by direct compression and IPA granulation. The reason for the observed variation in the release profiles among the formulations made by direct compression and by IPA granulation might be explained as follows. When the tablet came in to contact with the release media, the infiltration of the release media and thus the swelling process was little delayed in case of the formulations made by IPA granulation compared to the formulations made by direct compression. This might be due to the IPA (nonaqueous) granulated tablet matrices that exhibited resistance for the entry of the release media in to the tablet compared to directly compressed tablets. Thus, the swelling and formation of the hydrated gel layer was fast in case of directly compressed tablets than in IPA granulated tablets. It was also observed (visually) during in vitro release studies that the formation of swelled gel layer was very spontaneous with directly compressed matrix tablet, whereas, the IPA granulated tablets took some time in the formation of the hydrated gel layer.

The n values for the formulations ranged from 0.62 to 0.72 indicating that the release mechanism was anomalous non-Fickian. The release rate was fastest from H27 formulations with a K value of 0.287 $h^{-0.69}$ and $t_{50\%}$ 2.24 h. The release rate was slowest in case of H30 formulations with a K value of 0.122 $h^{-0.71}$ and $t_{50\%}$ of 7.31 h. The reason for anomalous non-Fickian diffusional release of isoniazid from directly compressed tablets remains same as for the IPA granulated formulations.

HPC (Klucel LF) formulations

The plots of percent cumulative released vs. time of isoniazid from 20% (H31), 40% (H32), 60% (H33), 80% (H34) and 100% (H35) HPC (Klucel LF) formulations are presented in Figure 6.8. The release was significantly dependent on the ratio of the polymer used; there was a decrease in the release as the polymer level was increased from 20% to 80%. Tablets containing HPC below 20% immediately disintegrated and

released the drug quickly; hence their release profiles are not given. The release was extended up to 4 h, 6 h, 8 h, 12 h and 16 h respectively in case of H31, H32, H33, H34, and H35 formulations respectively. The release profiles were fitted with several different kinetics models and data are shown in the Table 6.8. The nature of release found to be Higuchi's square root kinetics model as the plots of percentage drug released vs. square root of time found to be linear ('r' values found to be 0.941 to 0.959) at all polymer proportions. There was higher release during initial hours at all polymer ratios. The higher initial release might be due to the high solubility of the drug in the release media. Another reason might be the surface drug might have been released before the formation of hydrated gel layer. The release rate decreased with time because of the decrease in the drug available for the diffusion and reduction in the concentration gradient.

The release mechanism and kinetics of the release profiles were analyzed by Korsmeyer-Peppas model ($M_t / M_\infty = K. t^n$) up to 60% release. The values of K, n, $t_{50\%}$ (time for 50% of drug release) and r (correlation coefficient) obtained for various formulations are listed in Table 6.8. The n values for the formulations ranged from 0.60 to 0.76 indicating that the release mechanism was anomalous non-Fickian diffusion. The release rate was fastest from H33 formulations with a K value of 0.443 $h^{-0.60}$ and $t_{50\%}$ 1.22 h. The release rate was slowest in case of H35 formulations with a K value of 0.225 $h^{-0.76}$ and $t_{50\%}$ of 2.86 h. As the polymer level increased the release mechanism shifted from predominantly diffusion based release to diffusion and swelling/relaxation-based release. This might be due to the fact that at lower polymer proportion there might be more micropores present in the matrix that enhanced the diffusion of highly soluble isoniazid molecules thus the release mechanism was mainly by diffusion. But at higher polymer ratio the micropores might have been comparatively less (as substantial part of the matrix has been covered with the release retardant polymer) and the diffusion of the drug molecules was restricted. Thus, at higher polymer ratio the release was by the combination of both diffusion and swelling/erosion.

6.2.3.2. Effect of HPMC viscosity

Effect of HPMC viscosity was studied by making tablets using 60% HPMC of varying viscosity HPMC K100LV (100 cPs) (H4), K4M (4000 cPs) (H9), K15M (15,000 cPs)

(H14) and HPMC K100M (1,00,000 cPs) (H19). The results are depicted in the Figure 6.9. It can be observed from the plots of percent cumulative release vs. time for different formulations (varying in the viscosity of HPMC used) that as the polymer viscosity increased from 100 cPs (K100LV) to 4000 cPs (K4M) there was a slight decrease in the release. But there were no significant difference between the release profiles of the formulations made with K4M (4000 cPs), K15M (15000 cPs) and K100M (100000 cPs). The release profiles were analyzed for the similarity factor (f_2) values for assessment of statistical difference or similarity between the release profiles. The f_2 factor value observed to be 49.67 between K100LV and K4M formulations indicating the significant difference between the release profiles. Where as the f_2 factor values found to be; 77.83 between HPMC K4M and K15M formulations, 84.72 between HPMC K15M and K100M formulations and 69.70 between HPMC K4M and K100M formulations indicating insignificant difference between the release profiles of HPMC K4M, K15M and K100M formulations. The analysis of the f_2 factor values further proved that the effect of HPMC viscosity on release was only significant in low viscosity polymer formulations but not in high viscosity. Initial release was also high in all these formulations.

The effect of polymer viscosity was mainly due to the differences in their molecular weights. The molecular weights of HPMC K100LV, K4M, K15M, and K100M were reported to be 25 kDa, 95 kDa, 120 kDa and 250 kDa respectively (Gao et al. 1996). And there is a strong relationship exists between the polymer molecular weight (MW) and polymer disentanglement concentration ($C_{p.dis}$); $C_{p.dis}$ = 27000/MW (Lee and Peppas 1987). According to the relationship (equation), the $C_{p.dis}$ decreases with increasing MW and approaches a plateau at high MW. It was, however, reported that the change in the polymer disentanglement concentration between HPMC K100LV and other viscosity grades was appreciable leading to a higher release rates for the K100LV matrices. But the change in the $C_{p.dis}$ between HPMC K4M, K15M and K100M was so small that the matrix swelling and drug release profiles for these three HPMC formulations were indistinguishable. High solubility of isoniazid has made it unaffected by increased viscosity, particularly in case of high viscosity HPMCs. Other research groups have reported similar results that the drug release rate decreased with increasing molecular

weight for low molecular weight HPMCs and became independent of molecular weight for high molecular weight HPMCs (Ford et al. 1985, Feely and Davis 1988).

6.2.3.3. Effect of compression force

HPMC formulations

Release profile of isoniazid from tablet formulations made with 60% HPMC K100LV and HPMC K100M, compressed at three different hardness levels of 4, 7 and 11 kg/cm^2, are presented in Figure 6.10 and 6.11. It was observed for formulations with HPMC K100LV (H4) that the release rate is faster at lower compression force (hardness 4 kg/cm^2) compared to at 7 kg/cm^2. The release was extended up to 4 h at 4 kg/cm^2 compared to 10 h at 7 kg/cm^2. Where as, there were no significant differences between the release profiles of formulations compressed to get hardness of 7 kg/cm^2 and 11 kg/cm^2. In both cases the release was extended up to 10 h. On analysis of similarity factor, the f_2 factor value found to be 38.77 for the release profiles between the formulations with 4 kg/cm^2 and 7 kg/cm^2 hardness indicating that the release profiles were significantly affected by the compression force. But, the f_2 factor value found to be 66.29 between the formulations with 7 kg/cm^2 and 11 kg/cm^2 indicating the nonsignificant difference between the release profiles. The effect of compression force on the release profiles of formulations with 60% HPMC K100M (H19) is shown in the Figure 6.11. In this case also the release profiles followed the similar trend as that in case of HPMC K100LV formulations. The release was extended up to 6 h in case of formulations with hardness of 4 kg/cm^2 compared to the 16 h in case of formulations with 7 kg/cm^2 (f_2 = 35.90). There were no significant differences between the release profiles of the formulations with hardness of 7 and 11 kg/cm^2 (f_2 = 72.79). The results suggest that at low hardness level release is affected by hardness (4 to 7 kg/cm^2) but not at high level (above 7 kg/cm^2)

At lower applied compression force there might be insufficient tablet strength and greater level of porosity (void spaces within the matrix), which allowed a greater liquid penetration in to the matrix causing quick breakage and/or immediate solution of the drug within the matrix that enhanced the diffusivity of the drug out of the matrix. High

solubility of isoniazid further made the release faster. But, once the required minimum hardness was achieved, further increase in the hardness did not influence the release any more.

The n values ranged from 0.58 to 0.67 in case of HPMC K100LV (H4) matrix tablets indicating that the mechanism of release remained unaffected by the compression force (anomalous non-Fickian diffusion). The release rate was fastest from the formulations compressed at 4 kg/cm^2 (K = 0.34 h$^{-0.69}$) and, there were no significant differences between the K values of formulations compressed at 7 kg/cm^2 and 11 kg/cm^2 (0.321 h$^{-0.66}$ and 0.314 h$^{-0.67}$ respectively). The $t_{50\%}$ decreased to 1.25 h at hardness of 4 kg/cm^2, but there was no significant difference in the $t_{50\%}$ among the formulations compressed with 7 and 11 kg/cm^2 hardness (1.94 h and 1.95 h). The n values ranged from 0.58 to 0.69 in case of HPMC K100M (H19) matrix tablets indicating that the mechanism of release remained unaffected by the compression force (anomalous non-Fickian diffusion). The release rate was fastest from the formulations with 4 kg/cm^2 hardness (K = 0.34 h$^{-0.69}$) and, there were no significant differences between the K values of formulations with 7 kg/cm^2 and 11 kg/cm^2 (0.287 h$^{-0.58}$ and 0.246 h$^{-0.63}$ respectively) hardness. The $t_{50\%}$ decreased to 1.72 h at 4 kg/cm^2, but there was no significant difference in the $t_{50\%}$ among the formulations compressed at 7 and 11 kg/cm^2 (2.59 h and 3.05 h).

Carbopol 934P formulations

Release profile of isoniazid from wet granulated tablet formulations made with 60% Carbopol 934P, compressed at three different hardness levels of 4, 7 and 11 kg/cm^2, are presented in Figure 6.12. The compression force did not significantly affect the release profiles. The rate and extent of the drug release was almost similar at all three compression force levels (4, 7, and 11 kg/cm^2). Even the compression force applied to get the hardness of 4 kg/cm^2 was sufficient enough to extend the drug release up to 16 h (as in case of the formulations with hardness of 7, and 11 kg/cm^2). This was a very different observation from any other formulations (including rifampicin formulations) studied. The reason for such observation can be put forth as follows. It was already discussed that the exact nature of the compression force is highly material dependent and also depends on experimental factors such as compression speed and size and shape of the tooling (York

1979, Nokhodchi et al. 1996). However, as the porosity of the hydrated matrix is independent of the initial porosity, the compression force seems to have little influence on the drug release from Carbopol 934P. Also it was noticed that the formation of a hydrated gel layer was very spontaneous process in case of Carbopol 934P formulations. Thus, once the gel layer formation was fast then the initial porosity of the matrix seemed to have very little significance on drug release and hence the compression force. The similarity factor values of 80.44 and 82.15 between the release profiles of formulations with 4 and 7 kg/cm^2 and, 7 and 11 kg/cm^2 hardness respectively further demonstrated that the effect of compression force was nonsignificant in altering the release profiles.

The n values ranged from 0.55 to 0.57 indicating that the mechanism of release remained unaffected by the compression force (non-Fickian diffusion). The K values (0.332 $h^{-0.55}$, 0.308 $h^{-0.57}$ and 0.309 $h^{-0.57}$ respectively at 4, 7, and 11 kg/cm^2 hardness) and $t_{50\%}$ values (2.1 h, 2.32 h and 2.33 h respectively) indicated the insignificant difference between them. Thus, the release mechanism and release kinetics parameters both proved that the compression force had no significant effect on the release profiles.

Similarly, when release profiles were studied for tablet formulations made with 60% Carbopol 934P by direct compression with different hardness levels of same value, it was observed that that the release was higher at lower applied compression force (hardness 4 kg/cm^2) compared to 7 kg/cm^2 (Figure 6.13). At 4 kg/cm^2 hardness, release was extended up to 10 h compared to 20 h at 7 kg/cm^2 with f_2 value of 39.07. However, the release profiles found to be almost similar at 7 and 11 kg/cm^2 hardness levels even though there was slight reduction in the release rate and extension in the release profile at 11 kg/cm^2 was observed. The f_2 value found to be 61.58 indicating that there was no significant difference between the release profiles compressed at 7 and 11 kg/cm^2 hardness. Though no difference was found in wet granulation method, in case of directly compressed formulations a minimum hardness of 7 kg/cm^2 was required to achieve good controlled release. At low hardness level, tablet matrix was comparatively loose and allowed easy penetration of dissolution media along with quick disintegration that made release faster. But once the minimum hardness was achieved (7 kg/cm^2 in this case), further increase in the compression force did not seem to have a significant influence on the release. Hence, the drug release rates (profiles) found to be almost similar at 7 and 11 kg/cm^2 hardness.

The n values ranged from 0.71 to 0.80 indicating that the release mechanism remained unaffected by compression force. But the K values (0.181 $h^{-0.80}$ and 0.155 $h^{-0.72}$ respectively at 4, and 7 kg/cm^2 hardness) and $t_{50\%}$ values (3.58 h, and 5.08 h respectively at 4, and 7 kg/cm^2 hardness) have shown that the release profiles were significantly different at 4 and 7 kg/cm^2. However, the K values (0.155 $h^{-0.72}$ and 0.142 $h^{-0.71}$ respectively at 7, and 11 kg/cm^2) and $t_{50\%}$ values (5.08 h, and 5.87 h respectively at 7, and 11 kg/cm^2) have shown that there was no significant difference between the release profiles among the formulations with 7 and 11 kg/cm^2 hardness.

HPC (Klucel LF) formulations

Release profile of isoniazid from tablet formulations made with 80% HPC, compressed at three different hardness levels of 4, 8 and 12 kg/cm^2, are presented in Figure 6.14. It was observed that the release was higher at lower applied force (hardness 4 kg/cm^2) compared to 8 kg/cm^2 hardness. At 4 kg/cm^2 hardness the release was extended only up to 4 h compared to 12 h at 8 kg/cm^2 with f_2 value of 39.29. Similarly, after 8 h, ~ 95% of the drug has been released from the formulations compressed at 8 kg/cm^2 hardness compared to ~ 83% from 12 kg/cm^2 with f_2 value of 47.57. The reason for the decrease in the release with increase in the compression force is same as explained for the HPC formulations containing rifampicin.

The n values ranged from 0.60 to 0.70 indicating that the release mechanism remained unaffected by the compression force. But the K values (0.438 $h^{-0.60}$, 0.306 $h^{-0.70}$ and 0.260 $h^{-0.67}$ respectively at 4, 8 and 12 kg/cm^2) and $t_{50\%}$ values (1.24 h, 2.01 h and 2.57 h respectively at 4, 8, and 12 kg/cm^2) have shown that the release profiles were significantly different at 4, 8, and 12 kg/cm^2 hardness. The K and $t_{50\%}$ values both proved that the effect of compression force was significant in case of HPC formulations.

6.2.3.4. Effect of change in the release media

HPMC formulations

The release studies were carried out in 0.1 N HCl (pH 1.2) apart from pH 7.4 phosphate buffer for H3 and H13 formulations to know the effect of release media on release

profiles of isoniazid. The results are shown in the Figure 6.15 and 6.16 for H3 and H13 formulations respectively.

It can be seen from the Figure 6.15 that the drug release rate was higher in 0.1 N HCl compared to in pH 7.4 phosphate buffer for H3 (HPMC K100LV) formulations. The complete drug was released within 4 h in 0.1 N HCl compared to the release extension up to 6 h in pH 7.4 phosphate buffer. The f_2 value of 43.56 further demonstrated that the drug release was significantly higher in 0.1 N HCl compared to the release in pH 7.4 phosphate buffer. Similarly, the drug release observed to be higher in 0.1 N HCl than in pH 7.4 phosphate buffer in case of H13 (HPMC K15M) formulations. But the f_2 factor value of 53.36 demonstrated that there was no significant (statistical) difference among the release profiles of H13 formulations in 0.1 N HCl and pH 7.4 phosphate buffer. This might be due to the fact that the difference in the release profiles was only significant at later hours, i.e., after 4-5 h where almost 80-85% of the drug has been released. Where as, the regulatory guidelines specify that, not more than one point should be considered after 85% drug release while comparing the release profiles by f_2 metric comparison as the f_2 values are sensitive to the dissolution points (Shah et al. 1999). Also the f_2 factor value of ~ 50 did not specify that the release profiles are totally similar but there would be average difference of 10% at all time points exists between the release profiles of two batches taken for the comparison (Shah et al. 1998).

The observed difference in the release profiles of formulations with HPMC (H3 and H13) in 0.1 N HCl and in pH 7.4 phosphate buffer may be due to pH dependent solubility of HPMC or isoniazid. Although the solubility of isoniazid was little higher in 1.2 pH (326 ± 3.96) than in pH 7.4 phosphate buffer (274 ± 4.79), this was not statistically significant in causing any difference in the release rates as in both cases sufficient solubilities were there to maintain sink conditions. However, the release was higher in 0.1 N HCl (pH 1.2) than in pH 7.4 phosphate buffer in case of both HPMC formulations (H3 and H13). Probably this may be due to the fact that the HPMC release is reported to be higher in 0.1 N HCl than in pH 7.4 phosphate buffer or water (Siepmann et al. 1999, Siepmann and Peppas 2000). The reason for higher HPMC release in 0.1 N HCl than in pH 7.4 phosphate buffer might probably be due to differences in the osmotic pressure between these two media, difference in the solubility of HPMC in these media, and charge effects.

The exact analysis of the reason for such observation requires more detailed studies, which are beyond the scope of our present investigations.

The n values found to be 0.59 and 0.58 for the release profiles of H3 formulations in 0.1 N HCl and in pH 7.4 phosphate buffer respectively indicating the release mechanism remained unaffected by change in the release media. However, the K and $t_{50\%}$ values were different for H3 formulations in 0.1 N HCl (0.472 $h^{-0.58}$ and 1.10 h respectively) and in pH 7.4 phosphate buffer (0.396 $h^{-0.59}$ and 1.48 h respectively). The n values found to be 0.55 and 0.60 for the release profiles of H13 formulations in 0.1 N HCl and in pH 7.4 phosphate buffer respectively indicating that the release mechanism remained unaffected by change in the release media. However, the K and $t_{50\%}$ values were slightly different for H13 formulations in 0.1 N HCl (0.405 $h^{-0.60}$ and 1.42 h respectively) and in pH 7.4 phosphate buffer (0.370 $h^{-0.55}$ and 1.72 h respectively).

Carbopol 934P formulations

The effect of release media on release profiles of isoniazid from formulations with 60% Carbopol 934P (H24) made by wet granulation using IPA has been evaluated and presented in Figure 6.17. It was observed that the drug release rate was higher in 0.1 N HCl compared to in pH 7.4 phosphate buffer for H24 formulations with release extension of only up to 8 h in 0.1 N HCl compared to 16 h in pH 7.4 phosphate buffer. The f_2 factor value of 33.86 also further confirmed that.

The reason for the observed difference in the release profiles of Carbopol 934P formulations (H24) in 0.1 N HCl and in pH 7.4 phosphate buffer might be explained as follows. It has been already mentioned that the swelling of the anionic polymer (Carbopol 934P) is pH dependent. The polymer stars swelling as the pH increases above its pKa (5-6). At lower pH (0.1 N HCl release media), the polymer was not fully swollen and there were more areas of low microviscosity. Thus, at pH 1.2, the tablet had more number of pores in the matrix (regions of low microviscosity), as the tablet swelling was negligible. This might have resulted in the increased diffusion of the drug molecules (because of increased porosity and decreased tortuosity) and increase in the drug release. But in higher pH release media (pH 7.4 phosphate buffer), the ionization of the carboxylic acid groups of the Carbopol 934P might have resulted in the increased swelling. This has

resulted in the fewer and smaller regions of low microviscosity as the micropores have been closed due to swelling. Thus, the drug release was lower in case of pH 7.4 phosphate buffer than in 0.1 N HCl (pH 1.2).

The n values found to be 0.59 and 0.57 for the release profiles of H24 formulations in 0.1 N HCl and in pH 7.4 phosphate buffer respectively indicating the release mechanism remained unaffected by change in the release media. However, the K and $t_{50\%}$ values were different for H24 formulations in 0.1 N HCl (0.431 $h^{-0.59}$ and 1.29 h respectively) and in pH 7.4 phosphate buffer (0.308 $h^{-0.57}$ and 2.32 h respectively). These change in the K and $t_{50\%}$ values again proved that the release profiles were significantly affected by the release media pH.

Similarly, the effect of release media on release profiles of isoniazid from 60% Carbopol 934P formulations made by direct compression (H29) has been evaluated and presented in Figure 6.18. In this case also release was higher in 0.1 N HCl compared to in pH 7.4 phosphate buffer with release extension of only up to 8 h in 0.1 N HCl compared to 20 h in pH 7.4 phosphate buffer. The f_2 factor value of 23.66 also further confirmed that. The reason for the observed difference in the release profiles of directly compressed Carbopol 934P formulations (H29) in 0.1 N HCl and in pH 7.4 phosphate buffer is same as that for the formulations made by IPA granulation (H24).

The n values found to be 0.46 and 0.72 for the release profiles of H29 formulations in 0.1 N HCl and in pH 7.4 phosphate buffer respectively indicating the shift in the release mechanism. As we have already discussed there was negligible polymer swelling in 0.1 N HCl and thus the drug molecules were free to diffuse through the pores present in the matrix structure, along their concentration gradient, resulting in the purely Fickian diffusional release. The swelling phenomenon in pH 7.4 phosphate buffer closed these micropores available of the diffusion of the drug molecules and the molecules had to diffuse through the swelled gel layer. Hence the release was dependent on both diffusion and polymer swelling/relaxation process. The combination of these two factors resulted in the anomalous non-Fickian diffusion in pH 7.4 phosphate buffer. The K and $t_{50\%}$ values were different in 0.1 N HCl (0.421 $h^{-0.46}$ and 1.45 h respectively) and in pH 7.4 phosphate buffer (0.155 $h^{-0.72}$ and 5.08 h respectively). These change in the K and $t_{50\%}$

values again proved that the release profiles were significantly affected by the release media pH.

HPC (Klucel LF) formulations

The effect of release media on release profiles of isoniazid from formulations with 100% HPC (Klucel LF) (H35) has been evaluated and presented in Figure 6.19. In this case also release was higher in 0.1 N HCl compared to in pH 7.4 phosphate buffer with release extension of only up to 8 h in 0.1 N HCl compared to 16 h in pH 7.4 phosphate buffer. The f_2 factor value of 41.81 further confirmed that.

The n values found to be 0.59 and 0.76 in 0.1 N HCl and in pH 7.4 phosphate buffer respectively indicating a slight shift in the release mechanism. In 0.1 N HCl the release was predominantly by diffusion, whereas in pH 7.4 phosphate buffer, the release was predominantly by swelling/relaxation. The K and $t_{50\%}$ values were different in 0.1 N HCl (0.345 $h^{-0.59}$ and 1.93 h respectively) and in pH 7.4 phosphate buffer (0.225 $h^{-0.76}$ and 2.85 h respectively). These change in the K and $t_{50\%}$ values again proved that the release profiles were significantly affected by the release media pH.

6.2.4. Batch Reproducibility

The tablets showed low standard deviation values for the drug content, friability, weight variation and hardness from three different batches prepared separately (data not shown). These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility and absence of significant variations between batch-to-batch. No significant difference was observed in the release profiles of the formulations between different batches as indicated by the low standard deviation values of the percent cumulative release data at different time points obtained from the replicate release studies of the samples and from high similarity factor values. The f_2 values varied from 66.67 to 90.34 for release profiles of H3, H5, H8, H10, H13, H15, H18, H20, H22, H25, H28, H30, H33 and H35 formulations between original and reproduced batches (calculated from the average percent release at each time point with standard deviation values less than 3%). Thus, the reproducible physical properties in terms low standard deviation for each parameter from different batches and significantly similar release

profiles of isoniazid from all batches (proved by f_2 factor values) indicated that the formulation methodology employed (IPA granulation in case of all polymer formulations and direct compression in case of Carbopol 934P formulations) found to be suitable for manufacturing the good quality C.R. matrix tablets of isoniazid.

6.2.5. Stability Studies

The two best formulations from each batch of formulations, one with lower polymer proportion and another with higher, were selected for stability studies (Table 6.9). The drug content in triplicate was determined for each formulation by the UV spectrophotometric method described in chapter 3 and the observed degradation rate constants at different storage conditions and corresponding $t_{90\%}$ values are listed in Table 6.9. The isoniazid in matrix embedded tablets (in case of all polymer formulations) found to follow first order degradation as the plots of log percent drug content remaining vs. time found to be linear (with "r" value more than 0.971 in all cases and individual plots not given). The K_{deg} for isoniazid in various formulations ranged from 5.05×10^{-3} $month^{-1}$ to 6.64×10^{-3} $month^{-1}$ at CRT, 6.43×10^{-3} $month^{-1}$ to 8.31×10^{-3} $month^{-1}$ at 40 ± 2 °C and 8.70×10^{-3} $month^{-1}$ to 12.11×10^{-3} $month^{-1}$ at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 6.9). In all polymer formulations the degradation rate constant increased with increase in the polymer proportion. The $t_{90\%}$ values for isoniazid in various formulations ranged from 15.86 to 20.88 months at CRT, from 12.68 to 16.38 months at 40 ± 2 °C and from 8.70 to 12.11 months at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 6.9).

Isoniazid found to be more stable at CRT and less stable at 40 ± 2 °C/75 ± 5% RH in all formulations. Among the HPMC polymers, isoniazid was comparatively more stable in HPMC K100M formulations (Table 6.9). Isoniazid found to be comparatively less stable in formulations with HPMC K100LV (among HPMC). Among the HPC and Carbopol formulations, isoniazid found to be more stable in formulations with HPC. Among the Carbopol 934P formulations, IPA granulated tablets found to provide more stable environment for isoniazid (lower K_{deg} values) compared to directly compressed matrices. Among all the polymer formulations, isoniazid found to be comparatively more stable in formulations with HPMC K100M and HPC.

It was observed that with the raise in the temperature (from 25± 2 °C to 40 ± 2 °C) the K_{deg} values increased and $t_{90\%}$ values decreased in case of all formulations (in all polymer ratios). The K_{deg} values were higher at 40 ± 2 °C/75 ± 5% RH compared to 40 ± 2 °C in all the cases studied (Table 6.9). Thus, from present studies, it was observed that the humidity was the one of the most important parameter that affected the stability of isoniazid in all polymer formulations. It was also observed that the deleterious effect of humidity on the stability of the isoniazid was more pronounced than the temperature (as can be observed from the K_{deg} values and $t_{90\%}$ values at CRT, 40 ± 2 °C and 40 ± 2 °C/75 ± 5% RH in case of all formulation batches). Thus, the applicability of the Arrhenius relationship for the estimation of room temperature stability from extrapolated high temperature data found to be inappropriate (not justified). The increased K_{deg} values found at higher humidity condition again supported that the avoidance of aqueous granulation technology (use of either IPA granulation or direct compression) in the manufacturing of isoniazid matrix tablets was significantly beneficial in obtaining the stable C.R. matrix tablets of isoniazid.

The in vitro release profiles were studied as per the specifications enlisted in previous sections and compared with their respective initial release profiles. The in vitro release profiles of the formulations stored at CRT for 6 months were compared with their initial release profiles (0 time samples at CRT) with f_2 factor values. The f_2 factor values in all cases found to be more than 60 indicating that the isoniazid release profiles were significantly similar for zero time samples and 6 months samples (stored at CRT). Thus, the in vitro release characteristics were not significantly affected by the stability studies (storage at CRT) for about 6 months showing that the formulations were stable in terms of release characters.

6.3. Conclusions

The designed matrix tablets of isoniazid showed good physical properties indicating that the method of preparation of formulation is suitable and acceptable method for preparing good quality and reproducible matrix tablets of isoniazid. The tablet manufacturing method was relatively simple and can be easily adopted in conventional tablet manufacturing units on a commercial scale.

Increase in the polymer ratio, irrespective of the polymer type and viscosity grade, decreased the isoniazid release. Increase in the polymer viscosity from HPMC K100LV (100 cPs) to HPMC K4M (4000 cPs) resulted in the decrease in the isoniazid release, but beyond 4000 cPs (HPMC K4M) not significantly affected the release. The effect of compression force was more pronounced in case of formulations with HPC (Klucel LF). The Carbopol 934P formulations prepared by IPA granulation found to be more robust towards the effect of compression force. The release of isoniazid was higher in 0.1 N HCl compared to pH 7.4 phosphate buffer from all formulations. The isoniazid release was faster from the IPA granulated formulations than from the directly compressed tablets in case of Carbopol 934P.

The mechanism of isoniazid release found to be predominantly by non-Fickian diffusion in all formulations and at all polymer ratios.

The duration of isoniazid release varied from 6 h to 24 h depending on the polymer type, polymer ratio, polymer viscosity and processing techniques. The isoniazid release profiles found to follow Higuchi's square root kinetics or first order. The initial release was sufficiently higher in case of all formulations thus ruling out the need to have a loading dose. It has been reported that, to get best results, the controlled release formulations in case of isoniazid should contain 37% free isoniazid for attaining plasma drug level immediately, and 63% matrix embedded component (Eidus and Hodgkin 1975). Thus, from our present studies it was observed that most of the formulations studied produced good initial release followed by proper controlled release of the drug.

The low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility. The isoniazid and formulations were found to be stable as observed from the stability studies at different storage conditions. However, the degradation rate increased with increase in temperature and humidity. Thus, the avoidance of aqueous granulation technology (use of either IPA granulation or direct compression) in the manufacturing of isoniazid matrix tablets was found to be significantly beneficial in obtaining the stable C.R. matrix tablets of isoniazid.

It has been reported that (Eidus and Hodgkin 1975) the absorption of isoniazid continues throughout the GIT, thus a C.R. formulation of isoniazid can have complete absorption throughout the GIT and can be a substitute to multidose therapy. Thus, high dose of

isoniazid could be administered for longer duration of action without facing much of toxic or side effects. These controlled release formulations not only can optimize the drug blood levels and can minimize the first pass metabolism of isoniazid to its derivatives, which are reported to cause severe hepatotoxicity.

Table 6.1. Formula and physical properties of isoniazid matrix tablets prepared with HPMC K100LV.

Formulations	H1	H 2	H 3	H 4	H 5
Components *					
Drug (mg)	300	300	300	300	300
HPMC[a] (%)	20	30	40	60	80
Physical Properties					
Drug Content (% Label Claim)[b]	101.5 ± 1.7	102.0 ± 1.4	99.1 ± 1.2	99.8 ± 1.5	100.7 ± 1.6
Weight variation (%)[c]	± 2.3	± 1.9	± 1.8	± 2.8	± 2.5
Hardness (kg/cm^2)[d]	6.7 ± 0.8	6.5 ± 0.7	6.8 ± 0.8	6.5 ± 0.9	6.3 ± 0.5
Friability (%)	< 0.90	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 6.2. Formula and physical properties of isoniazid matrix tablets prepared with HPMC K4M

Formulations	H6	H 7	H 8	H 9	H 10
Components *					
Drug (mg)	300	300	300	300	300
HPMC[a] (%)	10	20	40	60	80
Physical Properties					
Drug Content (% Label Claim)[b]	100.3 ± 1.2	101.5 ± 0.9	99.8 ± 1.8	99.8 ± 1.5	99.6 ± 1.7
Weight variation (%)[c]	± 1.9	± 2.3	± 2.4	± 1.8	± 2.9
Hardness (kg/cm^2)[d]	6.3 ± 0.5	6.5 ± 0.6	6.9 ± 0.7	6.7 ± 0.4	6.4 ± 0.6
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 6.3. Formula and physical properties of isoniazid matrix tablets prepared with HPMC K15M

Formulations	H11	H 12	H 13	H 14	H 15
Components *					
Drug (mg)	300	300	300	300	300
HPMC[a] (%)	10	20	40	60	80
Physical Properties					
Drug Content (% Label Claim)[b]	99.1 ± 1.3	100.8 ± 1.5	101.6 ± 1.6	99.5 ± 1.7	102.3 ± 1.2
Weight variation (%)[c]	± 2.6	± 1.5	± 1.9	± 2.4	± 2.7
Hardness (kg/cm^2)[d]	6.6 ± 0.6	6.7 ± 0.8	6.7 ± 0.8	6.4 ± 0.6	6.9 ± 0.7
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 6.4. Formula and physical properties of isoniazid matrix tablets prepared with HPMC K100M

Formulations	H16	H 17	H 18	H 19	H 20
Components *					
Drug (mg)	300	300	300	300	300
HPMC[a] (%)	10	20	40	60	80
Physical Properties					
Drug Content (% Label Claim)[b]	102.0 ± 1.5	101.3 ± 1.3	98.9 ± 1.0	101.8 ± 1.6	99.2 ± 1.3
Weight variation (%)[c]	± 2.3	± 2.6	± 1.2	± 1.6	± 2.8
Hardness (kg/cm^2)[d]	6.5 ± 0.7	6.8 ± 0.4	6.8 ± 0.4	6.5 ± 0.5	6.7 ± 0.8
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 6.5. Formula and physical properties of isoniazid matrix tablets prepared with Carbopol 934P (IPA granulation)

Formulations	H21	H 22	H 23	H 24	H 25
Components *					
Drug (mg)	300	300	300	300	300
Carbopol[a] (%)	15	30	45	60	80
Physical Properties					
Drug Content (% Label Claim)[b]	100.9 ± 1.2	99.6 ± 1.7	101.6 ± 1.6	102.6 ± 1.2	99.4 ± 1.7
Weight variation (%)[c]	± 2.6	± 2.0	± 1.9	± 2.5	± 1.7
Hardness (kg/cm^2)[d]	6.6 ± 0.8	6.9 ± 0.5	6.6 ± 0.7	6.8 ± 0.8	6.9 ± 0.6
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 6.6. Formula and physical properties of isoniazid matrix tablets prepared with Carbopol 934P (Direct Compression)

Formulations	H26	H 27	H 28	H 29	H 30
Components *					
Drug (mg)	300	300	300	300	300
Carbopol[a] (%)	10	20	40	60	80
Physical Properties					
Drug Content (% Label Claim)[b]	98.8 ± 1.0	101.6 ± 1.4	102.2 ± 1.4	100. 9 ± 1.8	101.5 ± 1.3
Weight variation (%)[c]	± 1.9	± 2.3	± 2.9	± 1.8	± 2.7
Hardness (kg/cm^2)[d]	6.5 ± 0.4	6.7 ± 0.8	6.9 ± 0.5	6.8 ± 0.6	6.5 ± 0.9
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 6.7. Formula and physical properties of isoniazid matrix tablets prepared with HPC (Klucel LF)

Formulations	H31	H32	H33	H34	H35
Components *					
Drug (mg)	300	300	300	300	300
HPC[a] (%)	20	40	60	80	100
Physical Properties					
Drug Content (% Label Claim)[b]	101.8 ± 1.2	99.2 ± 1.6	99.7 ± 1.5	102. 3 ± 1.1	101.8 ± 1.7
Weight variation (%)[c]	± 2.8	± 2.0	± 2.3	± 2.6	± 1.6
Hardness (kg/cm^2)[d]	6.8 ± 0.6	6.6 ± 0.5	6.8 ± 0.8	6.8 ± 0.9	6.9 ± 0.4
Friability (%)	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [a] % w/w of the drug; [b] mean of triplicate with SD; [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 6.8. Release kinetics parameters for isoniazid C.R. formulations.

Formulations	Peppas model parameters				r^d for Zero Order	r^d for First Order	r^d for Higuchi's Kinetics
	n^a	K^b (h^{-n})	$t_{50\%}{}^c$ (h)	r^d			
H2	0.60	0.438	1.25	0.999	0.944	0.788	0.989
H3	0.59	0.396	1.48	0.999	0.898	0.957	0.997
H4	0.66	0.321	1.94	0.979	0.798	0.971	0.963
H5	0.70	0.282	2.26	0.986	0.812	0.992	0.970
H8	0.55	0.361	1.81	0.999	0.796	0.979	0.969
H9	0.59	0.301	2.34	0.998	0.737	0.936	0.941
H10	0.53	0.266	3.29	0.999	0.739	0.967	0.948
H12	0.64	0.368	1.61	0.999	0.905	0.968	0.995
H13	0.55	0.370	1.72	0.999	0.854	0.998	0.987
H14	0.60	0.296	2.39	0.993	0.762	0.966	0.955
H15	0.53	0.262	3.36	0.999	0.751	0.878	0.954
H17	0.63	0.359	1.39	0.989	0.844	0.957	0.982
H18	0.55	0.345	1.97	0.999	0.752	0.958	0.981
H19	0.58	0.287	2.59	0.998	0.786	0.951	0.966
H20	0.53	0.256	3.43	0.999	0.756	0.963	0.956
H22	0.68	0.388	1.46	0.987	0.753	0.989	0.946
H23	0.62	0.357	1.72	0.997	0.690	0.954	0.962
H24	0.57	0.308	2.32	0.992	0.824	0.978	0.980
H25	0.63	0.227	3.50	0.999	0.875	0.914	0.991
H27	0.69	0.287	2.24	0.999	0.870	0.958	0.999
H28	0.62	0.221	3.74	0.994	0.924	0.984	0.991
H29	0.72	0.155	5.08	0.997	0.889	0.980	0.986
H30	0.71	0.122	7.31	0.999	0.922	0.986	0.990
H33	0.60	0.443	1.22	0.999	0.776	0.993	0.959
H34	0.70	0.306	2.01	0.999	0.760	0.995	0.941
H35	0.76	0.225	2.86	0.997	0.795	0.979	0.954

[a] Diffusional exponent indicative of the release mechanism; [b] release rate constant; [c] time for 50 % of the drug release; [d] correlation coefficient.

Table 6.9. Stability studies of isoniazid C.R. formulations at different storage conditions.

Formulations	CRT		40 ± 2°C		40 °C/75% RH	
	K_{deg} x 10^3 ($month^{-1}$)*	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)*	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)*	$t_{90\%}$ (month)
H3	6.08	17.33	7.80	13.51	10.28	10.25
H5	6.64	15.86	8.31	12.68	11.00	9.58
H8	5.66	18.61	6.77	15.56	9.12	11.56
H10	5.88	17.92	7.07	14.91	9.50	11.09
H13	5.42	19.45	6.88	15.32	10.23	10.30
H15	5.73	18.38	7.18	14.67	10.89	9.68
H18	5.05	20.88	6.43	16.38	8.70	12.11
H20	5.28	19.96	6.62	15.91	9.06	11.63
H22	5.70	18.50	6.63	15.90	9.68	10.89
H25	6.06	17.40	6.93	15.20	10.43	10.10
H28	6.09	17.30	7.42	14.20	10.75	9.80
H30	6.27	16.80	7.81	13.50	12.11	8.70
H33	5.49	19.20	6.64	15.86	9.40	11.21
H35	5.73	18.40	7.03	14.99	9.76	10.80

* First order degradation rate constant based on average of triplicate assay values at five time points with C.V. less than 4.0% in all cases.

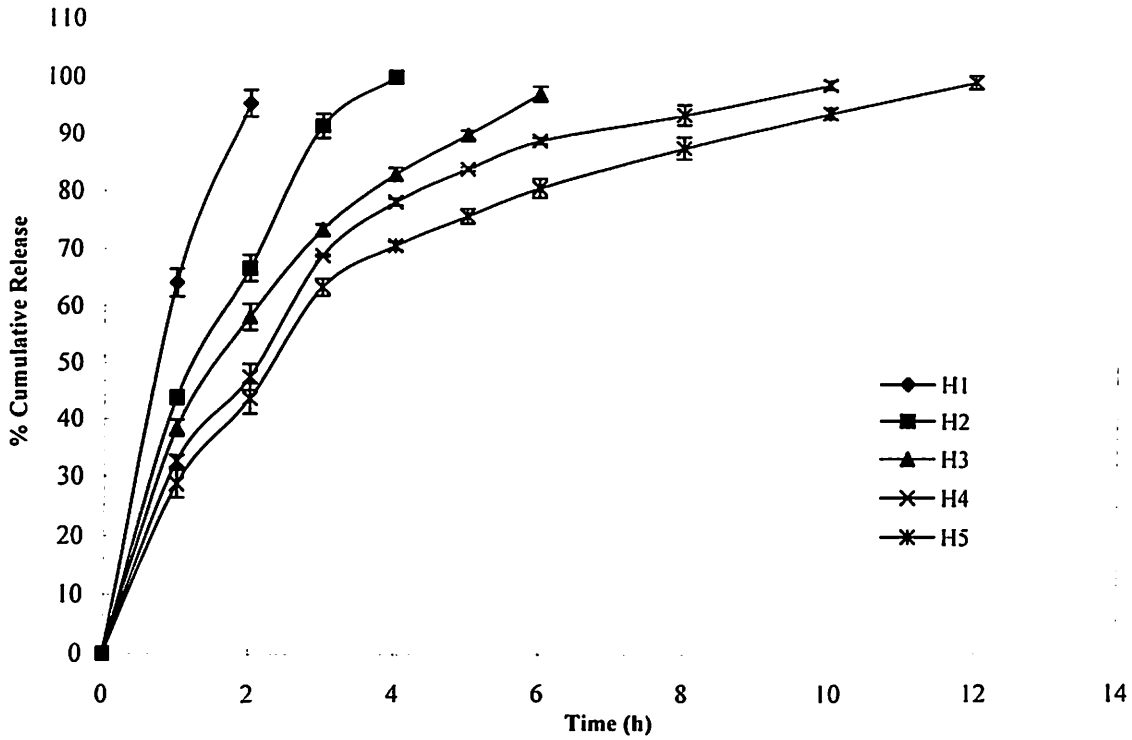

Fig 6.1. Comparative release profiles of isoniazid from HPMC K100LV formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

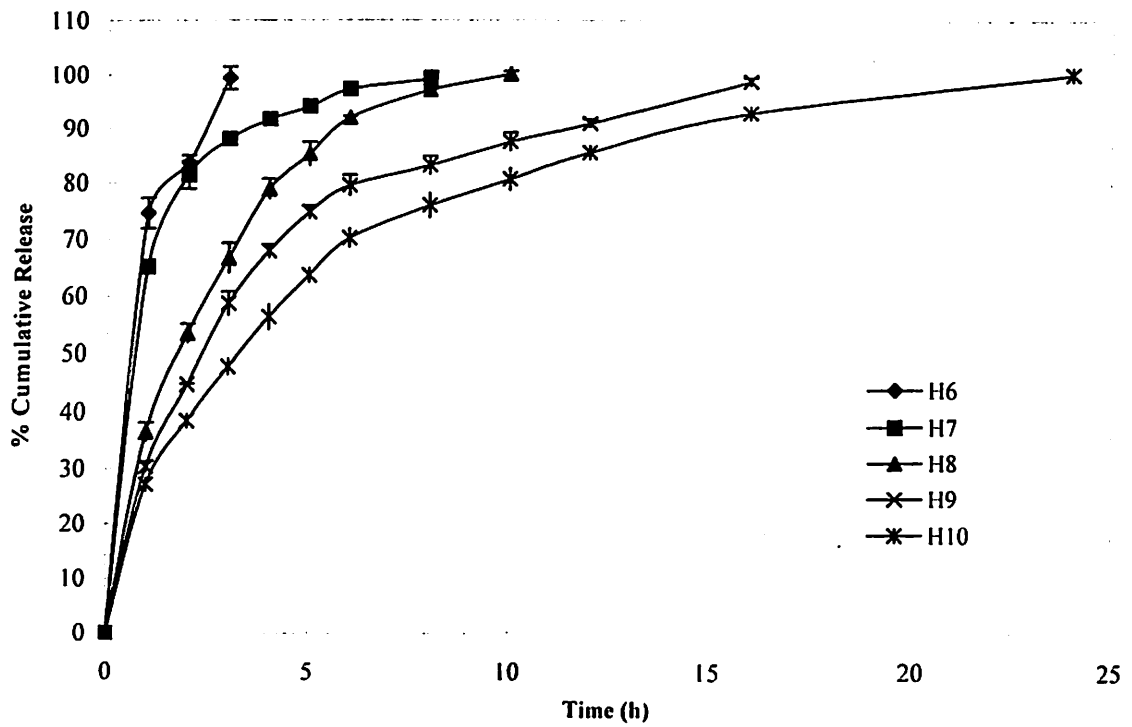

Fig 6.2. Comparative release profiles of isoniazid from HPMC K4M formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

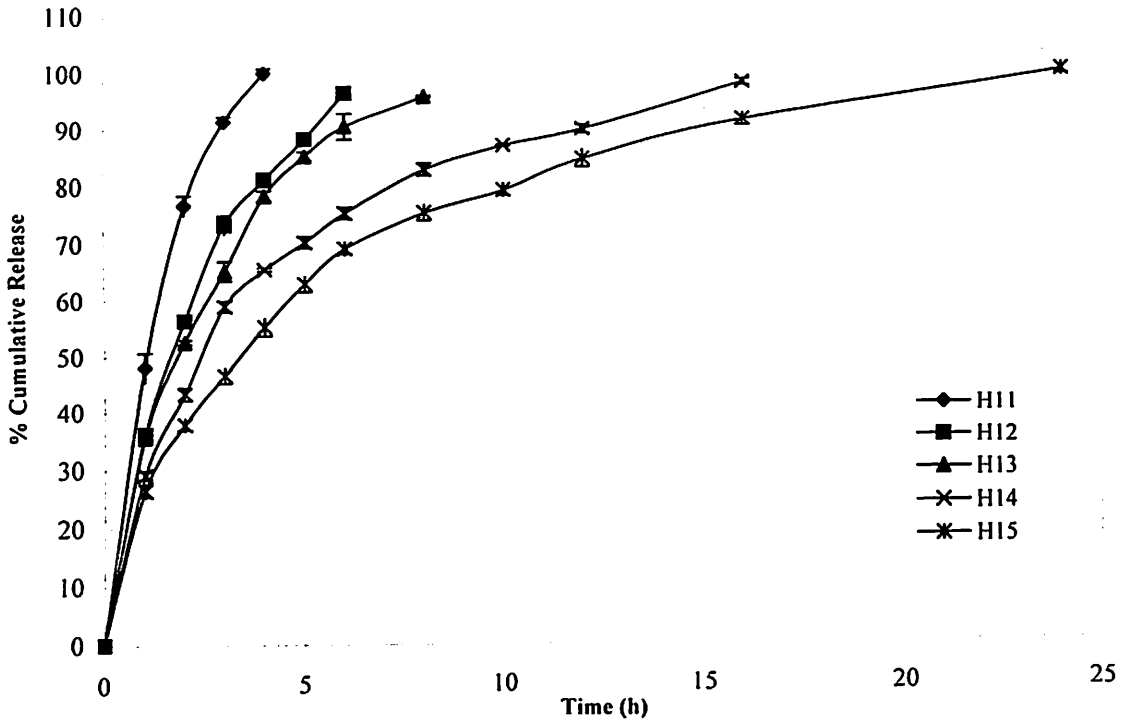

Fig 6.3. Comparative release profiles of isoniazid from HPMC K15M formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

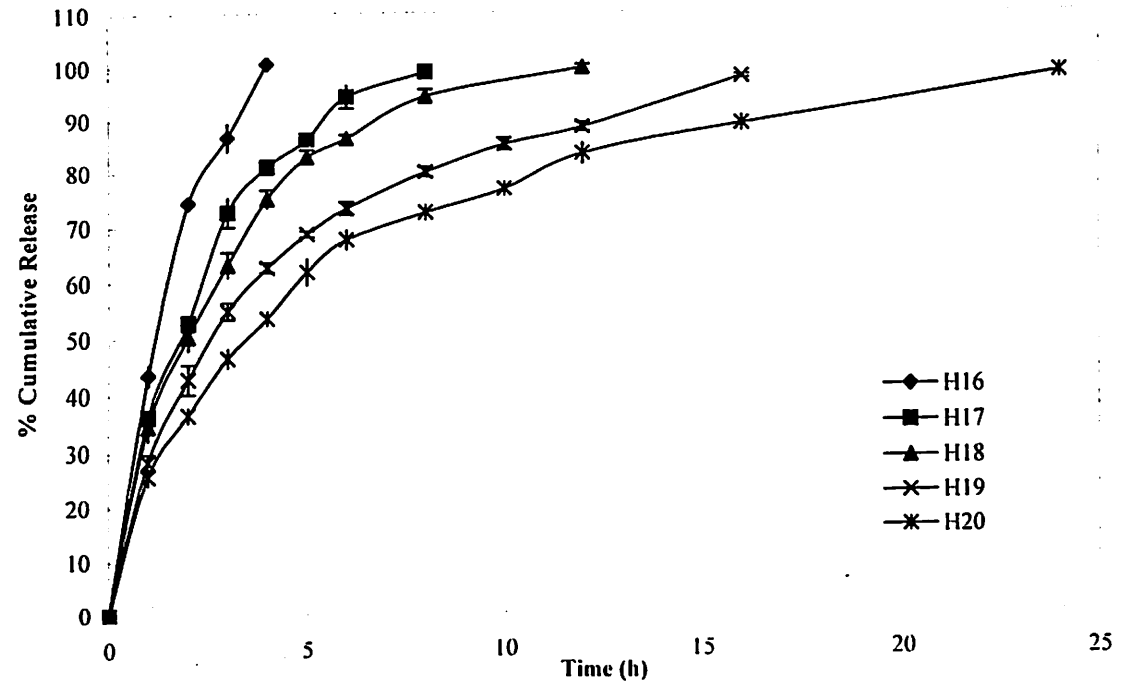

Fig 6.4. Comparative release profiles of isoniazid from HPMC K100M formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

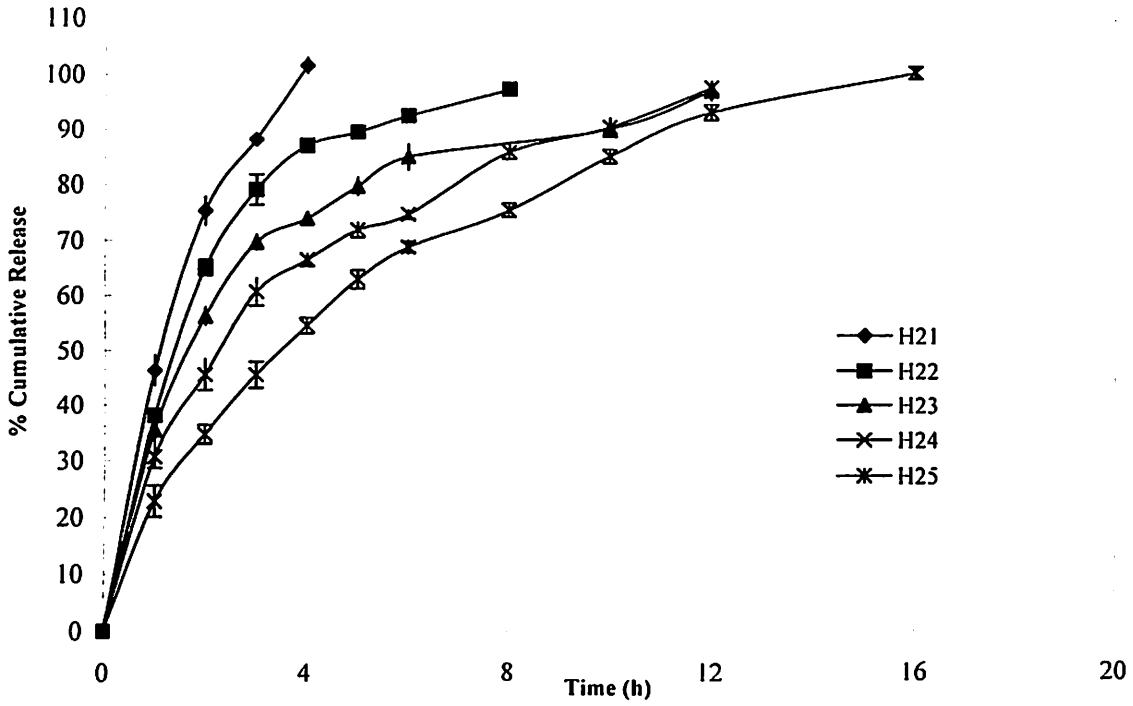

Fig 6.5. Comparative release profiles of isoniazid from Carbopol 934P (IPA granulation) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

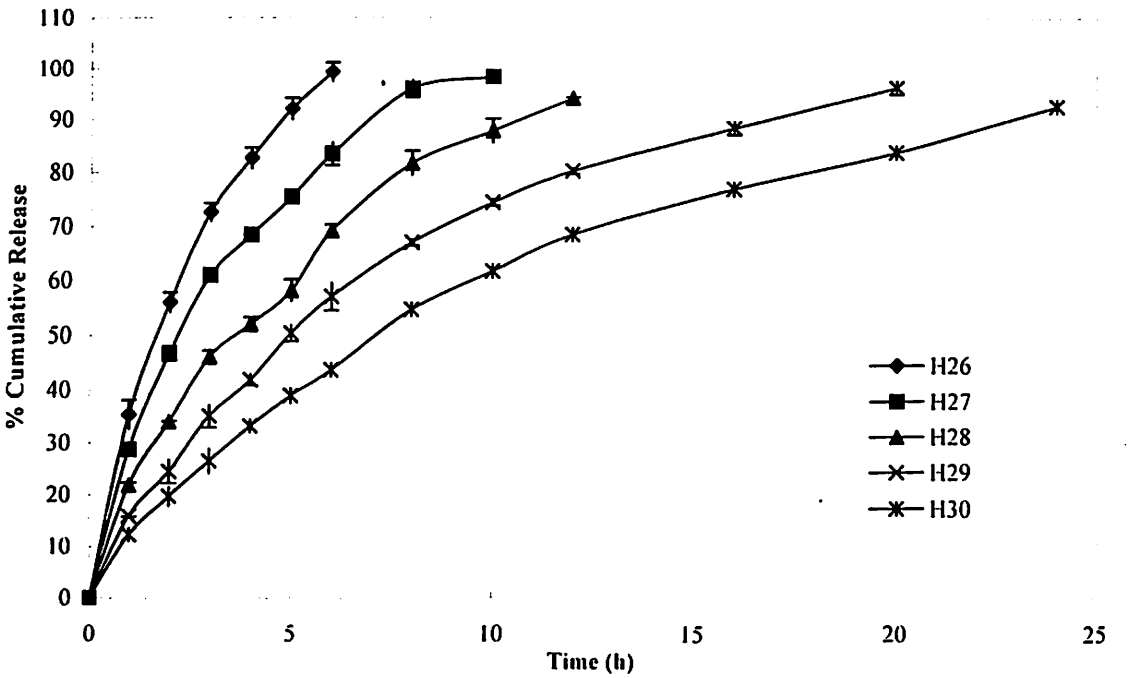

Fig 6.6. Comparative release profiles of isoniazid from Carbopol 934P (Direct compression) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

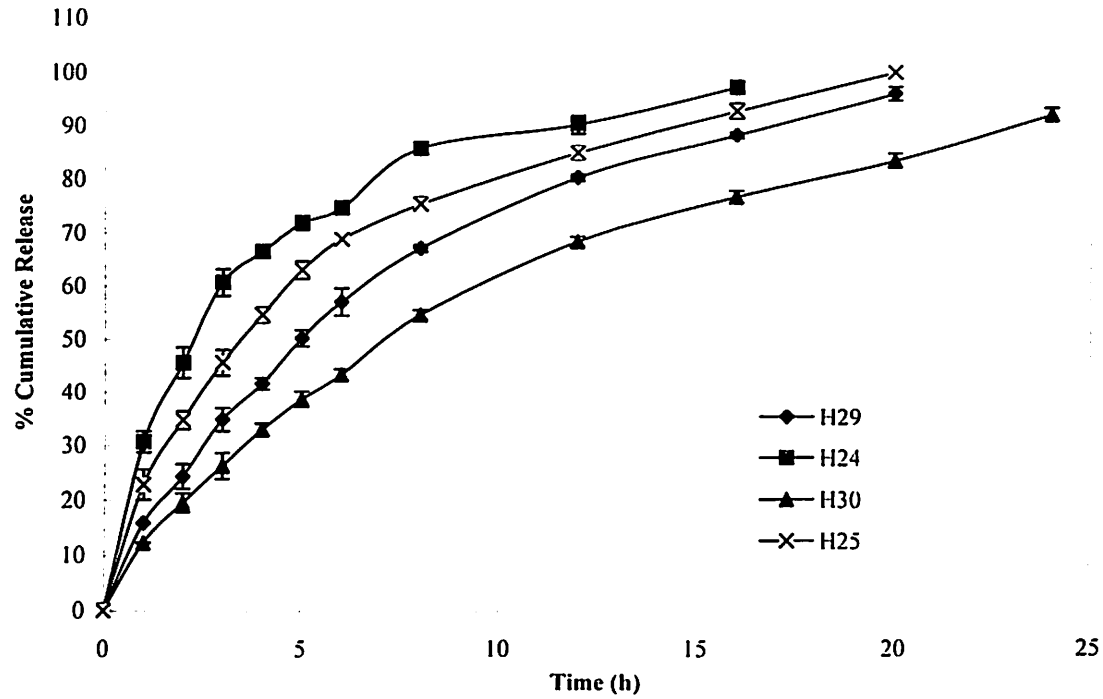

Fig 6.7. Effect of method of granulation on release profiles of isoniazid from Carbopol 934P formulations in pH 7.4 PO_4. Each data point represents the average of six tablets from three batches with S.D.

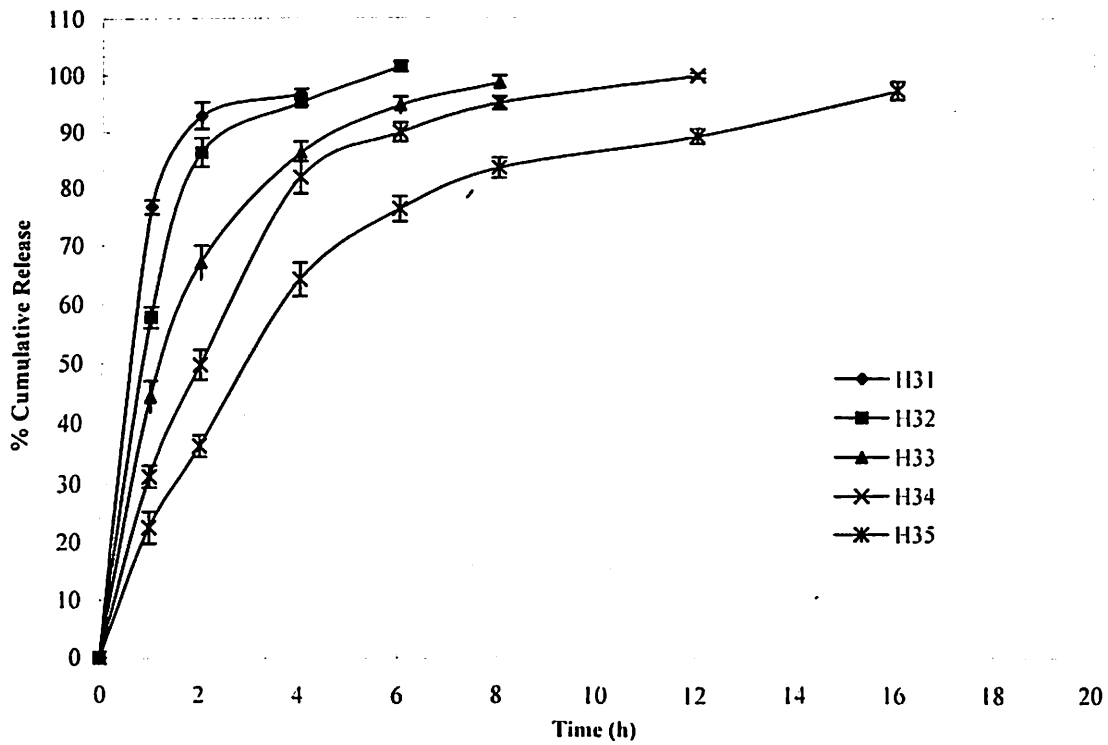

Fig 6.8. Comparative release profiles of isoniazid from HPC (Klucel LF) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

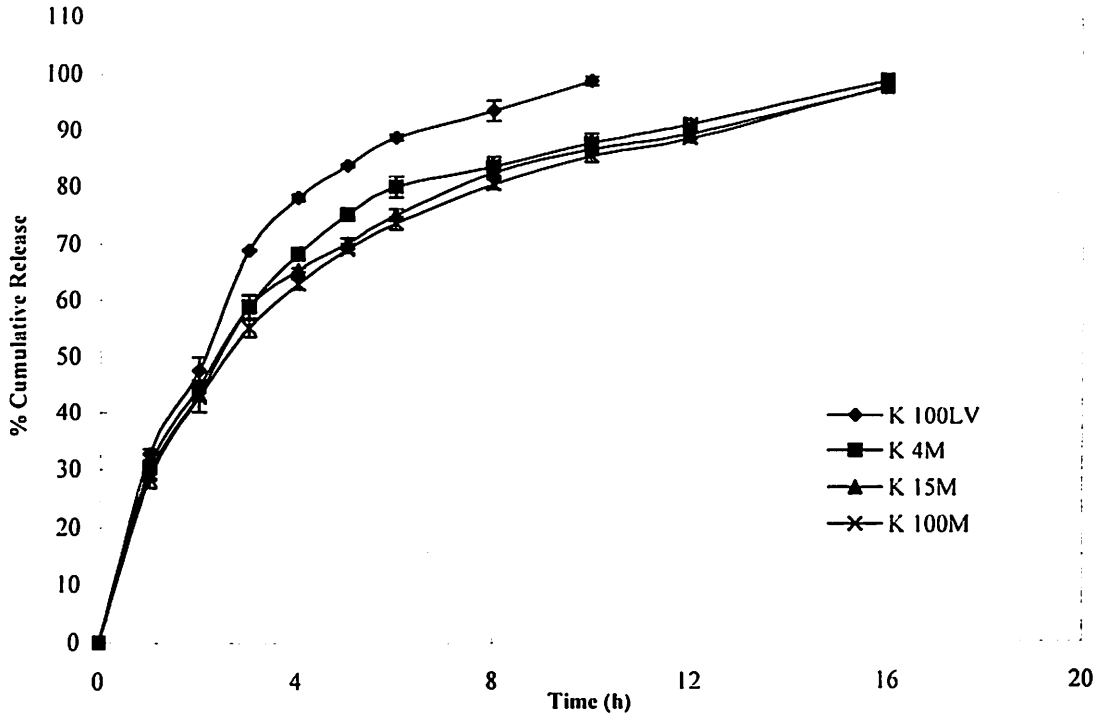

Fig 6.9. Effect of HPMC viscosity on isoniazid release profiles from 60% HPMC formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

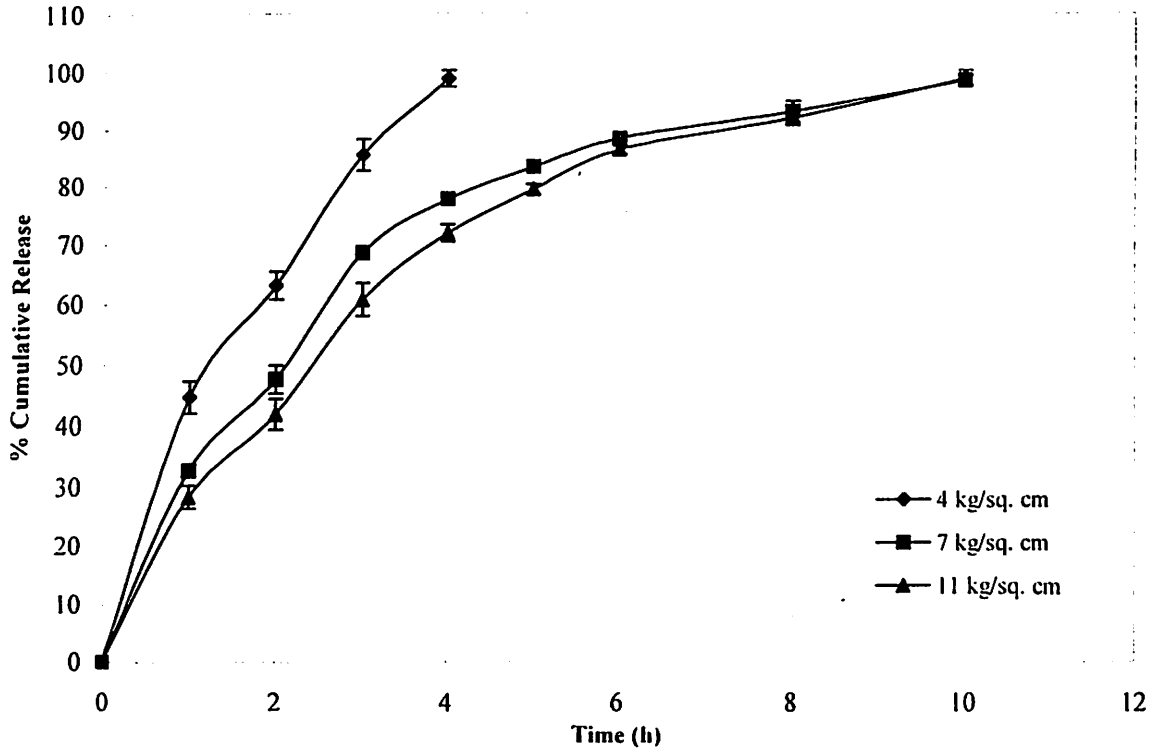

Fig 6.10. Effect of compression force on release profiles of isoniazid from HPMC K100LV (60%) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

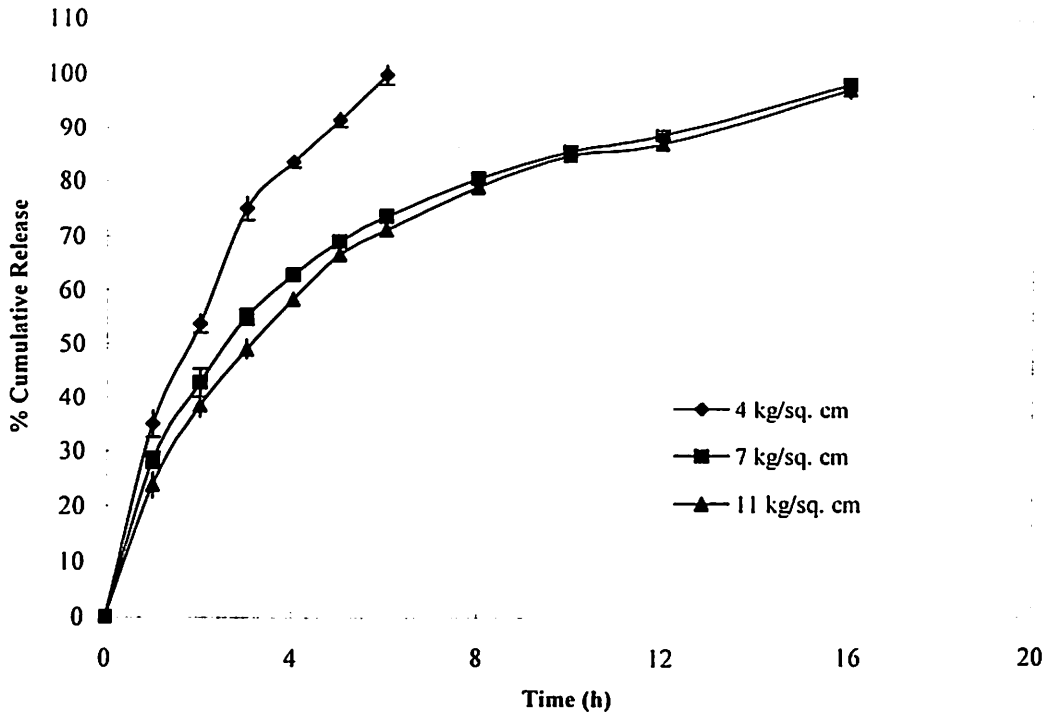

Fig 6.11. Effect of compression force on release profiles of isoniazid from HPMC K100M (60%) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

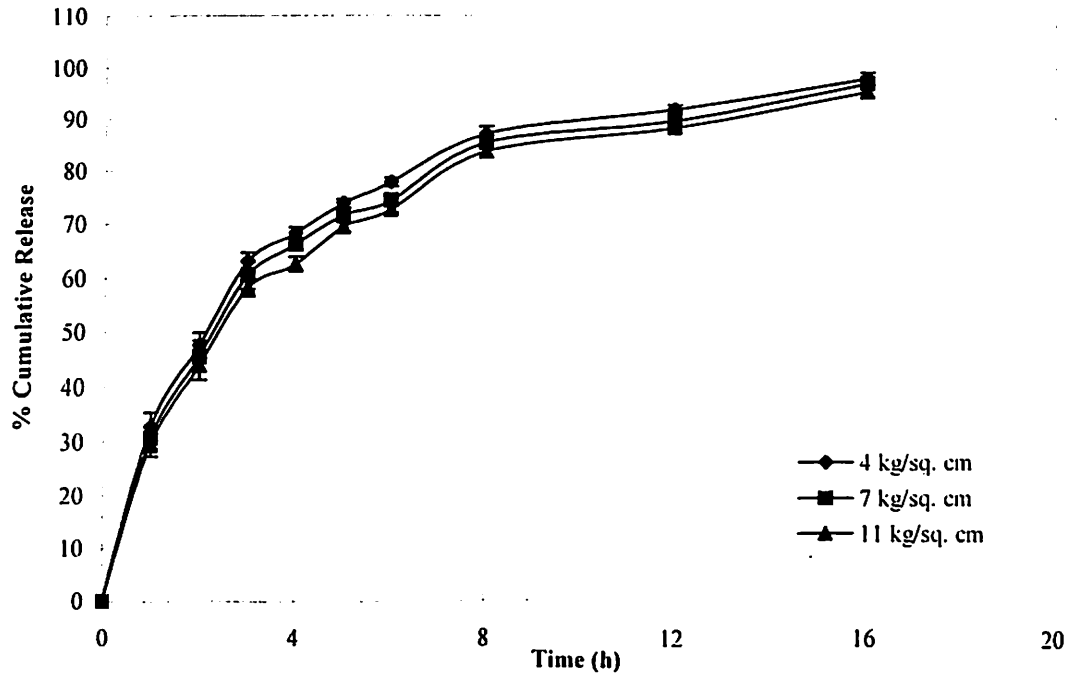

Fig 6.12. Effect of compression force on release profiles of isoniazid from 60% Carbopol 934P (IPA granulation) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

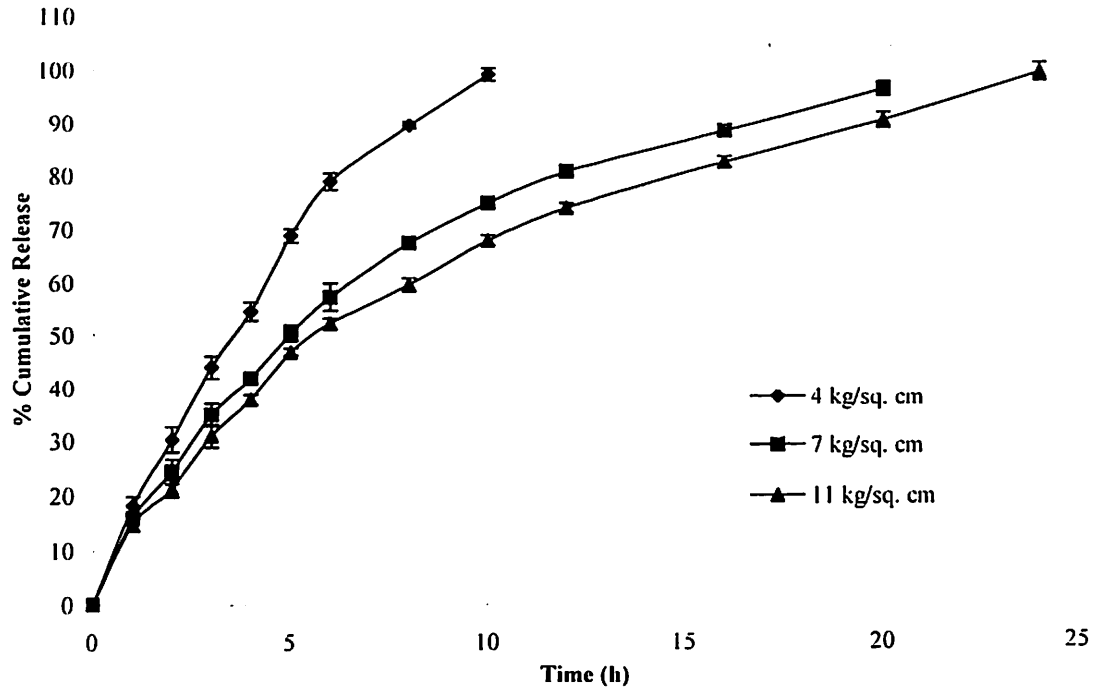

Fig 6.13. Effect of compression force on release profiles of isoniazid from 60% Carbopol 934P (Direct compression) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

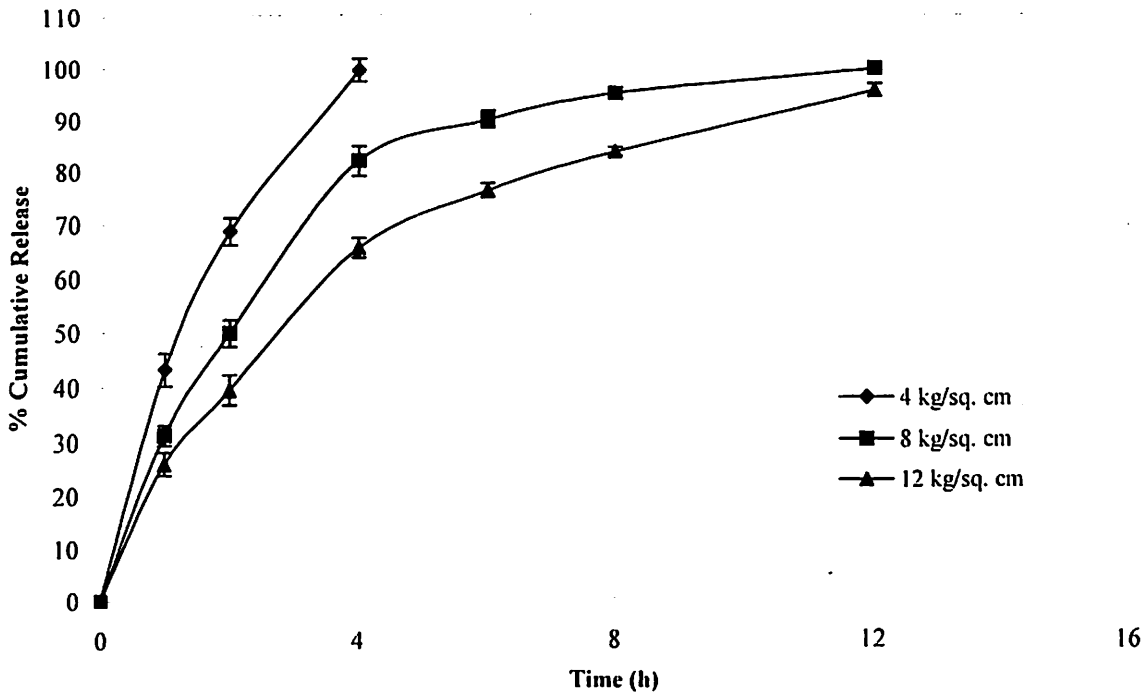

Fig 6.14. Effect of compression force on release profiles of isoniazid from 80% HPC (Klucel LF) formulations in pH 7.4 PO_4 (Each data point represents the average of six tablets from three batches with S.D.).

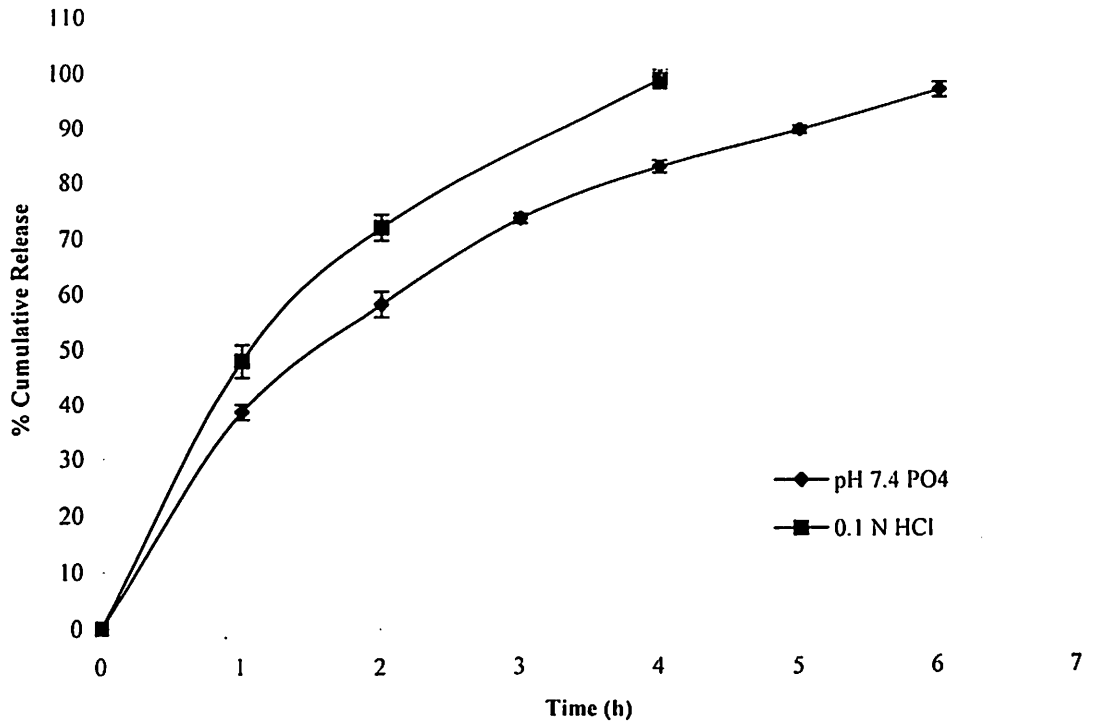

Fig 6.15. Effect of release media on release profiles of isoniazid from HPMC K100LV (40%) formulations (Each data point represents the average of six tablets from three batches with S.D.).

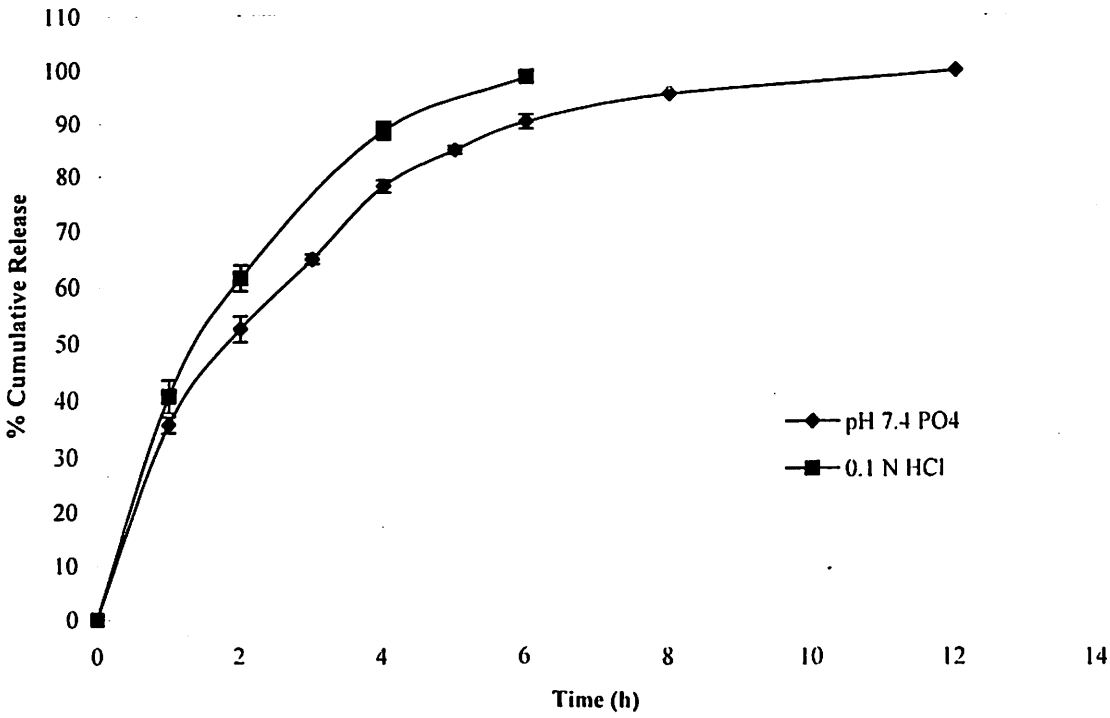

Fig 6.16. Effect of release media on release profiles of isoniazid from HPMC K15M (40%) formulations (Each data point represents the average of six tablets from three batches with S.D.).

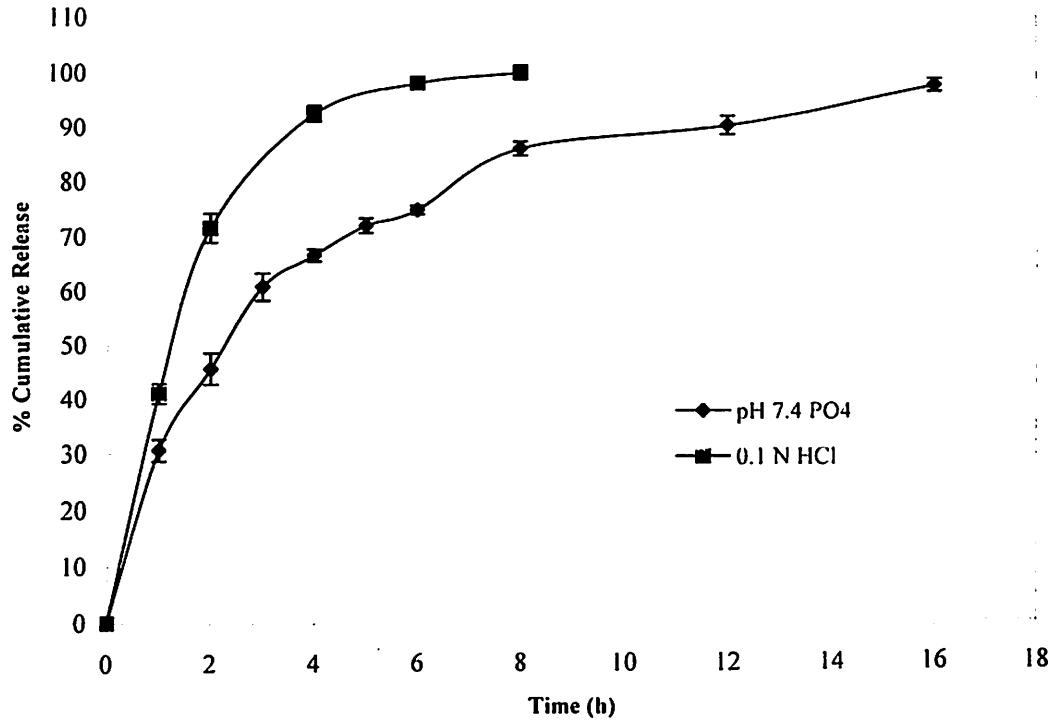

Fig 6.17. Effect of release media on release profiles of isoniazid from 60% Carbopol 934P (IPA granulation) formulations (Each data point represents the average of six tablets from three batches with S.D.).

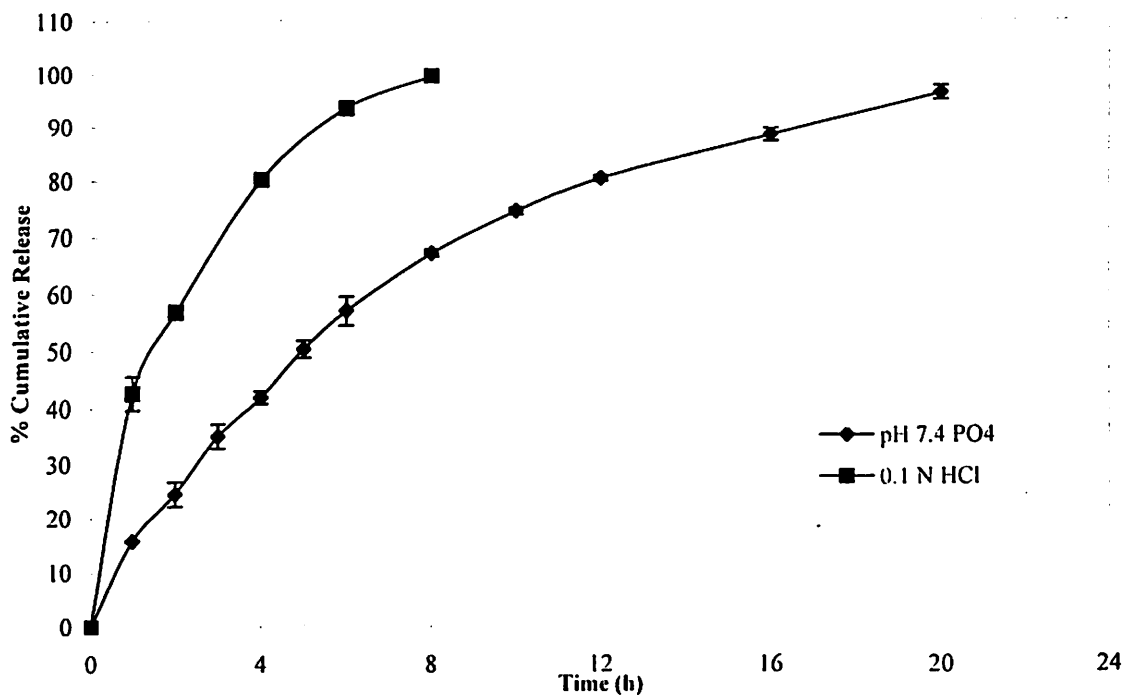

Fig 6.18. Effect of release media on release profiles of isoniazid from 60% Carbopol 934P (Direct compression) formulations (Each data point represents the average of six tablets from three batches with S.D.).

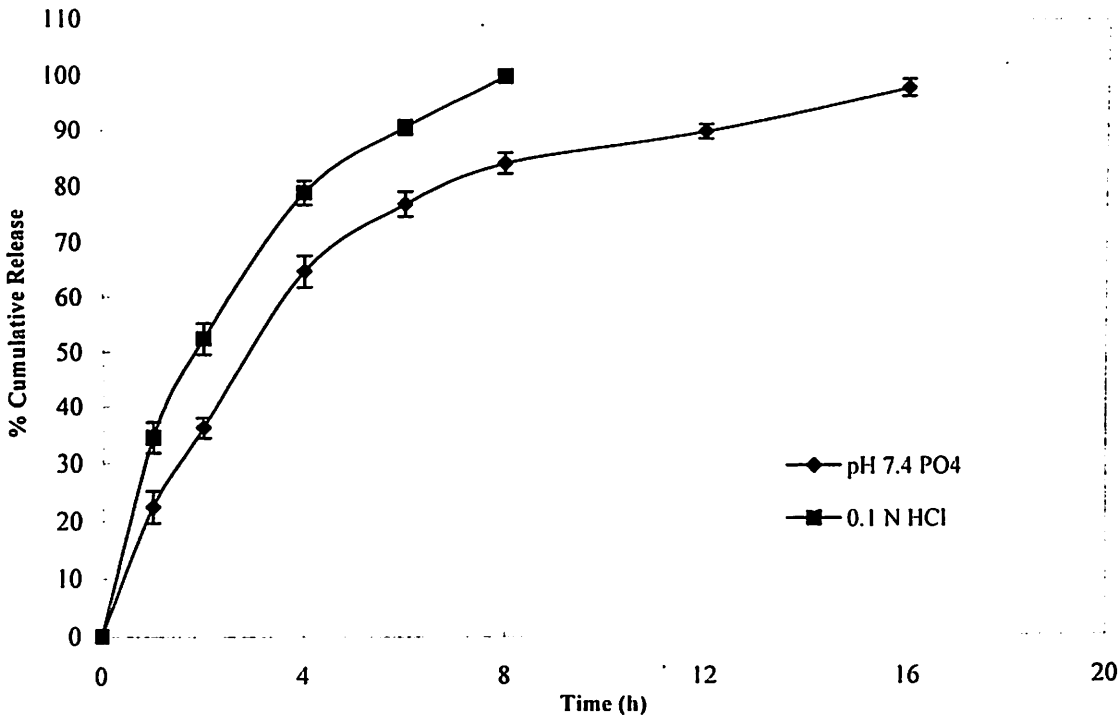

Fig 6.19. Effect of release media on release profiles of isoniazid from 100% HPC (Klucel LF) formulations (Each data point represents the average of six tablets from three batches with S.D.).

Chapter 7

Design and Studies of Oral Controlled Release Formulations of Rifampicin and Isoniazid Combination

Tuberculosis requires multidrug therapy for prolonged period of time that result in the increased incidence of toxic or side effects and poor patient compliance. Improper bioavailability of rifampicin and isoniazid from conventional anti-tubercular formulations is also matter of much concern. To overcome these drawbacks C.R. formulations containing rifampicin and isoniazid combination have been designed and studied.

7. 1. Experimental

Materials

Rifampicin and isoniazid were received as gift samples from the same sources as mentioned in chapter 3. Eudragit L100-55 was received as gift samples from Zydus Cadila Research Center, Ahmedabad. Ethyl cellulose was a gift sample from IPCA Labs Mumbai. All other polymers, chemicals and reagents received from the same sources as mentioned in chapter 5 and chapter 6.

Instruments/Equipments

All the instruments/equipments used were same as mentioned in chapter 5.

Analytical Method:

Analysis of drug samples was done as mentioned in chapter 3 for rifampicin and isoniazid combination by the UV visible spectrophotometric method.

7.1.1. Formulation of controlled release matrix tablets containing rifampicin and isoniazid combination

Some preliminary studies have been done before choosing suitable polymer combinations and their proportions. On the basis of experiments and results of C.R. formulations of individual drugs, specific formulations were designed and studied. Results of selected formulations are presented in this chapter.

7.1.1.1. Formulation of combination C.R. tablets with HPMC polymer

Accurately weighed quantity of isoniazid and HPMC K4M (passed through 60# mesh) were mixed uniformly and granulated with IPA, and dried in a tray drier at 60°C. To the dried granules was added weighed quantity of rifampicin (passed through 60# mesh) and mixed properly to assure uniform mixing. Finally this mixture was blended with talc (1% w/w) and magnesium stearate (1% w/w) and compressed in a 16 station tablet compression machine using 16/32" FFBE punches. Three batches of tablets were prepared for each formulation with each tablet contained 450 mg of rifampicin and 300 mg of isoniazid. The formula and the physical characteristics of the formulated tablets are given in Table 7.1.

7.1.1.2. Formulation of combination C.R. tablets with HPC polymer

The procedure of C.R. tablet manufacturing using HPC remains same as that for HPMC tablets. The formula and the physical characteristics of the formulated tablets are given in Table 7.2.

7.1.1.3. Formulation of combination C.R. tablets by bilayer technology

Controlled release bilayer tablets of rifampicin and isoniazid combination were prepared for comparative study. Three different tablet formulations were prepared containing separate layers of rifampicin (450 mg) and isoniazid (300 mg). Separately, two drugs were mixed with specific amount of retardant polymer combination of HPC, Eudragit (L100-55), and HPMC (K4M), Eudragit (L100-55) and granules were made. Tablet compression was carried out on a 16 station tablet compression machine using 16/32" FFBE punches.

First isoniazid granules were transferred in the die cavity and compressed at low compression force. Then rifampicin granules were filled in and comnpressed properly to get the tablets with hardness ranged between 8-9 kg cm^2 (The formula and the physical characteristics of the formulated tablets are given in Table 7.3).

7.1.2. Physical characterization of the tablets

Formulated tablets were subjected to different physical characterization studies (Table 7.1 to 7.3). The drug content of each batch of the formulated tablets was determined in triplicate. The weight variation was determined on 20 tablets using electronic balance (Afcoset). Tablet hardness was determined for minimum 6 tablets of each batch using Monsanto (Standard type) tablet hardness tester. Friability was determined with 20 tablets in a Cambell Electronic Friabilator for 5 min at 25 rpm.

7.1.3. In vitro release studies

Release studies were carried out using type 1 method (basket) at 37 ± 1° C with the stirring speed set at 100 rpm. The release medium was 350 ml SGF (pH 1.2) for initial 2 hours of the study, and then the total SGF media was replaced with 900 ml SIFsp (pH 6.8) in the later hours (3 to 24 h). At predetermined time intervals 10 ml of sample was withdrawn and replaced with fresh dissolution media. After appropriate dilutions, the samples were analyzed. Cumulative percent of drug released was calculated, and mean of six tablets from three different batches were used in the data analysis.

The in vitro release data was analyzed by using various kinetic models as reported in chapter 1. The following variations in tablet formulae were done and their effect on in vitro release rate, release mechanism and nature of release were studied.

7.1.3.1. Effect of polymer proportion

Tablets were made containing 20%, 40%, and 80% (w/w of isoniazid) of HPMC. However, the results are shown in the Figure 7.1 for 40% and 80% HPMC (RH2 and RH3) formulations as 20% polymer (RH1) released the whole amount of isoniazid within one hour. Tablets were made containing 40% (RH5), and 80% (RH6) of HPC. The results are depicted in the Figure 7.2.

To study the effect of addition of Eudragit L100-55, Eudragit L100-55 was added (60%) along with the HPMC K4M (80%) to isoniazid and granulated with IPA. The results are shown in the Figure 7.1. In case of HPC based tablets Eudragit was added along with the isoniazid and HPC and granulated with IPA. In this case (HPC), two formulations were

prepared, one with 60% HPC and 30% Eudragit and another with 80% HPC and 60% Eudragit. The results are shown in the Figure 7.3.

7.1.3.2. Effect of addition of EC

20% EC (w/w of isoniazid) was added along with the HPC (40% w/w of isoniazid) (RH7). The results are shown in the Figure 7.4.

7.1.3.3. Effect of change in the release media

For this study, formulations RH2 (40% HPMC K4M) and RH5 (40% HPC) were used. The release studies were done only in SIFsp for 24 h and same formulations were also subjected for the release studies in SGF (pH 1.2) for first 2 h followed by the release studies in SIFsp for 3^{rd} to 24^{th} h. The results are shown in the Figure 7.5 and 7.6 for HPMC K4M and HPC formulations respectively.

7.1.3.4. Effect of change in the manufacturing/formulation process

The study was carried out in case of formulations containing 80% and 60% (w/w of isoniazid) of HPMC K4M and Eudragit L100-55 respectively (RH4). In this case, rifampicin was mixed with isoniazid and this mixture was granulated with IPA instead of adding it extragranularly along with talc and magnesium stearate. The results are shown in the Figure 7.7.

The study was also carried out in case of formulations containing 80% and 60% (w/w of isoniazid) of HPC and Eudragit L100-55 respectively. Two small changes were done in the formulation processes, with the formula remaining same. In first case, instead of adding whole 60% (w/w of isoniazid) Eudragit L100-55 with isoniazid, only 30% was added and rest of the 30% (w/w of isoniazid) was added extragranularly along with rifampicin (RH10). In second case, rifampicin was granulated with IPA and mixed with isoniazid granules instead of adding it extragranularly along with talc and magnesium stearate (RH11). The results are shown in the Figure 7.8.

7.1.3.5. Effect of SGF media volume

For this study, a formulation containing 80% and 60% (w/w of isoniazid) of HPMC K4M and Eudragit L100-55 respectively as well as rifampicin granulated with IPA was selected (modified RH4 formulation). The SGF media volumes studied were 350 ml and 900 ml. The results are depicted in the Figure 7.9. The formulation containing 80% and 60% (w/w of isoniazid) of HPC and Eudragit L100-55 respectively as well as rifampicin granulated with IPA was also studied (RH11). The results are shown in the Figure 7.10.

7.1.3.6. Effect of presence of one drug on the release of other drug

The study was carried out for the formulations containing 80% and 60% (w/w of isoniazid) of HPC and Eudragit L100-55 respectively (RH10). In one case total isoniazid was replaced by rifampicin (RH12) and in other case total rifampicin was replaced by isoniazid (RH13). But, the care was taken to retain all other formulation and processing parameters same, so that any changes in the release characters/profiles should only attributed to the drug replacement. The results are shown in the Figure 7.11.

7.1.3.7. In vitro release studies from bilayer tablets

The results of release profiles are shown in the Figure 7.12, 7.13 and 7.14 respectively for RHB1, RHB2 and RHB3 formulations.

7.1.4. Batch Reproducibility

Batch reproducibility studies were carried out in a similar manner as performed for rifampicin C.R. formulations in chapter 5.

7.1.5. Stability Studies

Stability studies were carried out in a similar manner as performed for rifampicin C.R. formulations in chapter 5. The observed degradation rate constants and $t_{90\%}$ at different storage conditions are listed in Table 7.5.

7.2. Results and Discussions

7.2.1. Formulation of controlled release matrix tablets of rifampicin and isoniazid combination

It has already been discussed in preformulation studies that both rifampicin and isoniazid were sensitive to the moisture. It is also a known fact that the hydrolytic degradation of rifampicin enhances by presence of isoniazid. Thus, for both of these drugs, it has been decided to use either direct compression or nonaqueous granulation method for the preparation of the controlled release tablet formulations. It has already been observed, in case of rifampicin C.R. formulations, direct compression found to give better quality tablets with higher viscosity HPMC (K100LV, K4M and K15M), Carbopol 934P and HPC (Klucel LF) polymers. Where as, for the formulation of isoniazid C.R. tablets, nonaqueous granulation with IPA found to give better quality tablets with higher viscosity HPMC (K100LV, K4M, K15M and K100M) and HPC (Klucel LF) polymers.

So, same basic approach has been used in combined drug formulations also. The formulations prepared thus found to have good physicochemical properties (including hardness) (Table 7.1, and 7.2) and found to control the drug release for both the drugs when in vitro release studies were carried out. Further, to know the effect of processing techniques on release character, rifampicin was added along with the isoniazid and polymer and then granulated. Also rifampicin was separately granulated with IPA (with or without the polymer) and mixed with isoniazid granules (granulated separately along with the polymer, with IPA) and compressed to know its effect on the release profiles of both drugs.

The bilayered tablets prepared found to have good physicochemical properties (including hardness) (Table 7.3) and found to control the drug release for both the drugs when in vitro release studies were carried out.

As the drug doses (for isoniazid and rifampicin) were high, no attempt has been made to incorporate the excipients (such as microcrystalline cellulose, lactose, dicalcium phosphate, etc) other than controlled release polymers in to the matrices to increase the bulk further or to improve the granule properties. The use of polymer proportions also

restricted so as to make the formulations practically useful for the purpose of administration in terms of tablet height and thickness.

7.2.2. Physical characterization of the tablets

Physical appearance, tablet hardness, friability, weight variation, and drug content uniformity of all formulations were found to be satisfactory as can be observed from the data in Table 7.1, 7.2 and 7.3 for HPMC K4M, HPC (Klucel LF) and bilayer tablet formulations respectively. Hardness was found to be between 7 - 8 kg/cm^2 for HPMC K4M and HPC (Klucel LF) formulations. In case of bilayer tablet formulations the hardness was found to be between 8 - 9 kg/cm^2. The friability was less than 0.8% (w/w) in all cases. The formulated tablets showed very low weight variation and high degree of content uniformity, indicating that the method of preparation of formulation is an acceptable method for preparing good quality matrix tablets of rifampicin and isoniazid combination.

7.2.3. In vitro release studies

7.2.3.1. Effect of polymer and its proportion

HPMC formulations

Plots of percent cumulative drug released vs. time for HPMC K4M matrix tablet formulations, RH2 (40%) and RH3 (80%), are shown in the Figure 7.1. It can be observed from the graph that the increase in the polymer proportion resulted in decrease in the release for both rifampicin and isoniazid. Tablets containing HPMC K4M below 40% (w/w of isoniazid) did not significantly extended the release of isoniazid; hence their release profiles are not given. At the end of 24 h release studies, about 80% and 55% of the rifampicin was released from RH2 and RH3 formulations respectively. The isoniazid release was extended up to 12 h and 24 h respectively in case of RH2 and RH3 formulations. The release profiles were fitted with several different kinetics models as described in the methodology section. The correlation coefficient 'r' values for different release models are shown in the Table 7.4. The nature of rifampicin release was found to

be zero order in case of both the formulations as the percent drug released vs. time plots found to be linear (for rifampicin release). Where as, the isoniazid release found to follow Higuchi's square root kinetics model as the plots of percent cumulative drug released vs. square root of time found to be linear ($r \geq 0.930$). Since the drug release was less (for rifampicin) in the initial hours, for these formulations a separate loading dose would be required to attain the desired drug levels in the initial hour. In case of isoniazid, however, the results were quite different. There was very high initial release of isoniazid during initial hours. About 57% and 36% of isoniazid was released within 2 h from RH2 and RH3 formulations respectively. It was already discussed that for poorly soluble drug like rifampicin the erosional release would be dominant. For rifampicin the diffusional release would be very minimum, as the drug particles merely exist in the soluble form within the matrix. Thus, the rifampicin release was mainly because of the erosion matrix, which occurred at a constant rate resulting in the zero order release. The reason for initial higher release and decrease in the rate of isoniazid release with time can be explained as follows. As the drug (isoniazid) was highly soluble in the release media, the drug particles close to matrix surface might be released before the surrounding polymer reached the polymer disentanglement concentration. Within this time major amount of the drug might have been released resulting in the initial higher release in case of isoniazid. The decreasing rate of release with time for isoniazid was expected in a purely diffusional release due to increasing diffusional pathlength and decreasing diffusion coefficient of the drug as the release proceeded. There was a clear distinction between the isoniazid and rifampicin release profiles. This might be due to difference in their solubilities. Isoniazid being a highly soluble drug released at much faster rate compared to poorly soluble rifampicin. The presence of poorly soluble rifampicin drug particles along with isoniazid (in a same tablet matrix) didn't seem to decrease the initial release of isoniazid.

The release mechanism and kinetics of the release profiles were also analyzed by Korsmeyer-Peppas model up to 60% release. The values of K, n, $t_{50\%}$ (time for 50% of drug release) and r (correlation coefficient) obtained for various formulations are listed in Table 7.4. The n values for rifampicin release ranged from 0.86 to 0.89 indicating that the release mechanism was Case-II. Such mechanism of release mainly involves the swelling/relaxation and erosion contribution of the polymer matrix. These n values

showed that the rifampicin release was predominantly by erosion mechanism. The n values for isoniazid release ranged from 0.53 to 0.54 (nearly 0.5) indicating that the release mechanism was anomalous non-Fickian diffusion. In case of Higuchi's model, n is 0.5, thus the release profiles found to follow Higuchi's square root kinetics. It has already been stated that such mechanism of release was due to the combination of diffusion and swelling/relaxation. The difference in the release mechanisms between rifampicin and isoniazid was again because of difference in their solubilities. The release rate was high in case of RH2 (40%) formulations with K values of 0.046 $h^{-0.86}$ and 0.389 $h^{-0.54}$ respectively for rifampicin and isoniazid. The release rate was slow in case of RH3 (80%) formulations with K values of 0.029 $h^{-0.89}$ and 0.248 $h^{-0.53}$ respectively for rifampicin and isoniazid. The $t_{50\%}$ was low (15.77 h and 1.61 h respectively for rifampicin and isoniazid) in case of RH2 formulations compared to RH3 formulations (24.56 h and 3.77 h respectively for rifampicin and isoniazid).

Effect of addition of Eudragit L 100-55 was studied in RH3 formulations by adding 60% (w/w of isoniazid) Eudragit in the granulation stage along with isoniazid and HPMC K4M. The results are shown in the Figure 7.1. It can be observed from the Figure 7.1 that rifampicin release decreased in SGF and increased in SIFsp in presence of Eudragit. This might be due to pH dependent solubility of the Eudragit. Eudragit L100-55 is an anionic polymer based on methacrylic acid and methacrylic acid esters that dissolve above pH 5.5 by salt formation (Mehta et al. 2001, Khan et al. 1999). In SGF, as the polymer was insoluble it closed the pores available for the media infiltration in to the matrix. This caused the delay in the matrix hydration, swelling and ultimately erosion. Thus, the rifampicin release was slightly lower in presence of the Eudragit in SGF. When the release studies were continued in SIFsp the rifampicin release got enhanced in presence of the Eudragit. This might be because of the increased solubility and release of the Eudragit in SIFsp that resulted in the significant increase in the matrix porosity. Hence, there was increased penetration of the release media in to the tablet matrix and increased release of rifampicin through these matrix pores. Whereas addition of Eudragit produced different effect on isoniazid release. Isoniazid release was decreased both in SGF and SIFsp in presence of Eudragit. In SGF Eudragit acted as an insoluble mass that reduced the matrix porosity and thus decreased the diffusivity of isoniazid molecules.

The contribution of erosional release towards the total release mechanism was enhanced in presence of Eudragit for both isoniazid and rifampicin. This might be because of the fact that the Eudragit L100-55 is essentially a water insoluble polymer and erosion is the main mechanism of the drug release from the matrices prepared with this polymer (Ammar and Khalil 1997, Al-Taani and Tashtoush 2003). It can be observed from the Table 7.4 that the n value was 0.89 for RH3 and 1.02 for RH4 formulations indicating that the release mechanism of rifampicin was predominantly erosion based in presence of Eudragit. The n value found to be 0.53 for RH3 and 0.64 for RH4 formulations; here also the contribution of swelling/erosion towards isoniazid release became predominant in presence of the Eudragit.

HPC formulations

Plots of percent cumulative drug released vs. time for HPC matrix tablet formulations, RH5 (40%) and RH6 (80%), are shown in the Figure 7.2. It can be observed from the graph that the increase in the polymer ratio resulted in the decrease in the release for both rifampicin and isoniazid. Tablets containing HPC below 40% (w/w of isoniazid) didn't significantly extended the release of rifampicin and isoniazid; hence their release profiles are not given. The rifampicin release was extended up to 12 h and 24 h respectively from RH5 and RH6 formulations. The isoniazid release was extended up to 12 h and 16 h respectively in case of RH5 and RH6 formulations. The release profiles were fitted with several different kinetics models. The correlation coefficient 'r' values for different release models are shown in the Table 7.4. The 'r' values for all models (zero, first and Higuchi's square root kinetics) found to be poor, with comparatively higher value for first order. This might be due to the different release rate or profile of rifampicin in two different medias, SGF and SIFsp. This was probably due to the effect of pH on polymer. Hence, we decided to analyze nature of the release separately in two medias. It can be seen from the Figure 7.2 that there was initial higher release in SGF up to 2 h followed by a decrease in the release when the media changed to SIFsp. But in SIFsp the nature of rifampicin release was found to be zero order in case of both the formulations as the percent drug released vs. time plots found to be linear (r = 0.999 and 0.995 respectively for RH5 and RH6). Where as, the isoniazid release found to follow nearly first order

model, but showed different release profile in two media. In case of both the drugs, the initial release was higher indicating no necessity of incorporation of a separate loading dose to attain the desired drug levels in the initial hour. During initial hours about 79% and 40% of rifampicin was released within 2 h from RH5 and RH6 formulations respectively. Similarly, about 79% and 58% of isoniazid was released within 2 h from RH5 and RH6 formulations respectively. The detailed explanation for zero order release of rifampicin from HPC formulations remains same as that for HPMC formulations (RH2 and RH3), in brief it might be due to its poorly soluble nature that prevented its diffusional release. The reason for initial higher release and decrease in the rate of isoniazid from HPC formulations (RH5 and RH6) with time may be due to effect of pH (SGF and SIFsp) on HPC. In brief, the decreasing rate of release with time for isoniazid was expected in a purely diffusional release due to increasing diffusional pathlength and decreasing diffusion coefficient of the drug as the release proceeded. There was a clear distinction between the isoniazid and rifampicin release profiles (at 80% polymer ratio). This might be due to difference in their solubilities. Isoniazid being a highly soluble drug released at much faster rate compared to poorly soluble rifampicin.

The main difference in the in vitro release rate studies between HPMC and HPC formulations was observed in the initial release of rifampicin. It has been already discussed that the initial amount of rifampicin released was less in case of HPMC formulations. This might be due to the formation of thick hydrated gel layer once the tablet came in contact with the release media in case of high viscosity (4000 cPs) HPMC K4M formulations. This stable gel layer prevented the initial release of rifampicin because of the reduced tendency of the matrix to undergo erosion that prevented the initial release (which is erosional release) of rifampicin from HPMC based tablets. Where as, the HPC used was of low viscosity and was having less swelling tendency. Thus, the hydrated gel layer formed was not stable enough to resist erosion. Hence, during initial hours itself the matrix started eroding along with that substantial amount of the rifampicin has been released leading to higher initial release of rifampicin in case of HPC based formulations.

The release mechanism and kinetics of the release profiles were analyzed by Korsmeyer-Peppas model up to 60% release. The values of K, n, $t_{50\%}$ (time for 50% of drug release)

and r (correlation coefficient) obtained for various formulations are listed in Table 7.4. The n values for rifampicin release ranged from 0.74 to 0.89 indicating that the release mechanism was anomalous diffusion at low polymer ratio and Case-II at high polymer ratio. These n values showed that the rifampicin release was predominantly by erosion mechanism. The n values for isoniazid release ranged from 0.64 to 0.86 indicating that the release mechanism was anomalous non-Fickian diffusion and the contribution of swelling/relaxation towards release became prominent at high polymer ratio (RH6). Thus, the mechanism of release for rifampicin and isoniazid found to be combination of diffusion and erosion. At high polymer ratio closing of micropores and increase in the matrix tortuosity resulted in the decreased diffusion of the drug molecules that lead to the erosion dependent release at high polymer ratio. The release rate was high in case of RH5 (40%) formulations with K values of 0.358 $h^{-0.89}$ and 0.421 $h^{-0.86}$ respectively for rifampicin and isoniazid. The release rate was slow in case of RH6 (80%) formulations with K values of 0.198 $h^{-0.74}$ and 0.378 $h^{-0.51}$ respectively for rifampicin and isoniazid. The $t_{50\%}$ was low (1.47 h and 1.22 h respectively for rifampicin and isoniazid) in case of RH5 formulations compared to RH6 formulations (3.55 h and 1.73 h respectively for rifampicin and isoniazid). HPC behaved differently in SGF and SIFsp thus release characters were also different.

Effect of addition of Eudragit L100-55 was studied in two formulations, one with 60% HPC and 30% Eudragit L100-55 (RH8) and another with 80% HPC and 60% Eudragit L100-55 (RH9). The results are shown in the Figure 7.3. Rifampicin release was decreased in SGF and enhanced in SIFsp in presence of Eudragit from both RH8 and RH9 formulations. The f_2 factor values of 48.74 between RH6 and RH8 formulations and, 44.78 between and RH6 and RH9 formulations, for the release profiles of rifampicin showed that the rifampicin release profiles were significantly affected by the presence of Eudragit. But the f_2 factor value of 76.11 for the release profiles of rifampicin between RH8 and RH9 formulations indicated that there was no significant difference in the release profiles. Thus, the replacement of 20% HPC in case of RH6 formulations with 30% Eudragit L100-55 (in case of RH8) formulations was sufficient enough to decrease the initial release of rifampicin in SGF and, to obtain complete release (within 24 h) in SIFsp. The increase in the HPC ratio from 60% (RH8) to 80% (RH9) and Eudragit ratio

from 30% (RH8) to 60% (RH9) did not significantly changed the rifampicin release either in SGF or in SIFsp. The reason for the observed differences in the rifampicin release profiles in different release medias in presence of Eudragit from HPC formulations is similar to that explained in case of HPMC formulations. However, the effect was slightly different on isoniazid release. There was no significant difference in the isoniazid release profiles between RH6 and RH8 formulations (f_2 = 64.08). This might be due to increased solubility and dissolution of Eudragit L100-55 at higher pH conditions (SIFsp) that made the matrix more porous and more prone towards erosion. But there was significant difference in the isoniazid release profiles between RH6 and RH9 formulatios (f_2 = 39.35). Isoniazid release was decreased both in SGF and SIFsp from RH9 compared to RH6. The reason for such observation also remains same as explained for the release profiles of isoniazid from HPMC formulations in presence of Eudragit L100-55. The n values found to be 0.60 and 0.67 respectively for rifampicin release from RH8 and RH9 indicating that the release was due to combined effect of diffusion and erosion (anomalous non-Fickian diffusion). The isoniazid release was found to be by Fickian diffusion (n = 0.42) in case of RH8 and by non-Fickian diffusion (n = 0.63) in case of RH9 formulations. In this case also the contribution of swelling/erosion towards isoniazid release became predominant in presence of the Eudragit.

7.2.3.2. Effect of addition of ethyl cellulose

In this study 20% (w/w of isoniazid) of EC was added along with the HPC 40% (w/w of isoniazid) (RH7) and the effect on release was studied and compared with RH2 formulations (containing 0% EC). The results are shown in the Figure 7.4.

The rifampicin release was extended up to 24 h in case of RH7 formulations (containing 20% EC) compared to 12 h in case of RH2 formulations (containing 0% EC). Where as, isoniazid release was extended up to 16 h in case of RH7 formulations compared to 12 h in case of RH5 formulations. The rifampicin release during initial hours decreased in RH7 (~ 22% and 46% after 1st and 2nd h respectively) compared to RH2 (~ 34% and 79% after 1st and 2nd h respectively). But the reduction in the initial release of isoniazid from RH7 (~ 22% and 46% after 1st and 2nd h respectively) compared to RH2 (~ 43% and 64%

after 1st and 2nd h respectively) was significant only after 2nd h. The f_2 factor values of 30.46 and 48.49 for rifampicin and isoniazid release between RH7 and RH5 formulations indicated that the presence of EC significantly reduced the release of both rifampicin and isoniazid. Among two drugs, the rifampicin release was more affected by the presence of EC (refer f_2 factor values). This might be due to the fact that the release of rifampicin was mainly dependent on the matrix erosion (poorly soluble drug) and matrix erosion was hindered in presence of hydrophobic EC due to poor release media penetration in to the tablet matrix and decreased rate of hydration, swelling and ultimately erosion of the matrix. But isoniazid release was predominantly by diffusion (because of its high solubility), thus the presence of 20% EC did not drastically altered the gel layer strength or the barrier thickness resulting in the less influence on the diffusive release of the isoniazid.

The n value found to be 0.71 for rifampicin release indicating that the release was due to combined effect of diffusion and erosion, and, the contribution of the erosion being greater. The n value found to be 0.45 for isoniazid release indicating that the release was purely by Fickian diffusion. The presence of EC in the matrix structure might have reduced the swelling tendency and relaxation of polymer molecules thus reducing their contribution towards release, so the isoniazid release was purely by the Fickian diffusion through the pores present in the matrix structure. The K value decreased (0.243 $h^{-0.71}$) and $t_{50\%}$ increased (2.77 h) for rifampicin release in case of RH7 formulations compared to RH5. But there was no significant difference in the K value (0.438 $h^{-0.45}$) and $t_{50\%}$ (1.34 h) for isoniazid release between RH7 and RH5 formulations.

7.2.3.3. Effect of change in the release media on release profile

HPMC formulations

For this study, RH2 formulations (with 40% HPMC K4M) were selected. The release studies were done (i) in SIFsp for 24 h, (ii) in SGF (pH 1.2) for initial 2 h followed by SIFsp till 24 h. The results are shown in the Figure 7.5.

It can be seen from the Figure 7.5 that there was no significant difference in the release profiles of rifampicin due to change in media for first 2 h. Release in first 2 h and later

remained more or less same. The f_2 factor value of 68.54 between the rifampicin release profiles in SIFsp alone and in SGF followed by SIFsp for RH2 formulations showed that there was no significant difference in the release profiles.

There was marginal decrease in the release of isoniazid when initial release was also studied in SIFsp instead of SGF. The f_2 factor value (58.53) also showed insignificant difference in the release profiles in SGF and SIFsp. The results suggested that change in media for first 2 h would not change release of both rifampicin and isoniazid from formulations with HPMC K4M polymer.

HPC formulations

For this study, RH5 formulations were selected. The similar studies were carried out as that for the effect of release media in case of HPMC formulations (RH2). The results are shown in the Figure 7.6.

It can be seen from the Figure 7.6 that there was a significant decrease in the release profile of rifampicin as well as isoniazid, with more impact on rifampicin release, when initial release also studied in SIFsp media. The f_2 factor value of 9.75 for rifampicin also established the difference in release. The rifampicin release was extended from 12 h to more than 24 h when SGF was replaced with SIFsp for first 2 h. Only about 61% of the rifampicin released at the end of 24 h in single media of SIFsp. There was a huge difference in the initial amount of the rifampicin released also. In case of SIFsp only about 7% of the rifampicin was released within 2 h compared to about 79% in media change method. This might be due to strong dependence of rifampicin solubility on pH of the release media and effect of release media on polymer. Due to high solubility of rifampicin in pH 1.2, there was an initial burst release of rifampicin from low viscosity HPC (Klucel LF) formulation (at low polymer ratio) in SGF.

There was also a significant difference in the isoniazid release in changed media. Release was decreased when initial media changed from SGF to SIFsp. in the isoniazid release when release studies were done in SIFsp alone compared to media change method. The f_2 factor value of 30.86 for isoniazid release established the effect of media change on release. The isoniazid release was extended only up to 8 h in case SGF followed by SIFsp compared to 16 h in SIFsp alone. There was also a significant difference in the initial

amount of the isoniazid released. In case of SIFsp only about 49% of the isoniazid was released within 2 h compared to about 79% in SGF followed by SIFsp. This might again be due to difference in the rifampicin solubility in SGF and in SIFsp and effect of media pH on polymer (HPC). In a study of change of media on isoniazid release from isoniazid C.R. formulations (Figure 6.19), the release was appreciably decreased in 7.4 pH phosphate buffer compared to at 0.1 N HCl suggesting the effect of media on HPC polymer though the solubility of isoniazid was same. However, as change in release was much higher in combination formulation (Figure 7.6), probably the release in SGF was much higher due to excess solubility of rifampicin causing creation of large number of pores. Thus, the isoniazid release enhanced due to increased diffusion through the pores present in the matrix structure and enhanced erosion of the matrix. But the poorly soluble rifampicin hindered the isoniazid release by closing the micropores in the matrix structure and increasing the tortuosity in SIFsp.

The release mechanism for rifampicin was found to be Case-II (n = 0.89 in both release media studies). The K value was lower (0.042 $h^{-0.89}$) and $t_{50\%}$ was higher (15.83 h) for rifampicin when the release studies were done in SIFsp alone compared to media change method (K value 0.358 $h^{-0.89}$ and $t_{50\%}$ 1.47 h). There was a slight change in the release mechanism of isoniazid from almost Case-II release (n = 0.86) in media change method to non-Fickian release (n = 0.52) in SIFsp alone. This might be due to increased swellability and erosion of the matrix in SGF (because of the soluble rifampicin) that resulted in the relaxational controlled release (Case-II) in case of media change method. But in SIFsp alone, there were both diffusion and erosion mechanisms contributing towards the release of isoniazid. The K value was lower (0.314 $h^{-0.52}$) and $t_{50\%}$ was higher (2.43 h) for isoniazid when the release studies were done in SIFsp alone compared to media change method (K value 0.421 $h^{-0.86}$ and $t_{50\%}$ 1.22 h).

7.2.3.4. Effect of change in the manufacturing/formulation process

HPMC formulations

The release of both rifampicin and isoniazid decreased in case of formulations with intragranular rifampicin along with the isoniazid (modified RH4 formulations) (Figure

7.7). At the end of 24 h only ~ 48% and 52% of the rifampicin and isoniazid released respectively from the modified RH4 formulations compared to ~ 82% and 94% of the rifampicin and isoniazid respectively from RH4 formulations. The f_2 factor values of 38.21 and 28.83 for rifampicin and isoniazid release respectively between modified RH4 and RH4 formulations further proved that the variations in the formulation processing had a significant influence on the release of both isoniazid and rifampicin.

There was no change in the initial release (0-2 h). Release was changed in the later phase due to change in availability of rifampicin for diffusion an dissolution. Presence of rifampicin in intragranular space made its availability less with increased tortuosity and increased diffusional path length. However, the polymer matrix presented a lower resistance for the release media infiltration in formulations containing extragranular rifampicin leading to quicker polymer hydration, swelling and erosion. Thus, in case of RH4 formulations the erosional release of rifampicin was comparatively higher than that from modified RH4 formulations. The mechanism of rifampicin release found to be unaffected by the change in the formulation processing (n = 1.02 and n = 1.28 for RH4 and modified RH4 formulations respectively). However, the K value decreased (0.014 $h^{-1.28}$) and $t_{50\%}$ increased (16.28 h) in case of modified RH4 formulation compared to the K value (0.052 $h^{-1.02}$) and $t_{50\%}$ (11.34 h) for RH4 formulations. The decrease in K and increase in $t_{50\%}$ in case of modified RH4 again proved that the processing variable had a significant impact on rifampicin release.

The initial release of isoniazid was more drastically decreased compared to rifampicin when the formulation processing has been changed (from modified RH4 formulations). There was ~ 8% and 13% isoniazid was released from modified RH4 formulations after 1 and 2 h, compared to 16% and 27% from RH4 formulations. Thus, the presence of rifampicin inside the granulations along with the isoniazid significantly decreased the initial release of isoniazid. The possible explanation for such observations might be given as follows. In the modified RH4 formulation the isoniazid was present along with the rifampicin (within the granules). When the tablet matrix came in contact with the release media, the media penetrated in side the tablet and hydration of the polymer resulted in the swelling. The diffusion of isoniazid molecules through the hydrated gel layer was hindered because of the presence of the poorly soluble (compared to isoniazid) rifampicin

particles within the matrix. Here, the rifampicin acted merely as an insoluble filler or excipient in the matrix that reduced the porosity of the matrix and decreased the diffusivity of the isoniazid molecules. Thus, the isoniazid release decreased in the SGF in presence of intragranular rifampicin from modified RH4 formulations. The rate and extent of the reduction in the isoniazid release was much more pronounced in SIFsp release media. This might be due to very poor solubility of the rifampicin in SIFsp (compared to SGF). Thus, there might be more number insoluble rifampicin particles in the matrix structure at any time point compared to that in SGF. These insoluble rifampicin particles exhibited increased resistance to the diffusion of the isoniazid from the tablet matrix in to the SIFsp resulting in the decreased isoniazid release. The mechanism of isoniazid release found to be unaffected by the change in the formulation processing (n = 0.64 and n = 0.59 for RH4 and modified RH4 formulations respectively). However, the K value decreased (0.093 $h^{-0.59}$) and $t_{50\%}$ increased (17.34 h) in case of modified RH4 formulations compared to the K value (0.169 $h^{-0.64}$) and $t_{50\%}$ (5.47 h) for RH4 formulations. The decrease in K and increase in $t_{50\%}$ in case of modified RH4 again proved that the processing variable had a significant impact on isoniazid release.

HPC formulations

It can be observed from the Figure 7.8 that there was no significant difference in the release profiles of rifampicin between RH9 and RH10 formulations (f_2 = 72.31). Thus, the process variation in terms of the addition of Eudragit L100-55, totally inside the granules along with isoniazid or partly along with rifampicin extragranularly, did not significantly influence the release of rifampicin. Where as, the rifampicin release was decreased in case of RH11 formulations compared to RH9 in both SGF and SIFsp (f_2 = 45.46). Thus, the IPA granulation of rifampicin (in case of RH11) formulations significantly reduced its release compared to when it was present as a simple mixture (direct compression) extragranularly in case of RH9 formulations. The reason for such observation might be explained as follows. It has been already discussed that for a hydrophobic (poorly water soluble) drug like rifampicin, IPA granulation with HPMC (high viscosity) or HPC resulted in the decreased release rates and extension in the release compared to directly compressed tablets. This might be due to reduced release

media penetration in to the tablet because of the presence of insoluble drug granulated with nonaqueous IPA. This might have reduced the rate of tablet matrix hydration and erosion along with which the drug release decreased.

It can be seen from the Figure 7.8 that there was no significant difference in the release profiles of isoniazid between RH9 and RH10 formulations (f_2 = 57.83). The release profiles of isoniazid were almost same in SGF but, there was a slight decrease in the isoniazid release from RH10 formulations compared to RH9 in SIFsp. The isoniazid release from RH11 was lower compared to RH9 formulations (f_2 = 36.40). The reason for decrease in the isoniazid release from RH11 formulations compared to RH9 formulations might be again due to the intragranular presence of rifampicin and detailed explanation remains same as that discussed for the decrease in the rifampicin release from RH11 compared to RH9.

The n values found to be 0.68 for rifampicin release from RH10 indicating that the release was due to combined effect of diffusion and erosion (anomalous non-Fickian diffusion) and remained same as that for RH9. Where as, the mechanism of rifampicin release was shifted from non-Fickian release (n = 0.67) in case of RH9 formulations to Super Case-II (erosion controlled release with n = 1.12) in case of RH11 formulations. In case of RH9, the rifampicin was present extragranularly and thus there might be increased possibility of its escape through the matrix pores existed that lead to combined diffusion and erosion contribution in the release. Where as, in case of RH11, rifampicin was present within the granules (IPA granulations) thus, there were only two possibilities existed for the rifampicin release, either by the diffusion through the hydrated matrix layer or along with the matrix erosion. But, to diffuse through gel layer the drug should be in solubilized form, so, this possibility was limited for the rifampicin release. Thus, the only mechanism for the rifampicin release was by the erosion of the tablet matrix that lead to Super Case-II release mechanism in case of RH11 formulations. The n value for isoniazid release from RH10 found to be 0.53 indicating a non-Fickian release similar to RH9 formulations. Where as, the release mechanism shifted from non-Fickian diffusion (n = 0.63) in case of RH9 formulations to Fickian diffusion (n = 0.45) in case of RH11 formulations due to change in the formulation processing. This might be due to the reduced tendency of the matrix to swell and erode (because of reduced release media

infiltration) as the presence of IPA granulated rifampicin hindered the release media penetration and matrix hydration. Thus, the contribution of swelling/relaxation was negligible for isoniazid release in case of RH11 formulations resulting in the pure Fickian release profiles.

7.2.3.5. Effect of SGF media volume

HPMC formulations

It has been already discussed in the dissolution method development section that the volume of stomach fluid in the fasted state found to be as little as 20-30 ml (Dressman et al. 1998). And normally patient takes the formulation with a glass of water, then the total fluid volume of stomach in fasted state comes around 200-300 ml. Thus, it was decided to use 350 ml of SGF during initial hours of release studies to approximately simulate in vivo fasted state conditions. However, the study was carried out to know the effect of SGF media volume of the release profiles of rifampicin and isoniazid. For this study, modified RH4 formulations were selected. The SGF media volumes changed from 350 ml to 900 ml. The results are depicted in the Figure 7.9.

It can be observed from the graph that both rifampicin and isoniazid release was not significantly affected by the SGF media volume. The rifampicin and isoniazid release were extended up to 24 h in both the volumes. At the end of 24 h, ~ 46% and 48% of the rifampicin was released from modified RH4 formulations in the release studies containing 350 ml and 900 ml of SGF respectively. At the end of 24^{th} h, ~ 73% and 74.5% of the isoniazid was released from modified RH4 formulations in the release studies containing 350 ml and 900 ml of SGF respectively. The high f_2 factor values of 92.39and 81.95 respectively for rifampicin and isoniazid release between the 350 ml and 900ml SGF release studies proved that the SGF media volume had no significant influence on the release of both drugs.

The release mechanism and release kinetics parameters of rifampicin and isoniazid also remained unaffected by the change in the SGF media volume.

HPC formulations

For this study, RH11 formulation was selected. The SGF media volumes used for the study were same 350 ml and 900 ml as in earlier case. The results are shown in the Figure 7.10.

It can be observed from the graph that both rifampicin and isoniazid release was not significantly affected by the SGF media volume. The rifampicin and isoniazid release were extended beyond 24 h in both cases (when SGF media volume was 350 ml and 900 ml). At the end of 24^{th} h, ~ 80% and 81% of the rifampicin was released from RH11 formulations in the release studies containing 350 ml and 900 ml of SGF respectively. At the end of 24^{th} h, ~ 91% and 99% of the isoniazid was released from RH11 formulations in the release studies containing 350 ml and 900 ml of SGF respectively. The high f_2 factor values of 91.39 and 65.71 respectively for rifampicin and isoniazid release between the 350 ml and 900ml SGF release studies proved that the SGF media volume had no significant influence on the release of both drugs.

The release mechanism and release kinetics parameters of rifampicin and isoniazid also remained unaffected by the change in the SGF media volume.

7.2.3.6. Effect of presence of one drug on another drug release

The study was carried out in case of RH10 formulations. In one case total isoniazid was replaced by rifampicin (RH12) and in other case total rifampicin was replaced by isoniazid (RH13). But, the care was taken to retain all other formulation and processing parameters same, so that any changes in the release characters/profiles should only be attributed to the drug replacement. The results are shown in the Figure 7.11.

It can be observed from the Figure 7.11 that the rifampicin release occurred at a lower rate in case of RH12 formulations (containing rifampicin alone) compared to RH10 formulations (containing rifampicin in combination with the isoniazid). Although the release of rifampicin was extended up to 24 h in both cases (RH12 and RH10), from RH10 formulations there was complete rifampicin release within 24 h (about 100%) compared to about 92% from RH12. The f_2 factor value of 48.61 for rifampicin release profiles between RH10 and RH12 further proved that the rifampicin release was

significantly lower when it was present alone in the tablet matrix (RH12) compared to RH10. There was slight decrease in the initial release of rifampicin in case of RH12 formulations compared to RH10. The decrease in the rifampicin release from RH12 formulations was more pronounced in SIFsp. This might be due to the fact that the solubility of rifampicin in SGF was high enough to achieve sufficient initial release and thus present or absent of another soluble drug (isoniazid) didn't resulted in the significant change in the rifampicin release in SGF. But rifampicin was poorly soluble in SIFsp thus the presence of isoniazid (along with the rifampicin in the same matrix) resulted in the increased rifampicin release (due to increased matrix porosity and erosion) compared to when the total matrix was covered with the insoluble rifampicin.

In case of isoniazid release the results were exactly opposite. There was a decrease in the release profile of isoniazid from RH10 (when present along with the rifampicin) compared to RH12 (where isoniazid was present alone in the tablet matrix). The isoniazid release was extended up to 12 h in case of RH13 formulations compared to 16 h in case of RH10 formulations. The f_2 factor value of 48.28 for isoniazid release profiles (only in SIFsp) between RH10 and RH12 formulations further proved that the isoniazid release was significantly lower when it was present along with the rifampicin in the tablet matrix (RH10) compared to RH13. There was no significant difference in the isoniazid release profiles between RH10 and RH13 in SGF. But in SIFsp the isoniazid release was decreased in case of RH10 formulations compared to RH13. This might again be due to lower solubility of rifampicin SIFsp that decreased the isoniazid release from RH10 formulations; where as, the good solubility of rifampicin in SGF didn't significantly affect the isoniazid release.

There was no significant change in the release mechanism of rifampicin between RH10 ($n = 0.68$) and RH12 ($n = 0.75$) indicating that the mechanism of rifampicin release remained unaffected (non-Fickian diffusion). Similarly the mechanism of isoniazid release also remained unaffected (n value of 0.53 and 0.63 respectively for RH10 and RH13 formulations). The K value decreased (0.073 $h^{-0.75}$) and $t_{50\%}$ increased (12.98 h) for rifampicin in case of RH12 formulations. There was no significant difference between the K values of RH10 (0.225 $h^{-0.53}$) and RH13 (0.224 $h^{-0.64}$) formulations for isoniazid release. This might be because of the fact that the K value mainly depends upon the

initial drug release. If there is an increased (burst) initial release then the K value would be higher. Hence, in both formulations cases (RH10 and RH13), there was a good initial release of isoniazid that lead to no significant difference in the K values. Where as, there was a slight decrease in the $t_{50\%}$ in case of RH13 (3.50 h) compared to RH10 (4.56 h) for isoniazid release.

7.2.3.7. In vitro release studies from bilayer tablets

Plots of percent cumulative drug released vs. time for RHB2 formulations made with HPMC K4M are shown in the Figure 7.13. The isoniazid release was extended up to 24 h where as, at end of 24th h only about 40% of the rifampicin was released. The release profiles were fitted with several different kinetics models as described in the methodology section. The correlation coefficient 'r' values for different release models are shown in the Table 7.4. Both rifampicin and isoniazid release found to follow Higuchi's square root kinetics model as the plots of percentage drug released vs. square root of time found to be linear ('r' values are shown in the Table 7.4). The initial release was less in case of rifampicin indicating the necessity of incorporation of a loading dose. Where as, for isoniazid the initial release was higher indicating no necessity of incorporation of a separate loading dose to attain the desired drug levels in the initial hour. During initial hours about 10% and 34% of rifampicin and isoniazid were released respectively within 2 h from RHB2 formulations.

The reason for the decrease in the initial release of rifampicin from RHB2 formulations in SGF might be explained as follows. When the tablet came in contact with the release media the matrix hydration begun as the release media penetrated in side the matrix. The presence of soluble rifampicin (in SGF pH conditions) further enhanced the media penetration in to the matrix (or media up take by the matrix). This lead to a quicker hydration, swelling of the polymer and formation of stable gel layer. This gel layer (that has formed instantaneously) prevented the initial burst release of rifampicin and provided a resistant barrier for the diffusion of the rifampicin molecules in to the release media. Thus the rifampicin release was lower in SGF. The rifampicin release was lower also in SIFsp because of its low solubility in that media that lead to the decreased diffusion out of the matrix and thus decreased release. The reason for initial higher release and

decrease in the rate of isoniazid from RH11 formulations with time also remains same as that for HPMC (RH2 and RH3) and HPC (RH5 and RH6) formulations. The release of isoniazid was significantly higher than the rifampicin. This might be due to difference in their solubilities. Isoniazid being a highly soluble drug released at much faster rate compared to poorly soluble rifampicin. The n value for rifampicin release found to be 0.75 indicating that the release mechanism was non-Fickian anomalous diffusion with the contribution of erosion release being higher. The n value for isoniazid release found to be 0.56 indicating that the release mechanism was anomalous non-Fickian diffusion with the contribution of Fickian diffusional release being higher. There was a difference in the mechanism of release between rifampicin and isoniazid due to difference in their solubilities from HPMC bilayer tablets. The release of the insoluble/poorly soluble rifampicin proceeded predominantly by erosion; where as, the release of a soluble drug isoniazid mainly occurred by diffusion mechanism.

Plots of percent cumulative drug released vs. time for RHB1 formulations made with HPC are shown in the Figure 7.12. The rifampicin release was extended up to 24 h compared to 16 h for isoniazid. It can be seen from the Figure 7.12 that there was initial higher release in SGF up to 2 h followed by a decrease in the release when the media changed to SIFsp. But in SIFsp the nature of rifampicin release was found to be zero order in case of both the formulations as the percent drug released vs. time plots found to be linear ($r = 0.998$). Where as, the isoniazid release found to follow Higuchi's square root kinetics model as the plots of percentage drug released vs. square root of time found to be linear ('r' values are shown in the Table 7.4). In case of both the drugs, the initial release was higher indicating no necessity of incorporation of a separate loading dose to attain the desired drug levels in the initial hour. During initial hours about 30% and 31% of rifampicin and isoniazid were released respectively within 2 h from RHB1 formulations.

The detailed explanation for zero order release of rifampicin from RH11 formulations in SIFsp remains same as that for HPMC (RH2 and RH3) and HPC (RH5 and RH6) formulations, in brief it might be due to its poorly soluble nature (that prevented its diffusional release). But during initial hours as rifampicin was highly soluble in SGF, substantial amount of the rifampicin has been released before the hydration and swelling

of the tablet matrix leading to a higher initial release of rifampicin. The reason for initial higher release and decrease in the rate of isoniazid from RHB1 formulations with time also remains same as that for HPMC (RH2 and RH3) and HPC (RH5 and RH6) formulations. The release of isoniazid was significantly higher than the rifampicin. In SGF, there was no significant difference in the rifampicin and isoniazid profiles but in SIFsp, rifampicin release was lower than isoniazid. This might be due to difference in their solubilities in SIFsp. Isoniazid being a highly soluble drug released at much faster rate compared to poorly soluble rifampicin when the release studies were done in SIFsp. The n value for rifampicin release found to be 0.47 indicating that the release mechanism was non-Fickian anomalous diffusion. The n value for isoniazid release found to be 0.58 indicating that the release mechanism was anomalous non-Fickian diffusion. It has already been stated that such mechanism of release was due to the combination of diffusion and swelling/relaxation. Although there was significant difference in the solubility of rifampicin and isoniazid in SIFsp, the release mechanism found to be almost similar from HPC bilayer tablets. This might be due to the consideration of the drug release profiles only up to 60% release for the analysis by the proposed model. The initial release was higher in case of both rifampicin and isoniazid (because of good solubility of both drugs in SGF) and hence the release mechanism found to be same for both rifampicin and isoniazid.

Plots of percent cumulative drug released vs. time for RHB3 formulations made with both HPMC and HPC are shown in the Figure 7.14. The release of both drugs (rifampicin and isoniazid) was extended up to 24 h. It can be seen from the Figure 7.14 that there was initial higher release in SGF up to 2 h followed by a decrease in the release when the media changed to SIFsp. But in SIFsp the nature of rifampicin release was found to be zero order in case of both the formulations as the percent drug released vs. time plots found to be linear ($r = 0.994$). Where as, the isoniazid release found to follow Higuchi's square root kinetics model as the plots of percentage drug released vs. square root of time found to be linear ('r' values are shown in the Table 7.4). In case of both the drugs, the initial release was higher indicating no necessity of incorporation of a separate loading dose to attain the desired drug levels in the initial hour. During initial hours about 27% and 29% of rifampicin and isoniazid were released respectively within 2 h from

RHB3 formulations. At the end of 24th h about 97% and 99% of the rifampicin and isoniazid were released respectively. Thus, by the combination of suitable polymer with suitable drug (HPC with rifampicin and HPMC with isoniazid) it could be possible to manufacture the formulations with the sufficient initial release and controlled and complete release up to 24 h for both the drugs despite of the wide variations in their physicochemical properties.

The reason for the observed release profile of rifampicin from RHB3 formulations remains same as that for the RHB1 formulation. Similarly, the reason for the observed release profile of isoniazid from RHB3 formulations remains same as that for the RHB2 formulation. The n value for rifampicin and isoniazid release found to be 0.56 and 0.67 respectively indicating that the release mechanism was non-Fickian anomalous diffusion in case of both the drugs. Although there was significant difference in the solubility of rifampicin and isoniazid the release mechanism found to be almost similar from RHB3 bilayer tablets formulated with the combination of HPC (for rifampicin) and HPMC (for isoniazid). There was no significant difference between the release rates of rifampicin (K = 0.167 $h^{-0.56}$) compared to isoniazid (0.177 $h^{-0.67}$). The $t_{50\%}$ value was higher for rifampicin (7.15 h) compared to isoniazid (4.64 h). The K values again proved that the rifampicin and isoniazid release occurred almost at a similar rate from RHB3 bilayer tablet formulations even though both rifampicin and isoniazid differed significantly in their physicochemical properties.

7.2.4. Batch Reproducibility

Three batches of each formulation were prepared and their quality and respective release characters were evaluated under the same conditions as prescribed in previous sections. The physical properties of the tables from all three batches were evaluated in the same manner as for the original batch formulations. The tablets showed low standard deviation values for the drug content, friability, weight variation and hardness from three different batches prepared separately (data not shown). These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility and absence of significant variations between batch-to-batch. In vitro release data pertaining to reproducibility studies were compared by f_2 metric (similarity factor) values. No

significant difference was observed in the release profiles of the formulations between different batches as indicated by the low standard deviation values of the percent cumulative release data at different time points obtained from the replicate release studies of the samples and from high similarity factor values. The f_2 values varied from 66.98 to 88.01 for rifampicin release profiles of RH2, RH4, RH6, RH9, RHB1 and RHB2 formulations between original and reproduced batches (calculated from the average percent release at each time point with standard deviation values less than 4%). The f_2 values varied from 69.27 to 80.58 for isoniazid release profiles of RH2, RH4, RH6, RH9, RHB1 and RHB2 formulations between original and reproduced batches (calculated from the average percent release at each time point with standard deviation values less than 4%). Thus, the reproducible physical properties in terms low standard deviation for each parameter from different batches and significantly similar release profiles of rifampicin and isoniazid from all batches (proved by f_2 factor values) indicated that the formulation methodology employed (IPA granulation in case of isoniazid formulations and direct compression in case of rifampicin formulations) found to be suitable for manufacturing the good quality C.R. matrix tablets of rifampicin and isoniazid combination.

7.2.5. Stability Studies

The two best formulations from each batch of formulations were selected for stability studies (Table 7.5). The drug content in triplicate (for both rifampicin and isoniazid) was determined for each formulation by the UV spectrophotometric method described in the chapter 3 and the observed degradation rate constants and $t_{50\%}$ at different storage conditions are listed in Table 7.5. Both rifampicin and isoniazid in matrix embedded tablets (in case of all polymer formulations) found to follow first order degradation (with "r" value more than 0.971 and 0.978 respectively for rifampicin and isoniazid in all cases and individual plots not given). The first order degradation rate constants (K_{deg}) for rifampicin and isoniazid from different formulation batches are reported in Table 7.5. The K_{deg} values for rifampicin in various formulations ranged from 5.03×10^{-3} $month^{-1}$ to 6.32×10^{-3} $month^{-1}$ at CRT, 6.34×10^{-3} $month^{-1}$ to 8.00×10^{-3} $month^{-1}$ at 40 ± 2 °C and 10.89×10^{-3} $month^{-1}$ to 13.46×10^{-3} $month^{-1}$ at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 7.5). The $t_{90\%}$ values for rifampicin in

various formulations ranged from 16.67 to 20.95 months at CRT, from 13.17 to 16.62 months at 40 ± 2 °C and from 7.83 to 9.68 months at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 7.5). The K_{deg} values for isoniazid in various formulations ranged from 5.05 × 10^{-3} $month^{-1}$ to 6.06 × 10^{-3} $month^{-1}$ at CRT, 6.33 × 10^{-3} $month^{-1}$ to 7.41 × 10^{-3} $month^{-1}$ at 40 ± 2 °C and 9.04 × 10^{-3} $month^{-1}$ to 11.70 × 10^{-3} $month^{-1}$ at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 7.5). The $t_{90\%}$ values for isoniazid in various formulations ranged from 17.40 to 20.86 months at CRT, from 14.23 to 16.74 months at 40 ± 2 °C and from 9.01 to 11.66 months at 40 ± 2 °C/75 ± 5% RH (individual values for different formulations are presented in Table 7.5). In case of all polymer formulations (matrix tablets) the degradation rate constant increased with increase in the polymer proportion for both rifampicin and isoniazid.

Both rifampicin and isoniazid found to be more stable at CRT and less stable at 40 ± 2 °C/75 ± 5% RH in case of all formulations. Among the HPC and HPMC polymers, rifampicin was comparatively more stable in HPC formulations (Table 7.5). Among the HPC and HPMC polymers, isoniazid was comparatively more stable in HPMC formulations (Table 7.5).

It was observed that with the raise in the temperature (from 25± 2 °C to 40 ± 2 °C) the K_{deg} values increased and $t_{90\%}$ values decreased (for both rifampicin and isoniazid) in case of all polymer formulations (in all polymer ratios). The K_{deg} values were much higher at 40 ± 2 °C/75 ± 5% RH compared to 40 ± 2 °C in all the cases studied (Table 7.5) for both rifampicin and isoniazid. Thus, from our studies, it was observed that the humidity was the one of the most important parameter that affected the stability of both rifampicin and isoniazid in all polymer formulations. It was also observed that the deleterious effect of humidity on the stability of the rifampicin and isoniazid was more pronounced than the temperature (as can be observed from the K_{deg} values at CRT, 40 ± 2 °C and 40 ± 2 °C/75 ± 5% RH in case of all formulation batches). The increased K_{deg} values found at higher humidity condition again supported that the avoidance of aqueous granulation technology (use of either IPA granulation or direct compression) in the manufacturing of rifampicin and isoniazid combination matrix tablets was significantly beneficial in obtaining the stable matrix tablet formulations.

The in vitro release profiles were studied as per the specifications enlisted in previous sections and compared with their respective initial release profiles. The in vitro release profiles of the formulations stored at CRT for 6 months were compared with their initial release profiles (0 time samples at CRT) with f_2 factor values. The f_2 factor values in all cases found to be more than 56 indicating that both rifampicin isoniazid release profiles were significantly similar for zero time samples and 6 months samples (stored at CRT). Thus, the in vitro release characteristics were not significantly affected by the stability studies (storage at CRT) for about 6 months showing that the formulations were stable in terms of release characteristics.

7.3 Conclusions

The designed matrix tablets of rifampicin and isoniazid combination showed good and reproducible physical properties indicating that the methods of preparation of formulation are suitable and acceptable for preparing good quality matrix tablets. The tablet manufacturing method was relatively simple and can be easily adopted in conventional tablet manufacturing units in industries on a commercial scale.

Increase in the polymer ratio decreased the release of both rifampicin and isoniazid but isoniazid release was faster than rifampicin due to higher water solubility. Addition of the Eudragit L100-55 decreased the release of isoniazid in both SGF and SIFsp. But the presence of Eudragit L100-55 resulted in the decreased rifampicin release in SGF and increased in SIFsp. The release of both rifampicin and isoniazid were not affected by the change in the release media from HPMC, but formulations with HPC were sensitive to the change in the release media. The variation in the formulation processing technique found to have profound significance on the release profiles of both rifampicin and isoniazid. The release profiles of rifampicin and isoniazid were not significantly affected by the change in the SGF media volume. The presence of EC with HPC significantly reduced the release of both rifampicin and isoniazid. The presence of both rifampicin and isoniazid in a single C.R. matrix tablets found to have mutual influence on their individual release profiles.

The mechanism of rifampicin release from formulations with HPMC found to be Case-II (swelling/relaxational controlled). Rifampicin found to release predominantly by non-

Fickian diffusion from formulations with HPC. The mechanism of isoniazid release found to be predominantly by non-Fickian diffusion in all polymer formulations and at all polymer ratios.

In the present study a series of formulations were developed with different release rates and duration. The duration of rifampicin and isoniazid release could be by varying the polymer type, polymer ratio and processing techniques. The rifampicin release profiles found to follow zero order kinetics in case of HPMC. In case of formulations with HPC, there was an initial higher release in SGF followed by zero order release profiles in SIFsp for rifampicin. The isoniazid release profiles found to follow Higuchi's square root kinetics in case of all polymer formulations. The initial release was sufficiently higher for rifampicin from HPC thus ruling out the need to incorporate a separate loading dose. The initial release was sufficiently higher for isoniazid in all formulations. Thus, with the use of suitable polymer or polymer combinations and with the proper optimization of the processing technologies it was possible to design the C.R. formulations of rifampicin and isoniazid combination that could give the sufficient initial release and release extended up to 24 h for both the drugs despite of the wide variations in their physicochemical properties.

The rifampicin and isoniazid were found to be stable as found from the stability studies at different storage conditions. However, the degradation rate was comparatively higher at higher temperature and humidity conditions. Thus, the avoidance of aqueous granulation technology in the manufacturing of rifampicin and isoniazid combined matrix tablets was found to be significantly beneficial in obtaining the stable C.R. matrix tablets.

Table 7.1. Formula and physical properties of rifampicin and isoniazid combination matrix tablets prepared with HPMC K4M

Formulations	RH 1	RH 2	RH 3	RH 4
Components *				
Rifampicin (mg)[†]	450	450	450	450
Isoniazid (mg)	300	300	300	300
HPMC[a] (%)	20	40	80	80
Eudragit L100-55[a] (%)	-	-	-	60
Physical Properties				
Drug Content (R)[b]	102.5 ± 1.4	101.6 ± 1.7	99.0 ± 1.5	101.8 ± 1.9
Drug Content (H)[b]	99.3 ± 1.2	100.4 ± 1.8	102.4 ± 1.6	98.7 ± 1.1
Weight variation (%)[c]	± 2.6	± 2.9	± 1.9	± 3.1
Hardness (kg/cm^2)[d]	7.6 ± 0.6	7.8 ± 0.7	7.5 ± 0.8	7.9 ± 0.4
Friability (%)	< 0.8	< 0.8	< 0.8	< 0.8

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [†] added extragranularly; [a] % w/w of the isoniazid and added along with isoniazid during granulation; [b] % label claim (mean of triplicate with SD); [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 7.2. Formula and physical properties of rifampicin and isoniazid combination matrix tablets prepared with HPC (Klucel LF)

Formulations	RH 5	RH 6	RH 7	RH 8	RH 9
Components *					
Rifampicin (mg)[†]	450	450	450	450	450
Isoniazid (mg)	300	300	300	300	300
HPC[a] (%)	40	80	40	60	80
EC[a] (%)	-	-	20	-	-
Eudragit[a] L100-55 (%)	-	-	-	30	60
Physical Properties					
Drug Content (R)[b]	98.5 ± 1.3	102.2 ± 1.5	99.3 ± 1.8	101.9 ± 1.7	102.6 ± 1.1
Drug Content (H)[b]	102.4 ± 1.2	100.4 ± 1.9	102.6 ± 1.4	101.4 ± 1.1	98.8 ± 1.2
Weight variation (%)[c]	± 1.8	± 2.7	± 1.6	± 3.2	± 3.0
Hardness (kg/cm^2)[d]	7.7 ± 0.5	7.4 ± 0.7	7.8 ± 0.7	7.5 ± 0.8	7.6 ± 0.6
Friability (%)	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; [†] added extragranularly; [a] % w/w of the isoniazid and added along with isoniazid during granulation; [b] % label claim (mean of triplicate with SD); [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 7.3. Formula and physical properties of rifampicin and isoniazid combination matrix tablets (Bilayer tablets)

Formulations	RHB1		RHB2		RHB3	
Components*						
Drug layer	Rifampicin†	Isoniazid‡	Rifampicin†	Isoniazid‡	Rifampicin†	Isoniazid‡
Drug added (mg)†	450	300	450	300	450	300
HPMC[a] (%)	-	-	30	60	-	60
HPC[a] (%)	20	60	-	-	20	-
Eudragit[a] L100-55 (%)	20	20	20	20	30	30
Physical Properties						
Drug Content (%)[b]	102.6 ± 1.8	101.3 ± 1.9	99.3 ± 2.0	102.5 ± 1.7	100.6 ± 2.4	99.2 ± 2.3
Weight variation (%)[c]	± 2.9		± 3.5		± 3.2	
Hardness (kg/cm^2)[d]	8.3 ± 0.7		8.7 ± 0.5		8.5 ± 0.9	
Friability (%)	< 0.8		< 0.8		< 0.8	

* Also contains 1 % w/w of talc and 1 % w/w of magnesium stearate as additives; ˙ friability values were less than 0.8%; † mixed with the excipient components and directly compressed; ‡ mixed with excipient components, granulated with IPA and compressed; [a] % w/w of the drug; [b] % label claim (mean of triplicate with SD); [c] ± max % variation from the mean; [d] mean of 20 tablets.

Table 7.4. Release kinetics parameters for C.R. formulations of rifampicin and isoniazid combination.

Formulations	Peppas model parameters				r^d for Zero Order	r^d for First Order	r^d for Higuchi's Kinetics
	n^a	K^b (h^{-n})	$t_{50\%}{}^c$ (h)	r^d			
Rifampicin							
RH2	0.86	0.046	15.77	0.995	0.998	0.953	0.898
RH3	0.89	0.029	24.56	0.996	0.999	0.985	0.896
RH4	1.02	0.052	11.34	0.944	0.962	0.960	0.929
RH5	0.89	0.358	1.47	0.916	0.543	0.919	0.806
RH6	0.74	0.198	3.55	0.889	0.798	0.927	0.958
RH7	0.71	0.243	2.77	0.932	0.722	0.973	0.926
RH8	0.60	0.154	7.14	0.995	0.926	0.726	0.994
RH9	0.67	0.126	7.83	0.993	0.952	0.921	0.986
RH10	0.68	0.111	9.10	0.996	0.977	0.840	0.973
RH11	1.12	0.028	11.56	0.984	0.986	0.980	0.941
RH12	0.75	0.073	12.98	0.986	0.997	0.911	0.933
RHB1	0.47	0.186	8.14	0.939	0.926	0.934	0.984
RHB2	0.75	0.044	26.11	0.927	0.923	0.958	0.987
RHB3	0.56	0.167	7.15	0.973	0.930	0.918	0.993
Isoniazid							
RH2	0.54	0.389	1.61	0.996	0.697	0.966	0.930
RH3	0.53	0.248	3.77	0.998	0.803	0.904	0.973
RH4	0.64	0.169	5.47	0.990	0.869	0.993	0.990
RH5	0.86	0.421	1.22	0.999	0.544	0.974	0.822
RH6	0.51	0.378	1.73	0.962	0.675	0.986	0.911
RH7	0.45	0.438	1.34	0.960	0.608	0.945	0.867
RH8	0.42	0.363	2.15	0.984	0.820	0.932	0.982
RH9	0.63	0.229	3.46	0.972	0.859	0.955	0.981
RH10	0.53	0.225	4.56	0.980	0.929	0.863	0.995
RH11	0.45	0.213	6.56	0.990	0.890	0.988	0.996
RH13	0.64	0.224	3.50	0.995	0.921	0.924	0.984
RHB1	0.58	0.205	4.63	0.998	0.876	0.969	0.962
RHB2	0.56	0.214	4.52	0.985	0.847	0.948	0.986
RHB3	0.67	0.177	4.64	0.998	0.838	0.972	0.974

[a] Diffusional exponent indicative of the release mechanism; [b] release rate constant; [c] time for 50 % of the drug release; [d] correlation coefficient.

Table 7.5. Stability studies of rifampicin and isoniazid combination C.R. formulations at different storage conditions.

Formulations	CRT		40 ± 2°C		40 °C/75% RH	
	K_{deg} x 10^3 ($month^{-1}$)*	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)*	$t_{90\%}$ (month)	K_{deg} x 10^3 ($month^{-1}$)*	$t_{90\%}$ (month)
Rifampicin						
RH2	5.56	18.96	7.02	15.01	11.44	9.21
RH4	5.91	17.83	7.66	13.75	13.06	8.07
RH6	5.90	17.87	7.29	14.46	12.19	8.64
RH9	6.32	16.67	8.00	13.17	13.46	7.83
RHB1	5.03	20.95	6.34	16.62	10.89	9.68
RHB2	5.41	19.49	6.85	15.38	11.83	8.91
Isoniazid						
RH2	5.70	18.50	6.86	15.36	11.17	9.43
RH4	6.06	17.40	7.32	14.40	11.70	9.01
RH6	5.22	20.20	6.33	16.74	9.70	10.86
RH9	5.67	18.60	7.41	14.23	10.85	9.71
RHB1	5.05	20.86	6.71	15.70	9.04	11.66
RHB2	5.51	19.11	7.18	14.68	10.08	10.45

* First order degradation rate constant based on average of triplicate assay values at five time points with C.V. less than 4.0% in all cases.

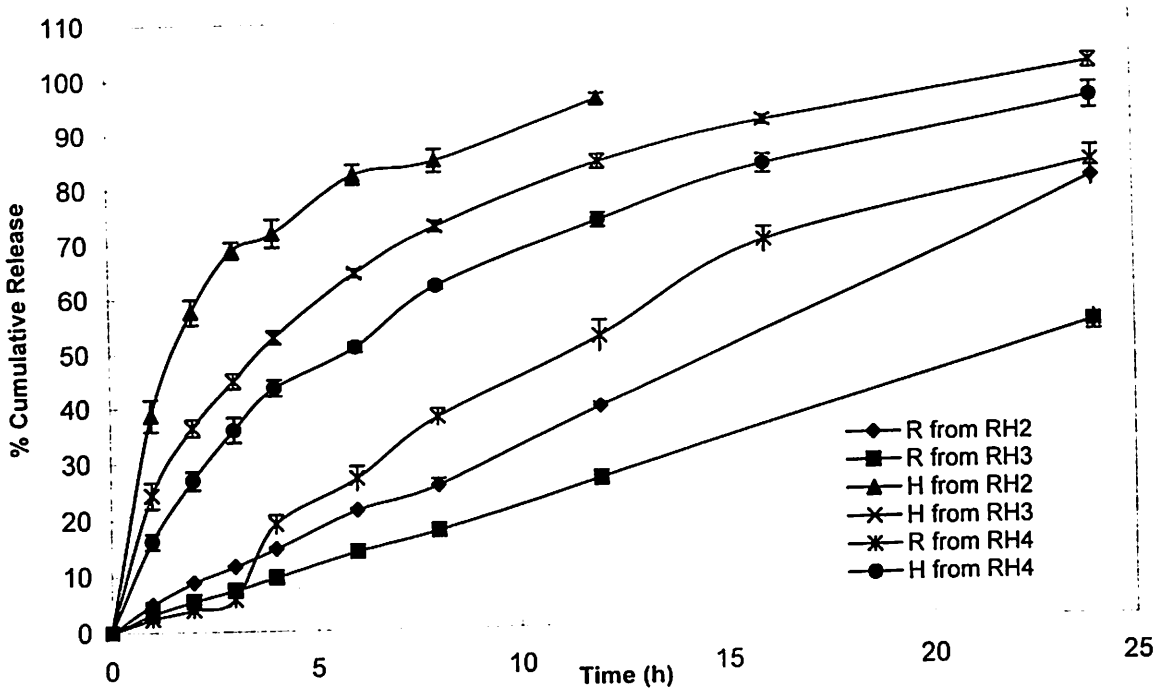

Figure 7.1. Comparative release profiles of rifampicin and isoniazid from RH2, RH3 and RH4 (HPMC K4M) formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

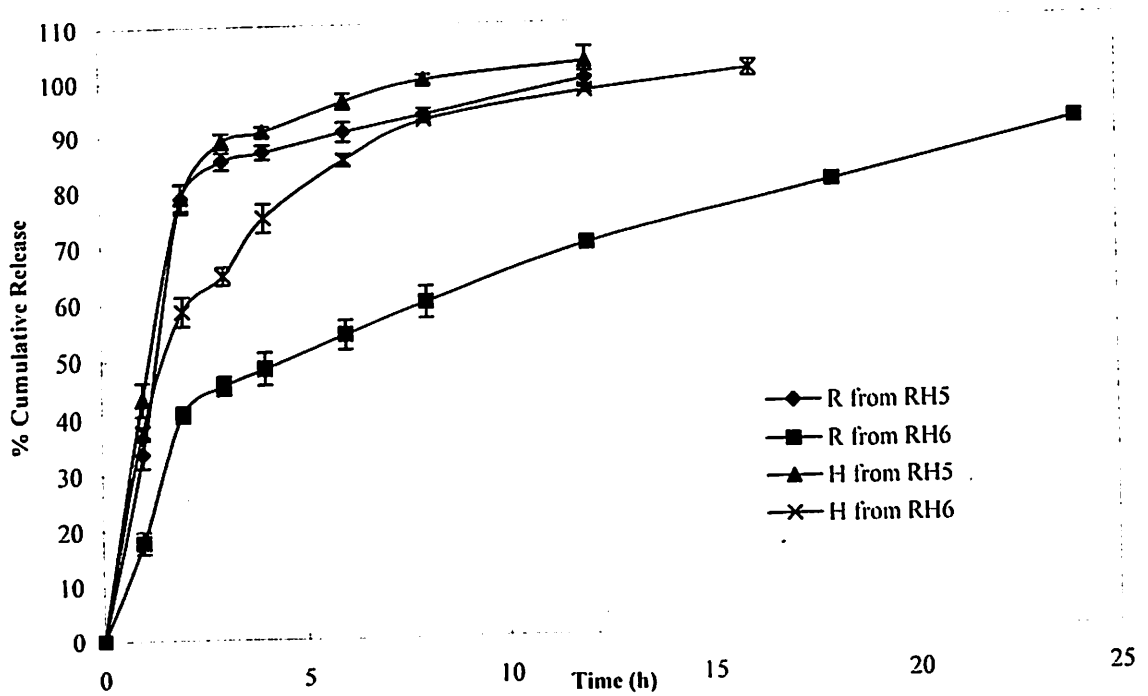

Figure 7.2. Comparative release profiles of rifampicin and isoniazid from RH5 and RH6 (HPC formulations) in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

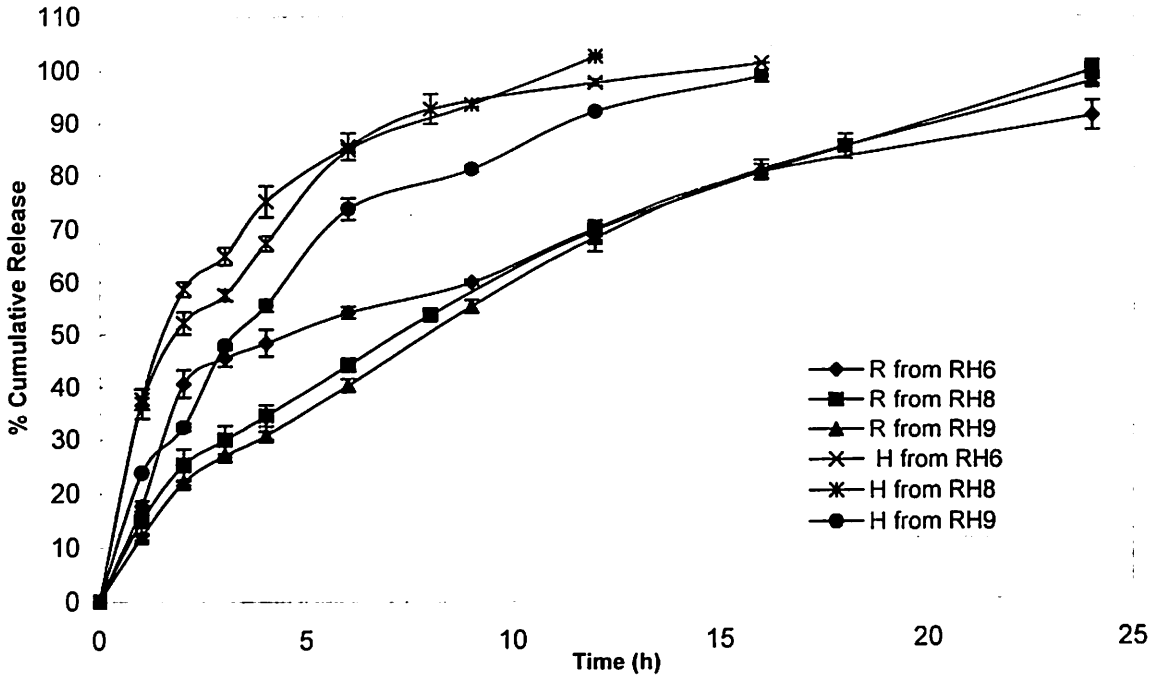

Figure 7.3. Effect of addition of Eudragit L10055 on release profiles of rifampicin and isoniazid from HPC formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

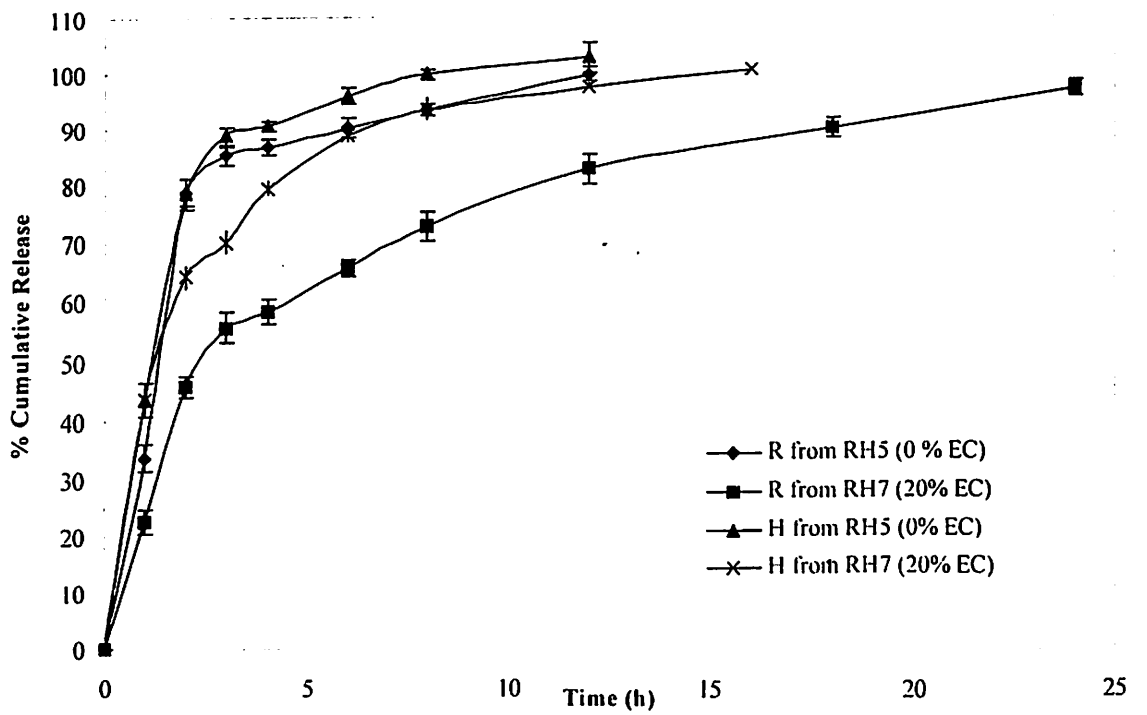

Figure 7.4. Effect of addition of EC on release profiles of rifampicin and isoniazid from HPC formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

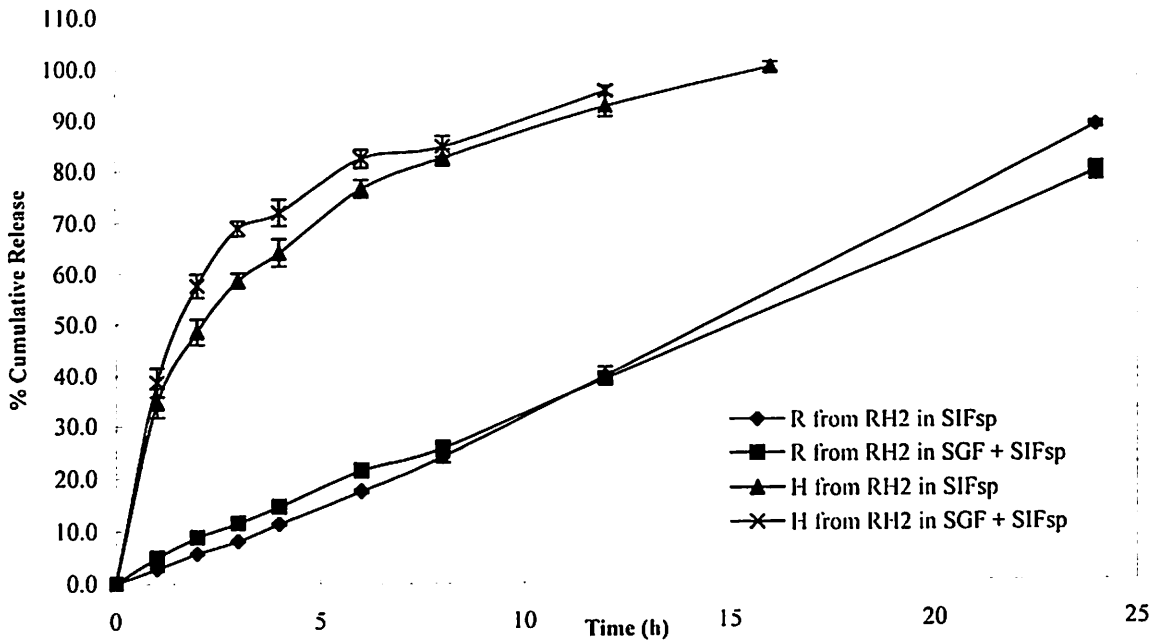

Figure 7.5. Effect of release media on release profiles of rifampicin and isoniazid from RH2 (HPMC K4M formulations) (Each data point represents the average of six tablets from three batches with S.D.).

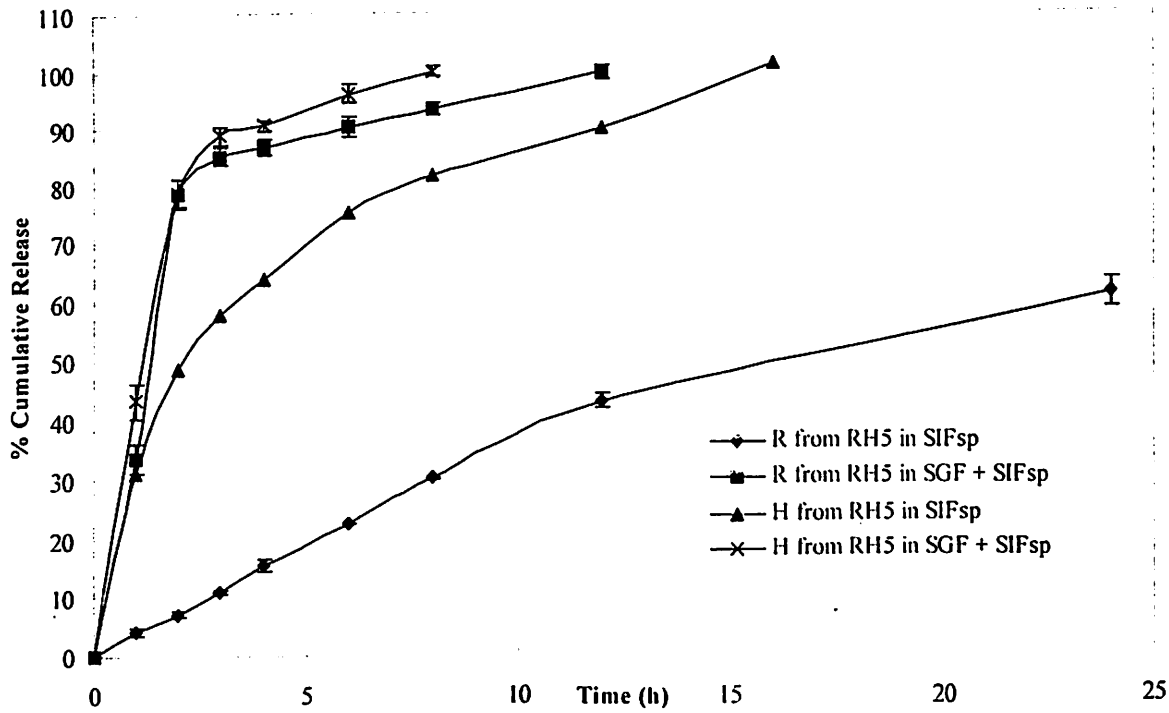

Figure 7.6. Effect of release media on release profiles of rifampicin and isoniazid from RH5 (HPC formulations) (Each data point represents the average of six tablets from three batches with S.D.).

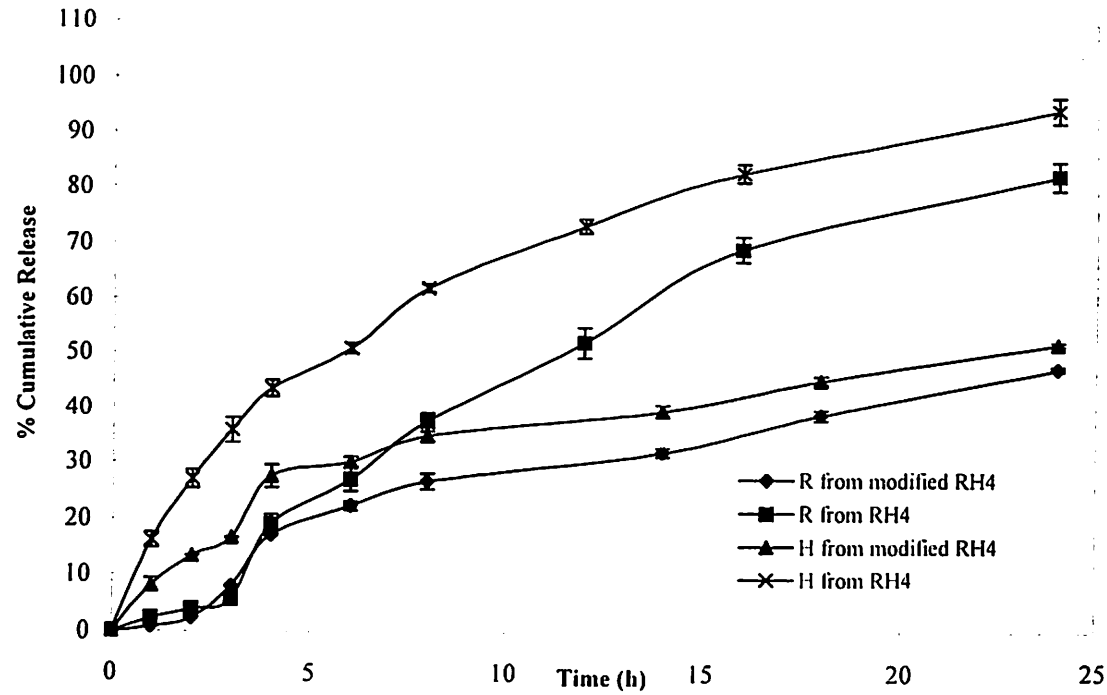

Figure 7.7. Effect of change in manufacturing process on release profiles of rifampicin and isoniazid from HPMC K4M formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

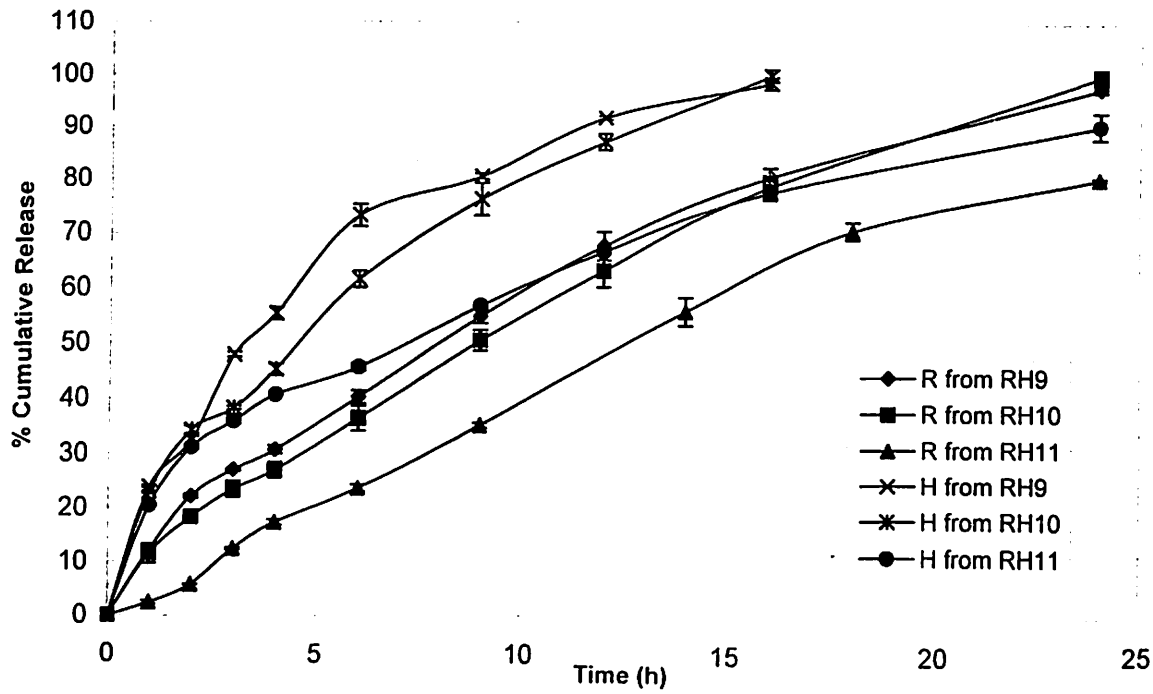

Figure 7.8. Effect of change in manufacturing process on release profiles of rifampicin and isoniazid from HPC formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

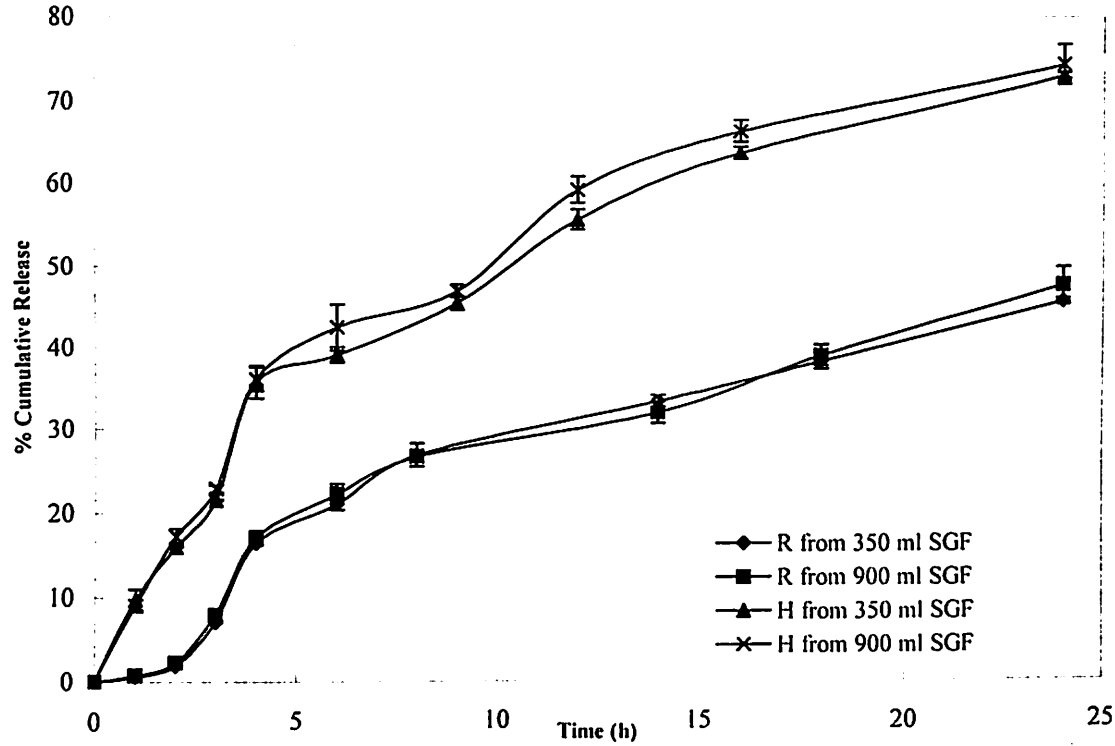

Figure 7.9. Effect of SGF media volume on release profiles of rifampicin and isoniazid from modified RH4 (HPMC formulations) (Each data point represents the average of six tablets from three batches with S.D.).

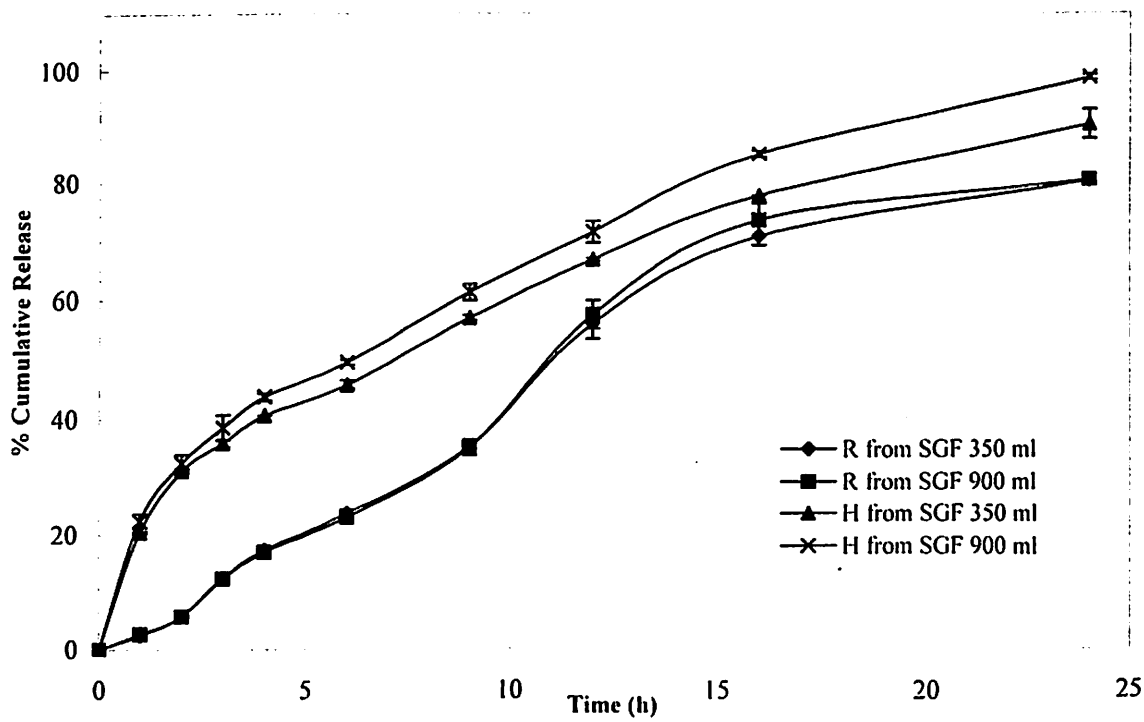

Figure 7.10. Effect of SGF media volume on release profiles of rifampicin and isoniazid from RH11 (HPC formulations) (Each data point represents the average of six tablets from three batches with S.D.).

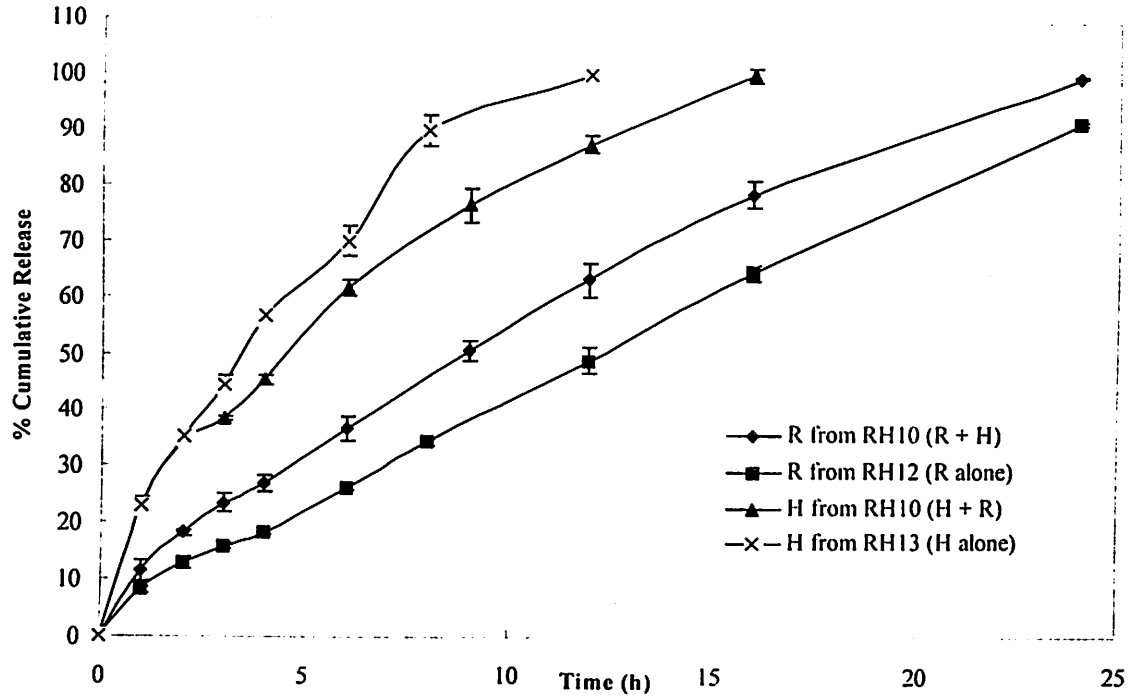

Figure 7.11. Effect of presence of one drug on the release of other drug from RH10 (HPC formulations) in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

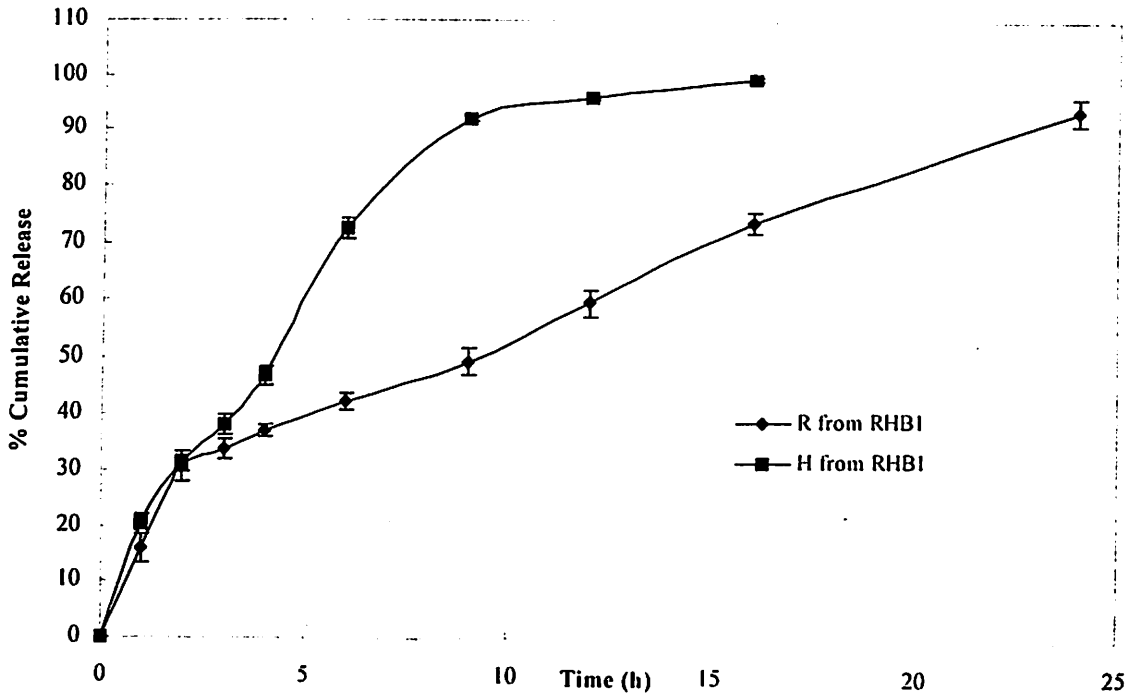

Figure 7.12. Comparative release profiles of rifampicin and isoniazid from RHB1 bilayer tablet formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

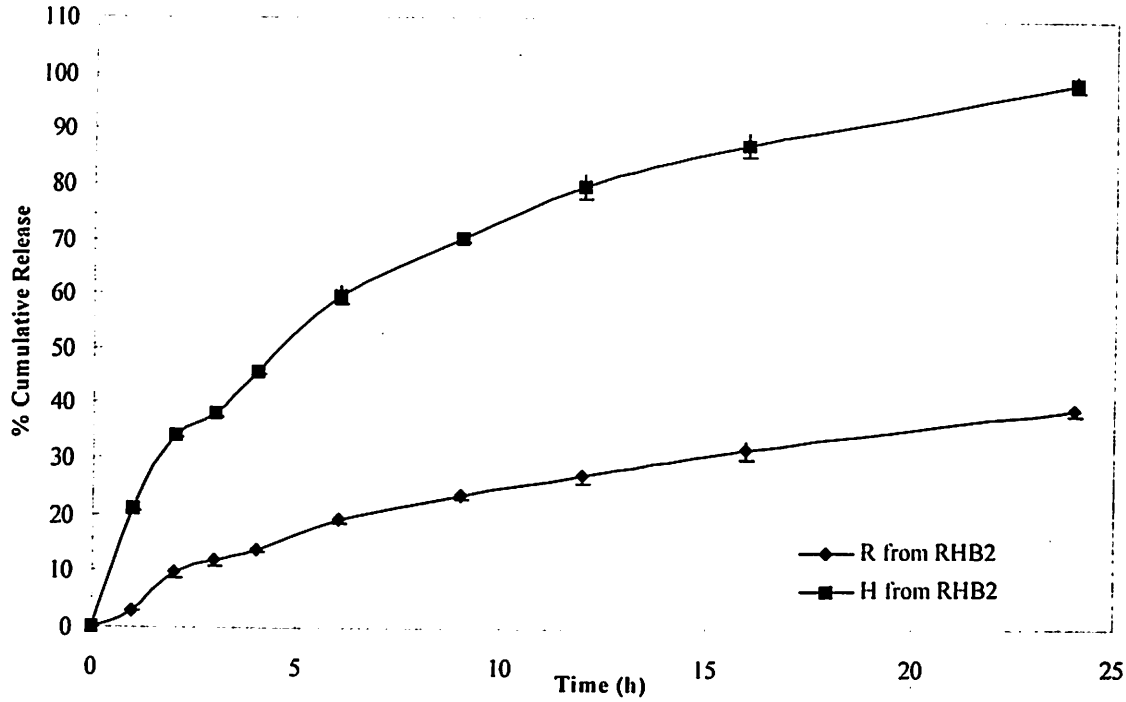

Figure 7.13. Comparative release profiles of rifampicin and isoniazid from RHB2 bilayer tablet formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

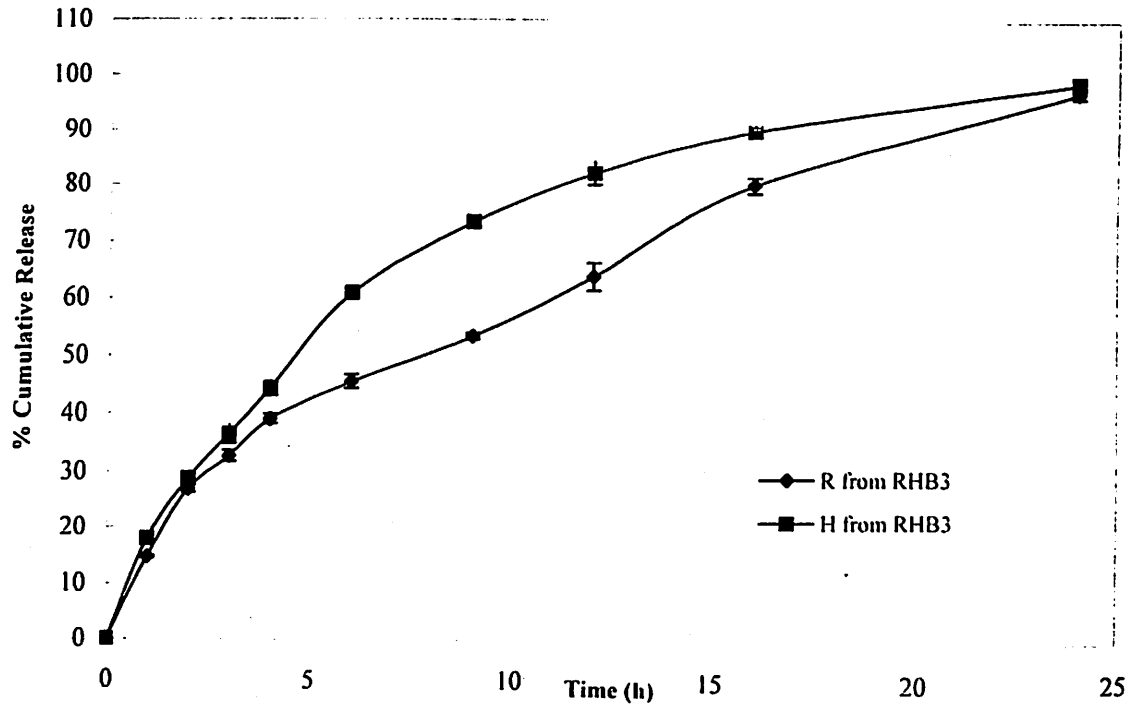

Figure 7.14. Comparative release profiles of rifampicin and isoniazid from RHB3 bilayer tablet formulations in SGF (0-2 h) followed by SIFsp (3-24 h) media (Each data point represents the average of six tablets from three batches with S.D.).

Chapter 8

Conclusions

8. CONCLUSIONS

Controlled release formulations are better over multidose delivery, particularly for long-term therapeutic effect. Thus, there is a scope of using single or multidrug controlled release formulations for the treatment of tuberculosis. In this project, work has been done on the design and evaluation of controlled release formulations of rifampicin and isoniazid individually or in combination using different polymers or polymer combinations at different ratios and by optimizing the various formulation factors and processing parameters.

To support various analytical works, simple visible or UV spectrophotometric methods have been selected or developed for the estimation of rifampicin and isoniazid, alone and in their combination. The developed methods were validated as per ICH guidelines and USP 2000. Methods were found to be highly useful.

Preformulation studies suggested that both rifampicin and isoniazid were stable in presence of most of the excipients used. The degradation rate was increased at high temperature and humidity. The degradation of both rifampicin and isoniazid in solution was significantly dependent on the pH. The degradation of rifampicin was higher at lower pH values and decreased as the pH of the solution increased, both in buffered or unbuffered. The degradation of the isoniazid was maximum at pH 2.0. Below and above pH 2.0, isoniazid was found to be more stable. Rifampicin showed a highly pH-dependent solubility profile. Rifampicin was more soluble at acidic and alkaline pH conditions with least solubility at pH 5.0. Dissolution media and conditions were optimized through experiment for rifampicin and isoniazid alone and in combination.

The designed matrix tablets of rifampicin and isoniazid, individually and in combination, found to possess good physical properties with insignificant batch-to-batch variations indicating that the methods used for the formulations were good and reproducible. The tablet manufacturing methods were relatively simple and can be easily adopted for commercial purpose.

The release of both rifampicin and isoniazid have been varied from 4 h to 24 h by increasing polymer proportion, varying polymer type and optimizing various other formulation and processing parameters. The nature of the rifampicin release was found to be either zero order or first order or following Higuchi's square root kinetics depending

on the nature of the polymer. The isoniazid release profiles found to follow Higuchi's square root kinetics in all formulations. Increase in the polymer ratio decreased the release rate in case of both drugs. Increase in the viscosity of the polymer decreased rifampicin release, but in case of isoniazid beyond 4000 cPs (HPMC K4M) there was no significant effect on release was observed. The effect of compression force was more pronounced in case of rifampicin formulations compared to isoniazid. Effect of compression force was more pronounced in rifampicin tablets with low viscosity grade HPMC and HPC. The release of rifampicin was faster from the directly compressed tablets than from the tablets made by wet granulation method. The isoniazid release was faster from the IPA granulated formulations than from the directly compressed tablets in case of Carbopol 934P formulations. The rifampicin release was higher in USP type II (paddle) method compared to USP type I (basket) method. Rifampicin formulations with low viscosity HPMC were more sensitive to the change in hydrodynamic conditions. The release of isoniazid was higher in 0.1 N HCl compared to pH 7.4 phosphate buffer.

In case of C.R. formulations of combined drugs with HPMC, the release was not affected by change in the release media, where as, release was affected in formulations with HPC. Addition of the Eudragit L100-55 decreased the release of isoniazid in both SGF and SIFsp media. But the presence of Eudragit L100-55 resulted in the decrease in the rifampicin release in SGF and increase in the rifampicin release in SIFsp. The variations in the processing techniques found to have profound significance on the release profiles of both rifampicin and isoniazid. The presence of both rifampicin and isoniazid in a single C.R. matrix tablets found to have mutual influence on their individual release profiles. The rifampicin and isoniazid formulations containing single or combination drugs were found to be stable on storage. However, moderate to negligible degradation was observed at higher temperature and humidity conditions for both rifampicin and isoniazid. Thus, the avoidance of aqueous granulation technology and use of either IPA granulation or direct compression in the manufacturing of rifampicin and isoniazid matrix tablets was found to be beneficial in manufacturing the stable C.R. matrix tablets.

In the present investigation, rifampicin and isoniazid combination controlled release formulations containing 80% HPC and 60% Eudragit, and bilayer matrix tablet formulation containing HPC and Eudragit for rifampicin and HPMC-Eudragit

combination for isoniazid found to be of good quality with proper release control. These formulations gave good initial release for both rifampicin and isoniazid and also, the release of both rifampicin and isoniazid were extended up to 18 h.

These formulations found to be promising and could further be considered for in vivo bioavailability studies in suitable animal models or human volunteers to assess their in vivo performance and bioavailability.

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Publications and Presentations made on this Doctoral Research Work

Journals

1. Hiremath, S. P., and Saha, R. N. Design and study of rifampicin oral controlled release formulations. Drug Delivery. 11(5):311-317. 2004.
2. Hiremath, S. P., and Saha, R. N. Studies on controlled release rifampicin tablets using hydroxypropyl methylcellulose matrices. Drug Delivery *(Communicated)*.
3. Hiremath, S. P., and Saha, R. N. Isoniazid controlled release formulations: Design and in vitro characterization *(Communicated)*.
4. Hiremath, S. P., and Saha, R. N. Design of controlled release tablet formulations of rifampicin and isoniazid combination *(Communicated)*.

Papers presented/accepted in conference/Symposium

1. Hiremath, S. P., and Saha, R. N. Design and study of controlled release matrix tablets of rifampicin and isoniazid using hydroxypropyl methylcellulose (HPMC). 2004 AAPS Annual Meeting and Exposition. Baltimore. MD. Nov 7-11. 2004.
2. Hiremath, S. P., and Saha, R. N. Studies on use of nonionic and anionic polymers in the design of controlled release formulations of rifampicin and isoniazid. 32nd Annual Meeting and Exposition of the Controlled Release Society. Florida. USA. June 18-22. 2005.
3. Hiremath, S. P., and Saha, R. N. Studies on isoniazid release from carbopol matrices. 5th Int. Symp. on Adv. in Tech. & Business Potential of NDDS. Mumbai. India. Feb 16-17. 2004.
4. Hiremath, S. P., and Saha, R. N. Design and study of oral controlled release formulations of rifampicin. 54th Indian Pharmaceutical Congress. Pune. India. Dec 13-15. 2002.

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