FORMULATION AND EVALUATION OF COLON TARGETED MINI TABLETS OF 5-FLUOROURACIL

A Thesis

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By

ANKIT KUMAR
41500133

Supervised By
Dr. Sachin Kumar Singh

Co-supervised By
Dr. Monica Gulati

LOVELY FACULTY OF APPLIED MEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY
PUNJAB, INDIA

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DEDICATED TO MY FAMILY

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Ankit Kumar

DECLARATION

I hereby declare that the present dissertation entitled "**Formulation and evaluation of colon targeted mini tablets of 5-Fluorouracil**" embodies the original research work carried out by me. It is further stated that no part of this dissertation has been submitted either in part or full for the award of any other degree of Lovely Professional University or any other University/Institution.

Ankit Kumar
Department of Pharmaceutical Sciences,
Lovely Professional University,
Jalandhar-Delhi G.T. Road,
Phagwara, Punjab 144411

CERTIFICATE

This is to certify that the present dissertation entitled "**Formulation and evaluation of colon targeted mini tablets of 5-Fluorouracil**" embodies the original research work carried out by **Ankit Kumar** under my supervision and guidance. It is further stated that no part of this dissertation has been submitted either fully or in part for any other degree of this or any other university.

Supervisor
Dr. Sachin Kumar Singh
Associate Professor
Dept. of Pharmaceutical Sciences,
Lovely Professional University,
Jalandhar-Delhi G.T. Road,
Phagwara, Punjab 144411

Co-Supervisor
Dr. Monica Gulati
Professor and Sr. Dean,
School of Pharmaceutical Sciences,
Lovely Professional University,
Jalandhar-Delhi G.T. Road,
Phagwara, Punjab 144411

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LIST OF ABBREVIATIONS

Abbreviated form	:	Full form
% RSD	:	Percentage Relative Standard Deviation
5-ASA	:	5-Aminosalicylic Acid
5-BU	:	5-Bromouracil
6-MP	:	6-Mercaptopurine
ADRs	:	Adverse Drug Reactions
AUC	:	Area Under Curve
AZA	:	Azathiopurine
BAM	:	Bacteriological Analytical Manual
BCS	:	Biopharmaceutics Classification System
BFM	:	Bifidobacteria Fermented Milk
BSA	:	Bovine Serum Albumin
$CaCl_2$	:	Calcium Chloride
Caco-2	:	Caucasian Colon Adenocarcinoma-2
CD	:	Crohn's Disease
CFU	:	Colony Forming Units
CG	:	Calcium Gluconate
CI	:	Compressibility Index
C_{max}	:	Maximum Plasma Concentration
CMTSP	:	Carboxy Methyl Tamarind Seed Kernel Polysaccharide
CO_2	:	Carbon dioxide
Conc.	:	Concentration
CPG	:	Calcium Pectinate Gel
CRC	:	Colorectal Cancer
CSA	:	Chitosan Acetate
CV	:	Cell Viability
D	:	Dose Administered

Abbreviated form	:	Full form
DAI	:	Disease Activity Index
DDD	:	Defined Daily Dose
DMEM	:	Dulbecco's Modified Eagle Medium
DMSO	:	Dimethyl Sulfoxide
DOE	:	Design of Experiment
DPD	:	Dihydropyrimidine Dehydrogenase
DR	:	Drug Release
EC	:	Ethyl Cellulose
FBS	:	Fetal Bovine Serum
FTIR	:	Fourier Transform Infrared Spectroscopy
FTM	:	Fluid Thioglycolate Medium
GIT	:	Gastro Intestinal Tract
HCL	:	Hydrochloric Acid
HETP	:	Height Equivalent to Theoretical Plate
HFS	:	Human Faecal Slurries
HM	:	High Methoxylated
HQC	:	Higher Quality Control Concentration
HR	:	Hausner's Ratio
IAEC	:	Institutional Animal Ethics Committee
IBD	:	Inflammatory Bowel Disease
IBS	:	Irritable Bowel Syndrome
ICH	:	International Council for Harmonisation
Ils	:	Interleukins
LD_{50}	:	Lethal Dose 50
LM	:	Low Methoxylated
LOD	:	Limit of Detection
LOQ	:	Limit of Quantification
LQC	:	Lower Quality Control Concentration

Abbreviated form	:	Full form
MAO	:	Myeloperoxidase Activity
MEM	:	Minimum Essential Medium
min.	:	Minute
mM	:	Millimolar
MQC	:	Middle Quality Control Concentration
MRT	:	Mean Residence Time
MTT	:	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole)
NaOH	:	Sodium hydroxide
NOCC	:	N,-O-Carboxy methyl Chitosan
PB WRCC	:	Probiotics Without Rat Caecal Content
PC	:	Probiotic Culture
PCMGG	:	Partially Carboxymethylated Guar Gum
QC	:	Quality Control
RCC	:	Rat Caecal Content
RH	:	Relative Humidity
RP-HPLC	:	Reverse Phase-High Performance Liquid Chromatography
Rpm	:	Rotations Per Minute
SFZ	:	Sulfasalazine
SGF	:	Simulated Gastric Fluid
SIF	:	Simulated Intestinal Fluid
$t_{1/2}$	:	Half Life
TEA	:	Triethanolamine
T_{max}	:	Time to reach maximum concentration in plasma
TPP	:	Tripolyphosphate
UC	:	Ulcerative Colitis
USP	:	United State Pharmacopoeia
UV-Visible	:	Ultra-Violet Visible

Abbreviated form	:	Full form
WRCC	:	Without Rat Caecal Content
%	:	Percentage
µL	:	Microliter
^{0}C	:	Degree Celsius
Cm	:	Centimeter
cm^{-1}	:	Centimeter inverse
cm^{2}	:	Centimeter square
G/g	:	Gram
g/ml	:	Gram per milliliter
H	:	Hour
Kg	:	Kilogram
Kg/cm^{2}	:	Kilogram per centimeter square
M	:	Molarity
mAU	:	Milli absorbance units
Mg	:	Magnesium
mg/g	:	Milligram per gram
mg/Kg	:	Milligram per kilogram
mg/mL	:	Milligram per millilitre
mL	:	Millilitre
mL/min	:	Millilitre per minute
ml^{-1} min	:	Millilitre per minute
Mm	:	Millimeter
N	:	Normality
Ng	:	Nanogram
ng/mL	:	Nanogram per millilitre
Nm	:	Nanometer
r^{2}	:	Determination coefficient
v/v	:	Volume by volume

Abbreviated form	:	Full form
w/v	:	Weight by volume
w/w	:	Weight by weight
λ_{max}	:	Absorbance maximum
M	:	Micron
µg/mL	:	Microgram per millilitre
Mm	:	Micrometer
Pb	:	Bulk density
Pt	:	Tapped density

LIST OF APPENDICES

ABSTRACT

The present study was conducted for the development of polysaccharide based oral colon targeted mini-tablets containing 5-Fluorouracil (5-FU) for its site-specific release. The colon targeted 5-FU mini tablets were co-administered with probiotics which serve to constantly replenish microflora of colon that gets damaged during cancer as well as due to side effect of 5-FU. Guar gum and pectin were used as polysaccharides to accomplish colon specific release and Eudragit S100 was used as pH dependent polymer as core and coating materials for 5-FU mini tablets. The results of dissolution studies indicated that un-coated tablets, containing Eudragit® S100 (1 g), guar gum (1.35 g), pectin (1.35 g) showed the least drug release in first 5 h. Hence, they were further coated with polymers. Ternary phase diagram was reported for the first time to optimize the coating of tablets. The site-specific release of coated tablets was confirmed by dissolution studies, wherein, the formulation containing Eudragit® S100, pectin and guar gum in the ratio of 04:03:03 w/w, showed less than 10 % drug release in initial 5h and immediate burst release (100 %) within 5^{th} to 10^{th} hour. The results are found to be similar for mini tablets with probiotics, however, between 5^{th} to 10^{th} hours, the rate and extent of drug release was found superior as compared to tablets administered without probiotics. For better insights and correlation, the pooled dissolution samples at predetermined time intervals were also subjected for cell line studies (MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole)] assay) on Caco-2 cell lines. The results indicated that more than 90 % cells were viable in all the cases till 5^{th} hour. The significant ($p < 0.05$) decline in cellular viability was observed in case of mini tablets whose dissolution study was conducted with Rat Caecal Contents (RCC) as compared to those whose dissolution study was conducted without RCC. The pharmacokinetic studies showed a lower as well as delayed plasma concentration in the coated formulations endorsing the original hypothesis of reduction in drug exposure of the non-target sites. The C_{max} reached in plasma for coated mini tablets with and, without probiotics was 104.55 ± 14.98 ng/mL (0.104 µg/mL) and 119.56 ± 14.22 ng/mL (0.119 µg/mL) respectively. It is, therefore, concluded that mini tablets of 5-FU prepared using

Eudragit® S100 and colon specific polysaccharides with concomitant administration of probiotics offer an efficient, scalable and cost-effective treatment of colon cancer.

Keywords**:** 5-FU; Colon targeted delivery; Mini tablets; Dissolution; Caco-2 cells; Pharmacokinetics.

Chapter 1
Introduction

1. Colonic diseases

Colonic diseases like Ulcerative Colitis (UC), amoebic colitis, Crohn's Disease (CD) and colon cancer are reported to be on the rise due to deterioration in the dietary and life style habits (1). This has led to an increase in the interest of formulators in colon targeted drug delivery systems (2). Effectiveness of the agents used for the treatment of the colonic diseases can be improved by their local delivery to the colon (3). In colon targeting, absorption of the drugs from stomach and small intestine is minimized while the absorption from the lumen of the large intestine is enhanced. In recent years, several novel delivery systems have been designed to deliver drugs quantitatively to the colon and subsequently trigger the release of active agents therefrom (4-9). The colonic route of drug delivery may also be used for the systemic administration of drugs. In some cases, like protein and peptide drugs, whose degradation takes place by various enzymes in the upper small intestine, colonic route can prove to be very effective.

1.1. Prevalence rate of colonic diseases world-wide

The incidences related to the diseases associated to colon are increasing rapidly around the globe (10). Prevalence rates are higher in developed countries in comparison to those in developing countries. However, the increase in incidence rates are higher in the newly industrialized countries of Africa, Asia and North America including Brazil and Taiwan (11). Colder-climate regions and urban areas have a greater rate of Inflammatory Bowel Disease (IBD) than those in warmer climates and rural areas. Globally, the incidences of IBD are 0.5-24.5 cases per 100,000 person-years for UC and 0.1-16 cases per 100,000 person-years for CD (11). Overall, the prevalence for IBD is 396 cases per 100,000 persons annually (11).

Among the colonic diseases, colorectal cancer happens to be the 3rd most commonly occurring cancer in men and the 2nd most in women. Over 1.8 million new cases were reported in the year 2018. The global burden of Colorectal Cancer (CRC) is expected to

be increased by 60 %, i.e., more than 2.2 million new cases and 1.1 million deaths by 2030.

1.2. Drugs used to treat Inflammatory Bowel Diseases (IBDs)

1.2.1. Drugs used to treat Ulcerative Colitis (UC) and Crohn's Disease (CD)

The patients with mild to moderate UC are started on treatment with oral Sulfasalazine (SFZ) with daily doses up to 4-6 g (12). SFZ is a prodrug in which the active moiety 5-Amino Salicylic Acid (5-ASA) is linked to sulfapyridine by diazo bond (13). The bacteria in the colon cleave this diazo bond and release 5-ASA and Sulfapyridine (14). The anti-inflammatory activity of SFZ is attributed to 5-ASA. Major drawbacks of the drug are at effective doses it also causes significant adverse effects, which include nausea, headache, vomiting, anorexia, dyspepsia and some serious side effects like agranulocytosis, pancreatitis, haemolytic anemia and megaloblastic anaemia. The patients with allergy to sulfa drugs cannot be recommended for the treatment with SFZ. SFZ also inhibits foliate absorption. Therefore folic acid supplements should be prescribed along with the drug (15).

Another commonly used aminosalicylate is mesalamine. This molecule does not contain sulfapyridine and therefore, the associated adverse effects are overcome. However, mesalamine has been implicated in causing nephrotoxicity (16). The renal toxicity of 5-ASA is dependent on the dose and duration of treatment (17).

Corticosteroids are used to treat moderate to severe relapses of UC as they have anti-inflammatory effect. They inhibit transcription of Interleukins (IL's) and metabolism of arachidonic acid and, also stimulate apoptosis of lymphocytes in lamina propria of large intestine (18). Corticosteroids are also associated with short as well as long term adverse effects.

Azathioprine (AZA) and 6-Mercaptopurine (6-MP) are purine anti-metabolites, which have found place in therapy of UC. These drugs are used in the patients in whom steroids cannot be used. The associated adverse effects in this case also become a limiting factor

in their use (15). Cyclosporine is another immunosuppressant which has proven to be effective in treatment of UC (13). But again its use is associated with toxicity and oral cyclosporine use has been associated with long term failure (15).

Infliximab is an anti-Tumor Necrosis Factor (TNF) monoclonal antibody. It has strong anti-inflammatory properties. Its use is associated with 4 to 5 times increase in risk of tuberculosis, anaphylactic reactions, fever, joint pain & myalgia (15).

1.2.1.1. Limitations of present therapies

- Dose dependent adverse effects of SFZ have been reported. Long term treatment with the drug possesses the adverse reactions like headache, gastric pain, nausea etc., and the frequency of the development of the side-effects is 1 in 3 patients. The newer Aminosalicylates, i.e., mesalalzine, olsalazine and balsalazide can cause mild to transient side effects when compared to SFZ (15).
- Short term side effects of corticosteroids are more of cosmetic nature like acne, moon face, face oedema, as well as mood fluctuations, glucose intolerance etc. Long term side effects include cataracts, osteoporosis, myopathy and, suppressed immune response and increased vulnerability towards the infections. Budesonide is one of the preferred corticosteroids but it is poorly absorbed and has limited bio-availability due to extensive first-past metabolism (15).
- Thiopurines like AZA and 6-MP cause distressing side effects like bone marrow depression, anorexia, nausea, vomiting and opportunistic infections (15, 19).
- Methotrexate causes nausea, vomiting, diarrhoea, stomatitis. Its use is also associated with serious adverse effects like hepatotoxicity, pneumonitis (19).
- The side-effects of cyclosporine therapy include tremors, paresthesia, malaise, headache, atypical liver functions etc. (19).

1.2.2. Drugs used to treat CRCs

Food and Drug administration (FDA) has approved anticancer drugs for the treatment of CRCs. For the treatment of CRCs, monotherapy as well as combination therapy is being used. Some of the drugs that are routinely used include 5-Fluorouracil (5-FU), avastin,

cetuximab, capecitabine, etc. Various drugs and their combinations used to treat CRCs are depicted in Figure 1-1 (20).

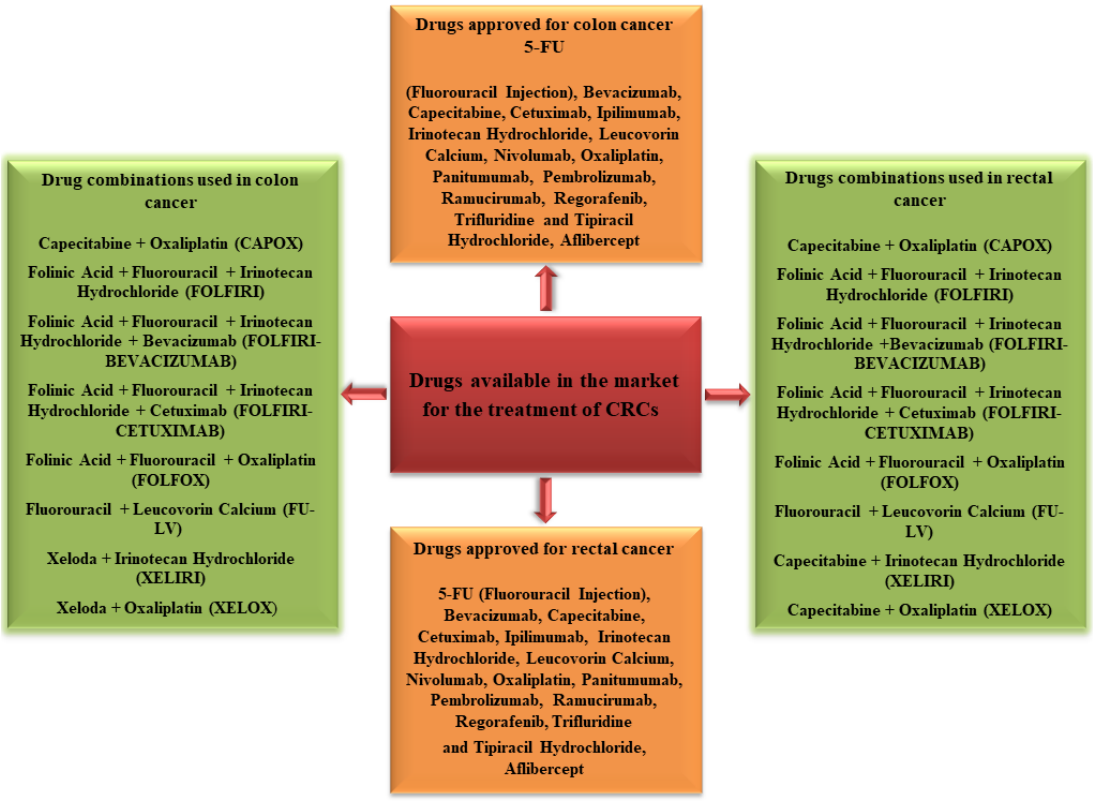


Figure 1-1: Drugs available in the market for the treatment of CRCs.

1.3. Approaches used to target the drug to the colon

The most widely used approaches to formulate the colon-specific drug delivery systems include, timed release systems, pressure-controlled systems, osmotically controlled systems, pH sensitive polymer coating and prodrugs (6, 21-24). The mechanisms involved in these delivery systems are shown in Figure 1-2. Use of the mentioned approaches is, however, limited by a number of inherent disadvantages (25-28). The best alternative approach for colon specific drug delivery is the application of carriers, especially those which gets digested by the action of colonic microflora. These carbohydrate carriers include pectin and its salts, chondroitin sulphate, amylose, inulin, guar gum and many more (4, 29-31).

An abrupt increase of the bacterial population and associated enzymatic activities in the ascending colon represents an exclusive and non-continuous event, independent of GI transit time and pH (32-34).

Approaches used world-wide to target the drug to the colon

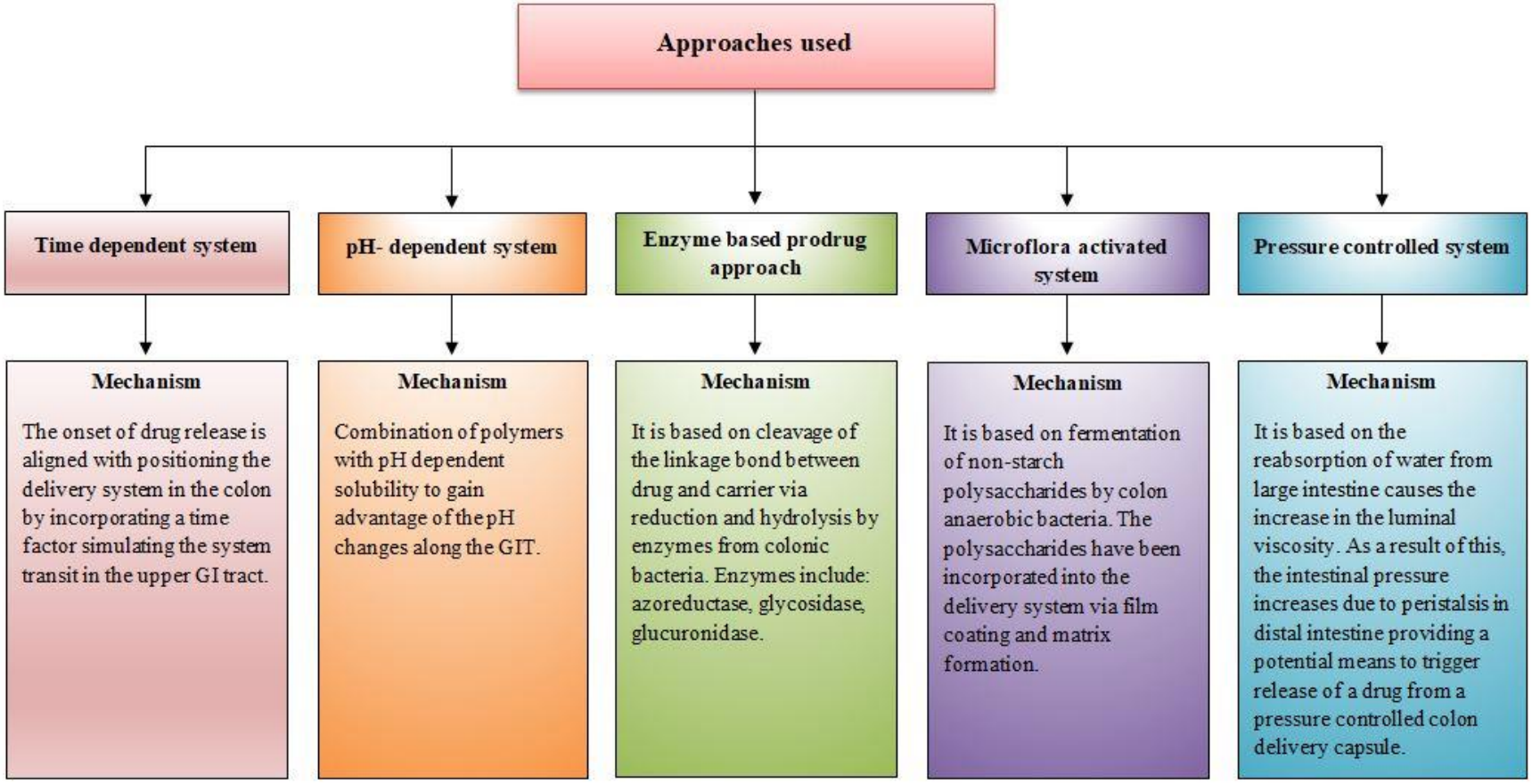


Figure 1-2: Approaches used to target the drug to colon and their mechanism.

1.4. Limitations of reported formulations to target the drug to the colon

The available strategies used to treat the colonic diseases such as UCs, CDs and colon cancer mainly consist of sustained release, delayed release oral delivery systems and intravenous injection. These available strategies suffer from one or more limitations as they do not successfully reach the target area of the colon. The oral delivery of medications through sustained or delayed release systems for the treatment of colonic diseases tend to expose the larger and healthy area of intestine which further leads to adverse effects as well as incomplete treatment due to the lower availability of the active constituent at the site of disease. As a result, the complications related to the management of disease enhance with the prolongation of the treatment thereby reducing the patient compliance. On the other hand, in case of intravenous injections, the drug is distributed systemically to the entire body through blood and poses a higher risk of toxicity to the non-target sites. Surgical removal of the cancerous tissue is the last resort in such cases. The surgical excision of the colon mainly involves the removal of the affected part of the colon. This is costly, requires prolonged hospitalization and most importantly involves the risk of metastasis.

1.5. Need for the development of colon targeted delivery systems

Though the use of polysaccharide based colon targeted delivery systems is widely reported, the approach could not be translated into commercial formulation due to an inherent drawbacks of the systems used. The drugs that are used for CRC are generally cytotoxic in nature and kill the natural microbiota in the colonic milieu. As the mechanism of release of drugs from polysaccharide based delivery systems is completely dependent on the action of microbiota, there is a compromise in the release of drug from the subsequent doses. This renders the therapeutic maneuver ineffective.

To overcome these complications, development of a novel delivery system is desired which can deliver the drug to the colonic site. The targeted delivery of the drugs will not only improve their effectiveness of the treatment by enhancing the availability of drug at local site of colon but will also reduce the adverse effects associated with the drugs by lowering their systemic availability. In addition to this approach that has been reported earlier, the formulation will be co-administered with probiotics in order

to get triple benefits of probiotics that include, enhancing the release of drug at the colonic site by utilizing the polysaccharide coat thereby improving availability of drug at colonic site, maintenance of gut microbiota becomes mandatory. This, in turn improves the therapy by reducing the side effects associated with drug such as diarrhoea.

Hence, in the present study, efforts have been made towards development of polysaccharide based mini tablets containing 5-FU to provide site specific release at the colonic milieu. The major reasons for development of mini tablets are, ease of administration of drug to experimental animals to generate pre-clinical data and reduction in amount of excipients used as the polysaccharide swell upon exposure to water which makes coating difficult. *In vitro* dissolution studies, *in vitro* cell line studies using Caucasian colon adenocarcinoma-2 (Caco-2) cells and pharmacokinetic studies conducted on the prepared formulation are indicative of its efficacy.

Chapter 2
Review of Literature

Delivery of drugs through oral route is most preferred because of its high patient compliance (3). However, from conventionally administered oral dosage formulations, the drug enters the systemic circulation. This, in turn, leads to exposure of non-target sites to the drug, leading to side effects. Site-specific drug delivery offers several advantages including low dose, less dosing frequency, increased therapeutic activity, and a decrease in side effects (35). The drugs that are specific for colonic diseases should remain unabsorbed in the upper Gastro Intestinal Tract (GIT). The dosage regimens for diseases requiring delayed drug release such as nocturnal asthma and angina etc., can also be optimized by colon specific drug delivery (36). Various colonic diseases including IBD that includes both CD and UC, Irritable Bowel Syndrome (IBS), polyps, diverticulitis, and infectious diseases can be effectively treated by targeted delivery (6). Factors affecting the release of drug in the colon include its residence time, presence or absence of food in GIT, pH of the colon, gastric emptying time, and nature and number of colonic microflora (3).

2.1. Need for targeted drug delivery

Targeted drug delivery systems are used to deliver the drug to specific/required site for desired therapeutic effect. This results in decrease in the required dose, thereby reducing the adverse effects of the drugs. At the same time, these delivery systems ensure increase in the efficacy of the drug (37). They ensure an increase in the drug concentration at the required site of action while reducing in the other tissues.

Thus, the advantages of the targeted drug delivery systems can be summarized as:

- Increase in the concentration of the drug at desired site of action
- Reduction in the exposure of non-target sites, therefore, reduction in side effects
- Reduction in the required dose

2.2. Colon targeted drug delivery

Colon drug delivery has gained increasing importance not just for the treatment of colonic diseases, but also for the delivery of proteins and peptides like insulin (6).

Colon as a target site has several advantages like almost neutral pH, low peristalsis, longer transit time, lesser digestive enzymes activity and presence of large amount of enzymes produced by colonic microbiota (36, 38). Various systems are available for colonic drug delivery which include colon susceptible linkage of drug with carrier, pH sensitive polymer intended for coating, time dependent release systems and enzymatically controlled drug delivery (39). Enteric coating systems are most commonly used for colonic drug delivery, their major limitation being that they do not allow reproducible drug release. The disadvantage of time dependent release systems lies in the fact that any variation in gastric emptying time leads to drug release in small intestine, i.e. before reaching colon. The most convenient approach for site specific drug delivery is enzymatically controlled delivery systems (40). To improve the specificity of release certain type of polysaccharides are used such as pectin, chitosan, dextran, guar gum, inulin etc. (6, 41). Various oral colon targeted delivery systems prepared so far are enlisted in Table 2-1.

Table 2-1: Research work reported so far on the development of colon targeted drug delivery systems.

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
1.	Metronidazole	Pectin	Microspheres	*In vitro* studies were carried out in rat caecal content and drug release rate were calculated. It was inferred that bacterial polysaccharide facilitated the release of the drug.	(42)
2.	Ibuprofen	Carboxy Methyl Tamarind Seed Kernel Polysaccharide (CMTSP)	Pellets	Studies conducted in presence of rat caecal content. The higher viscosity of CMTSP than TSP indicated its suitability to be developed as a sustained release formulation.	(43)
3.	Metronidazole	Partially carboxymethylated guar gum and Calcium gluconate (PCMGG:CG) in the ratios of 10:0, 10:1, 7.5:1, 5:1, 1:1, 1:3, 1:5	Tablets	Crosslinking of Ca^{+2} with PCMGG facilitated water penetration and changed the viscosity of the gel resulting in the change of its swell and erosion characteristics.	(44)
4.	Metronidazole	Chitosan: Xanthan Gum (1:1)	Tablets	Bio adhesion was performed on sheep duodenum using a modified two arm method. The formulation showed sustained release. Strong bio adhesion was obtained when compared to commercially available tablet.	(45)
5.	Metronidazole	Guar Gum	Tablets	Rat caecum studies were carried out. The tablets released 20 % of the loaded metronidazole in simulated colonic fluid indicating the applicability of guar gum.	(25)
6.	Metronidazole	Guar gum, Xanthan gum and 10 % starch paste(Guar Gum and Xanthan Gum 100:0, 65:35, 55:45, 35:65 and 0:100	Tablets	It was found not to be suitable as it degraded into 2-3 pieces at the end of the dissolution study.	(46)
7	Metronidazole	Calcium Pectinate	Emulsion Gel Beads	Emulsion gelation method was used to prepare floating drug delivery system. The emulsion of calcium pectinate gel beads showed rapid release when compared to the conventional available forms.	(47)

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
8.	Metronidazole	Chitosan	Nanoparticle	The nanoparticles were formed with chitosan to Tripolyphosphate (TPP) weight ratios of 3:1, 4:1, 5:1, 6:1, and 7:1 respectively. Drug release at 12 h was obtained in a sustained manner.	(48)
9.	Metronidazole	Pectin	Beads	The performed study showed the use of pectin as an internal cross linking agent to prepare calcium pectinate beads. It has shown promising results as a carrier to improve drug entrapment in the beads. With this, sustained release of water soluble drugs was achieved.	(49)
10.	Metronidazole	Pectin	Microspheres	Conjugate technique was used to synthesize the prodrug. Emulsion dehydration technique was utilized for the preparation of microspheres. Drug showed optimum and desired release from the microspheres.	(50)
11.	Metronidazole	Thiolated Tamarind seed polysaccharide.	Gels	Esterification of the hydroxyl group of drug with thioglycolic acid was done. An increase in mucoadhesion was observed.	(51)
12.	Metronidazole & Paracetamol.	Guar gum and Xanthan gum.	Tablets	*In vitro* drug release studies in human fecal slurries were carried out in USP type 1 dissolution apparatus. Based on the results, 250 mg of xanthan and guar gum was found to give the optimum formulation.	(52)
13.	Metronidazole	Chitosan and carbapol 934P (50:150)	Tablets	3^2 factorial design was used in designing this formulation. It was formulated with a ratio of 50:150 which showed the best degradation in simulated colonic fluids.	(53)
14.	Albendazole	Guar Gum	Tablets	This study emphasizes the applications of guar gum as a carrier for the development of colon targeted drug delivery systems. The concomitant administration of metronidazole with tinidazole was found to interfere in the degradation process of guar gum by colonic bacteria, when administered as a matrix tablet of guar gum.	(54)
15.	Albendazole	Guar Gum	Tablets	*In vivo* evaluation of the formulation was carried out on healthy	(55)

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
				human volunteers and a delayed T_{max} and absorption time were observed.	
16.	Albendazole	Chitosan	Microcapsules	Formulations with chitosan showed maximum entrapment efficiency. The microscopic evaluation of the micro capsules revealed that they were porous and wavy. Simulation was performed using rat caecal contents.	(56)
17.	Albendazole	Dextrin	Tablets	Different concentrations of dextrin were used to prepare tablets of albendazole. Wet granulation technique was used for the preparation and different IPQC tests were performed. The dissolution profile showed sustained release pattern of drug.	(57)
18	Albendazole	Chitosan	Beads	Simple ionic gelation method was used for preparation of Alg-CS beads. They showed excellent sustained release activity.	(58)
19.	Albendazole	Chitosan	Microparticles	Spray drying method was used in the preparation of microparticles. Formulation showed a marked increase in the oral bioavailability of the drug.	(59)
20.	Albendazole	Chitosan	Microparticles	Albendazole-chitosan microparticles were formulated by the aid of ionic interaction technique. They showed remarkable increase in the dissolution profile of the loaded drug in comparison to the release profile of pure API.	(60)
21.	Albendazole	Chitosan	Microspheres	Microspheres of albendazole were prepared by optimizing the ratio of chitosan, pectin and carboxymethylcellulose. The formulated microspheres showed consistent morphology, better yield, entrapment efficiency and drug release properties. The formulated microspheres had shown 10 folds increase in the bioavailability of albendazole,	(61)
22.	Tinidazole	Pectin	Microbeads	Calcium alginate beads of tinidazole were prepared by ionotropic gelation process and further coated with Eudragit® S100 by utilizing	(62)

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
				solvent evaporation technique. Formulation with 10 % TWG was found to be the optimized. Dissolution study was performed in the rat caecal content. The microbeads showed 70.83 % of drug release in 24 h.	
23.	Tinidazole	Guar gum	Tablets	*In vivo* studies of the prepared tablets showed no drug release in the stomach or small intestine. The elimination half-life of tinidazole was found to be around 7 h following the administration of the tablet.	(63)
24.	Tinidazole	Guar gum	Micro capsules	2^4 factorial design was incorporated to optimise the formulation parameters. *In vitro* studies were performed in pH 1.2, 4.5 and 6.8 buffers. Release of the drug was found in accordance of Korseymeyer Peppas model.	(64)
25.	Tinidazole	Chitosan	Micro capsules	Two different type of micro-capsules were prepared, i.e., alginate micro capsules and chitosan micro capsules. Chitosan microcapsules showed slower drug release. *In vitro* drug release in the dissolution medium containing rat caecal content was higher in comparison to that in conventional dissolution medium.	(65)
26.	Tinidazole and theophylline	Chitosan	Hydrogels	Tinidazole and theophylline were incorporated into the matrix of the hydrogel formed by using chitosan. The drug release kinetics was in accordance with the Korsemeyer-Peppas model.	(66)
27.	Mesalamine	Chitosan	Tablets	Chitosan was utilized to retard the drug release from the prepared tablets. Prepared tablets were coated with Eudragit® S100. Application of chitosan in combination with pH dependent polymer, showed remarkable decrease in the drug release profile of the tablets.	(67)
28.	Mesalamine	Guar Gum and Starch paste	Tablets	A 3^2 factorial design approach was utilized for the optimization of formulation parameters of tablets. Tablets were coated with Eudragit® S100. The enteric coating alone had failed to target the	(68)

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
				drug release to the colonic site.	
29.	Mesalamine	Chitosan and Eudragit® S100.	Microspheres	Ionic gelation technique was used for the formulation of mesalamine loaded microspheres. Good percentage yield and drug loading was observed. Encapsulation efficiency was also found to be good. The ratio of 1:3 of chitosan: guar gum was found to be optimum for the formulation of microspheres.	(69)
30.	Mesalamine	Locust Bean and Eudragit® S100	Microspheres	Ionic gelation method was used for the preparation of microspheres of mesalamine using $CaCl_2$ cross-linked with glutaraldehyde. The microspheres were coated using Eudragit® S100. In this study locust beans were administered orally to induce enzymes for biodegradation. Eudragit® S100 coated microspheres showed the release of drug in accordance with Korsmeyer-Peppas.	(70)
31.	Mesalamine	Gellan Gum	Beads	Ionotropic gelation method was used in the preparation of beads. Polymethacrylate base coating was carried out by dip coating method to retard the drug up to colonic pH. The *in vitro* dissolution study had shown a prolonged drug release in the colon.	(71)
32	Mesalamine	Sodium alginate, ratio of triglyceride and monostearate	Nanoparticles	Nanoparticles of mesalamine were formulated and characterized for their shape and surface morphology. Maximum entrapment efficiency was found to be at 72.71 %. Studies were carried out in the medium containing human and rat caecal contents.	(72)
33.	Mesalamine	Guar Gum, Eudragit® S100 and Pectin	Tablets	The research work employed guar gum as a carrier for Mesalamine. $BaSO_4$ was incorporated as an X-ray imaging material to check the movement of tablets in GIT. The results demonstrated that the drug was released in the colon site by bypassing the upper part of GIT.	(73)
34.	Mesalamine	Apple Polysaccharide	Mini tablets	Apple polysaccharide was used in the preparation of mini tablets. Different antioxidants and pre oxidation parameters were determined. Combination along with probiotics was used. The	(74)

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
				results were ratified with macroscopic scoring. Better results in the treatment were observed in the formulated administered with probiotics in comparison to which of devoid of probiotics.	
35.	Mesalamine	Guar gum and Xanthan Gum.	Microsphere	Microspheres of mesalamine were prepared with prebiotics and probiotics Dissolution studies were performed in simulated colonic fluid. A sustained release of drug was observed from the microspheres.	(75)
36.	Sulfasalazine	Guar Gum and Eudragit®	Spheroids	Pharmacokinetic evaluation was done for concomitant administration of probiotics with the formulated spheroids of sulfasalazine. Experiments in rats showed delayed T_{max} and decreased C_{max} values of the drug.	(76)
37.	Sulfasalazine	Pectin and Guar Gum	Liquisolid	Box Behnken design was utilized to optimize the methodology of this formulation. Rat, human and goat caecal contents showed significant drug release for the liquisolid compacts release.	(77)
38.	Sulfasalazine	Guar gum	Spheroids	Spheroids were prepared by the extrusion-spheronisation technique and were coated with guar gum. The probiotics had shown remarkably higher drug release in the dissolution medium in comparison to the medium devoid of probiotics.	(78)
39.	5-Flurouracil	Guar Gum	Tablets	Direct compression method was used to prepare disintegrating tablets of 5-FU. Different percentages of compression coat of guar gum were applied over the tablets. *In vitro* drug release studies revealed that 80 % coating of guar gum is likely to prove the targeted release of drug to the colon.	(79)
40.	5-Flurouracil	Guar Gum	Microspheres	Emulsion polymerization method was used to develop the microspheres. Cell line study was performed on Human HT 29 cells. It was concluded based on the results of this study, that guar gum could be used as a potential polymer to target the drug release to	(80)

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
				colon.	
41.	5-Flurouracil	Guar Gum and Xanthan Gum.	Nanoparticle	It was reported that colorectal cancer was the 3rd most reported cancer in humans. The prepared nanoparticles were evaluated in rat caecal content. The result of the studies indicated that the microflora present in the GIT was damaged due to the administration of 5-FU.	(81)
42.	5-Flurouracil	Chitosan	Microspheres	Glutaraldehyde was used as a cross linking agent. Microspheres were coated with Eudragit® S100 using solvent evaporation method in the ratio of 1:100. Drug content in different parts of GIT was calculated. An amount of 84 % of drug was found in colon. Thus, the prepared formulation might be a suitable alternate for the colonic drug delivery of therapeutics.	(82)
43.	5-Flurouracil	Apigenin	Liposome	Apigenin, a naturally occurring flavonoids was used synergistically along with 5-Flurouracil. It was tested on xenograft model of nude mice. Treatment was highly significant with both models ($P < 0.0001$).	(83)
44.	5-Flurouracil	Pectin	Microspheres	Emulsion dehydration technique was employed in the preparation of microspheres. With varying ratios of pectin, microspheres of varying mean diameters were formulated. Release rate was found to be 66.32 % in the colon of albino rats.	(84)
45.	5-Flurouracil	Eudragit® S100 Coated Citrus Pectin	Nanoparticles	HT - 29 cells were employed in the study. Sulforhodamine assay was performed. Galectin 3 receptor was used as a ligand which was found to increase cell targeting of nanoparticles.	(85)
46.	5-Flurouracil	Alginate/chitosan/kappa – carrageenan	Microbeads	Three different types of formulations were prepared, i.e., Alg, Alg/Cs, Alg/Cs/Kc. Microbeads were tested for biodegradability and particle size analysis. The formulated microbeads were found biodegradable and nontoxic.	(86)
47.	5-Fluorouracil	Guar gum or xanthan gum	Microspheres	The targeted delivery of drugs to the colon for the treatment of colon	(87)

Sr. No.	Drug Used	Polymer/ratio of polymer	Type of Formulation	Outcome	References
				specific disease with improved efficacy and decreased toxicity associated with the drug molecules was observed.	
48.	Rhubarb (Herbal Drug)	Pectin	Tablets	65 % w/w coating of ES 100 was employed and the amount of polymer and drug was a detrimental factor in formulation studies. Site specific release was observed.	(88)
49.	Methotrexate	Guar gum	Microspheres	Microspheres were prepared by emulsification method. Mean diameter was found between 12.4 ± 1.02 µm to 16. 5 ± 1.22 µm. Glutaraldehyde and guar gum concentrations were found to have a crucial role to play in controlling the release rate of the drug, as around 79 % of drug was released in colon.	(89)
50.	Methotrexate	Xanthan Gum	Tablets	Xanthan gum was used to prepare the tablets to deliver the drug at the colonic site. Drug release profile was observed in accordance with the Korseymeyer Peppas model.	(90)
51.	Cisplatin	Hyaluronan	Nanoparticles	Microbeads were prepared by electrospray technology. DMH was used to induce tumors in rats. Serum creatinine was significantly increased. It was also inferred that cisplatin nanoparticles can treat nephrotoxicity *in vivo*.	(91)
52.	Doxorubicin	Dextran	Nanoparticles	Nanoparticles of doxorubicin loaded with cisplatin were prepared and tested on Autochthonous tumor model. From the performed study, it was observed that the prepared formulation blocks the tumor growth in colorectal carcinoma and also inhibits the metastasis of 4T1 mammary carcinoma cells.	(92)

Four different approaches have been widely reported until now for targeting of drugs to colon by oral route. These include:

- pH based approach (93)
- GI transit time (94)
- GI pressure differences (95)
- Microflora activated systems (25)

In pH based approach, a pH sensitive polymer (e.g. Eudragit® S100) or a combination of such polymers (e.g. Eudragit® L100 & Eudragit® S100) is used for delivery of drugs specifically to the colon (96). Colonic delivery can also be used to deliver macromolecules such as protein and peptide drugs (e.g., Insulin) because of the low abundance of proteolytic enzymes in the colon (96). The intestinal transit time plays a vital role in delivery of drugs to the colon. The transit time of dosage form depends on the disease state, time of administration, presence or absence of food and/or even on time of administration like dawn and dusk. In pathological conditions such as UC, shorter transit times were observed when compared to healthy subjects (97).

Moreover, a delayed transit has been observed during sleep and larger dosage forms (e.g., capsules) transit faster than smaller ones (e.g. dispersed particles) (98). Two time- dependent systems, namely, Pulsincap® and Time Clock®, have been developed with the lag time of 8 and 9.2 h, respectively (6).

Pressure based approach depends on the intestinal pressure differences due to strong peristaltic waves in the colon. Because of the reabsorption of water in the colon, the viscosity of its contents increases. As a result of this, a raise in pressure is observed, which causes the rupture of pressure-controlled colon delivery capsules, resulting in release of drug, specifically in the colon (6).

Colon contains a number of anaerobic microorganisms that may be utilized as a triggering factor for delivering drugs specifically to the colon. Predominantly the colon contains *Clostridium, Bacteroides, Lactobacillus,* and *Bifidobacteria* species. The colonic microflora hydrolyses various polysaccharides such as guar gum, pectin, chitosan, calcium alginate, calcium pectinate, dextran, ethyl cellulose etc. Coating the

formulations with these polysaccharides will provide site-specific drug delivery since these microorganisms are uniquely endemic to colon (41).

2.2.1. Polysaccharides used for colon targeted drug delivery

Use of certain polysaccharides enhances the colon specificity for formulations as they are metabolized exclusively by the colonic microflora. Different carbohydrate polymers used for developing colon targeted drug delivery systems include (99):

- Starch,
- Cellulose
- Arobinogalactan
- Alginic acid
- Agar
- Chitin
- Pectin
- Guar gum
- Hyaluronic acid
- Gellan gum
- Pullunan

Chemically modified carbohydrate polymers are:

- Guar gum (Acetyl derivative)
- Arabinogalactan (Tethering with folic acid)
- Pectin (Oxidized citrus pectin)
- Chitosan (Folate complex prepared)
- Dextran (Methylated and succinic derivative prepared)

Polymers of plant origin such as gums and mucilage have array of pharmaceutical and cosmeceutical applications. A number of modifications can be done to these natural polymers to make them suitable for drug delivery and competent enough with existing synthetic polymers (100).

Chemically modified polysaccharides help in the reduction of drug release in the gut. However, the degradation kinetics and drug release from these gums and mucilages is

dependent on number of formulation and process variables. Hydrogel formulations are designed to provide site specific release of drug to colon. Their release is also dependent on the composition and physicochemical characteristics of dosage form. Hence, the solubility of these polysaccharides alone or in combination at colonic pH is required to be tested. The combination of polysaccharides depends on the nature of the release mechanism such as erosion of film coat or their swelling degradation of polysaccharide in the GIT (99).

In one study various blends of kollicoat SR30D and chitosan were coated on the tablet core and investigated for swelling and controlled drug release in various GI fluids. The authors also studied *in vivo* degradation of tablets in rats. The authors also studied *in vivo* degradation of tablets in rats. The results revealed that composition coating affected the swelling behavior and release rate. Moreover, the coat was susceptible to enzymatic degradation. The results of pharmacokinetic data presented delayed T_{max} decreased C_{max} and extended the Mean Residence Time (MRT) (101).

In one of the studies 5-ASA was compression coated with chitosan acetate (spray dried) and ethyl-cellulose. The swelling behavior of tablet compressed coated with chitosan acetate alone and with mixture of ethyl-cellulose/chitosan acetate in various GI fluid (0.1 N HCl, phosphate buffer (pH 6.8) and acetate buffer (pH 5.0) was evaluated. The effect of various parameters such as coating polymer ratio, stirring speed of paddle, pH of dissolution medium, caloric enzyme and use of super disintegrant on drug release was evaluated. The results revealed that both the polymers, i.e., ethyl cellulose and chitosan acetate in the ratio of 87.5and 12.5 % provided no drug release till first 5 h and about 90 % drug release within 12 h. This system was found to be controlled (due to swelling of ethyl-cellulose and diffusion of drug from its matrix) and well as pH dependent due to disintegration of chitosan acetate at higher pH , that allowed complete drug release (102).

The effect of variation in type of pectin and process variables used to prepare MS microspheres on its entrapment and release was investigated. The results indicated better stability of MS microspheres upon coating as compared to uncoated one during storage. Furthermore, the reduction in cross linking time between drug and polymer

and higher concentration of calcium chloride improved the entrapment efficiency. Whereas, the condition was found opposite for the drug release. However, no effect of pectin and time of cross linking was observed on drug`s release rate. The proteolytic enzymes that were added in the colonic medium failed to provide degradation of microsphere coat. It was concluded that the MS prepared using 2.5 % w/v of calcium chloride was adequately modulated the release of drug by modulation of pH and control on transit time, thus avoidance of drug release at gastric site. 100 % drug release was observed once the formulation reached the colon (103).

A clinical cross over study carried out for metronidazole (500 mg) loaded in immediate release and guar based colon targeted tablets. The study showed immediate appearance of C_{max} was delayed by 9 h (T_{max}) in case of metronidazole loaded in colon targeted tablets upon oral administration. This indicated the potential of guar gum in retarding the drug release in upper GIT, i.e., stomach and small intestine and complete delivery of drug upon reaching to colon. Moreover, the C_{max} appeared in colon was 25.76 mg/mL and C_{max} appeared for immediate release tablet was 37.26 mg/mL indicating poor absorption of drug from colonic site. Thus making the drug for local action (8).

Guar gum based multilayer colon targeted tablets of metronidazole were prepared by compression coating and compared with its matrix tablet. About 50 % drug release was observed from the matrix tablet during dissolution study, whereas 25 - 44 % drugs got released from multilayer tablets in stomach followed by small intestine. However, the multilayer compression coated tablet released only 1 % metronidazole in first 5 h, indicating the potential of developed multilayered compression coated guar gum based formulation to target colon rest of the drug got released in a sustained manner after 5 h indicating local and sustained release of drug at colonic site (25).

Compression coated fast disintegrating tablets of 5-FU using varying ratio of (60 %, 70 % and 80 %) of guar gum were prepared and reported by Krishaniah, *et al.*, (2002). The *in vitro* dissolution studies indicating maximum 4 % drug release in gastric fluid. Upon subjecting the tablet in medium containing rat caecal contents (4 % w/v) after 5 h, the tablets coated with 60, 70 and 80 % guar gum showed 70, 55 and 41 % drug

release respectively. Hence, the authors concluded that the tablet containing 80 % guar gum was found best for local action to target colon (79).

The design of colon targeted delivery system based on polysaccharides depends on degradation of these polysaccharides by the colonic bacteriae. These include "β-glucosidase, β-galactsidase, dextranase, Xylomerase, Pectinase, Amylase" etc. The basic principle includes fermentation of polysaccharide core followed by degradation of matrix. Example of polysaccharide include pectin, chitosan, guar gum, cyclodextrin and inulin (36).

Bovine serum albumin loaded colon targeted calcium pectinate based beads were prepared. The beads were subjected for dissolution study in various gastro intestinal medium simulating gastric intestinal and colonic fluids. The results of *In vitro* dissolution study revealed no drug release for first 5 h in gastric and intestinal medium. Upon reaching to colonic site after 5 h, a sudden release was observed for bovine serum albumin. This indicated the potential of cross linking of serum albumin with calcium alginate that led to target it to colon (104).

Colon targeted indomethacin spheres were prepared using ionic-gelation method by dispersing indomethacin in methoxy pectin solution and dropping this dispersion slowly to calcium chloride solution. Several process variables such as presence of hardening agent, type of pectin were found to affect spherical bead size, drug loading and morphology. The *in vitro* dissolution study indicated the formulation to be colon targeted as less than 10 % drug got released in first 5 h in upper GIT. Rest all drug got released after 5 h resulting in colon specific delivery of indomethacin (105).

Sulfasalazine loaded calcium-alginate-N,-O-carboxymethyl Chitosan (NOCC) beads were formulated, coated with chitosan to target the drug to colon. Drug (1 % w/v) was added to alginate solution, previously prepared by adding calcium-alginate-NOCC beads to 1.5 % w/v solution of chitosan. The prepared beads were evaluated for swelling properties, drug content, entrapment efficiency, and drug release studies. The swelling degree of air and freeze-dried beads at pH 1.2 was considerably low as compared to the swelling degree at pH 6.8. The swelling degree at pH 1.2 and pH 6.8 was 2.8 and 46, respectively. The encapsulation efficiency of uncoated alginate beads

was relatively higher, i.e. 65 % as compared to coated-chitosan beads, i.e., 60 %. The drug release was less in acidic pH due to less solubility of drug in acidic media. Burst release was not observed when the formulation was introduced to alkaline media. Coating with chitosan reduced the drug release in acidic and small intestine fluid, i.e., only 40 % of encapsulated drug was found to be released after 5 h (106).

Pectinate gel microspheres of sulfasalazine, prepared by ionotropic gelation methods, were characterized for influence of different formulation parameters upon their morphology, rheological analysis, and release kinetics. SFZ microspheres were prepared by adding 10 mL of drug solution, i.e., drug in 4 % of Low Methoxylated (LM) or High Methoxylated (HM) pectin or a combination of both (LM-HM) to 40 mL of calcium chloride ($CaCl_2$)-glutaraldehyde solution, while continuous stirring. The resulted microspheres were allowed to harden for half-an-hour, followed by filtering, washing, and drying these beads for 96 h. The prepared beads were evaluated for their morphology (using scanning electron microscope), swelling studies, entrapment efficiency, water content, and drug release kinetics. Rheological studies indicated LM-HM-SSZ could be a promising polymer for colon delivery of the drug due to its apparent lower viscosity than that of LM-SSZ and HM-SSZ. Both LM-SSZ and HM-SSZ showed irregular shape when examined for their morphology, on the other hand, spherical shape beads were observed for LM-HM-SFZ formulation. Amount of water was found to be similar in case of all formulation regardless of their degree of esterification. The swelling index of HM-LM-SFZ was higher in both Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) as compared to that of LM-SFZ, whereas, HM-SFZ formulation produced sticky mass, therefore, swelling behavior was not studied. The entrapment efficiency was found to be close to 99 % in all the formulations. The cumulative percentage of drug release was found to be higher in case of HM-SFZ that that of LM-SFZ and LM-HM-SFZ. The amount of drug release from LM-HM-SFZ was less than 10 % after first 2 h in SGF. Comparatively lesser drug release was observed from this formulation in SIF too. Hence, it was concluded that formulation containing a combination of these polymers could be able to provide colon-specific delivery of drugs (107).

In vivo studies were performed for the directly compressed SFZ tablets prepared using natural polysaccharides, i.e., guar gum and pectin. These tablets were evaluated for their anti-inflammatory effect against chemically induced ulcerative colitis in rats. The anti-inflammatory effect of these tablets was compared to that of pure 5-ASA. *In vitro* dissolution studies showed a negligible drug release up to 6 h in SGF and SIF, however, a constant drug release was achieved after addition of media comprising colonic-microflora. Guar gum coated SFZ tablets reduced the inflammation up to 90 % as compared to untreated and Sham group animals. Pectin coated tablets reduced the inflammation up to 82 % against untreated and Sham group animals. Therefore, it was apparent that guar gum was relatively superior in reducing inflammation than pectin. It was also found that both the polysaccharide-coated formulations were superior than pure drug, i.e., 5-ASA in treating inflammation (108).

Two different types of formulations of SFZ spheroids were performed to target the drug to colon, one coated with guar gum alone and another coated with a mixture of guar gum and Eudragit® S 100. The prepared spheroids were evaluated for *in vitro* and *in vivo* studies. They also studied the effect of co-administration of probiotics on the therapeutic performance of SFZ spheroids. The percentage drug loading of spheroids was found to be between 95-100 % and the mean diameter of the prepared spheroids was 1.0-1.5 mm. The formulations containing high amount of guar gum showed least drug release in initial 5 h, i.e., B4 and B5 which containing 15 %, 20 % of guar gum, respectively. However, B10 containing 15 % w/w guar gum and 15 % w/w Eudragit® S100 also showed less drug release in the first 5 h. Hence, these 3 batches were selected for further studies. After 24 h of the dissolution study in presence of 4.5 % w/v rat caecal contents, batches 4 and 5 showed similar dissolution profile. Stability studies indicated no significant difference between fresh spheroids and aged spheroids. *In vivo* studies including Disease Activity Index (DAI), macroscopic scoring, and determination of Myeloperoxidase Activity (MAO) were performed. All the treatment groups showed a decrease in DAI. After 14 days of continuous administration of probiotics with SFZ spheroids, even a dose as small as 100 mg/kg was also proven effective in reducing the inflammation and damage to mucosal epithelium. A decrease in MPO activity was observed in all the treatment groups. All

the *in vivo* studies indicated that the therapeutic efficacy of SFZ spheroids was increased by combination with probiotics. Thus, they concluded that therapeutic efficacy of formulations prepared by coating with polysaccharides could be enhanced by co-administration of drug with probiotics, which helps in maintenance of colonic microflora that are required to degrade these polysaccharide coatings (78).

2.2.2. Dissolution testing of polysaccharide based colon targeted formulations

Dissolution testing is the most commonly employed *in vitro* evaluation technique for predicting the *in vivo* drug release kinetics from orally administered dosage forms including the modified release delivery systems (1, 2). There are 2 predominant challenges involved in the designing of dissolution methods for site specific delivery systems. First challenge involves creating a micro-environment that is a physiological replica of the biological milieu at the site of drug release. Second major challenge lies in simulating the conditions that the drug delivery system is likely to encounter during its transit from mouth to target site (109). The more diverse the milieus encountered by the formulation, the more complex the dissolution method becomes. Due to the distal location of colon, the colon targeted oral delivery systems traverse the longest path coming across the most varied milieus. Designing a suitable biorelevant medium, therefore, has proved to be a complex task. The situation is further complicated by the varied approaches that have been used for achieving colon targeting. The most commonly used strategies to formulate colon-specific drug delivery systems include timed release systems, prodrugs, pH sensitive polymer coating and colonic microflora activated delivery systems (6, 21-24). Among these, the microflora activated delivery systems have been found to be quite promising. An abrupt increase in the number of microbiota and the resultant increase in enzymatic activities in the ascending colon represent an exclusive and non-continuous event independent of GI transit time and pH (4, 5, 25, 34).

Human colon represents a dynamic and ecologically diverse environment, comprising over 400 distinct species of bacteria with a population of 10^{11} to 10^{12} CFU/mL of colonic contents. These bacteria produce wide spectrum of reductive and hydrolytic enzymes, which are responsible for many biorelevant processes like carbohydrate and protein metabolism (2). Hence, in order to simulate the colonic milieu *in vitro*, the

methodology should be designed in such a way that it involves the microbiota of colon. At the same time, the method should be convenient, inexpensive and reproducible.

The methods that have been reported for evaluation of colon targeted delivery systems include triggering by enzymes (24, 103, 110-118), rat caecal contents (54, 99, 117, 119-129), human faecal slurries (130-135), and multi stage compound culture system (1, 2, 136). The fact that despite a large number of research reports on microbially triggered delivery systems for colonic delivery, no such product is commercially available, can be attributed to the lack of availability of a pragmatic dissolution method to evaluate the products during their development phase. Table 2-2 enumerates various dissolution methodologies reported so far to evaluate the drug release from microbially triggered colon-specific drug delivery systems. Their advantages and limitations are also stated in the Table 2-2.

The only dissolution medium used so far, that has been found to be the closest to meet these criteria is the one that uses colonic contents of rodents and thus, has been employed most frequently by the researchers. However, even this media suffers from a number of limitations like lack of reproducibility, cumbersomeness of procedure and most importantly, high cost and sacrifice of laboratory animals (1). The dissolution method using human faecal slurries is the next best option.

Probiotics are the products containing live micro-organisms that confer wide health advantage in human beings. There have been a few reports where the probiotic culture has been shown to mimic the colonic milieu in terms of the presence of polysaccharide metabolizing bacteria leading to perpetual generation of the relevant enzymes. *Lactobacilli* culture has been shown to release drug from soy-polysaccharide/ethyl cellulose films meant for colon targeted delivery (134, 137). Various strains of *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* have been demonstrated to metabolize polysaccharides like guar gum, pectin and amylose etc. (138-140).

Table 2-2: Dissolution methods used for the evaluation of polysaccharide-based colon targeted drug delivery systems.

S. No.	Polysaccharide used in formulation	Components used in dissolution medium	Drug	References	Advantages	Limitations
			Dissolution media employing enzymes			
1.	Guar gum	Galactomannanase	Dexamethasone, budesonide	(24)	1. Simple and convenient.	The method is not predictive of the *in vivo* performance of the delivery system because:
2.	Guar gum	Galactomannanase	Indomethacin	(110)	2. Higher reproducibility of results.	1. Non-predictive for *in vivo* performance of formulation.
3.	Amylose and ethyl cellulose	Amylases	5-Amino salicylic acid	(111)	3. Comparatively less expensive.	2. Non-replenishment of enzyme *in vitro* due to absence of microbiota.
4.	Chondroitin and chitosan	Chondroitinase	Bovine Serum Albumin (BSA)	(112)	4. No involvement of sacrifice of animals.	3. Lack of universality of enzymes for polysaccharide to degrade the coat. They are specific to specific polysaccharide.
5.	Pectin	Pectinase	Theophylline	(103)	5. Can be used as quality control tool. But its use is limited to specific polysaccharide.	4. In *in vivo*, there are many enzymes to degrade the coating of polysaccharide present in formulation but during *in vitro* dissolution studies, single enzyme is used.
6.	Pectin	Pectinase	5-Amino salicylic acid	(113)		
7.	Pectin–Eudragit® complex	Pectinolytic enzymes	Theophylline	(114)		
8.	Combination of cellulosic/acrylic/ calcium pectinate	Pectinolytic enzymes	Theophylline	(115)		
9.	Lactulose–Eudragit® combination	ß-Glucose-oxidase	Mebeverine	(116)		
10.	Combination of Chitosan–Eudragit® S100	Extracellular enzymes	Satranidazole	(117)		

S. No.	Polysaccharide used in formulation	Components used in dissolution medium	Drug	References	Advantages	Limitations
11.	Combination of guar gum–Eudragit®	β-Mannanase	Lansoprazole	(118)		
			Dissolution media employing rat caecal contents			
12.	Pectin	2	5-Amino salicylic acid	(112)	1. Highly significant to be used as biorelevant media. 2. Universal applicable to polysaccharide based delivery systems.	Good to predict *in vivo* performance of drug but fails as routine quality control tool due to requirement of larger number of animals to be sacrificed daily.
13.	Calcium pectinate	1.25	Indomethacin	(119)		
14.	Guar gum	4	Indomethacin	(120)		
15.	Guar gum	2 and 4	Satranidazole	(122)		
16.	Guar gum	2 and 4	Ornidazole	(99, 123)		
17.	Guar gum–xanthan gum mixture	2	5-Fluorouracil	(117)		
18.	Guar gum	4	Albendazole	(54, 124)		
19.	Chitosan/guar gum/inulin	4	Diltiazem	(125)		
20.	Dextran	4	Budesonide	(126)		
21.	Guar gum	4	Quercetin	(127)		

S. No.	Polysaccharide used in formulation	Components used in dissolution medium	Drug	References	Advantages	Limitations
22.	Pectin–ethyl cellulose combination	4	5-Fluorouracil	(128)		
23.	Chitosan	2	5-Fluorouracil	(129)		
			Dissolution media employing human faecal slurries			
24.	Dextrin	4	Ibuprofen	(130)	1. Highly significant to be used as biorelevant media. 2. Universal applicable to polysaccharide based delivery systems.	1. Variation in results between batches. 2. Limitation of having control on volunteer. 3. Inter subject variability in microflora. 4. The collection and application of faecal slurries is expensive process.
25.	Amylose/ethyl cellulose	10	5-Aminosalisylic acid	(131, 133, 141)		
26.	Nutriose	5	5-Aminosalisylic acid	(134, 135, 142)		

2.3.Colonic Microflora, Prebiotics, Probiotics and Synbiotics

2.3.1. Colonic Microflora

Colon is a sterile organ at birth, but it provides ideal conditions for bacterial growth, i.e., anaerobic, warm and moist atmosphere. Thus, rapid bacterial growth occurs after birth. There are reports that approximately 500 species of bacteria inhabit the colon. These play important role in maintaining human health. Major population of the colonic micro-organisms comprises of bacteria, though some fungi and protozoa do exist in colon (143, 144). The number of microbes is 10^{11} per gram of colonic contents as compared to 10^{0-2} in proximal small intestine (145).

The commensal bacteria play important role in maintaining the health of an individual. They prevent the predominance of pathogenic bacteria as they compete with them for space and food. They also produce certain molecules which prevent the colonization by pathogenic bacteria (146). The healthy microflora is considered to be the one, which has significant population of bifidobacteria and lactobacilli (147). These are considered to be healthy as these 2 genera do not contain any known pathogens. These bacteria mainly cause carbohydrate fermentation and produce short chain fatty acids which have positive effect on host health. On the other hand, bacteria like bacterioids and clostridia lead to protein metabolism and generate substances like ammonia, sulphides, phenols, thiols, indols, and amines, which do not promote human health (148).

2.3.2. Prebiotics

A prebiotic can be defined as ‘a non-indigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one of a limited number of bacteria in the colon and thus improves host health.’ (149). Prebiotics have gained importance as they are believed to influence the composition of colonic microflora, such that health promoting microorganisms predominate. They are also cheaper as compared to probiotics and are expected to produce similar kind of beneficial effect on health like probiotics. The examples of prebiotics are: Inulin, fructo-oligosaccharides, galacto-oligosaccharides, soya-oligosaccharides, xylo-oligosaccharides, isomalto-oligosaccharides & pyrodextrins (150).

Studies have shown that prebiotics, even when taken in small amounts in diet, promote the growth of bifidobacteria and lactobacilli. Both of these genera contain health promoting bacteria (151).

2.3.3. Probiotics

The products containing live organisms that have been shown to confer health advantage in human beings are termed probiotics. They have been shown to provide benefit in various disorders like diarrhoea, IBD and IBS (152). Many studies have shown probiotics to be useful in cancer therapy, e.g., Lactic acid bacteria cause changes in colonic microflora, boost the immune system of host and is associated with antioxidant and anti-proliferative activity. All these properties account for its beneficial effect on colon, bladder, gastric, liver and breast cancers (153).

The literature suggests that colonic bacteria reduce mucosal inflammation by decreasing the permeability of intestine (154). The microflora generate certain metabolites which increase the integrity of gut mucosa (155, 156). They also provide competition to the pathogenic species of bacteria (157).

2.3.4. Synbiotics in IBDs

There are number of studies which indicate that commensal bacteria play a role in development of IBD. When the experimental animal models of IBD are housed in germ free atmosphere, they stay healthy; but when the colonization with bacteria occurs they develop inflammation (158, 159). In UC, dysbiosis, i.e., an imbalance between harmful and beneficial bacteria occurs. Overall decrease in *Bactroides vulgates* was reported in UC (160). Another study reported the reduction in the number of bacteriodes species, *Eubacterium* species, *Lactobacillus* species and Bifidobacteria, whereas there was increase in *E.coli* and clostridia in IBD patients (161). At some point the immune tolerance to colonic microflora is broken (162) and the host activates immune reaction against the colonic bacteria. This causes inflammation of mucosa which is characteristic of UC (143).

There is evidence for the beneficial effects of probiotics in maintaining remission in patients of UC. In the controlled clinical trials, it was found that *E. coli* Nissle (1917) was similar in efficacy to mesalazine (163). The use of *E. coli* Nissle (1917) enemas

in left sided UC was found to be efficacious (164). In another study the beneficial effect of *Lactobacillus plantarum 299v* was demonstrated in UC patients (165).

An uncontrolled, open trial was conducted by researchers for a period of 12 months. In the treatment group patients were given a probiotic preparation consisting of 3 strains of bifidobacteria, 4 strains of lactobacilli and *Streptococcus salivarius* to UC patients. The results suggested that probiotic preparation helped in maintaining remission in the patients suffering from UC (166).

In another randomized clinical trial Bifidobacteria-Fermented Milk (BFM) was given as a dietary supplement to UC patients. The data reflected significant benefit in the symptoms of disease in BFM group compared with control group (167).

It is important to note that the role of probiotics in treating cancer has been explored in our previous research work wherein probiotics have been co-administered with 5-FU microspheres. The microspheres of 5-FU were prepared using guar and xanthan gum as prebiotics and co-administered with probiotics to rats. The results indicated better site-specific release of drugs in rats when the microspheres were co-administered with probiotics as compared to their administration alone. The side effects of 5-FU such as diarrhea were also reported to be minimized with this approach. However, the major limitations of this approach were less drug loading, time consuming process, poor stability on storage and difficulty to scale up (21).

2.4. Mini tablets

Mini-tablets have a diameter in the range of 1.0-3.0 mm flat or concave tablets (168). Mini-tablets, used in the form of multi-unit doses, exhibit several advantages over. First, the production process is simple and reproducible, resulting in a flexible and accurate dosage. Second, mini-tablets allow high drug loading, with a wide range of drug release modes, such that release rates can be slightly adjusted based on the need. Finally, mini-tablets help overcome some issues, such as difficulty in swallowing and reduce adverse effects of combined medications.

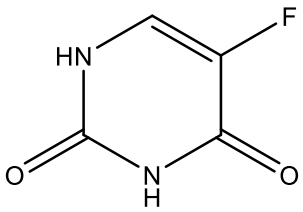
2.4.1. Advantages of mini tablets

Advantages of mini tablets are listed below:

- Single unit compressed dosage form
- Easy to administer
- Enhanced physico-mechanical stability
- Improved drug loading
- Efficient coating can be achieved
- Easy to scale up
- Less quantity of excipients is required and stable upon storage

2.5. Drug profile

2.5.1. 5-Fluorouracil (5-FU)

Parameters	Details
Chemical structure	 5-Fluoro Uracil
Molecular formula	$C_4H_3FN_2O_2$
IUPAC name	5-fluoro-1,2,3,4-tetrahydropyrimidine-2,4-dione
Molecular weight	130.08
Appearance	Crystalline white powder
Aqueous solubility and BCS class	5-6 mg/mL, BCS class III
Bioavailability	28-100 % (based upon formulation)
Melting point	283˚C
Half life	10-20 min
Lethal dose (LD) 50	230 mg/kg (Oral, mice)
Drug category	Antineoplastic agents (Antimetabolites, Pyrimidine analogues).
Pharmacological indication	For the treatment of actinic keratosis, neck and cervical cancer, and various types of gastrointestinal cancers.
Defined Daily Dose (DDD)	Not specified, as the doses used vary substantially due to wide range of types and the severity of cancers. In addition to that it is recommended to be used in combination therapies.
Marketed formulation	A. Intravenous injections (50mg/mL, 2.5 g/50mL, 5.0 g/100mL) B. Topical cream (5 mg/g) C. Topical solution (0.5 g/10mL, 1.25 g/10mL)
Common Adverse Drug Reactions (ADRs)	A. Cardiotoxicity B. Encephalopathy C. Dihydropyrimidine Dehydrogenase (DPD) deficiency

Chapter 3
Rationale of the study

Drugs meant for various colonic disorders when administered in the form of conventional dosage forms like tablets and capsules show various side effects like diarrhoea, nausea, myalgia, flatulence etc. along with many more serious side effects. At times, the side effects become so prominent that they become a limiting factor for continuation of therapy.

Therefore, an inventive approach to overcome these undesirable side-effects is available in the form of oral colon targeted drug delivery systems designed by a combination of natural as well as synthetic polymers. The natural polysaccharides coating on the core of the drug consisting of polymer which can be digested only by bacteria in the colon are supplemented with a coating of synthetic polymer that will ferry the core through the gastric as well as the upper intestinal milieu. Such dual coating is expected to provide targeting of the drug to the colon. This will lead to lower the side effects. Moreover, therapeutic effects of the drug will be enhanced as almost the entire drug will be delivered at the affected segment of bowel.

In most of the diseases of colon, the diversity as well as number of commensal microflora gets affected. The situation is further worsened by the fact that most of the drugs used for the colonic disorders alter the normal microfloral picture of colon. Both these factors when combined shift the balance of microflora from gram +ve to gram –ve (due to decreased colonization resistance) which increases the chances of secondary infections. Use of the improved oral colon targeted drug delivery system which comprises of unique combination of targeted drug delivery system, i.e., tablets comprising the drug, natural polysaccharide and synthetic polymer along with probiotics in a single, pharmaceutically acceptable dosage form will be designed where the polysaccharide used in targeting will also serve as prebiotics.

Advantages of above system include: The system serves bifunctional role, firstly, it provides targeting of drug to target site due to which side-effects like inflammation and diarrhoea are reduced or eliminated altogether, and secondly, it provides prebiotics in the form of coating polysaccharides. The dual coating with

polysaccharide and synthetic enteric coating polymer ensures the release of the drug in the colonic milieu only. Co-administration of probiotics along with the drug delivery system leads to a replenishing effect on colonic microflora which had been damaged or lost due to attack of the drug and due to disease's pathophysiological conditions.

5-FU is the most preferred drug that is being practiced to treat colon cancer. It is available in the market in the form of intravenous injection. Oral formulations of 5-FU are unavailable in market due to its major side effects such as inflammation of the mouth, appetite loss, nausea, vomiting, low blood cell counts, hair loss, and inflammation of the skin. Hence, in the present study oral colon targeted mini tablets coated with pH sensitive polymers as well as polysaccharide have been proposed that could be able to deliver the drug directly at the colonic site. The site-specific release will minimize the dose required for 5-FU to treat colon cancer as well as the associated side effects such as oral inflammation, nausea and vomiting can be minimized. The advantage of mini tablets includes, ease of administration from patient perspective and less requirement of excipients for administering low dose potent drugs (e.g., 5-FU) from industrial perspective. These tablets will be co-administered with probiotics to restore colonic microflora and site-specific release of drug.

3.1. Objectives of the study

Following are the three goals expected to be achieved through the designed formulation:

1. Colon targeting of the drug to bypass the side-effects due to less systemic bioavailability of drug.
2. Provide probiotics to replenish colonic microflora for successful release from subsequent doses.
3. To supply prebiotics in the form of guar gum for the thriving of probiotics as well as normal colonic microflora.

Chapter 4
Experimental Work

4.1. Materials

4.1.1. Chemicals

The chemicals used in the present study are given below in Table 4-1.

Table 4-1: List of chemicals along with their manufacturers.

Materials	Manufacturer
5-Fluorouracil	MOLYCHEM Pvt. Ltd., India.
Eudragit® S-100	Evonik Rohm GmbH, Pharma Polymers, Germany.
Guar gum	LOBA Chemie, Pvt. Ltd., India.
HCl	Central Drug House Pvt. Ltd., India.
Magnesium stearate	SD fine chemicals Ltd., India.
Millipore water (Prepared in-house)	Bio-Age Equipment Ltd., India.
Pectin	LOBA Chemie, Pvt. Ltd., India.
pH buffer tablets	LOBA Chemie, Pvt. Ltd., India.
Potassium dihydrogen orthophosphate buffer	LOBA Chemie, Pvt. Ltd., India.
Probiotics	BIOMIX-I, Unique Biotech, India.
Sodium hydroxide	LOBA Chemie, Pvt. Ltd., India.
Talc	Qualikems Fine Chem Pvt. Ltd., India.
Triethanolamine	Central Drug House Pvt. Ltd., India.

4.1.2. Equipment and Machines

Equipment and machines used in the presented study are listed in Table 4-2.

Table 4-2: List of equipment and machines along with their manufacturers.

Equipment & Machines	Model and manufacturer
Centrifugation machine	LALCO 230/02, D.P. Lab Instruments, India.
Dissolution rate test apparatus	DS 800 (Manual) LABINDIA, India.
Disintegration rate test apparatus	DT 1000, LABINDIA, India.
Electronic Weighing balance	AX 200, Shimadzu, Japan.
Hot air oven	LT-90, Labtherm India, India.
HPLC	LC-20AD Shimadzu Co. Ltd., Japan.
IR-spectrophotometer	8400 S, Shimadzu Co. Ltd., Japan.
Magnetic stirrer	REMI 2 MLH, Rajendra Electronics Ltd., India.
Micropipette	Perfitt, India.
Millipore filter	0.45 µm, Millipore, Germany.
Monsanto hardness tester	MHT-20, Campbell Electronics, India.
Pan Coater	Navyug India, India.

Equipment & Machines	**Model and manufacturer**
pH meter	Phan, LABINDIA, India.
Roche type friabilator	LABINDIA, India.
Sieves	Sieve No. 20, Bhushan Engineering and Scientific Traders, India.
Stability chamber	CHM 10S, REMI, India.
Syringe filter (0.22µm)	Millipore, Germany.
Multi punch tablet compression machine	Trover, Pharmamec, India.
Tissue homogenizer	REMI, Rajendra Electronics Ltd., India.
Ultrasonication Bath	ATS-02, Athena technology, India.
UV-visible spectrophotometer	UV-1800, Shimadzu, Japan.
Vacuum filtration pump	Millipore, India.
Vernier Calliper	LABINDIA, India.

4.2. Methods

4.2.1. Analytical method development using RP-HPLC

4.2.1.1. Determination of absorbance maxima of 5-Flurouracil (5-FU)

The absorbance maxima of 5-FU was determined by using Ultra Violet (UV)-visible double beam spectrophotometer (UV-1800, Shimadzu, Japan). 5-FU (10 mg) was weighed accurately using analytical weighing balance (AX 200, Shimadzu, Japan) and transferred to a clean and dried volumetric flask of 10 mL containing 5 mL of triple distilled water. The volumetric flask was sonicated for 10 min using ultrasonication bath (ATS-02, Athena technology, India). The volume of the prepared solution was made up to 10 mL using triple distilled water. The above prepared solution was analyzed by UV-visible spectrophotometer to determine the absorbance maxima of 5-FU.

4.2.1.2. Estimation of 5-FU by RP-HPLC

The RP-HPLC system consisted of a mobile phase delivery pump (LC-20 AD; Shimadzu, Japan), a photodiode array detector (SPDM20A; Shimadzu, Japan), a 20 µL loop fitted with manual Rheodyne injector and LC Solution software. A C-18 reverse-phase column (Nucleodur C18, 250 mm × 4.6 mm i.d., 5µ) was utilized as stationary phase for the estimation of 5-FU, using 0.05 M potassium dihydrogen orthophosphate buffer containing 0.1 % Triethanolamine (TEA), as mobile phase. The flow rate was 1.2 mL min^{-1} and detection wavelength was 266 nm. Standard dilutions (2, 4, 6, 8 and 10 µg/mL) were prepared in mobile phase and analyzed. Isocratic elution was carried out.

Preparation of potassium dihydrogen orthophosphate buffer (0.05 M): Potassium dihydrogen orthophosphate buffer solution was prepared as per the procedure reported by Ciccoline, *et al.,* 2004.

a) Potassium dihydrogen orthophosphate (3.4 g) was dissolved in volumetric flak of 500 mL.

b) TEA (0.5 mL) was added to the above prepared solution and the final volume was made up to 500 mL by using triple distilled water.

c) The pH of the above prepared buffer solution was identified to be 6.8.

d) The above prepared solution was filtered through 0.45 µm nylon filter and sonicated for 10 min.

4.2.1.3. Preparation of calibration curve of 5-FU by using RP-HPLC

4.2.1.3.1. Preparation of standard working solutions of 5-FU

5-FU (10 mg) was accurately weighed and transferred to a standard volumetric flask of 10 mL, containing 5 mL of triple distilled water. The solution was sonicated for 10 min using the ultrasonication bath (ATS-02, Athena technology, India). The final volume of the solution was made up to 10 mL using triple distilled water (solution A) in order to get the strength of 1000 µg/mL. The sample was further diluted 100 times using triple distilled water to obtain a solution of 10 µg/mL concentration (solution B).

4.2.1.3.2. Development of calibration curve

In order to prepare the calibration curve of 5-FU, aliquots (2, 4, 6, 8 and 10 mL) were withdrawn from the solution B and diluted up to 10 mL using triple distilled water to achieve the concentration of 2, 4, 6, 8 and 10 µg/mL. The linearity of the method was assessed by analyzing the above prepared standard dilutions of 5-FU. About 25 µL of each of the 5 working standard solutions was injected to the RP-HPLC. The calibration curve was developed by plotting the graph between mean peak areas versus concentration of standard solutions. The study was carried out 5 times and mean data was recorded at a wavelength of 266 nm.

4.2.1.4. Validation of analytical method

The developed analytical method was validated as per International Council for Harmonization (ICH) Q2 (R1) guideline (169). The performance of system was checked by the measurement of system suitability parameters. To check the system suitability, 6 replicate injections of standard solutions (6 µg/mL) of 5-FU were injected to HPLC. The Height Equivalent to Theoretical Plate (HETP), theoretical plate, theoretical plate/meter, tailing factor and peak purity index were also measured.

4.2.1.4.1. Linearity and range

The calibration curve was developed by plotting the graph between mean peak area of 5 replicates versus corresponding concentrations of 5-FU, and the regression equation was recorded.

4.2.1.4.2. Accuracy

The accuracy of the developed method was assessed through calculation of absolute recovery of the drugs from the mobile phase. The dilutions were made at 3 different levels, i.e., lower quantified concentration [LQC (80 %)], medium quantified concentration [MQC (100 %)], and high quantified concentration [HQC (120 %)] of method concentration (i.e., 6 µg/mL). To carry out this, suitable aliquots of 4.8, 6, and 7.2 mL aliquots were withdrawn from 10 µg/mL of standard stock solution and transferred individually into 10 mL standard volumetric flask. These were further diluted by triple distilled water to achieve the LQC, MQC and HQC. Percentage absolute recovery was calculated by dividing the actual recovery of drug to their theoretical concentration and multiplying them by hundred (Eq.1). The study was carried out 5 times and mean data were recorded.

$$Absolute\ \%\ recover = \frac{Actual\ concentration\ recovered}{Theoritical\ concentration} X\ 100 \ldots\ldots\ldots\ldots\ldots Eq.1$$

4.2.1.4.3. Precision

Precision studies were carried out in terms of repeatability and intermediate precision. Repeatability was tested by injecting 5 times the samples of LQC, MQC, and HQC on the same day and under same experimental conditions. The intermediate precision was evaluated by determining LQC, MQC, and HQC samples 5 times on each of 3 different days (inter-day) as well as by the 3 different analysts (inter-analyst) under the same experimental conditions. The mean data were recorded, and percentage relative standard deviation (% RSD) was calculated. The method was considered as precise if the obtained % RSD was found to be less than 2 %.

4.2.1.4.4. Robustness

Robustness is generally carried out to check the effect of small changes on the precision of developed method. The study was carried out by varying the flow rate of

mobile phase (0.8, 1.0, and 1.2 mL/min) and absorbance maxima of 5-FU (264, 266 and 268 nm). Five replicates of method concentration (6 µg/mL) were injected and their effect on variation in the area of obtained peak, recovery (%), and retention time was observed. The mean and percentage relative standard deviation were calculated.

4.2.1.4.5. Estimation of LOD and LOQ

LOD and LOQ were determined by standard deviation of response (sigma) and slope of calibration curve (S). Standard deviation of Y intercepts of regression line was used as standard deviation. Eq. 2 and 3 for LOD and LOQ, respectively, are as follow:

$$LOD = \frac{3.3\,\sigma}{S} \ldots\ldots\ldots\ldots\ldots Eq.2$$

$$LOD = \frac{10\,\sigma}{S} \ldots\ldots\ldots\ldots\ldots Eq.3$$

4.3. Preparation of mini tablets of 5-FU

4.3.1. Selection of polymers

For the preparation of mini tablets of 5-FU, the various colon targeted polymers were selected based upon the reports of scientific literature (3, 21, 54). The selected polymers for the preparation of mini tablets of 5-FU are given in Table 4-3.

Table 4-3: Selected excipients and their role in the preparation of mini tablet.

S. No.	Polymer	Role
1.	Eudragit® S100	Core preparation and coating of core
2.	Guar gum	Core preparation and coating of core
3.	Pectin	Core preparation and coating of core
5.	Magnesium stearate	Core preparation
6.	Talc	Core preparation

4.3.2. Preformulation studies

Preformulation studies of the 5-FU and the excipients were carried out as per the standard procedure reported in the literature (170). Initially the drug and excipients mixtures were subjected for drug excipient compatibility studies. After the confirmation of no physicochemical interaction between the drug and selected excipients, various pre-formulation parameters such as visual observation for color,

odor, taste, bulk density, tapped density, flow property by an angle of repose, Carr's (compressibility) index and Hauser's ratio were checked. The methods used for the same are reported below.

4.3.2.1. Drug-excipient compatibility studies

All the excipients were mixed individually with 5-FU in 1:1 ratio and packed in clean and dry petri plates covered with perforated aluminum foil. The petri plates were stored in humidity-controlled cabinet that was set at a temperature of 37 ± 0.5ºC and a relative humidity of 65 ± 5 % for a time period of 14 days. The design protocol of the study and coding given to the API and excipients are given in Table 4-4 and Table 4-5. Physical evaluation for samples was done by visual observation on day 0, day 7th and day 14th for any possible physical interaction. In addition to this, the infra-red spectrums of all the 12 samples mentioned in Table 4-5 were recorded. Selected excipients individually and in combination with the drug (as reported in section 4.3.1) were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) model 8400S of Shimadzu, Japan on day 0 and day 14th of the study for any possible chemical interaction. The samples was placed on the samples stage and covered with the sample holder. The infrared spectrum was recorded in the wave number range of 4000-400 cm^{-1} using FTIR spectrophotometer and the characteristic bands were recorded (171).

Table 4-4: Coding of API and excipients.

Sr. No.	Compound	Code
1.	5-Fluorouracil	D
2.	Eudragit® S100	E1
3.	Guar gum	E2
4.	Pectin	E3
5.	Magnesium stearate	E4
6.	Talc	E5

Table 4-5: Protocol of drug-excipient compatibility study.

Sr. No.	Code	Retest time					
		0^{th} day		7^{th} day		14^{th} day	
		Visual	**IR**	**Visual**	**IR**	**Visual**	**IR**
1.	D	√	√	√	X	√	√
2.	E1	√	√	√	X	√	√
3.	E2	√	√	√	X	√	√
4.	E3	√	√	√	X	√	√
5.	E4	√	√	√	X	√	√
6.	E5	√	√	√	X	√	√
7.	D+E1	√	√	√	X	√	√
8.	D+E2	√	√	√	X	√	√
9.	D+E3	√	√	√	X	√	√
10.	D+E4	√	√	√	X	√	√
11.	D+E5	√	√	√	X	√	√
12.	D+E1-5	√	√	√	X	√	√

4.3.2.2. Characterization of physical mixture of drug and excipients

4.3.2.2.1. Angle of repose

The angle of repose of prepared blend of formulations was measured using fixed funnel and standing cone method (172). A funnel was fixed with its tip 7 cm above a plain paper placed on a flat horizontal surface. The orifice of the funnel was sealed with thumb and the blend was transferred into the funnel. Then the powder was allowed to flow through the funnel and the mean diameter of the powder pile was measured. The tangent angle (α) was calculated using formula given in Eq. 4.

$$tan\ \alpha = \frac{2h}{D} \dots\dots\dots\dots\dots Eq.4$$

Where, "h" is the height of pile of the powder and "D" is the diameter of the base of pile of powder.

4.3.2.2.2. Bulk density (ρb)

Bulk density was determined by transferring the powder mixture into a measuring cylinder and gently tapping it to adjust the upper surface of powder (172). The volume occupied by the powder was recorded as "Vb". The weight of powder was measured and recorded as "M". Bulk density was calculated by using formula given in Eq. 5.

$$\rho b = \frac{M}{Vb} \dots\dots\dots\dots\dots Eq.5$$

4.3.2.2.3. Tapped density (ρt)

The tapped density of the powder blend was determined by transferring the powder mixture into a measuring cylinder and then the measuring cylinder was tapped 100 times (172). The volume obtained was recorded as "Vt". The weight of powder was measured and recorded as "M". Tapped density was calculated by using the formula given in Eq. 6.

$$\rho t = \frac{M}{Vt} \dots\dots\dots\dots\dots Eq.6$$

4.3.2.2.4. Compressibility Index (CI)

CI was calculated by using formula given in Eq. 7.

$$C.I. = \frac{Bulk\ density - Tapped\ density}{Bulk\ density} X\ 100 \ldots\ldots\ldots\ldots\ldots Eq.7$$

4.3.2.2.5. Hausner's ratio (HR)

Hausner's ratio was determined by using formula given in Eq. 8.

$$H.R. = \frac{Bulk\ volume}{Tapped\ volume} \ldots\ldots\ldots\ldots\ldots Eq.8$$

4.3.3. Compression of mini tablets of 5-FU

5-FU mini tablets were prepared by using direct compression method. Each of the ingredients mentioned in Table 4-6 (as per the individual batch formula) was weighed accurately using electronic weighing balance (AX 200, Shimadzu, Japan) and mixed homogenously using 'V' cone blender. The uniformly mixed batches of powder blend were compressed using multi-punch compression machine (Trover, Pharmamec, India) fitted with a tablet punch of 4 mm diameter. Ten different batches of mini tablets (B1-B10) were prepared by varying the formula composition. The unit formula composition of the batches of mini tablets is given in Table 4-6.

Table 4-6: Batch formula composition of mini tablets.

S. No.	Ingredients	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
						Weight (g)					
1.	5-FU	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.	Eudragit® S100	1.50	1.65	1.60	1.60	1.55	1.40	1.30	1.20	1.10	1.00
3.	Guar gum	1.20	1.05	1.10	1.00	1.00	1.00	1.15	1.25	1.30	1.35
4.	Pectin	1.00	1.00	1.00	1.10	1.20	1.30	1.25	1.25	1.30	1.35
5.	Mg stearate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
6.	Talc	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Note: Each batch formula is calculated based upon the requirement of a number of 100 mini tablets.

4.3.4. Characterization of prepared mini tablets of 5-FU

All the compressed mini tablet batches (B1-B10) were subjected to various parameters post compression evaluation. These include assay, uniformity of weight and content, friability and hardness testing, tablet diameter and thickness.

4.3.4.1. Content uniformity

Ten mini tablets were selected randomly from each of the prepared batches individually to perform the content uniformity test. The tablets were crushed individually using mortar pestle and the powder was dissolved in 100 mL of 0.1 M potassium dihydrogen orthophosphate buffer of pH 6.8 in the volumetric flasks of 100 mL capacity. The flasks were subjected for sonication until the crushed powder was completely dissolved. All the dispersions were filtered through 0.45 μm membrane filter (Millipore, Germany) to separate the insoluble matter and the drug content was estimated by using RP-HPLC (LC20 AD, Shimadzu, Japan). The study was carried out in triplicate and the mean response of each sample was recorded (173).

4.3.4.2. Uniformity of weight

Twenty mini tablets were selected randomly from each of the prepared batches to perform the uniformity of weight. These were weighed on an electronic weighing balance and the mean weight was calculated. Percentage weight variation was calculated and recorded (173).

4.3.4.3. Friability testing

Ten pre-weighed mini tablets were selected randomly from each of the prepared batches individually and placed in the drum of the friabilator (Roche Friabilator). The friabilator was rotated at 25 rpm for 4 min. After the completion of stipulated time, the mini tablets were subjected to dusting by a microfiber cloth and the final weight of the mini tablets was measured and recorded. The percentage loss in weight was calculated and taken as a measure of friability. The experiment was carried out in triplicate and mean data was recorded.

4.3.4.4. Hardness testing

The average breaking strength of mini tablets were determined by Monsanto hardness tester. Ten mini tablets were selected randomly and their strength was tested. The mean response of hardness was recorded.

4.3.4.5. Diameter and thickness

Average diameter and thickness of the prepared mini tables were measured using Vernier Caliper. The process was performed in triplicate and the mean diameter and thickness of the mini tablets were recorded.

4.3.4.6. Disintegration test of mini tablets

One tablet in each of the 6 tubes of disintegration rate test apparatus was placed and the disk was placed on each of the tablet. Simulated gastric medium maintained at a temperature of 37 ± 2˚C was used to carry out the study. The instrument was operated at the standard range of cycles (30-31 cycle/min). The study was carried out in triplicate (174).

4.3.4.7. Assay of mini tablets

Twenty mini tablets from the prepared batch were weighed and crushed using mortar pestle. An equivalent amount to 1 tablet was weighed accurately and transferred in 250 mL of 0.1 M potassium dihydrogen orthophosphate buffer of pH 6.8. The solution was stored for 5 h with intermittent shaking. These were then subjected to dispersion by using magnetic stirrer at 500 rpm in order to break the swollen polysaccharide matrix. The resultant dispersion was filtered through 0.45 μm membrane filter to separate the insoluble matter. Suitable dilutions were prepared to bring the concentration of the filtered solution within the range of calibration plot. The 5-FU content in the samples was estimated by analyzing the filtrate using HPLC at the wavelength 266 nm. The percentage drug loading was calculated according to the formula given in Eq. 9.

$$\%\ Drug\ Loading = \frac{Sample\ area\ x\ Standard\ dilution}{Standard\ area\ x\ sample\ dilution}\ x\ 100 \ldots\ldots\ldots\ldots\ldots Eq.\ 9$$

4.3.4.8. *In vitro* dissolution study of mini tablets of 5-FU

In vitro dissolution study of the prepared mini tablets was carried out according to the procedure reported by Singh, *et al.*, 2015 (175). Gradient pH dissolution method was used to evaluate the drug release behavior from prepared mini tablets of 5-FU. USP dissolution apparatus II (paddle type) was used at a rotation speed of 50 ± 4 rpm and a temperature of 37 ± 0.5 ºC. One mini tablet containing 5-FU was added to each of the 6 dissolution vessels containing dissolution media. For the first 2 h the dissolution study of the 5-FU tablets was carried out in 200 mL of 0.1 N HCl buffer (pH 1.2). Then the volume of dissolution media was made up to 900 mL using phosphate buffer to maintain a pH of 6.8. The study was continued in the media for next 3 h. An aliquot of 5 mL was withdrawn from each of the dissolution vessels at 0.5, 1, 2, 3, 4 and 5 h respectively. The withdrawn medium was replaced by using fresh medium to maintain sink condition. The withdrawn samples were filtered using syringe filter (0.45 µm). The samples were analyzed at 266 nm by using RP-HPLC. All studies were performed in hexaplicate and the mean of response was recorded (78, 175). This study was repeated for all the prepared batches (B1-B10).

4.4. Coating of mini tablets of 5-FU

Since batch B10 has shown the least drug release profile in first 5 h, it was selected for coating.

4.4.1. Preparation of coating solution

4.4.1.1. Eudragit® S100 solution

The coating solution of Eudragit® S100 was prepared by dispersing the required amount of Eudragit® S100 in solvent system prepared by using 1:1 v/v ratio of acetone and isopropyl alcohol in an iodine flask. The dispersion was subjected for overnight stirring on a magnetic stirrer at room temperature to obtain a clear solution of Eudragit® S100. The final solution was utilized for the coating on mini tablets.

4.4.1.2. Guar gum solution (1 % w/v)

The coating solution of guar gum was prepared by dissolving the 1.0 g guar gum in 100 mL of distilled water. The dispersion was kept overnight for swelling of guar gum. The swollen dispersion was subjected to homogenization using a tissue homogenizer. The homogenized solution was filtered to remove any undissolved traces using muslin cloth and the final obtained solution was used for coating of mini tablets.

4.4.1.3. Pectin (1 % w/v)

The coating solution of pectin was prepared by dissolving the 1.0 g pectin in 100 mL of distilled water. The dispersion was kept overnight for swelling of guar gum. The swollen dispersion was subjected to homogenization using a tissue homogenizer. The finally obtained solution was used for coating of mini tablets.

4.4.2. Selection of coating ratios for the coating of mini tablets

All the 3 coating solutions viz. Eudragit® S100, guar gum and pectin were utilized in varying ratio to coat the tablets in order to achieve site specific release (Table 4-7) using ternary phase diagram using. Triplot software was used to prepare ternary phase diagram. A total of 27 coating ratios were decided for the coating of mini tablets to retain the drug release. The coating ratios of polymers were adjusted between 1:10 to 10:1. The internal ratio of pectin and guar gum was varied in 3 ratios, i.e., 1:1, 1:2 and 2:1. Less than 10 % drug release in 5 h was kept as key parameter to optimize the formulation. All the tablets were coated using a pan coater. The temperature of dryer was maintained at 60℃ and the speed of coater was maintained at 50 rpm.

Table 4-7: Coating ratios selected for the coating of mini tablets (B10).

Batch No.	% w/w coating ratios and coating material			
	Eudragit® S100	**Pectin: Guar gum**	**Pectin: Guar gum**	**Pectin: Guar gum**
C1-C3	01	4.5:4.5 (C1)	03:06 (C2)	06:03 (C3)
C4-C6	02	04:04 (C4)	2.7:5.3 (C5)	5.3:2.7 (C6)
C7-C9	03	3.5:3.5 (C7)	2.3:4.7 (C8)	4.7:2.3 (C9)
C10-C12	04	03:03 (C10)	02:04 (C11)	04:02 (C12)
C13-C15	05	2.5:2.5 (C13)	1.7:3.3 (C14)	3.3:1.7 (C15)
C16-C18	06	02:02 (C16)	1.3:2.7 (C17)	2.7:1.3 (C18)
C19-C21	07	1.5:1.5 (C19)	01:02 (C20)	02:01 (C21)
C22-C24	08	01:01 (C22)	0.7:1.3 (C23)	1.3:0.7 (C24)
C25-C27	09	0.5:0.5 (C25)	0.7:0.3 (C26)	0.3:0.7 (C27)

4.4.3. Dissolution study of coated batches of mini tablets

For the evaluation of the dissolution behavior of the prepared formulation in the fecal matter of rats, rat caecal contents were used. Sprague Dawley rats of either sex, weighing 250-300 g were procured from NIPER, Mohali, India. Approval was received from the Institutional Animal Ethics Committee (IAEC Protocol Number: LPU/IAEC/2019/48). All the animals were kept in standard laboratory conditions at 25 ± 2°C. Light and dark cycles of 12 h were maintained. Animals were fed on rat chow diet and water was provided *ad libitum*. For acclimatization of the animals, they were kept in the laboratory for 10 days before starting the study.

The dissolution studies for all the tablets were carried out for initial 5 h as mentioned in section 4.3.4.8. Twenty min prior to the end of the 5th hour, 3 rats were sacrificed and their caecal contents were isolated by incision of the abdomen. The caecal contents were accurately weighed and 15 g of the content was transferred into 300 mL of phosphate buffer of pH 6.8. It is important to note that 300 mL of the phosphate buffer used for the making of 5 % w/v rat caecal slurry was bubbled with CO_2 for 15 min before the addition of caecal content. To each of the 3 dissolution vessels, 100 mL of caecal slurry (4.5 % w/v) was added and the pH of the medium was adjusted to 7.4 at the end of

5th hour of the study. Final concentration of caecal contents present in dissolution medium (1000 mL) thus became 0.45 % w/v. The dissolution study thereafter was carried out up to 24 h under constant supply of CO_2 in order to mimic the anaerobic conditions prevailing inside the colon. Five mL samples were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 24 h respectively from the dissolution medium and were filtered using membrane filter of 0.45 µm. The samples prepared were then analysed at 266 nm by using HPLC. It is pertinent to add that the withdrawn volume of 5 mL was replaced with fresh 5 mL of medium for maintaining the sink condition. The study was performed in triplicate and mean data was recorded (74, 175).

4.4.3.1. Dissolution studies of optimized batch of mini tablets

4.4.3.1.1. Dissolution testing of mini tablet in probiotic based culture medium

4.4.3.1.1.1. Development of probiotic culture-based dissolution method

4.4.3.1.1.2. Preparation of probiotic based culture medium

In order to evaluate the *in vitro* dissolution testing for polysaccharide-based colon specific drug delivery, probiotic based fluid thioglycolate medium was prepared. The composition of probiotic (BIOMIX-I, Unique Biotech, Hyderabad, India) used in the present study is shown in Table 4-8.

Table 4-8: Composition of 325 mg of probiotic blend.

S.No.	Composition	Bacterial Count (billion cfu)
1	*Lactobacillus acidophilus*	0.75
2	*Lactobacillus rhamnosus*	0.75
3	*Bifidobacterium longum*	0.75
4	*Bifidobacterium bifidum*	0.50
5	*Saccharomyces boulardii*	0.10
6	Fructo Oligo Saccharide	100 mg

Fluid Thioglycolate Medium (FTM) was prepared according to the formula specified in the FDA Bacteriological Analytical Manual (BAM) (176) that conforms to harmonized with United States Pharmacopoeia (USP) requirement (177). The composition of FTM is

shown in Table 4-8. Accurately weighed 8.94 g of the medium was dispersed in 300 mL of purified water and heated with frequent agitation to completely dissolve the medium. The prepared medium was sterilized by autoclaving at 15 lbs. pressure at 121°C for 15 min, cooled and stored in dark place below 25°C. Media were inoculated by adding 325 mg of probiotic blend (BIOMIX-I) and incubated for 48 h in an anaerobic glove box at 35°C.

4.4.3.1.1.3. Dissolution studies using probiotic culture

The dissolution studies of mini tablets of batch B10C10 were carried out for initial 5 h as mentioned in section 4.3.4.8. At the end of the 5th hour, pH was adjusted to 7.4 and the media was purged with CO_2 for 10 min to remove undissolved oxygen and to maintain anaerobic conditions inside the medium. Then 100 mL of probiotic culture in FTM was added and the final volume was made up to 1000 mL in each flask. The study was continued up to 24 h, under continuous purging of CO_2. Five mL samples were withdrawn at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 24 h respectively from the dissolution medium. The removed medium was replaced by the fresh one. The withdrawn samples were filtered using 0.45 µm membrane filters and suitable dilutions were made. The diluted solutions were injected in HPLC and analyzed at 266 nm. All the studies were performed in triplicate and the mean data were recorded. For the negative control, the dissolution study was conducted in a manner exactly as reported above except for the addition of FTM.

4.4.3.1.2. *In vitro* drug release using 5.0 % w/v human fecal slurries

The dissolution studies for the mini tablets of batch B10C10 were carried out for initial 5 h as mentioned in section 4.3.4.8. At the end of 5th hour, the media was purged with CO_2 for 10 min to remove undissolved oxygen and to maintain anaerobic conditions. Then 5 % w/v of freshly prepared fecal slurries were added to the dissolution medium, pH of the dissolution medium was adjusted to 7.4 and the study was continued up to 24 h. The media was continuously purged with CO_2 throughout the studies. The final fecal content in dissolution medium (1000 mL) was 0.5 % w/v. At the end of time periods,

5.0 mL samples were withdrawn at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 24 h respectively from the dissolution medium and replaced by the fresh medium. The withdrawn samples were filtered through 0.45 µm membrane filters and suitable dilutions were made. The diluted solutions were injected in HPLC and analyzed at 266 nm. All the studies were repeated 3 times and the mean data was recorded. For the negative control, the dissolution study was conducted in a manner exactly as reported above except for the addition of fecal slurries.

4.4.3.1.3. Dissolution studies using 4.5 % w/v rat caecal contents

The protocol for dissolution study for batch B10C10 has already been discussed in section 4.4.3. For the negative control, the dissolution study was conducted in a manner exactly as reported above except for the addition of caecal slurries.

4.5. *In vitro* cell line studies

This study was conducted to evaluate the effect of co-administration of probiotic on dissolution profile of coated mini tablets. In order to evaluate this, the study was conducted in 2 different steps on Caco-2 cells.

Step 1 - The study was conducted in 6 dissolution vessels. To 3 dissolution vessels mini tablets (B10C10) were added with probiotics (500 mg) while in other 3, they were added without probiotics. The dissolution study was conducted without caecal contents till 24 h. Samples (5 mL) were withdrawn at time intervals of 1, 3, 5, 8, 10, 12, 18 and 24 h, respectively. The withdrawn medium was replaced by fresh one in order to maintain sink condition.

Step 2 - The study was conducted in 6 dissolution vessels. To the 3 dissolution vessels, mini tablets (B10C10) were added with 500 mg probiotics and other 3 vessels without probiotics. The dissolution study was conducted with caecal contents till 24 h. Samples were withdrawn at time intervals of 1, 3, 5, 8, 10, 12, 18 and 24 h, respectively. The withdrawn medium was replaced by fresh one in order to maintain sink condition.

Note: The dissolution medium with or without rat caecal contents and placebo tablets (coated similarly as B10C10) were also subjected for cell line studies at similar time intervals as control.

"MTT assay was carried out on the basis of viability of cells that release succinate dehydrogenase which reduces tetrazolium salt of MTT to produce formazan. Initially, the cell count was adjusted to 1.0×10^5 cells/mL by making suspension in Dulbecco's Modified Eagle Medium (DMEM) having 10 % Fetal Bovine Serum (FBS). Then, the suspension was further cultured by adding 100 µL of suspension in 96 well plate for 24 h. Then the cells were centrifuged and pellets were suspended in the maintenance media containing 100 µL of all the withdrawn dissolution samples separately and incubated at 37℃ for 48 h in the presence of 5 % CO_2. After 48 h, sample solutions were centrifuged and pellets were re-suspended in MEM-PR solution containing 20 µL of MTT and incubated at 37℃ in the presence of 5 % CO_2. After 2 h, 100 µL of Dimethyl Sulfoxide (DMSO) was added to dissolve the formed formazan and absorbance was noted at 540 nm (178)". The percentage viability of cells was calculated using formula given in Eq. 10.

$$\%\ cell\ viability = \frac{Mean\ OD\ of\ individual\ test\ group}{Mean\ OD\ of\ control\ group}\ X\ 100 \ldots\ldots\ldots\ldots\ldots Eq.10$$

4.6. Bioanalytical method development and pharmacokinetic studies

In order to perform the pharmacokinetic study of 5-FU in a suitable animal model, a simple, accurate and robust bioanalytical method was required to be developed using RP-HPLC.

4.6.1. Method development of 5-FU using mobile phase and rat plasma

Similar chromatographic conditions as mentioned in section 4.2.1 were used to develop bioanalytical method. However, the linearity range was developed in the range of 50-250 ng/mL in order to estimate the plasma samples.

4.6.1.1. Preparation of standard stock solution of 5-FU in mobile phase

5-FU (100 mg) was accurately weighed and transferred to 100 mL standard volumetric flask containing 20 mL of mobile phase. The solution was sonicated for 5 min and volume was adjusted using mobile phase to get a concentration of 1000 µg/mL (Solution C). Aliquot of 10 mL was withdrawn in a 100 mL standard volumetric flask and volume was adjusted to 100 mL by using mobile phase to get a concentration of 100 µg/mL (Solution D). Further, 10 mL aliquot was withdrawn in 100 mL standard volumetric flask and volume was adjusted to 100 mL by using mobile phase to get a concentration of 10 µg/mL (Solution E). From above prepared solution E, 10 mL of aliquots was withdrawn in 100 mL standard volumetric flask and volume was adjusted to 100 mL by using mobile phase to get a concentration of 1 µg/mL (1000 ng/mL) (Solution F). The overview of the prepared dilutions is shown in Figure 4-1.

4.6.1.2. Preparation of stock solution of internal standard in mobile phase

5-BU (10 mg) was accurately weighed and transferred to 100 mL volumetric flask. The solution was sonicated for 15 min and volume was adjusted to 100 mL using mobile phase to get the solution of 100 µg/mL concentration (Solution G). Aliquot of 10 mL was withdrawn in a 100 mL standard volumetric flask and volume was adjusted to 100 mL by using mobile phase to get the final solution of 10 µg/mL concentration (Solution H). The prepared dilutions are shown in Figure 4-2.

4.6.1.3. Development of calibration curve in mobile phase (nanogram level)

In order to prepare the calibration curve for 5-FU, aliquots (0.5, 1.0, 1.5, 2.0 and 2.5 mL) were withdrawn from the solution F in a 10 mL volumetric flask and then diluted up to 10 mL using mobile phase to achieve the concentration of 50, 100, 150, 200, and 250 ng/mL. The dilutions were injected to HPLC for development of calibration curve. About 25 µL of each of the 5 working standard solutions were injected into the RP-HPLC. The study was carried out 5 times and mean data was recorded at a wavelength of 266 nm.

4.6.1.4. Development of calibration curve in plasma (nanogram level)

In order to prepare the calibration curve for 5-FU, aliquots (0.5, 1.0, 1.5, 2.0 and 2.5 mL) were withdrawn from the solution F in a 10 mL volumetric flask. To this, 1 mL of solution H (5-BU, 10 µg/mL) was added and followed by the addition of 0.5 mL of rat plasma. To this mixture 1 mL acetone was added and the contents were centrifuged at 35000 *g* for 15 min. The supernatant was collected and evaporated. All the samples were reconstituted up to 10 mL using mobile phase in order to achieve the concentration of 50, 100, 150, 200, and 250 ng/mL and injected to HPLC for analysis of drug at 266 nm. The scheme for dilution is shown in Figure 4-3.

4.6.2. Method validation

The method was validated for accuracy, precision, robustness, LOD and LOQ. The validation the levels of LQC, MQC and HQC were kept as 120 ng/mL, 150 ng/mL and 180 ng/mL, respectively. The procedure for method validation was followed as described in section 4.2.1.4. The dilutions were found accurate when the percentage recovery was found between 95 to 105 % and précised when the % RSD was found between 2 as per ICH Q2 (R1) guidelines (169).

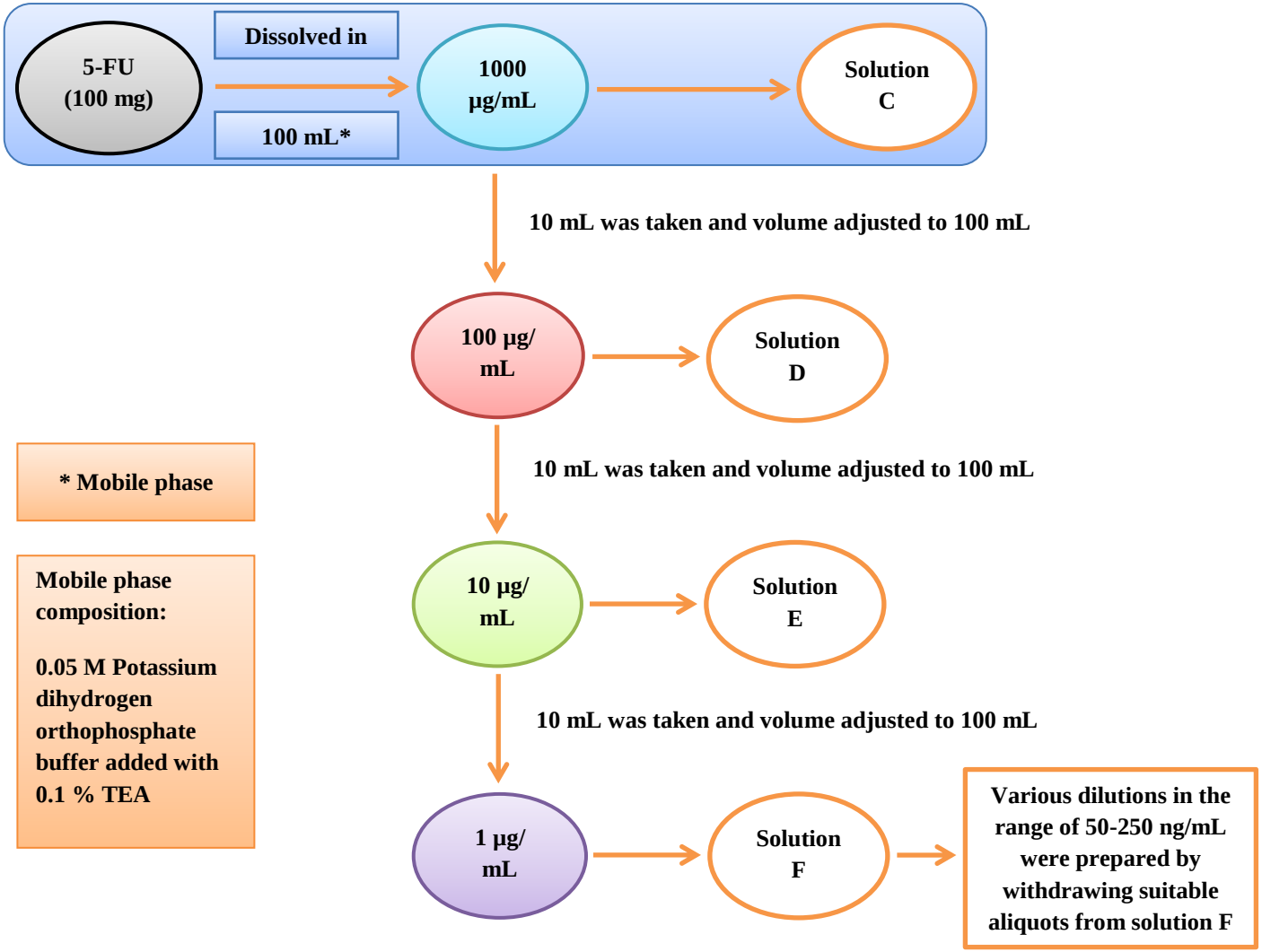


Figure 4-1: Diagrammatic representation of the dilutions prepared for 5-FU in mobile phase in the range of 50-250 ng/mL without plasma.

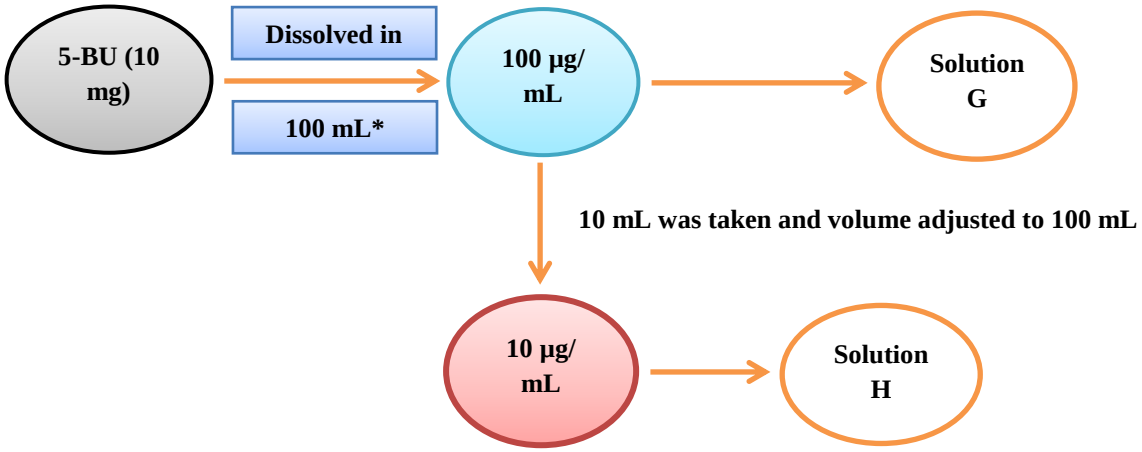


Figure 4-2: Diagrammatic representation of the dilutions prepared for 5-BU.

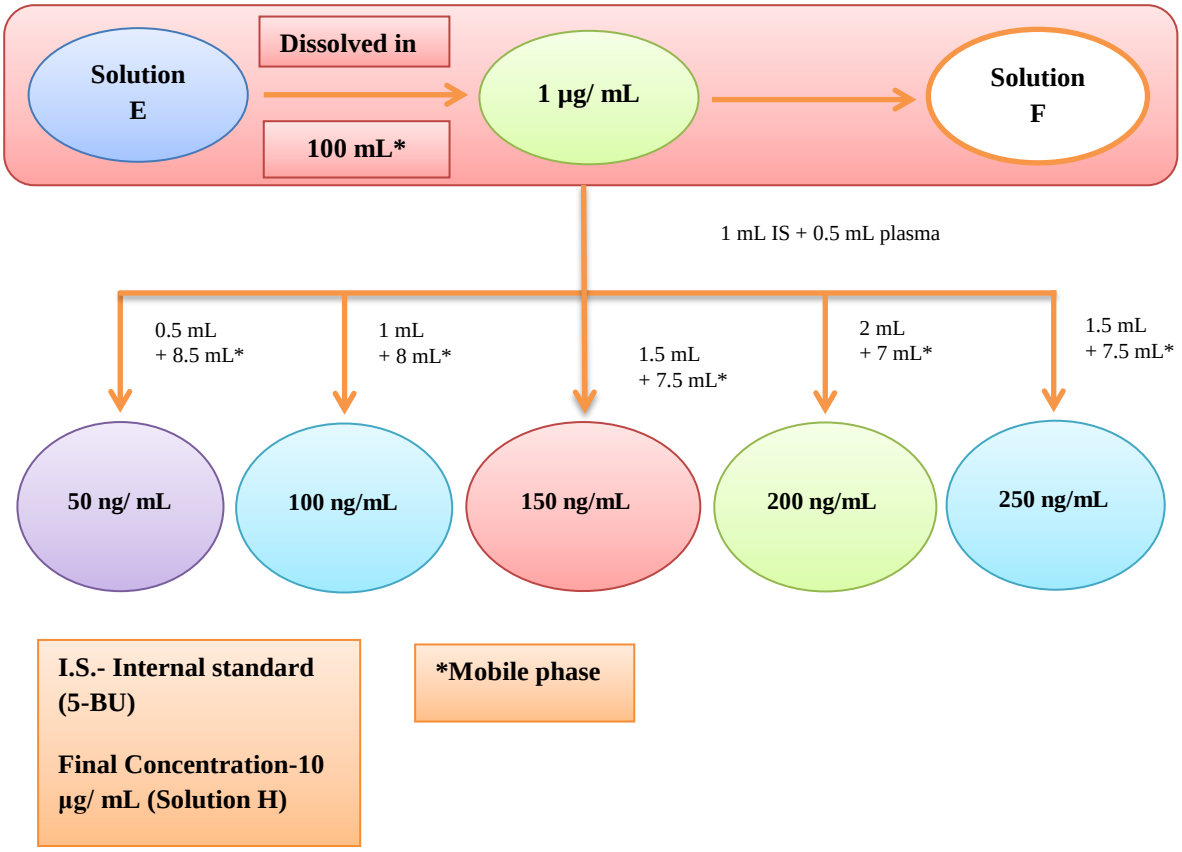


Figure 4-3: Diagrammatic representation of the dilutions prepared for 5-FU in mobile phase in the range of 50-250 ng/mL with plasma.

4.6.3. Method specificity

Method specificity was determined by injecting the following samples to HPLC using the developed method:

a. 5-FU alone
b. 5-FU + Plasma
c. 5-FU + mixture of excipients used for tablet formulation
d. 5-FU + rat caecal contents
e. 5-FU + human fecal slurries
f. 5-FU + probiotic culture

Absence of any unwanted/additional peak(s) at the retention time of 5-FU indicated about specificity of method.

4.7. Pharmacokinetic studies

The protocol number for study was LPU/IAEC/2019/48. The parallel study was carried out using 18 rats that were divided into 3 groups, each group containing 6 rats. A cross over study with Latin square design was followed, wherein each group was subjected to all the 3 different treatments with a washout period of 7 days. The animals were fastened for 8 h prior to administration of drug. The standard recommended food was provided after 3 h of completion of study in order to avoid food effect in the initial studies. The groups are given below and Latin square design is described in Table 4-9.

Treatment 1: Uncoated mini tablets of 5-FU

Treatment 2: Coated mini tablets of 5-FU with probiotics

Treatment 3: Coated mini tablets of 5-FU without probiotics

In all the cases, blood samples (0.2 mL) were collected at 0, 1, 3, 5, 7, 9, 18 and 24 h from rats of all the groups in vials containing potassium oxalate as anticoagulant. The blood samples were mixed well, centrifuged at 35000 *g* for 15 min and the plasma was transferred to 5 mL vials, capped tightly and processed as mentioned in section 4.6.1.4. The area under the curve (AUC_{0-t} and $AUC_{0-\infty}$) was calculated by using PK solver (2.0) software. The relative oral bioavailability was calculated by the formula given in Eq. 11.

$$Relative\ bioavailability\ (Fr) = \frac{(AUC)test\ \times\ Dstd}{(AUC)std\ \times\ Dtest} \quad \ldots\ldots\ldots\ldots\ldots Eq.11$$

Where, AUC – Area under the curve; D – Dose administered.

Table 4-9: Treatment schedule.

Day	Group 1	Group 2	Group 3
1	Treatment 1	Treatment 2	Treatment 3
	Washout for 7 days		
8	Treatment 2	Treatment 3	Treatment 1
16	Treatment 3	Treatment 1	Treatment 2

4.8. Stability studies

The mini tablets were kept for stability studies for 6 months at 25±0.2ºC/65±5 % relative humidity and 40±0.2ºC/75±5 % relative humidity in a stability chamber (CHM 10S, Remi, India). The results of assay and dissolution of aged tablets were evaluated and compared with freshly prepared mini tablets. The dissolution study was conducted in rat caecal contents as per the procedure mentioned in section 4.4.3. The dissolution profiles of aged and fresh samples were compared using student 't' test and model independent analysis (f2 value). The values were found to be significant if 'p' value was less than 0.05 and profiles were found similar if 'f2' value was more than 50 (179).

4.9. Statistical Analysis

The obtained results from the experiment were expressed as mean ± SD. The graphs were plotted using Graph Pad Prism version 7.0 (Graph Pad software INC., CA, USA). The results were compared using analysis of variance and student 't' test. The $P < 0.05$ (wherever, applicable) value indicated significant difference in the results.

Chapter 5
Results and Discussion

5.1. Analytical method development using RP-HPLC

5.1.1. Determination of absorbance maximum of 5-FU

The observed absorbance maximum (λmax) of 5-FU was found at 266 nm. The same λmax was utilized for further analytical method development process. The obtained UV spectrum of 5-FU is shown in Figure 5-1.

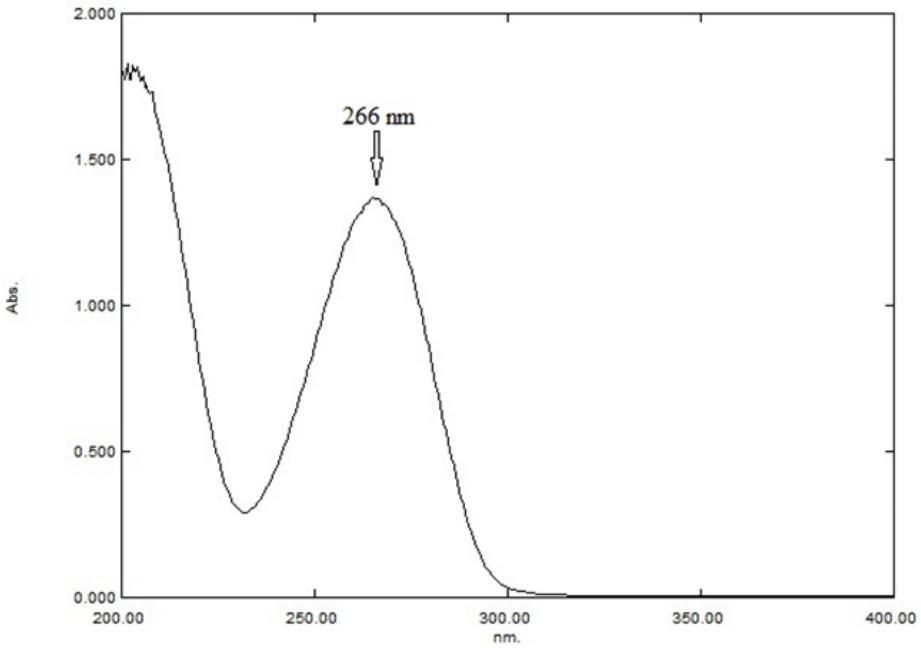


Figure 5-1: UV spectrum of 5-FU.

5.1.2. Selection of mobile phase for the estimation of 5-FU

For the estimation of 5-FU, different trials were performed by changing the composition of mobile phase such as methanol-potassium dihydrogen orthophosphate buffer (10 mM, pH 6.8) in ratios of 70:30 and 65-35 at a flow rate of 0.8 mL/min, in ratio of 65:35, 60:40 and 55:45 at a flow rate of 0.4 mL/min, potassium dihydrogen orthophosphate buffer (50 mM, pH 6.4) added with 0.1 % v/v TEA at a flow rate of 1.0 mL/min. The final chromatograms of 5-FU is shown in Figure 5-1.

Out of the performed trials, the trial conducted with the mobile phase potassium dihydrogen orthophosphate buffer (50 mM) containing 0.1 % v/v TEA showed better

results in terms of sharpness of peak and absence of fronting or tailing. The retention time obtained for 5-FU was found to be 7.636 min (Figure 5-1). This method was further validated for various parameters that are discussed in section 5.1.3.

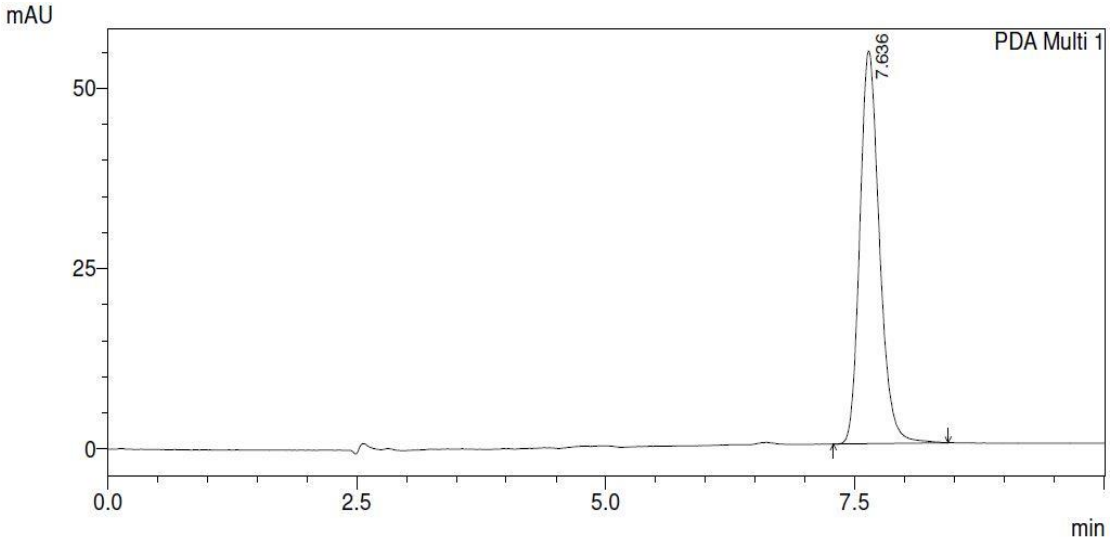


Figure 5-1: Chromatogram of 5-FU in potassium dihydrogen orthophosphate buffer (50 mM) containing 0.1 % TEA, flow rate 1.2 mL/min.

5.1.3. Validation of RP-HPLC method

5.1.3.1. Linearity and Range

The calibration curve was developed by plotting the graph between concentration and mean peak area. The curve was found to be linear in the range of 2-10 µg/mL with a determination co-efficient (r^2) of 0.999 (Figure 5-2).

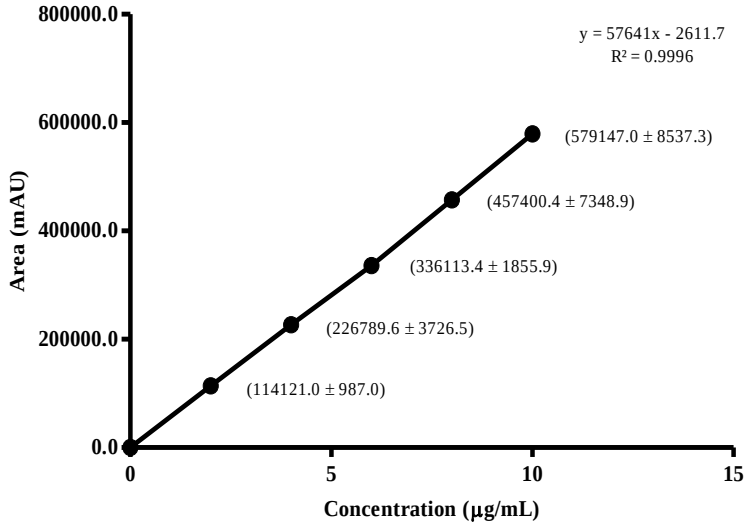


Figure 5-2: Calibration curve of 5-FU.

5.1.3.2. Accuracy

The accuracy of the developed method was assessed by determining the mean percentage recovery of the LOQ, MQC and HQC solutions in mobile phase. The data revealed that for all the 3 levels, the mean percentage recovery in mobile phase was within the fixed limits of 95-105 % (Table 5-1). The accuracy of developed method was verified by percentage relative standard deviation which was less than 2 %. The results of precision study are summarized below in Table 5-1.

Table 5-1: Results of accuracy study.

Levels	Concentration of standard solution (µg/mL)	Actual mean concentration (µg/mL)	% Recovery	SD	% RSD	Mean % recovey ± SD
LQC	4.8	4.65	96.87	0.018	0.39	97.38 ± 1.07
MQC	6	5.80	96.67	0.016	0.28	
HQC	7.2	7.10	98.61	0.017	0.25	

5.1.3.3. Precision

The precision of developed method was evaluated by calculating the percentage relative standard deviation for the 5 determinations of LQC, MQC and HQC solutions at interday, intraday and interanalyst level under the same experimental conditions. The observed percentage relative deviation was found to be less than 2 % for all the samples. The results of precision study are summarized below in Table 5-2.

Table 5-2: Results of precision studies.

Parameters	Levels	Conc. (µg/mL)	Analytical response (area, injections)					Mean (N = 5)	SD	% RSD
			1	2	3	4	5			
Repeatability (Intraday precision)										
	LQC	4.8	261537	269160	260333	264533	261091	263330.80	3627.90	1.40
	MQC	6	329074	322689	321821	321669	320028	323056.20	3498.89	1.09
	HQC	7.2	403341	403793	403368	405443	411993	405587.60	3682.54	0.91
Intermediate precision (Interday)										
Day 1	LQC	4.8	263767	268581	266419	265093	268901	266552.20	2210.05	0.83
	MQC	6	335937	338629	334677	337235	334089	336113.40	1855.99	0.55
	HQC	7.2	409092	403711	401537	400572	402485	403479.40	3345.51	0.83
Day 2	LQC	4.8	268166	263043	267974	267588	262304	265815.00	2887.20	1.09
	MQC	6	331842	332900	341562	332230	339908	335688.40	4569.26	1.36
	HQC	7.2	405432	407958	408082	411012	405213	407539.40	2365.47	0.58
Day 3	LQC	4.8	261537	269160	260333	264533	261091	263330.80	3627.90	1.40
	MQC	6	329074	322689	321821	321669	320028	323056.20	3498.89	1.09
	HQC	7.2	403341	403793	403368	405443	411993	405587.60	3682.54	0.91
Intermediate precision (Intraday)										
Analyst 1	LQC	4.8	264231	262121	263120	265317	258360	262629.80	2669.98	1.02
	MQC	6	334927	333972	333671	334237	325104	332382.20	4095.03	1.23
	HQC	7.2	378130	383741	390211	391042	392584	387141.60	6062.73	1.56

Analyst 2	LQC	4.8	255146	263023	257104	257082	255344	257539.80	3202.22	1.24
	MQC	6	341942	332101	342171	332180	331894	336057.60	5477.81	1.63
	HQC	7.2	395512	397238	408103	410097	404910	403172.00	6503.44	1.61
Analyst 3	LQC	4.8	260517	255130	251303	252513	254041	254700.80	3562.98	1.40
	MQC	6	319784	321669	320922	330098	321002	322695.00	4193.49	1.30
	HQC	7.2	395231	403225	402942	415116	411531	405609.00	7841.43	1.93

5.1.3.4. Robustness

Robustness of developed method was studied by varying the flow rate (0.8, 1 and 1.2 mL/min) of mobile phase and the detection wavelength (264, 266 and 268 nm). The observed percentage relative deviation was found to be less than 2 % for all the samples, indicating that the developed method was satisfactorily robust and the responses were unaffected by these changes. The results of the robustness study are summarized below in Table 5-3.

Table 5-3: Results of robustness studies of various parameters tested for 5-FU.

Variables	**Value**	**Conc. (µg/mL)**	**Mean peak area**	**SD**	**% RSD**	**Mean R_t (min)**	**SD**	**% RSD**
Flow rate (mL/min)	0.8	6	407271.60	3876.19	0.95	8.94	0.11	1.22
	1	6	336113.40	1855.99	0.55	7.58	0.07	0.94
	1.2	6	243530.60	2753.34	1.13	6.02	0.05	0.93
Wavelength (nm)	264	6	337290.00	1709.90	0.51	7.58	0.07	0.94
	266	6	336113.40	1855.99	0.55	7.58	0.07	0.94
	268	6	334823.20	4155.27	1.24	7.58	0.07	0.94

5.1.3.5. System suitability

System suitability parameters, i.e., Height Equivalent to Theoretical Plate (HETP), theoretical plate, theoretical plate/meter and, tailing factor of peak and peak purity index were calculated for the analytical method. The results of the study are summarized below in Table 5-4.

Table 5-4: Results of system suitability parameters.

Parameters	Value
HETP	17.07
Theoretical plate	8788.27
Theoretical plate/meter	58588.48
Tailing factor	1.22
Peak purity index	1.00

5.1.3.6. LOD and LOQ

The LOD and LOQ were found to be 0.25 µg/mL and 0.83 µg/mL respectively.

5.1.3.7. Method specificity

In order to find out specificity of developed method, 100 µg/mL solution of 5-FU (1 mL) was mixed with 1 mL of each of the excipients' solutions (0.1 % w/v) that were used to prepare mini tablets, i.e., Eudragit® S100, guar gum, pectin, magnesium stearate and Talc. The solution was filtered using 0.45 µm Whatman filter paper and then diluted to 10 mL using mobile phase. Then it was injected to HPLC. The chromatogram showed absence of any additional peak at the retention time of 5-FU (Figure 5-3). This indicated that the method was specific for detecting 5-FU only.

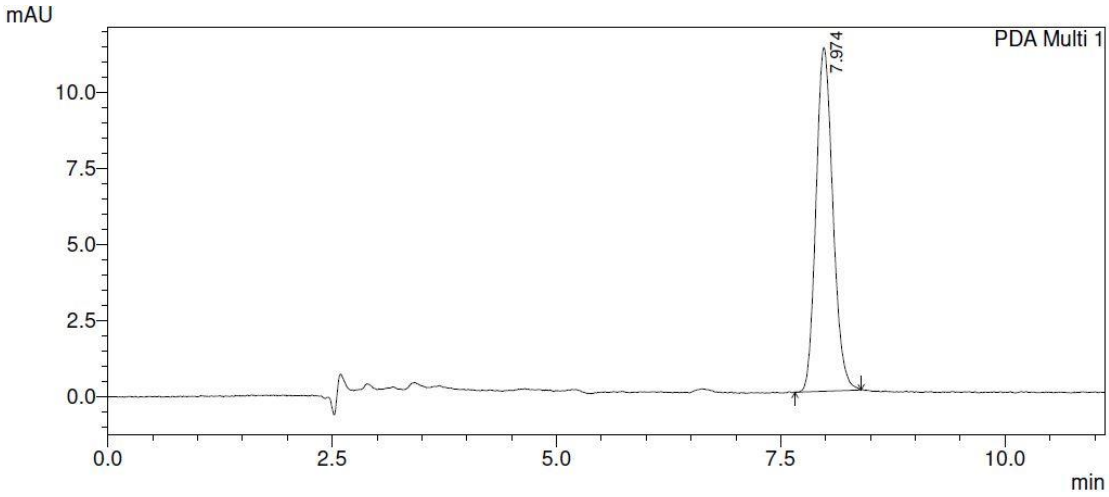


Figure 5-3: Chromatogram of 5-FU along with excipients**.**

5.2. Pre-formulation studies

5.2.1. Drug excipient compatibility studies

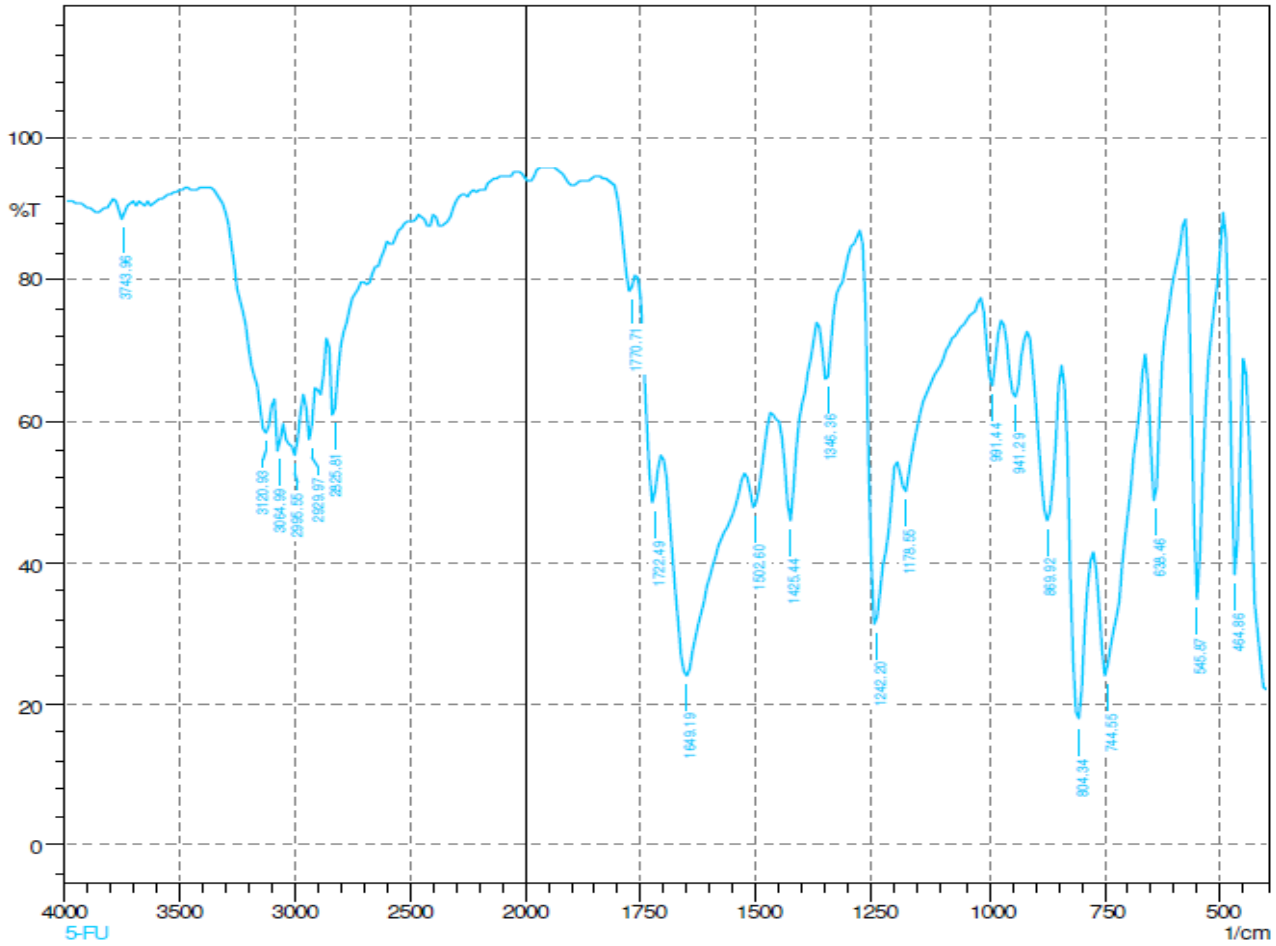
The study was carried out for 14 days and the samples were visually observed for any possible color change, lump formation and their flow properties. The results are shown in Table 5-5. The results indicated no change in physical appearance of the powders. Moreover, the angle of repose was found to be within the pharmacopoeial limits (174).

Further drug excipient compatibility studies were assessed through IR studies. Major vibrational peaks of 5-FU were found at 3747.93 cm^{-1}, 1649.19 cm^{-1}, 1242.20 cm^{-1} and 1502.60 cm^{-1} belonging to N-H stretch, C=0 stretch, C-N stretch and C=C (in plane bending), respectively.

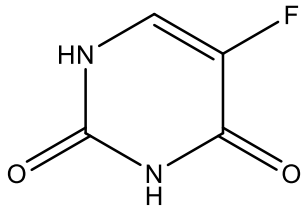
Table 5-5: Results of drug-excipient compatibility study.

S. No.	Code	0 day			7th day			14th day		
		Colour	**Initial appearance**	**Angle of repose (Θ) (Mean ± SD)**	**Colour change**	**Lump formation**	**Angle of repose (Θ) (Mean ± SD)**	**Colour change**	**Lump formation**	**Angle of repose (Θ) (Mean ± SD)**
1.	D	White	No lump	5.03 ± 0.39	No	No	5.11 ± 0.66	No	No	5.33 ± 0.34
2.	E1	White	No lump	5.77 ± 0.60	No	No	6.90 ± 0.35	No	No	6.84 ± 0.17
3.	E2	White	No lump	31.30 ± 0.54	No	No	31.29 ± 1.13	No	No	31.91 ± 0.41
4.	E3	White	No lump	26.99 ± 0.43	No	No	27.50 ± 0.69	No	No	27.72 ± 0.32
5.	E4	Whitish brown	No lump	31.48 ± 0.49	No	No	31.96 ± 0.86	No	No	33.07 ± 0.56
6.	E5	White	No lump	45.60 ± 0.87	No	No	46.28 ± 0.97	No	No	46.42 ± 0.60
7.	D+E1	White	No lump	6.72 ± 0.36	No	No	7.32 ± 0.30	No	No	7.38 ± 0.18
8.	D+E2	White	No lump	29.72 ± 0.35	No	No	30.04 ± 0.24	No	No	29.25 ± 0.22
9.	D+E3	White	No lump	24.34 ± 0.51	No	No	25.05 ± 0.17	No	No	25.11 ± 2.45
10.	D+E4	Whitish brown	No lump	30.28 ± 0.30	No	No	30.36 ± 0.13	No	No	30.13 ± 0.10
11.	D+E5	White	No lump	40.17 ± 0.19	No	No	40.25 ± 0.30	No	No	40.70 ± 0.26
12.	D+E1-5	Whitish brown	No lump	28.25 ± 0.95	No	No	28.43 ± 0.58	No	No	28.27 ± 0.53

5.2.1.1. IR spectra of 5-FU and excipients

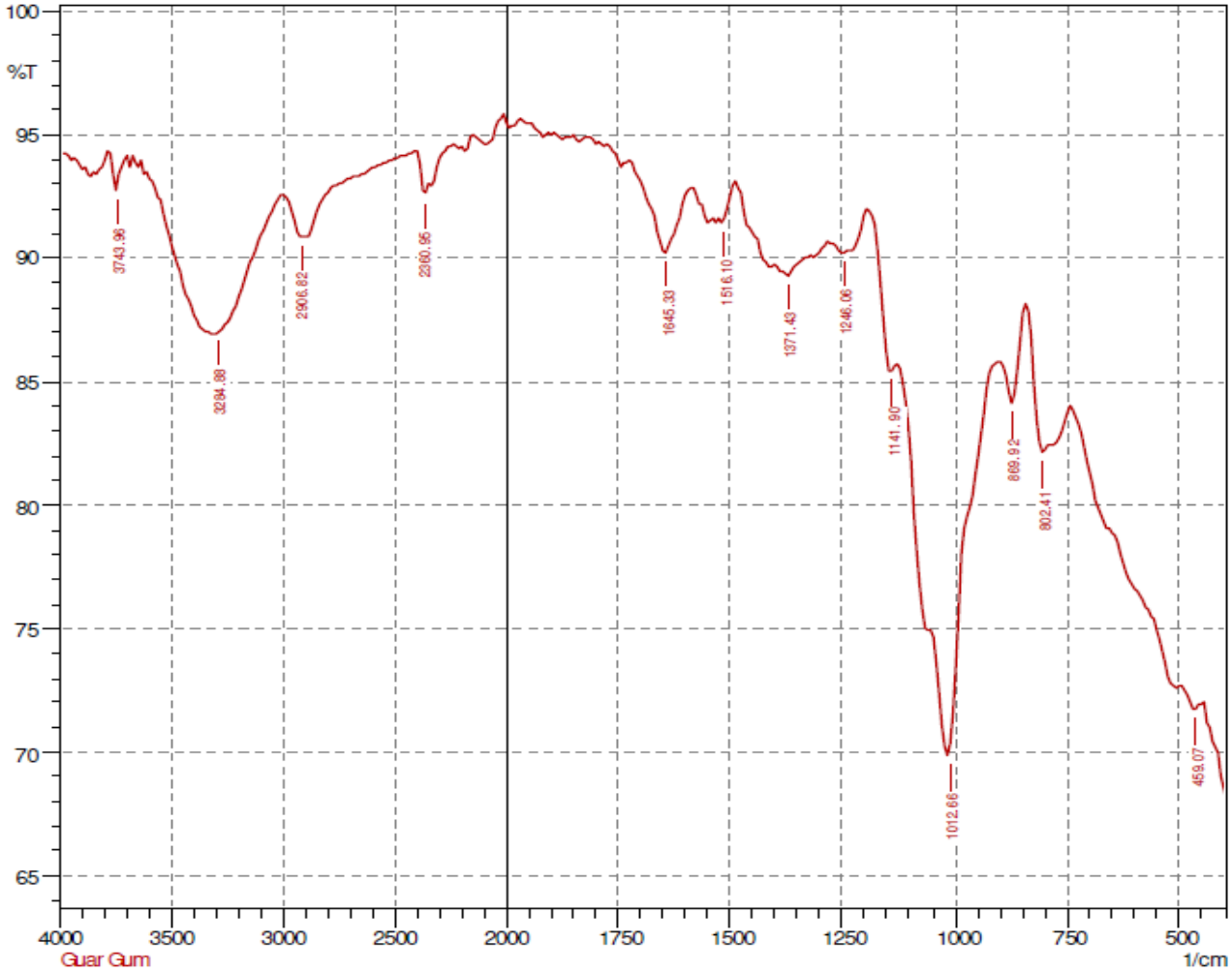


S. No.	Functional Group	% Intensity
1	N-H	3747.93
2	C=O	1649.19
3	C=C	1502.60
4	C-N (Bending)	1242.20

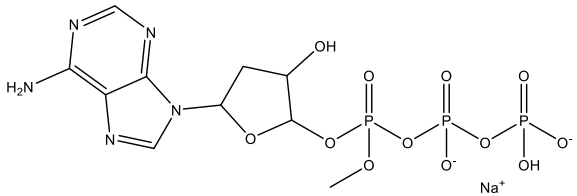


5-Fluoro Uracil

Figure 5-4: IR spectra of 5-FU.



S. No.	Functional Group	% Intensity
1	NH_2	3743.96
2	OH	3284.89
3	C=N	1645.33
4	C-N	2360.96
5	COC	1516.10
6	COC (Bending)	1246.06



disodium;[[[5-(6-aminopurin-9-yl)-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxidophosphoryl] hydrogen phosphate

Figure 5-5: IR spectra of Guar Gum.

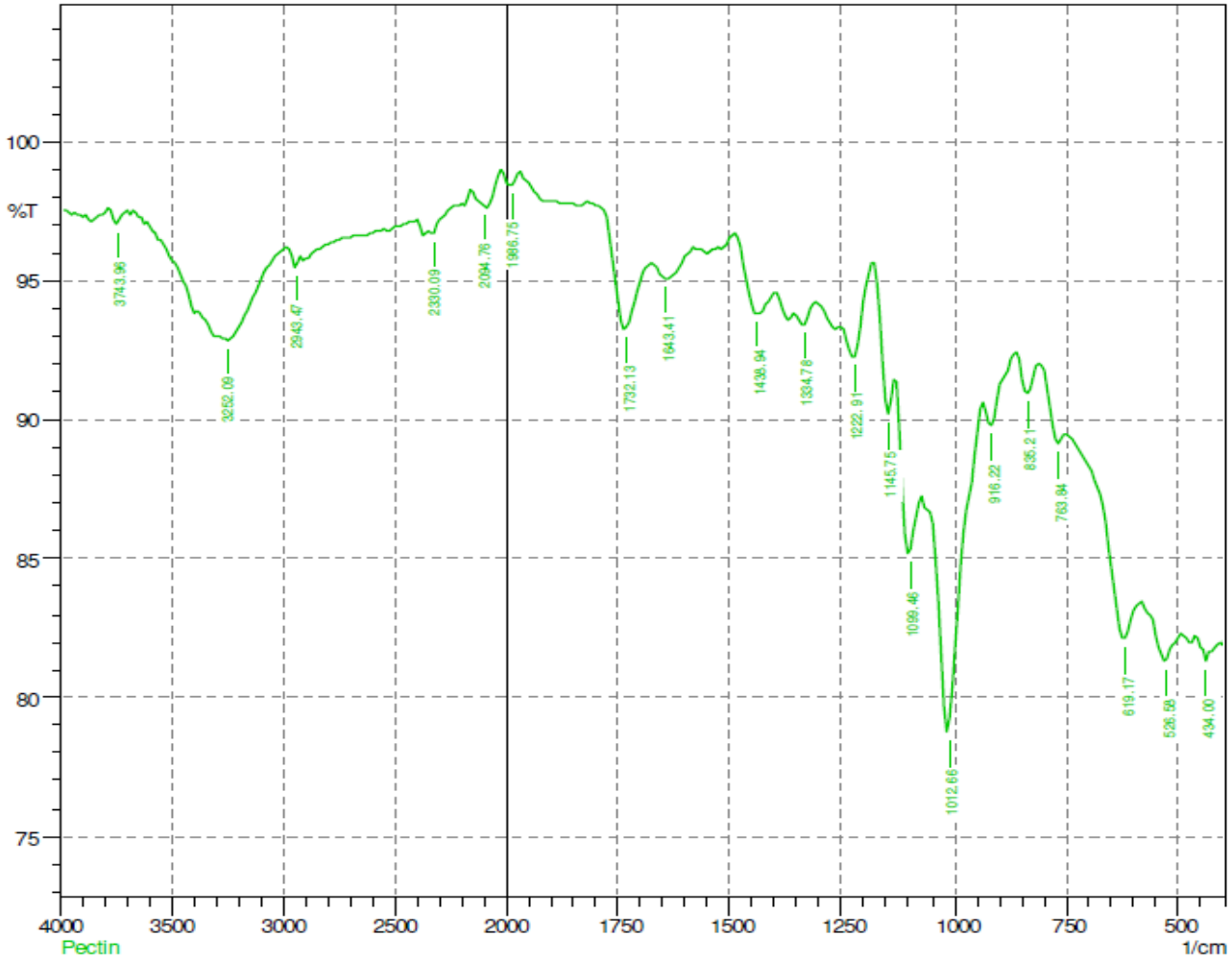
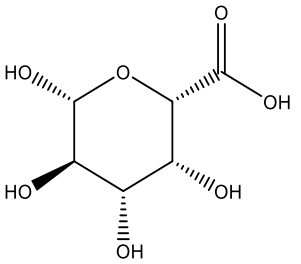


Figure 5-6: IR spectra of Pectin.

S. No.	Functional Group	% Intensity
1	OH	3252.09
2	C=O	1732.76
3	COC	1438.94
4	COC (Bending)	1222.91



(2S,3R,4S,5R,6R)-3,4,5,6-tetrahydroxyoxane-2-carboxylic acid

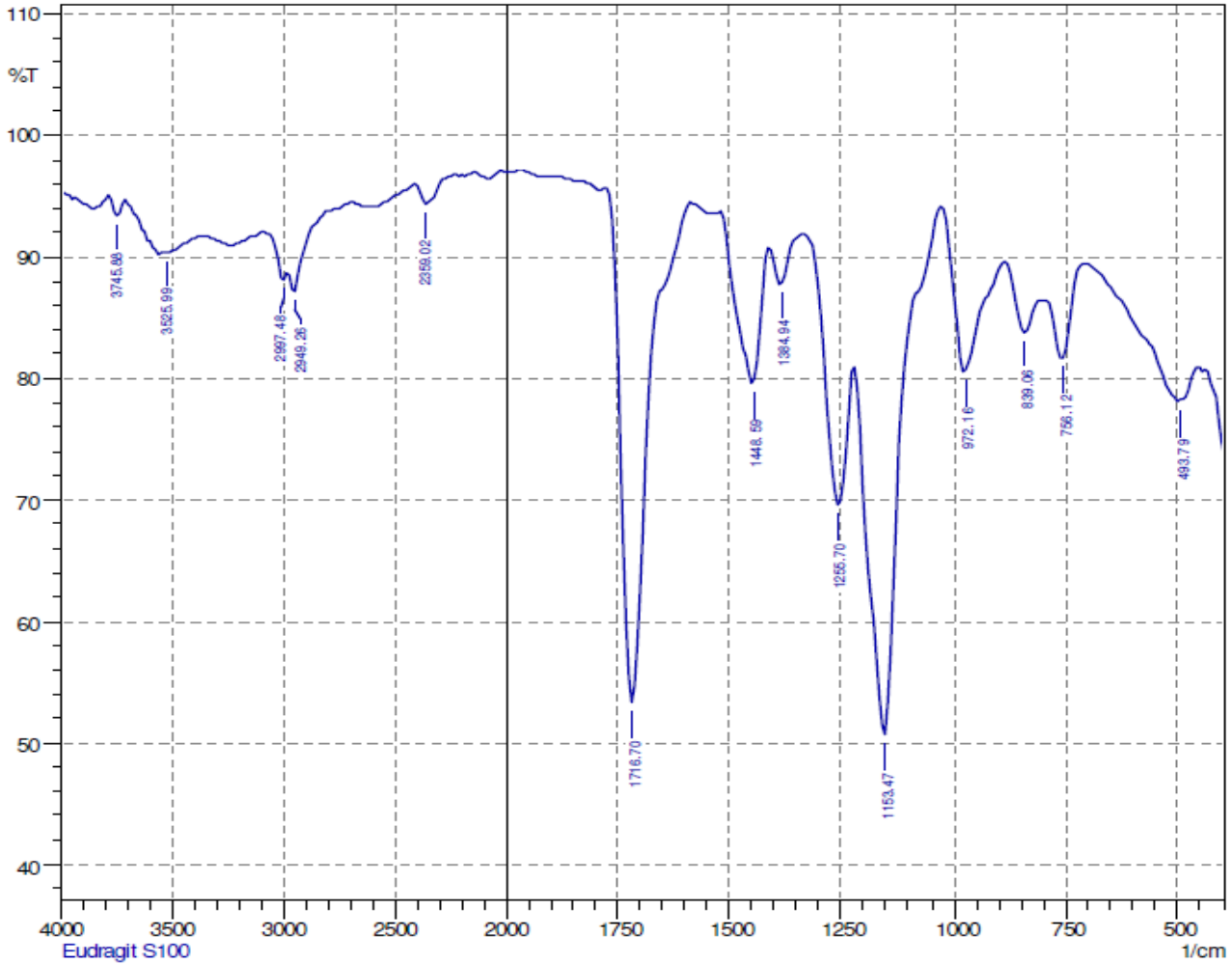
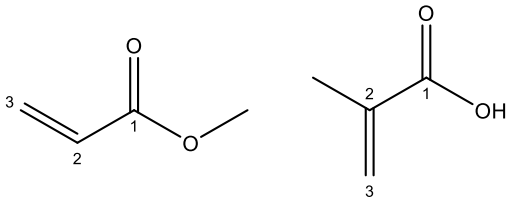
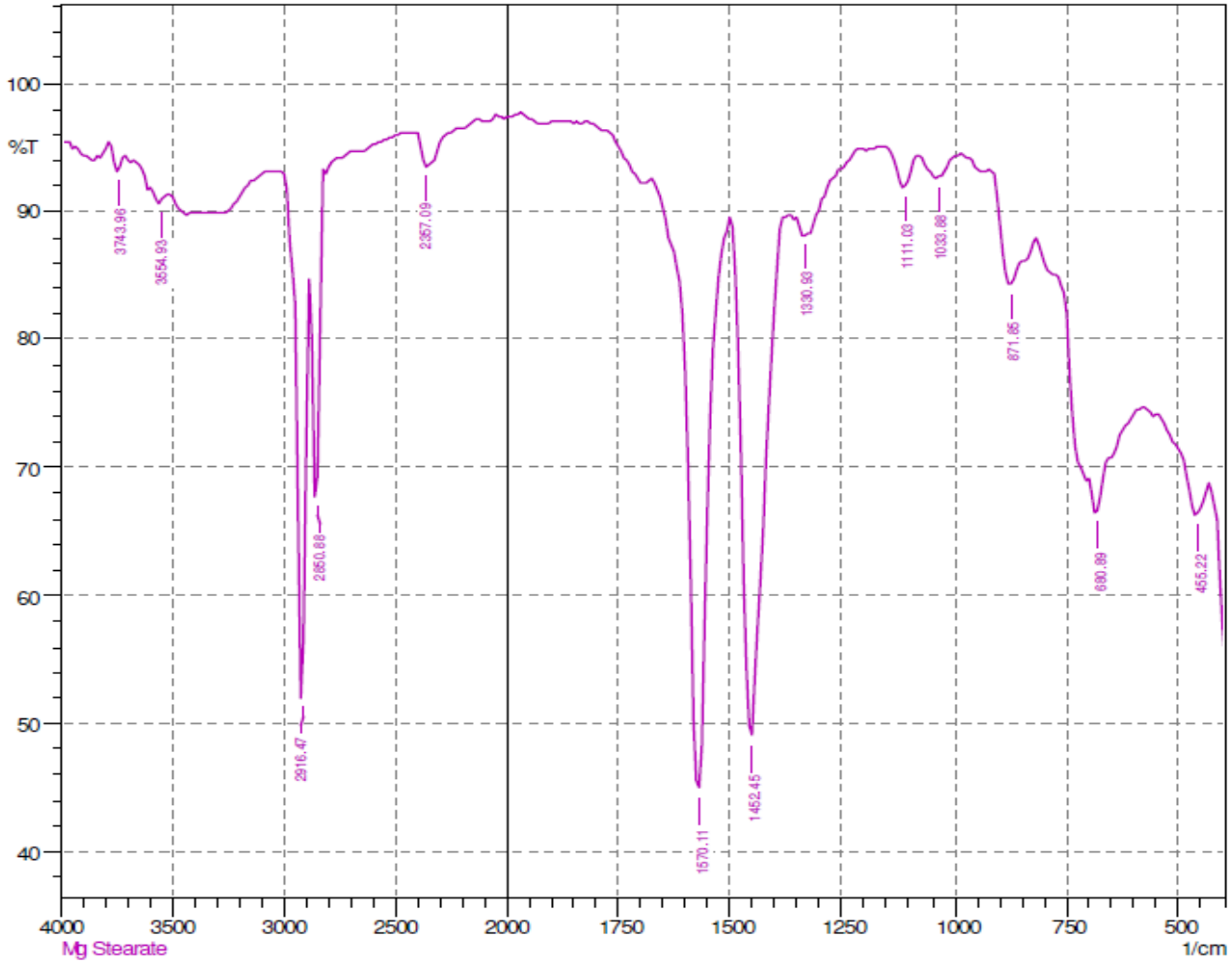


Figure 5-7: IR spectra of Eudragit® S100.

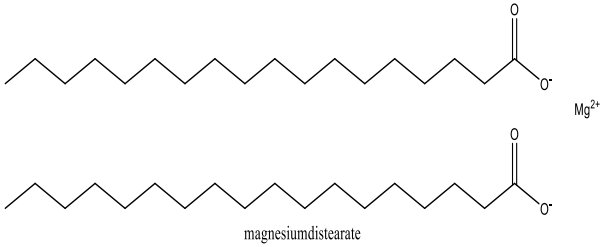
S. No.	Functional Group	% Intensity
1	C=O	1716.70
2	C=C	2997.46
3	CH	2946.26
4	OH	3525.49
5	COC	1153.47



Methyl prop-2-enoate; 2 methylprop-2-enoic acid

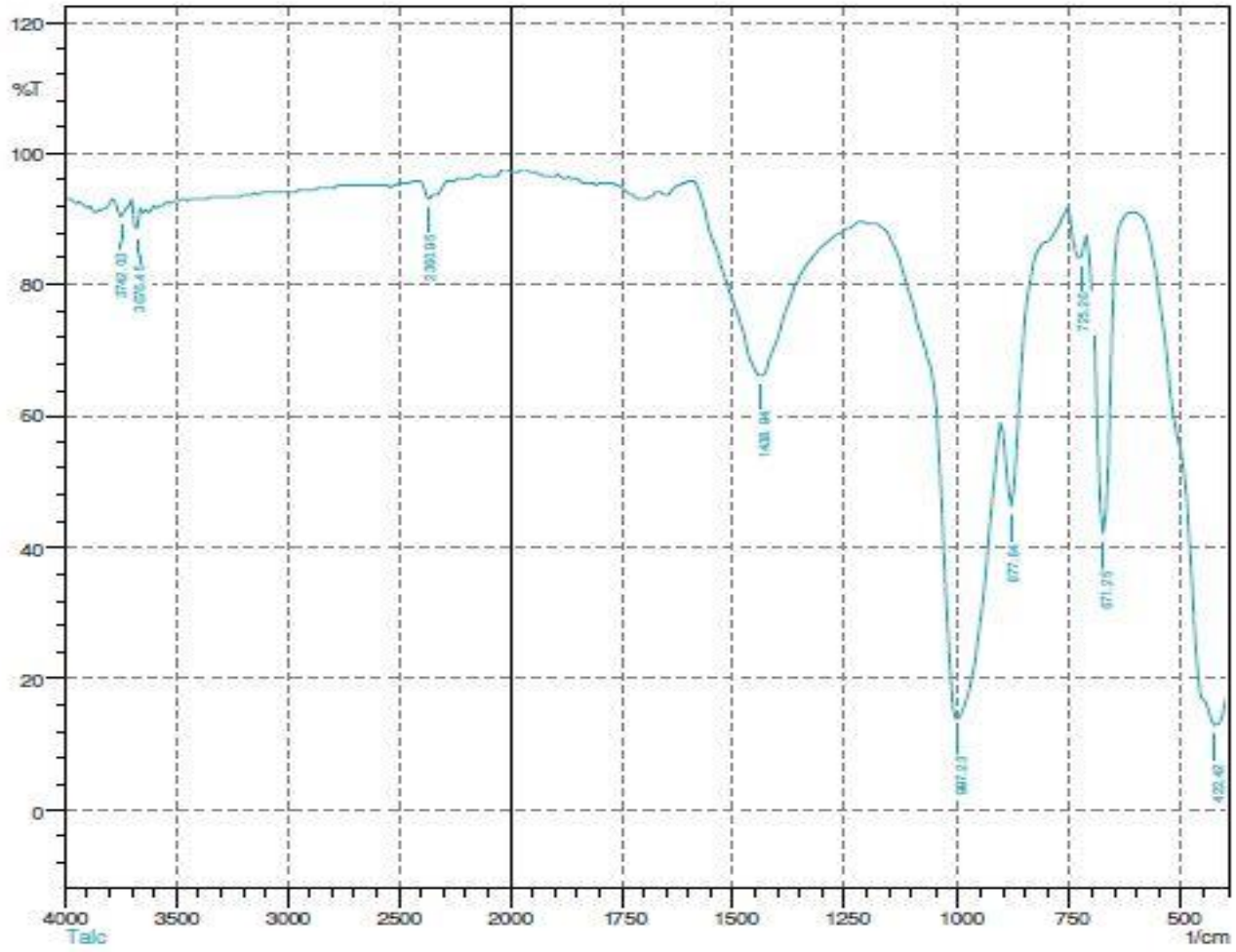


S. No.	Functional Group	% Intensity
1	C=O	1570.11
2	C-O	1330.93

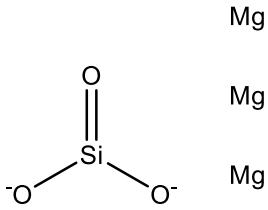


magnesiumdistearate

Figure 5-8: IR spectra of Magnesium stearate.



S. No.	Functional Group	% Intensity
1	S=O	1438.94



trimagnesium;dioxido(oxo)silane

Figure 5-9: IR spectra of talc.

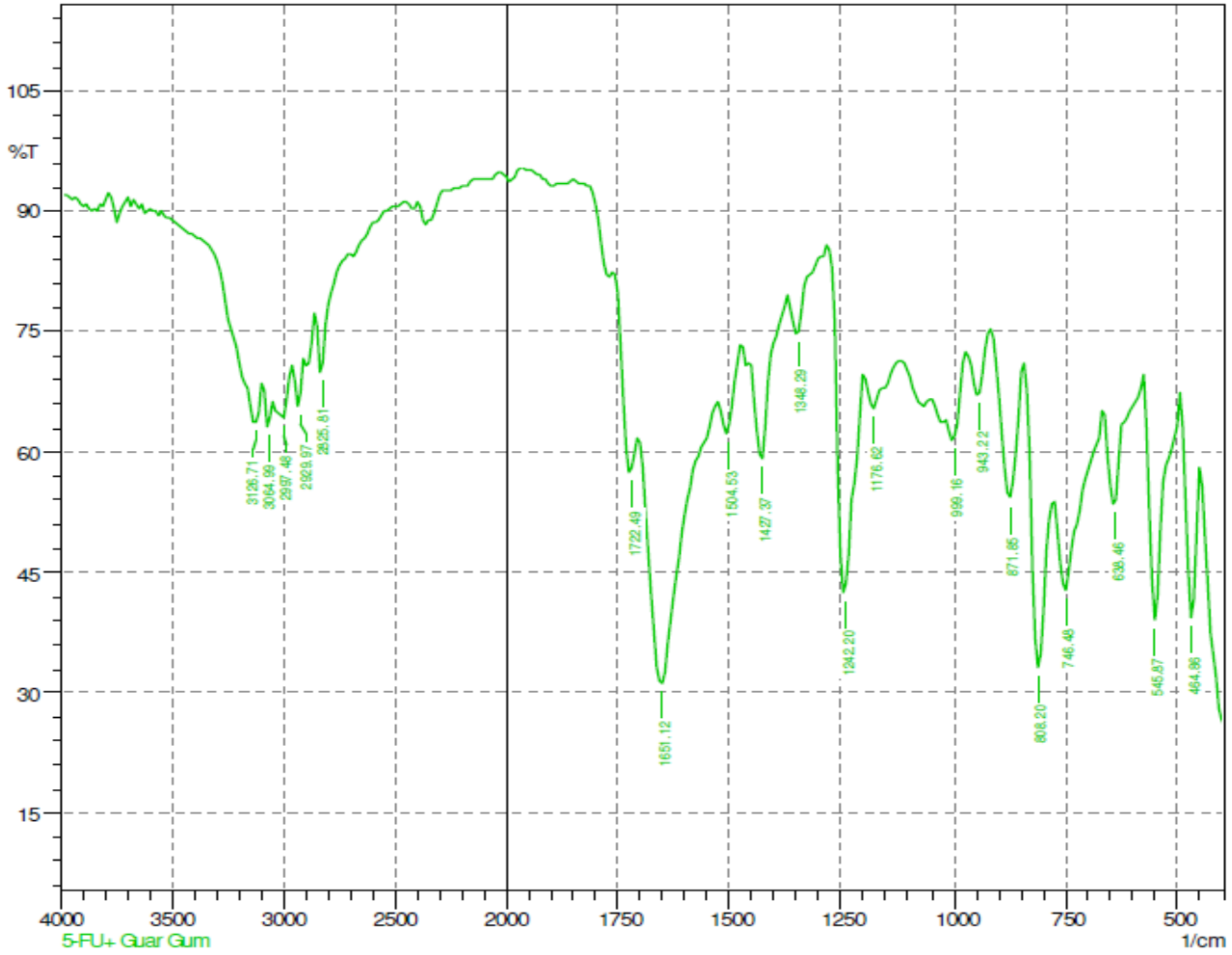


Figure 5-10: IR spectra of binary mixture of 5-FU and Guar gum.

Conclusive remark: The major characteristic peaks of 5-FU remain unchanged and identical to that of in the IR spectra of pure 5-FU. Moreover, the characteristic peak of guar gum also remains unchanged. This indicates that, there was no chemical interaction between 5-FU and guar gum. Apart from this, the results also revealed that 5-FU was stable with guar gum and no identifiable chemical degradation occurred in either of the drug and excipient.

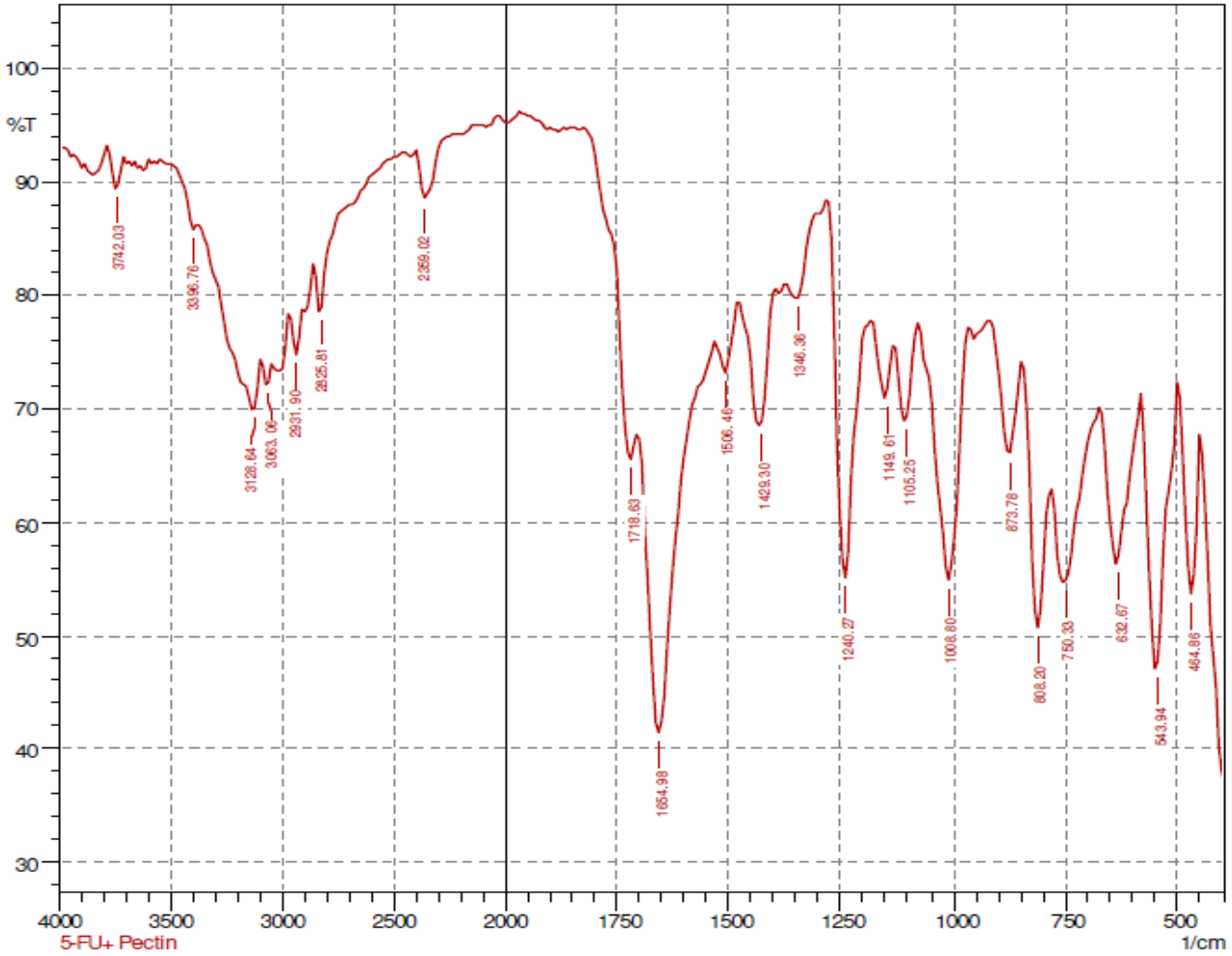
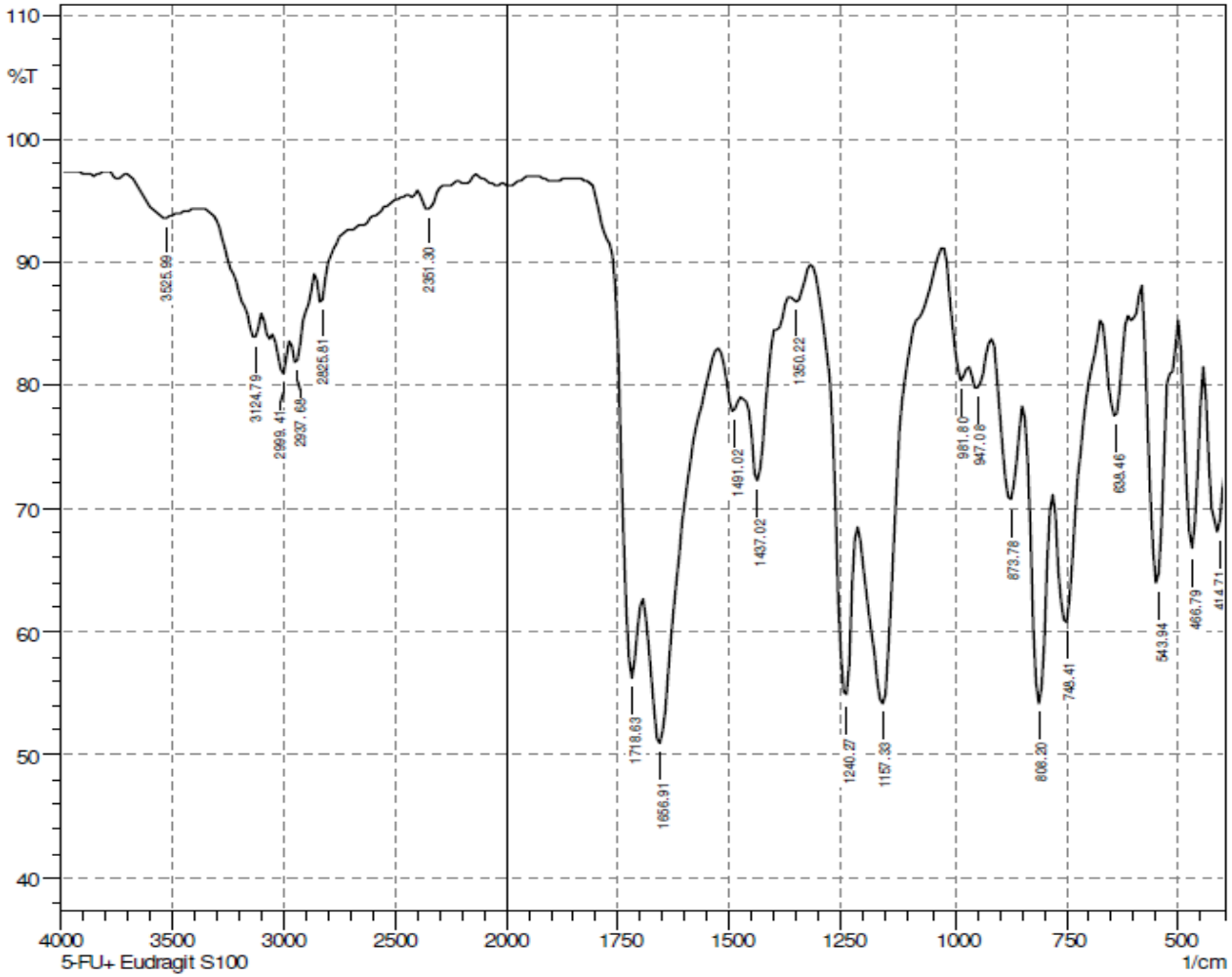


Figure 5-11: IR spectra of binary mixture of 5-FU and Pectin.

Conclusive remark: The major characteristic peaks of 5-FU remain unchanged and identical to that of in the IR spectra of pure 5-FU. Moreover, the characteristic peak of pectin also remains unchanged. This indicates that, there was no chemical interaction between 5-FU and pectin. Apart from this, the results also revealed that 5-FU was stable with pectin and no identifiable chemical degradation occurred in either of the drug and excipient.



Conclusive remark: The major characteristic peaks of 5-FU remain unchanged and identical to that of in the IR spectra of pure 5-FU. Moreover, the characteristic peak of Eudragit® S100 also remains unchanged. This indicates that, there was no chemical interaction between 5-FU and Eudragit® S100. Apart from this, the results also revealed that 5-FU was stable with Eudragit® S100 and no identifiable chemical degradation occurred in either of the drug and excipient.

Figure 5-12: IR spectra of binary mixture of 5-FU and Eudragit® S100.

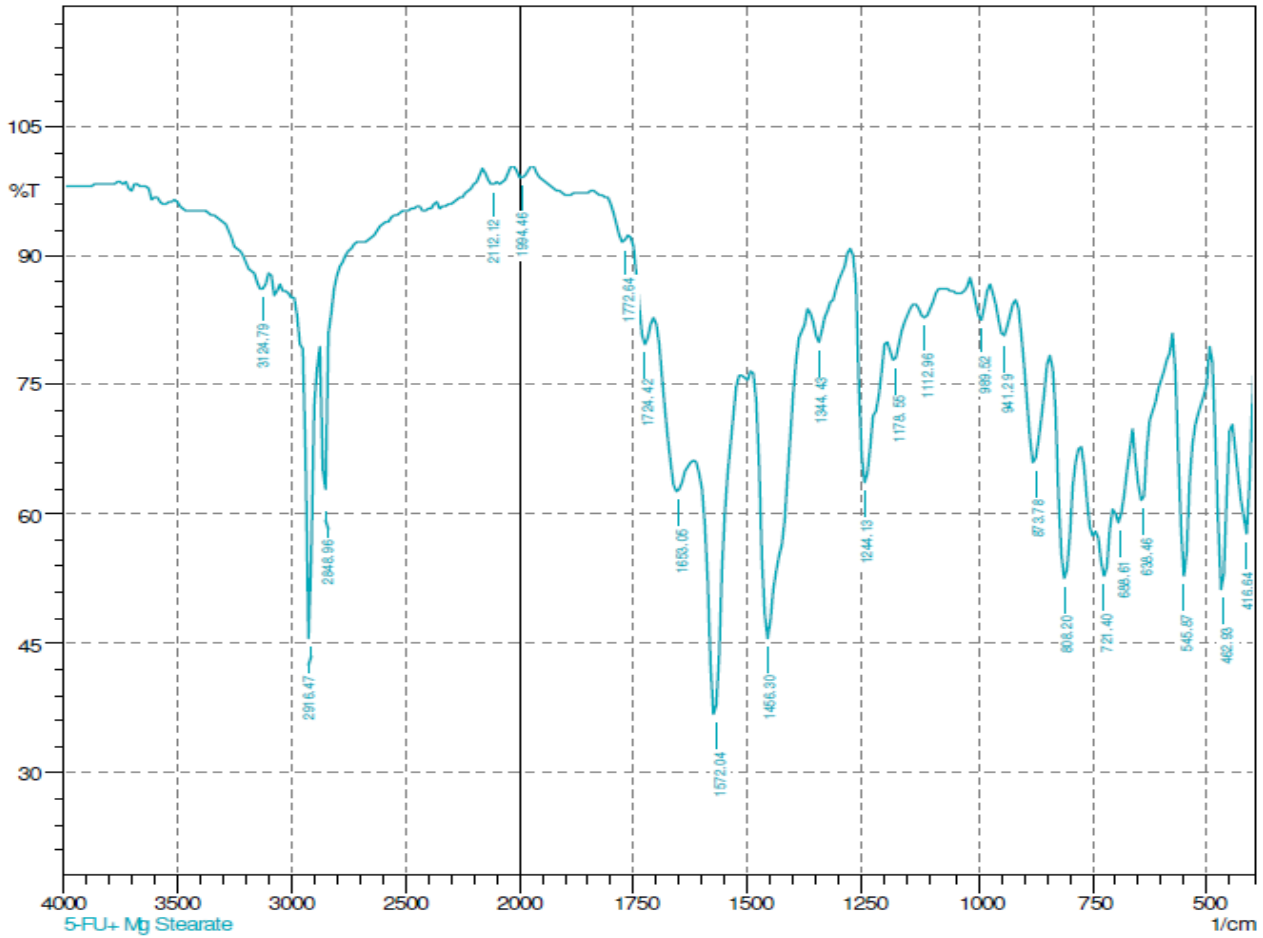


Figure 5-13: IR spectra of binary mixture of 5-FU and Mg. Stearate.

Conclusive remark: The major characteristic peaks of 5-FU remain unchanged and identical to that of in the IR spectra of pure 5-FU. Moreover, the characteristic peak of Mg stearate also remains unchanged. This indicates that, there was no chemical interaction between 5-FU and Mg stearate. Apart from this, the results also revealed that 5-FU was stable with Mg stearate and no identifiable chemical degradation occurred in either of the drug and excipient.

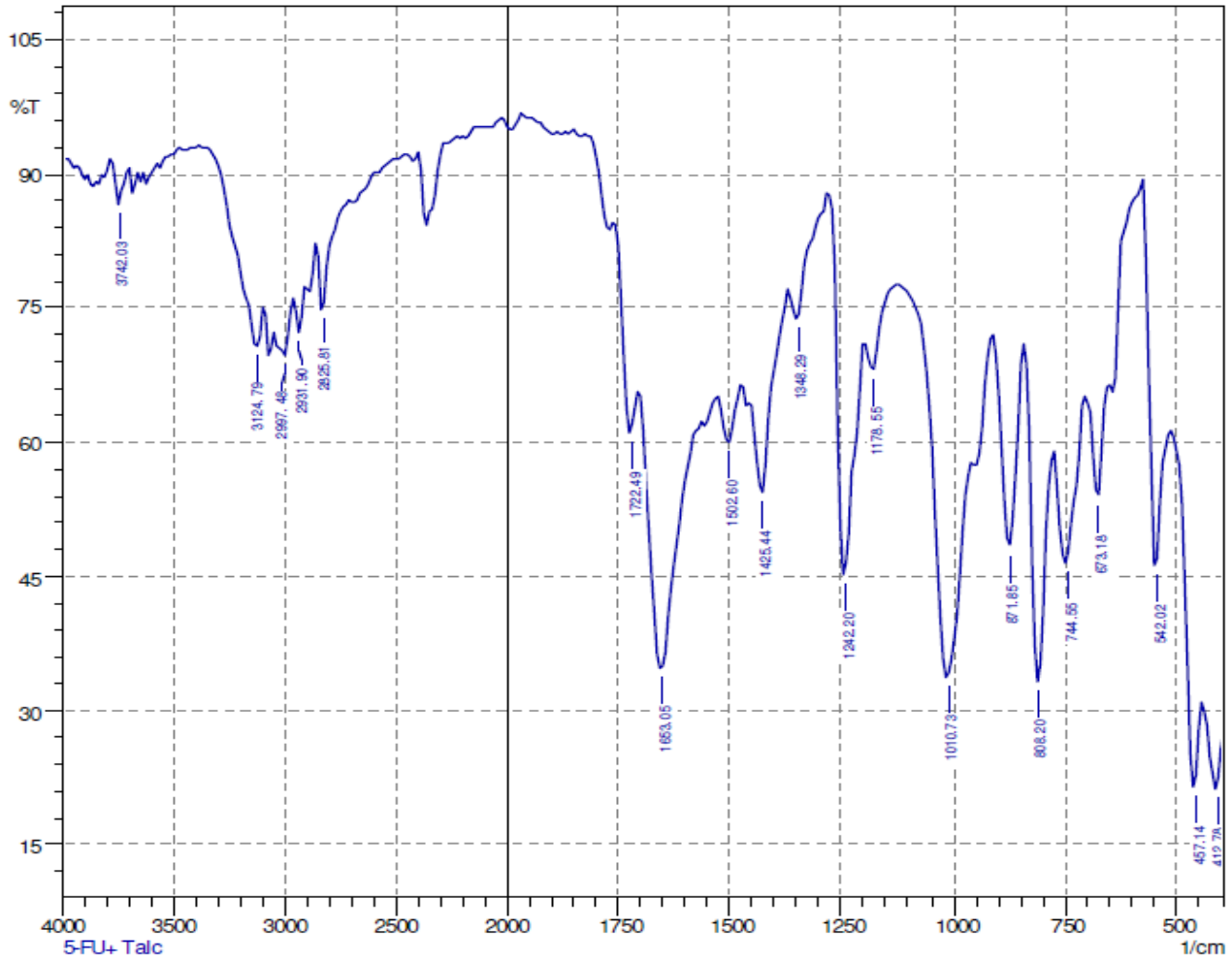


Figure 5-14: IR spectra of binary mixture of 5-FU and Talc.

Conclusive remark: The major characteristic peaks of 5-FU remain unchanged and identical to that of in the IR spectra of pure 5-FU. Moreover, the characteristic peak of talc also remains unchanged. This indicates that, there was no chemical interaction between 5-FU and talc. Apart from this, the results also revealed that 5-FU was stable with talc and no identifiable chemical degradation occurred in either of the drug and excipient.

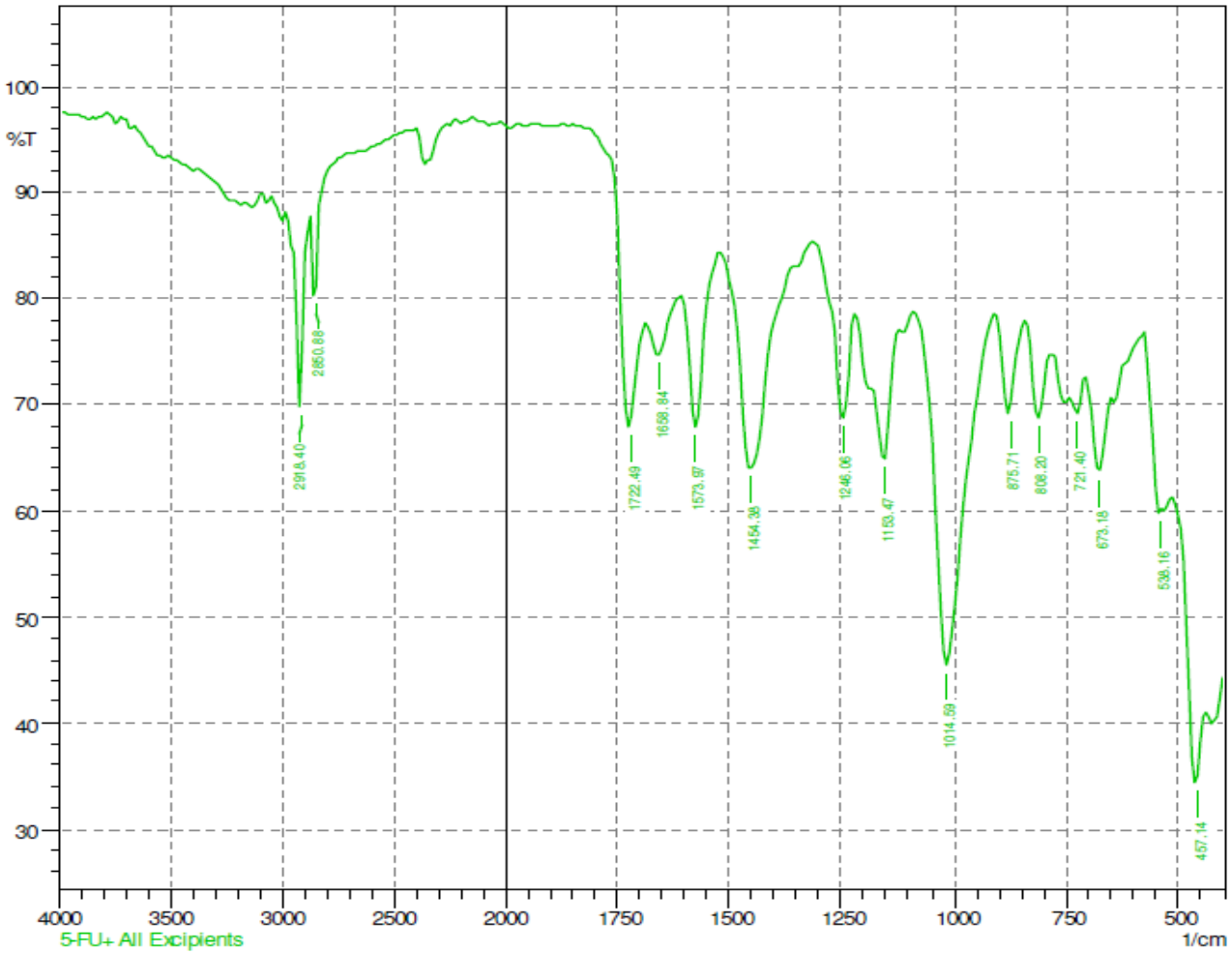


Figure 5-15: IR spectra of the mixture of 5Fu and all the excipients.

Conclusive remark: The major characteristic peaks of 5-FU remain unchanged and identical to that of in the IR spectra of pure 5-FU. Moreover, the major characteristic peaks the excipients also remain unchanged. This indicates that, there was no chemical interaction between 5-FU and talc. Apart from this, the results also revealed that 5-FU was stable with all the excipients used and no identifiable chemical degradation occurred in either of the drug and excipients.

5.2.2. Pre-compression studies

The results of pre-compression studies are summarized below in Table 5-5.

Table 5-5: Results of pre-compression study.

Batch	Angle of repose (º)	Bulk Density (g/cc)	Tapped Density (g/cc)	Carr's index (%)	Hausner's ratio
B1	34.55 ± 0.74	0.37 ± 0.02	0.45 ± 0.02	17.87 ± 0.54	1.22 ± 0.04
B2	33.32 ± 1.32	0.36 ± 0.01	0.44 ± 0.02	18.2 ± 0.55	1.22 ± 0.04
B3	32.06 ± 0.71	0.35 ± 0.03	0.42 ± 0.04	15.13 ±0.92	1.18 ± 0.05
B4	30.95 ± 0.47	0.36 ± 0.05	0.43 ± 0.03	16.2 ± 0.87	1.19 ± 0.05
B5	34.47 ± 0.46	0.39 ± 0.03	0.48 ± 0.05	19.58 ± 1.01	1.24 ± 0.06
B6	35.02 ± 0.22	0.39 ± 0.03	0.50 ± 0.06	20.8 ± 1.27	1.26 ± 0.05
B7	35.64 ± 0.48	0.40 ± 0.01	0.52 ± 0.05	21.92 ± 1.07	1.28 ± 0.05
B8	35.9 ± 0.86	0.41 ± 0.05	0.54 ± 0.04	24.07 ± 0.86	1.31 ± 0.06
B9	36.43 ± 0.52	0.42 ± 0.04	0.55 ± 0.06	23.63 ± 1.21	1.31 ± 0.06
B10	37.52 ± 0.63	0.42 ± 0.03	0.55 ± 0.07	25.00 ± 1. 37	1.33 ± 0.07

5.3. Post-compression studies

The obtained results of post-compression studies are summarized in Table 5-6.

Table 5-6: Results of post-compression studies of mini-tablets.

Batch	Hardness (kg/cm^2)	Weight variation (%)	Friability (%)	Disintegration time (min)	Thickness (mm)	Diameter (mm)	Assay (%)
B1	2.48 ± 0.14	47.40 ± 1.96	0.73 ± 0.19	0.55 ± 0.02	1.68 ± 0.005	5.05 ± 0.01	98.84 ± 1.32
B2	2.68 ± 0.09	48.45 ± 1.98	0.57 ± 0.14	1.05 ± 0.02	1.78 ± 0.01	5.06 ± 0.01	99.63 ± 1.46
B3	2.65 ± 0.07	48.70 ± 1.70	0.57 ± 0.15	1.06 ± 0.03	1.82 ± 0.01	5.05 ± 0.01	99.73 ± 1.35
B4	2.55 ± 0.08	47.40 ± 1.53	0.61 ± 0.16	0.55 ± 0.05	1.68 ± 0.01	5.05 ± 0.01	99.12 ± 2.14
B5	3.04 ± 0.06	50.35 ± 1.95	0.32 ± 0.08	1.14 ± 0.02	1.96 ± 0.005	5.06 ± 0.01	98.31 ± 1.49
B6	2.85 ± 0.10	48.60 ± 1.93	0.43 ± 0.11	1.11 ± 0.02	1.81 ± 0.01	5.06 ± 0.01	99.65 ± 2.31
B7	2.96 ± 0.09	47.00 ± 1.45	0.37 ± 0.09	1.10 ± 0.03	1.61 ± 0.01	5.05 ± 0.01	96.27 ± 1.91
B8	3.02 ± 0.12	47.30 ± 1.97	0.29 ± 0.07	1.15 ± 0.01	1.63 ± 0.01	5.06 ± 0.01	98.06 ± 1.31
B9	3.06 ± 0.07	46.90 ± 1.94	0.27 ± 0.07	1.13 ± 0.09	1.58 ± 0.01	5.05 ± 0.01	97.97 ± 1.14
B10	3.27 ± 0.19	49.95 ± 1.87	0.16 ± 0.04	1.16 ± 0.07	1.93 ± 0.01	5.05 ± 0.02	99.78 ± 1.38

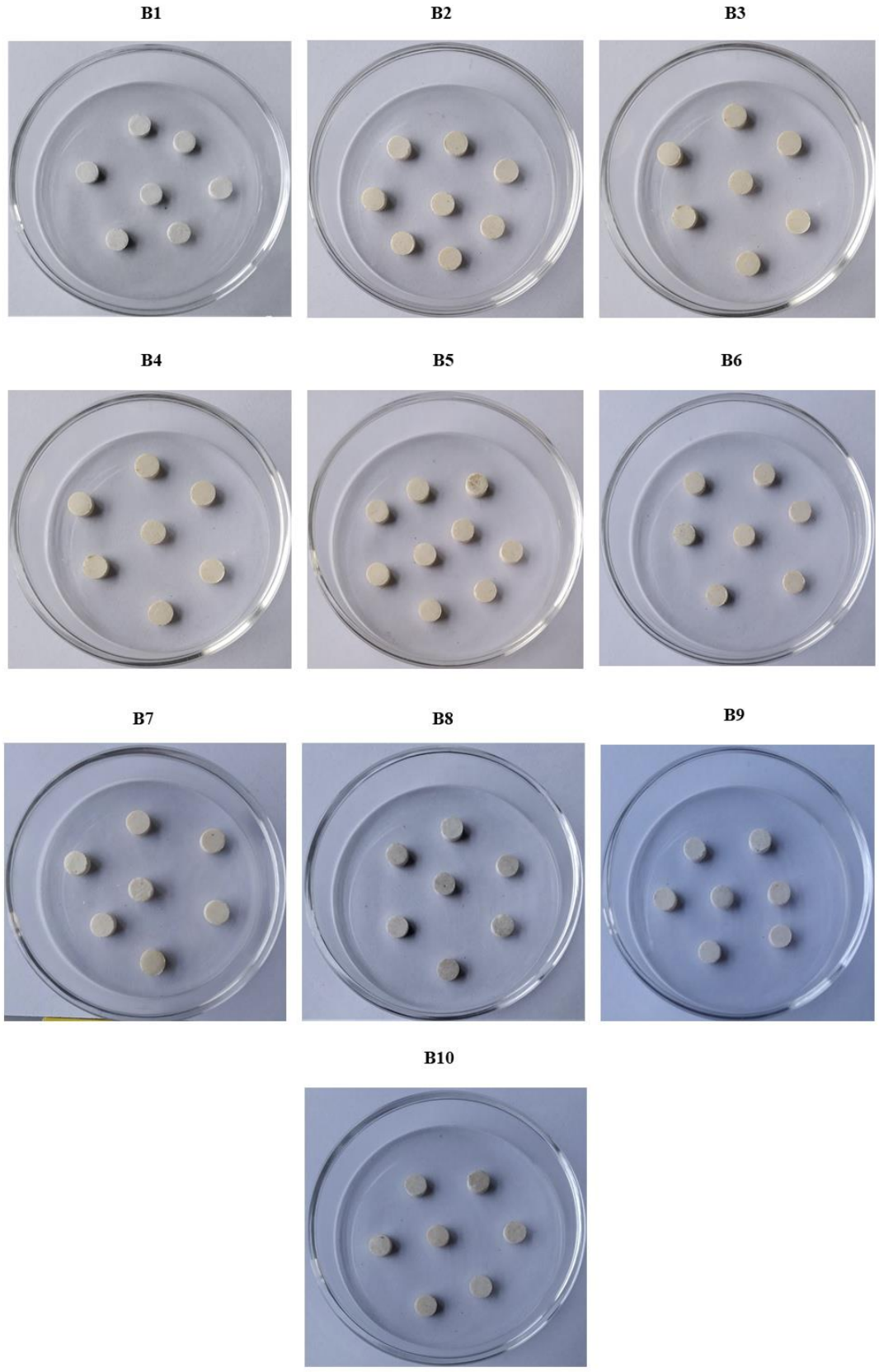


Table 5-7: Uncoated tablets (B1-B10).

5.3.1. *In vitro* dissolution studies of uncoated tablets

The results of pre and post compression parameters revealed that all the parameters of all formulations were within the pharmacopoeial limits. However, they differ in their dissolution profile. Hence, in order to select the best batch that could provide site specific release of 5-FU in the colon, the dissolution study has been performed initially in conventional dissolution medium for 24 h. It was important to note that the purpose of the study was to evaluate the optimum formulation that could restrict the drug release to less than 10 % in the first 5 h. The study revealed that maximum dissolution rate was achieved with B1 (73.16 %) and B4 (67.22 %) in 5 h. This was followed by batch B2 (59.22 %), B3 (56.18 %), B6 (52.19 %), B7 (46.23 %), B5 (43.23 %), B8 (42.17 %), B9 (41.64 %), and B10 (35.28 %).

Since, the drug release for all the formulations was more than 30 % in first 5 h, it was decided to coat the selected formulation further to restrict the drug release in the first 5 h (i.e., less than 10 %). Among formulations (B1-B10), B10 have shown the lowest dissolution rate in 5 h, hence, it was selected for further coating. Dissolution profiles of all the prepared batches are shown in Figure 5-16.

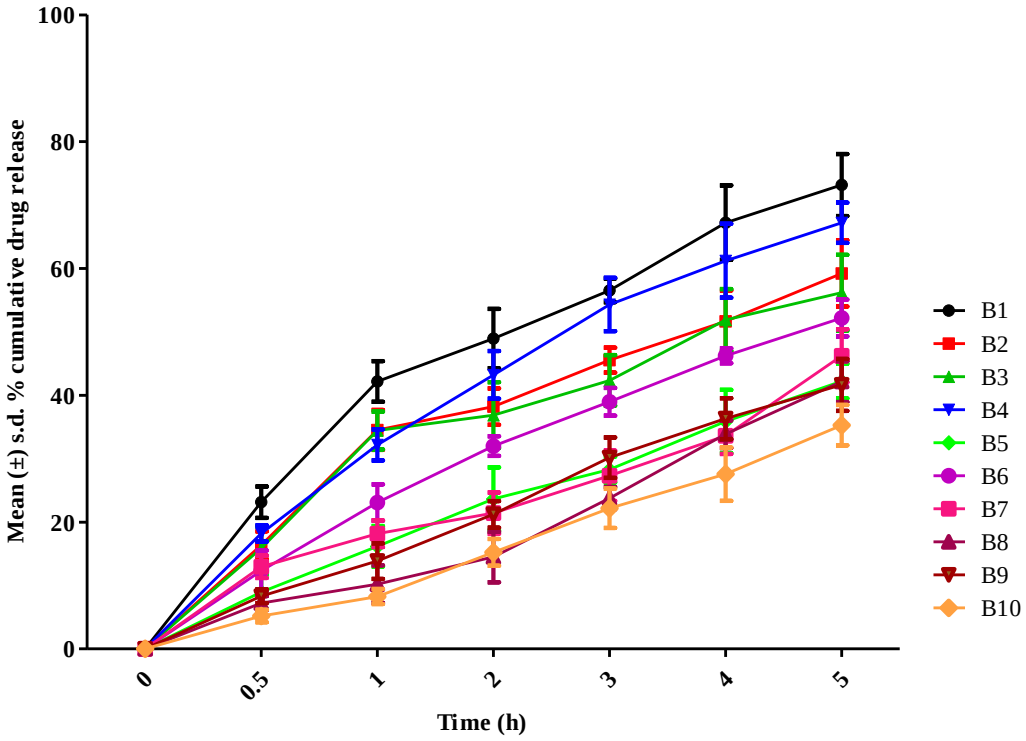


Figure 5-16: Mean (± s.d.) cumulative drug release profile of uncoated 5-FU tablets.

5.3.2. *In vitro* dissolution studies of coated tablets

A total of 27 different coating compositions were used by varying the ratios of Eudragit® S100, guar gum and pectin using ternary phase diagram (Figure 5-17). The formulations that showed less than 10 % drug release were taken into consideration. It was observed that out of 27 formulations, only 3 formulations, B10C1 (9.5 %), B10C10 (3.60 %), and B10C11 (8.40 %) were able to restrict the drug release to less than 10 % in first 5 h. Total 6 formulations viz. B10C2 (10.30 %), B10C3 (12.80 %), B10C12 (12.60 %), B10C13 (15.50 %), B10C19 (15.50 %) and B10C20 (18.30 %), were able to restrict the drug release to 10-20 % in first 5 h. The results of two way ANOVA indicated that the 'P' value of dissolution profile was found significant ($p < 0.05$) between 2-7 h for B10C10 as compared to all the formulations, except B10C1 and B10C11. It was observed that coating of polysaccharides played major role in restricting the drug release. Among them pectin was found to be more effective as compared to guar gum. The results of dissolution profiles of formulation B10C1 to B10C27 are shown in Figure 5-17.

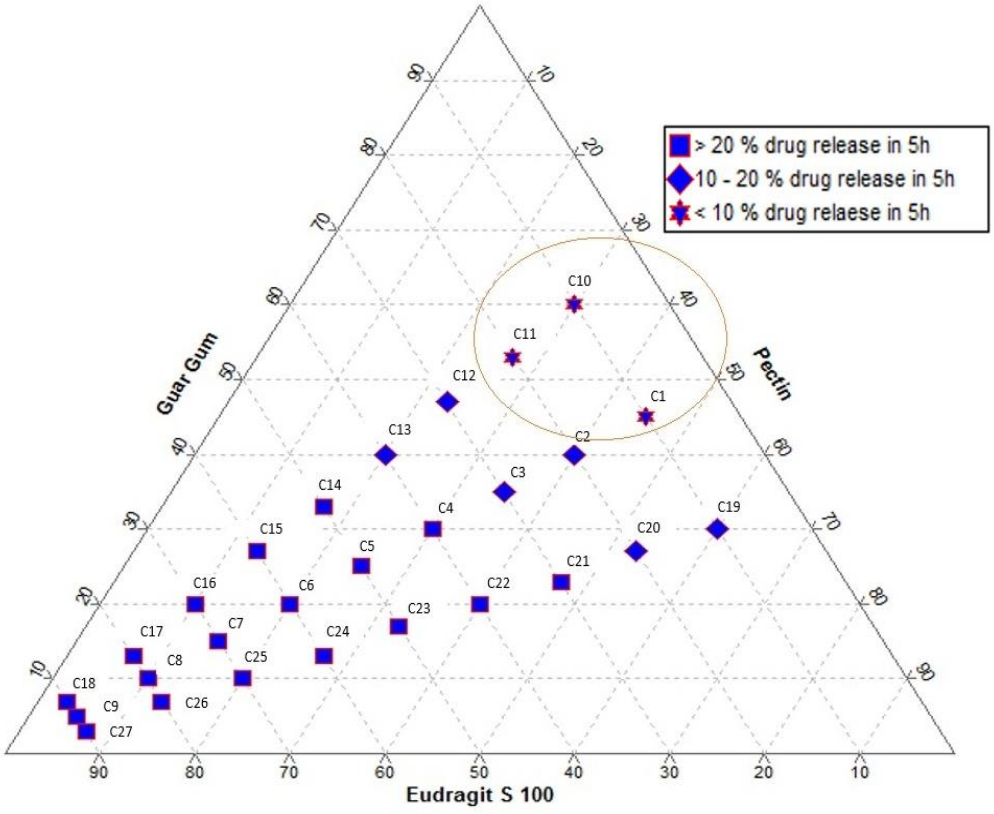


Figure 5-17: Ternary phase diagram indicating drug release in 5 h from formulations B10C1 to B10C27 (i.e., C1-C27). Note: The red circle indicates the desirable coating ratio zone.

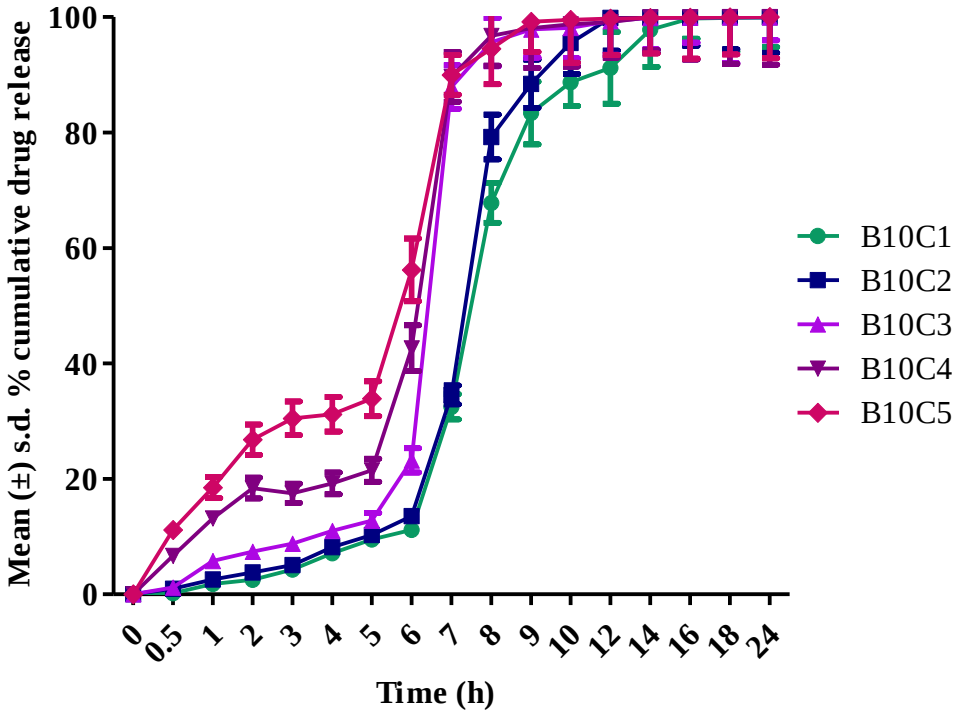


Figure 5-18: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C1-B10C5).

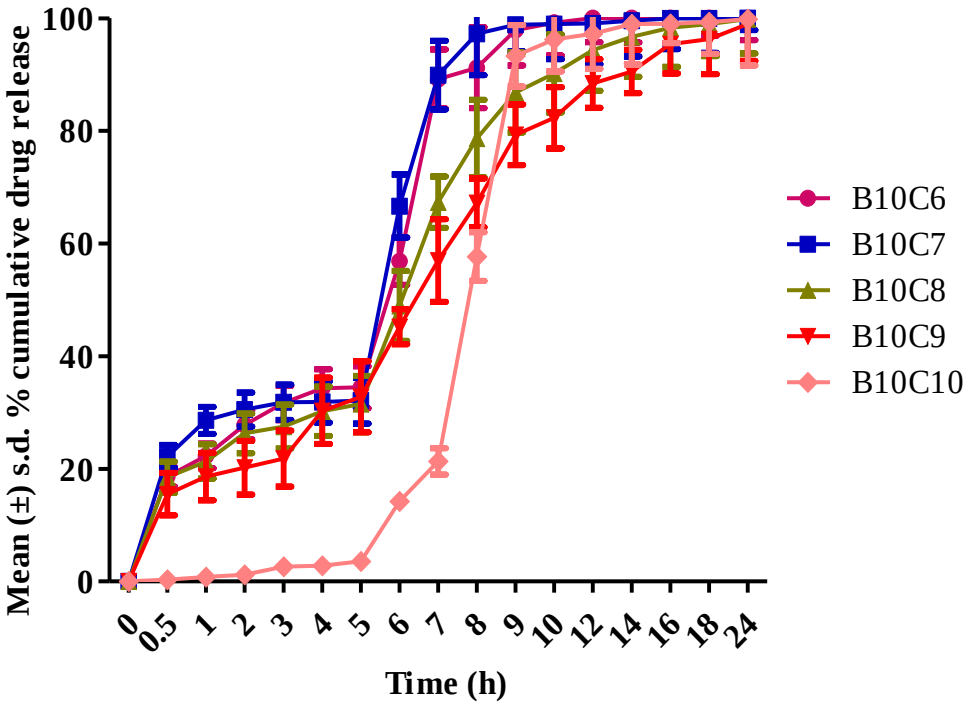


Figure 5-19: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C6-B10C10).

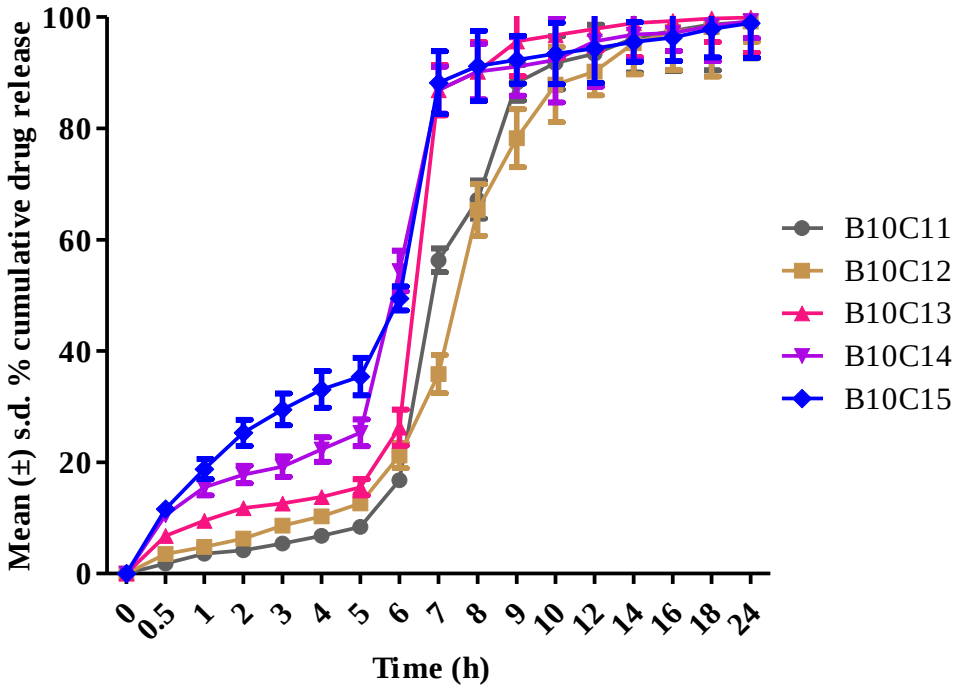


Figure 5-20: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C11-B10C15).

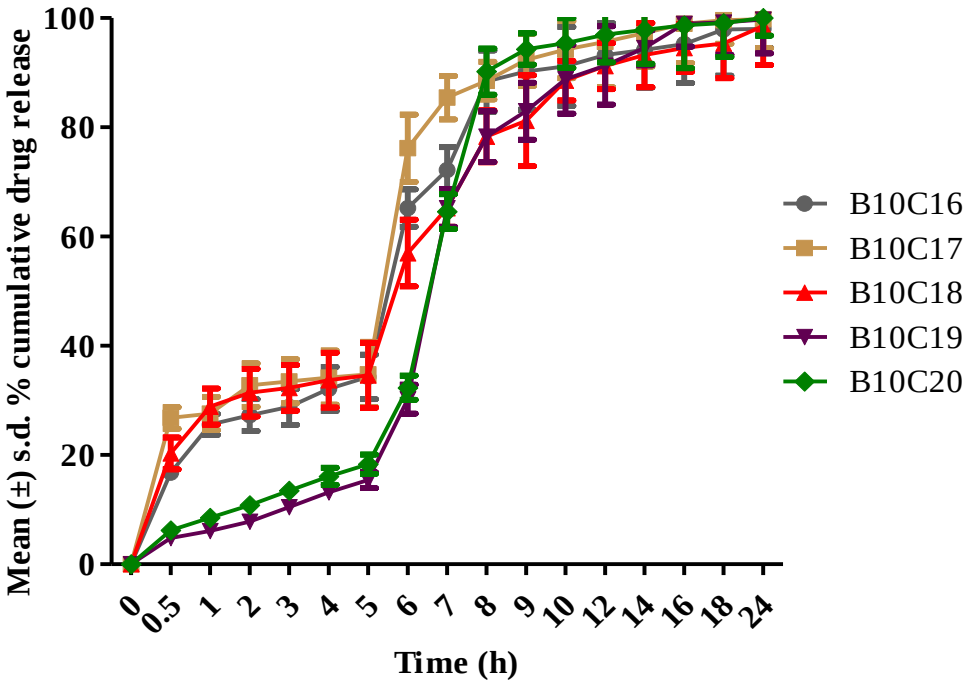


Figure 5-21: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C16-B10C20).

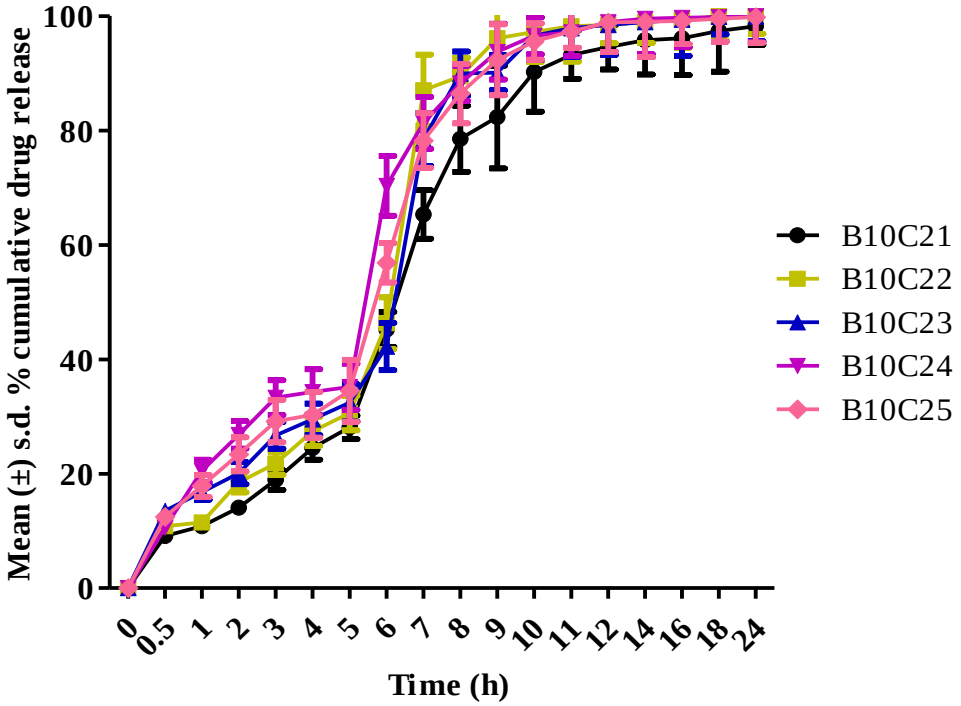


Figure 5-22: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C21-B10C25).

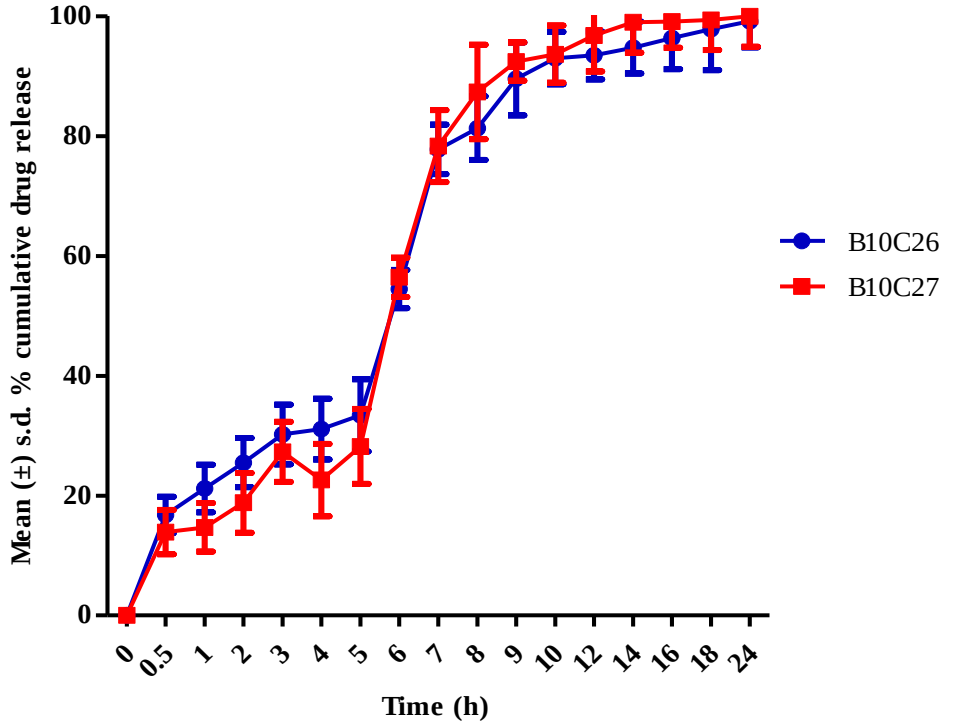


Figure 5-23: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C26 and B10C27).

5.3.3. Dissolution studies of optimized batch mini tablets

The image of B10C10 mini tablets is shown in Figure 5-24.

Figure 5-24: Final batch (Batch B10C10) of coated mini-tablets.

The results of dissolution studies indicated that batch B10C10 has shown better restriction of drug release, i.e., less than 10 % in first 5 h. Hence, it was taken for further evaluation. In order to evaluate the release profile of formulation and effect of co-administration of probiotics on site specific release, the study was conducted in 3 different colon specific media cited in literature, i.e., by addition of rat caecal contents (4.5 % w/v), probiotic culture medium and human fecal slurry (5 % w/v). In case of dissolution profile of 5-FU in rat caecal contents, 14.22 % and 21.34 % release were observed in vessels containing mini tablets alone and 28.36 % and 78.98 % release were observed in vessels containing mini tablets and probiotics at 6^{th} and 7^{th} hour (Figure 5-25). It is pertinent to add here that 100 % drug release within 10 h was observed in vessels containing mini tablets and probiotics, whereas in vessels containing mini tablets alone it was observed at 24 h. However, the release was more than 90 % for 5-FU present in mini tablets within 9 h. This indicated that addition of probiotics along with mini tablets increased the dissolution rate of 5-FU. Although significantly less ($p < 0.05$) extent of drug release was observed from the media without rat caecal contents, but here also the spiking of probiotics accelerated the dissolution rate of 5-FU. The major reason behind this could be the presence of guar gum and pectin in the core as well as in the coat that served as prebiotic and acted as substrate for probiotics to get activated. Similar results were observed in the other 2 studies also where probiotic culture and human faecal slurries were used in the

dissolution medium. In case of dissolution profile of 5-FU in human fecal slurries, 15.35 % and 20.32 % release were observed in vessels containing mini tablets alone and 25.54 % and 66.22 % release were observed in vessels containing mini tablets and probiotics at 6th and 7th hour Figure 5-26. In case of dissolution profile of 5-FU in probiotic culture medium, 14.22 % and 21.34 % release was observed in vessels containing mini tablets alone and 28.36 % and 78.98 % release was observed in vessels containing mini tablets and probiotics at 6th and 7th hour respectively Figure 5-27. The results of dissolution profiles of B10C10 were found significant ($p < 0.05$) between 8-24 h containing microbiota as compared to its profile obtained without microbiota. In case of rat caecal contents, the dissolution profiles obtained for B10C10 with probiotics was found significantly higher ($p < 0.05$) between 6-8 h as that of B10C10 subjected for dissolution study alone. In case of human faecal slurries, this significant increase in drug release was observed at 7 and 8 h. Interestingly, in case of probiotic culture, the increase in drug release was found significantly higher between 6-10 h, when B10C10 was co-subjected for dissolution studies with probiotics.

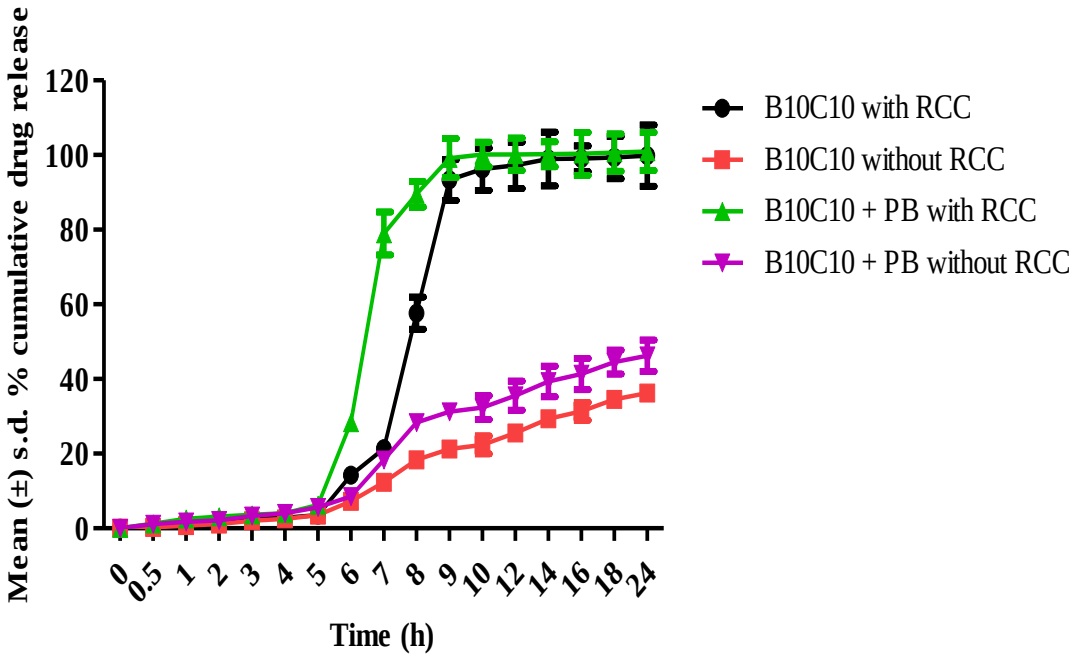


Figure 5-25: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C10) with and without probiotics in Rat Caecal Contents (RCC).

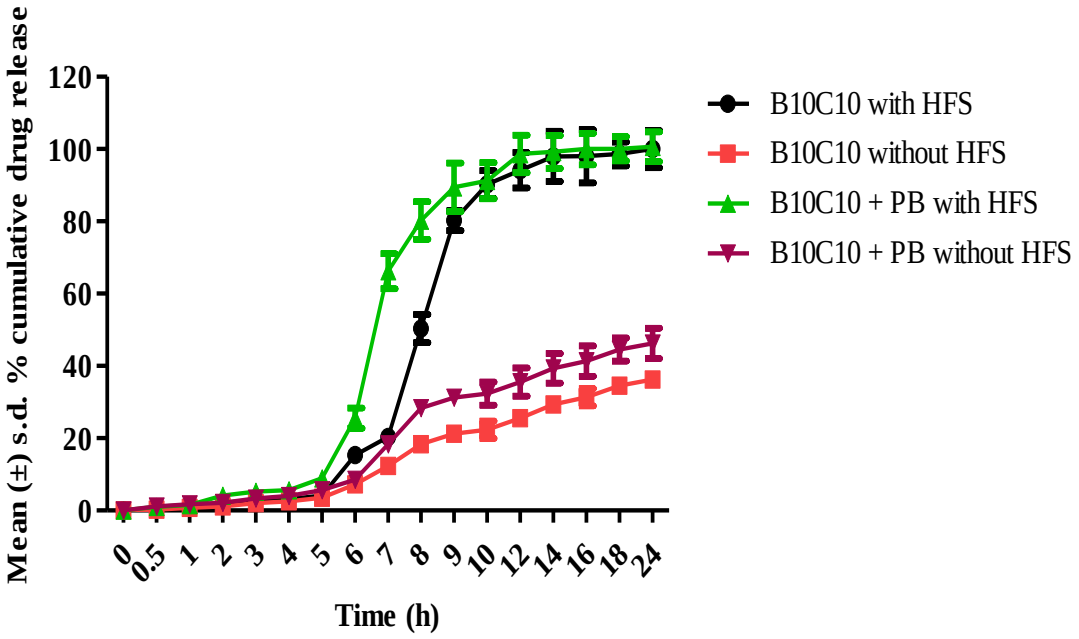


Figure 5-26: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C10) with and without probiotics in Human Fecal Slurries (HFS).

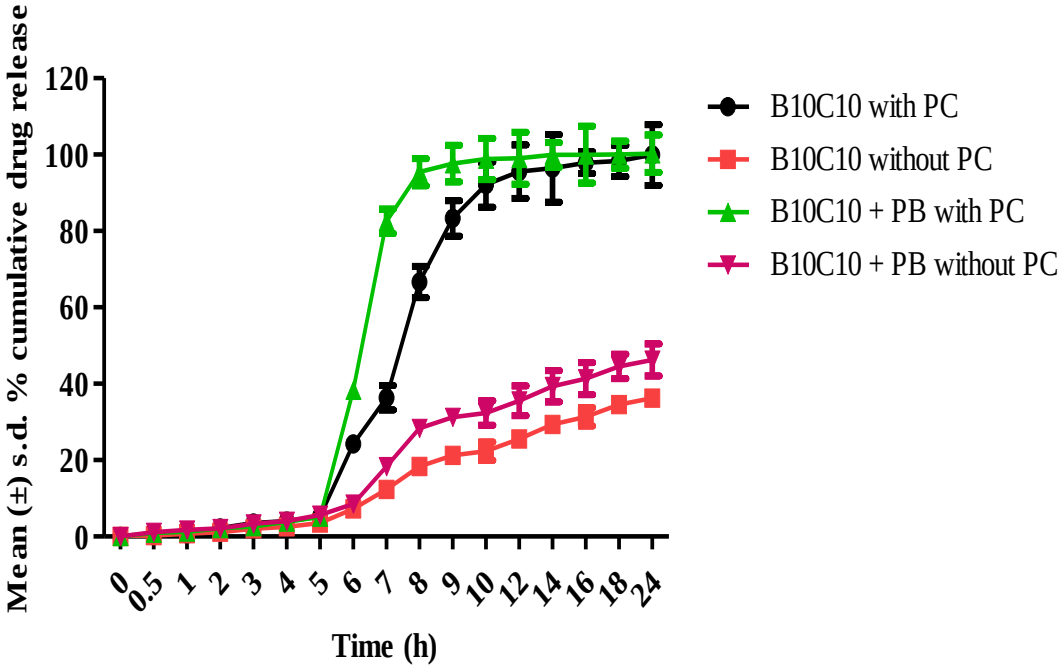


Figure 5-27: Mean (± s.d.) cumulative drug release profile of coated 5-FU tablets (batch B10C10) with and without probiotics in Probiotic Culture (PC) medium.

5.4. Cell line studies

In order to confirm the site-specific release, the studies were conducted in 2 different steps as mentioned in section 4.5. The results indicated that more than 90 % cells were viable in all the cases till 5th hour. The significant ($p < 0.05$) decline in cellular viability was observed in case of mini tablets whose dissolution study was conducted with rat caecal contents as compared to those whose dissolution study was conducted without rat caecal contents. The results are shown in Figure 5-28 and Figure 5-29. An overlay of entire cell line study is presented in Figure 5-30. At the end of 8th hour, 71.35 % and 81.63 % cells were found viable in case of mini tablets treated with and without probiotics in absence of rat caecal contents, whereas, 42.65 % and 48.33 % cells were found viable in case of mini tablets treated with and without probiotics in presence of rat caecal contents. At the end of 10th hour, 67.66 % and 77.66 % cells were found viable in case of mini tablets treated with and without probiotics in absence of rat caecal contents, whereas, 32.78 %, and 40.55 % cells were found viable in case of mini tablets treated with and without probiotics in presence of rat caecal contents. This indicated that the drug release was restricted by the polymer matrix and coat for 5 h in all the cases. Once they came in contact with rat caecal contents, the digestion of core and coat took place, and all the drug got released with complete disintegration between 5 to 10 h. This could be understood by significant increase of cell death in case of B10C10 and B10C10 + PB in rat caecal contents as 5-FU is highly cytotoxic. About 70 % of cell death was observed upon complete release of drug, i.e., concordance to previous reports (180). For better insight, the results of dissolution studies and cell viability studies were correlated and comparative table is presented wherein the percentage cell viability, percentage drug release and the amount of drug released have been correlated with respect to time (Table 5-8). The results were found opposite in case of mini tablets which were not exposed to rat caecal contents and more than 50 % cells were found viable till 24 h. The results of ANOVA indicated significant increase in cell death in case of B10C10 and B10C10 co-subjected with probiotics in rat caecal contents between 5-24 h as compared to those not exposed to rat caecal contents. However, in case of B10C10 and B10C10 co-subjected with probiotics, the significant decrease in cell death was observed with B10C10 with probiotics at 8th hour. It is also important to note that the percentage cell

viability rate and extent were found less for the mini tablets in absence of probiotics, with or without caecal contents. Hence, the results of cell line studies and dissolution studies (Figure 5-25) clearly confirmed the effect of co-administration of probiotics with mini tablets on site specific release of drug. It is important to note that, no cell death was observed in case of blank dissolution medium with or without rat caecal contents as well as placebo. To explore further, the formulations were subjected to pharmacokinetic studies.

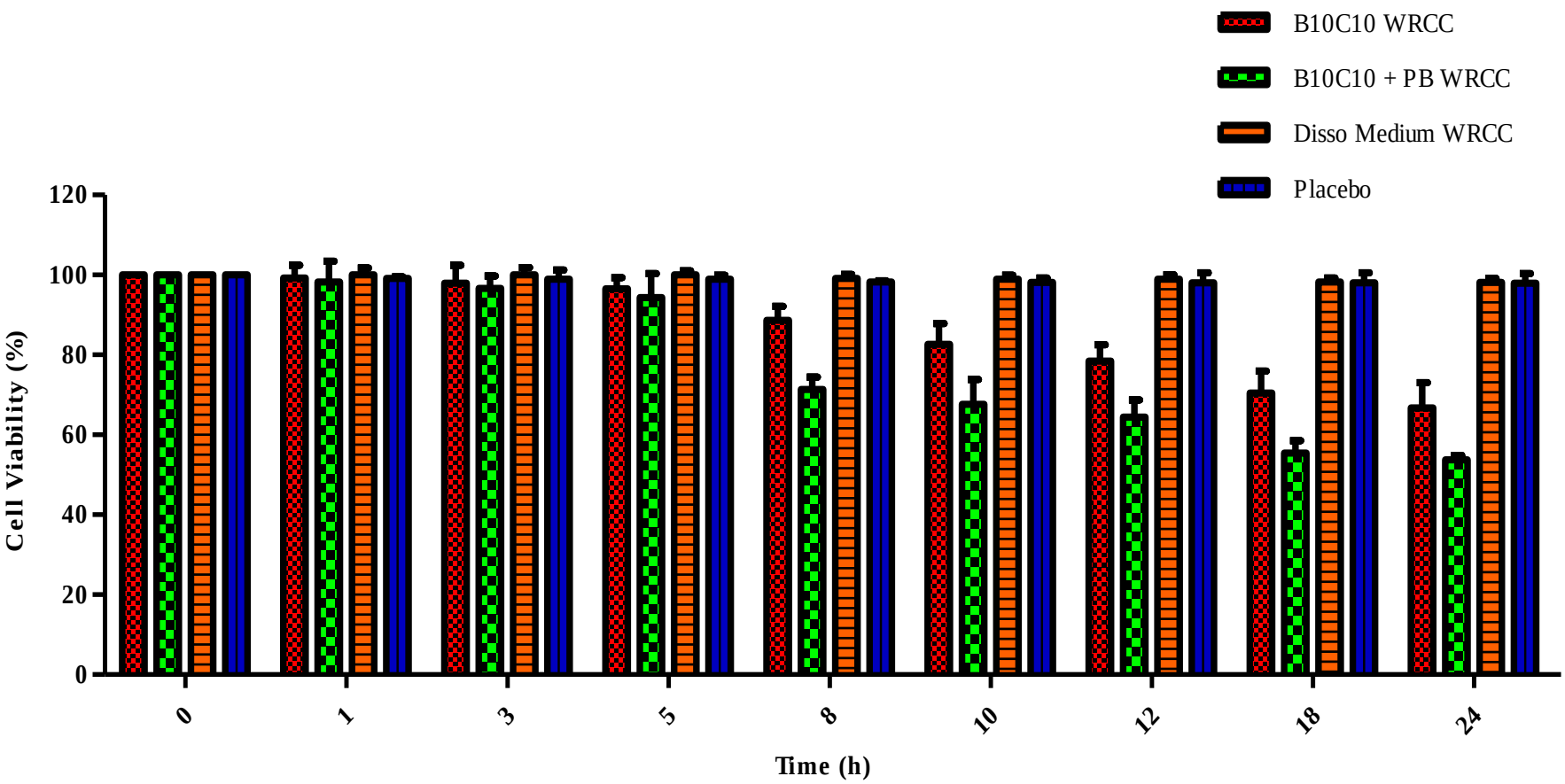


Figure 5-28: Results of cell line study (% cell viability) for mini tablets in WRCC.

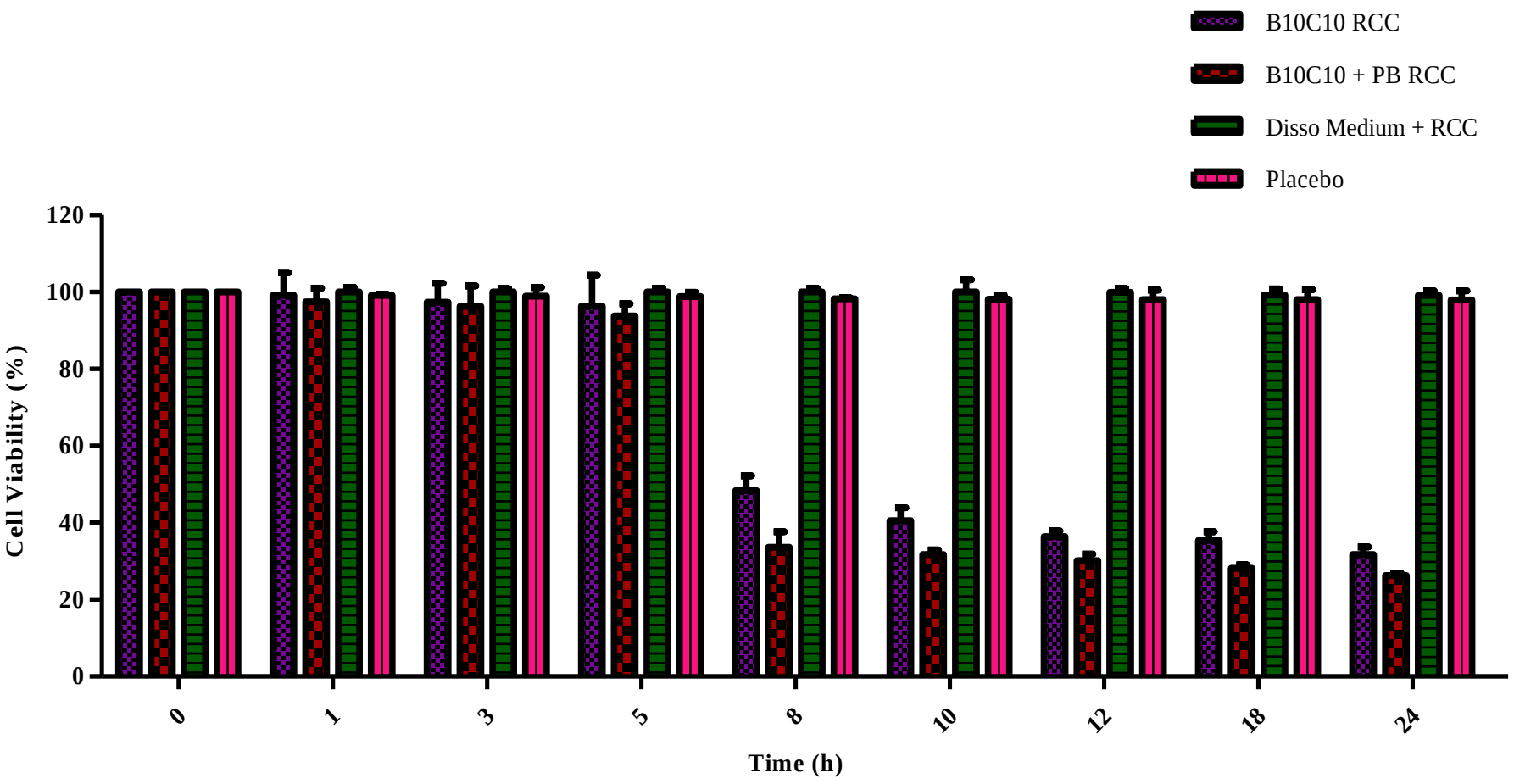


Figure 5-29: Results of cell line study (% cell viability) for mini tablets in RCC.

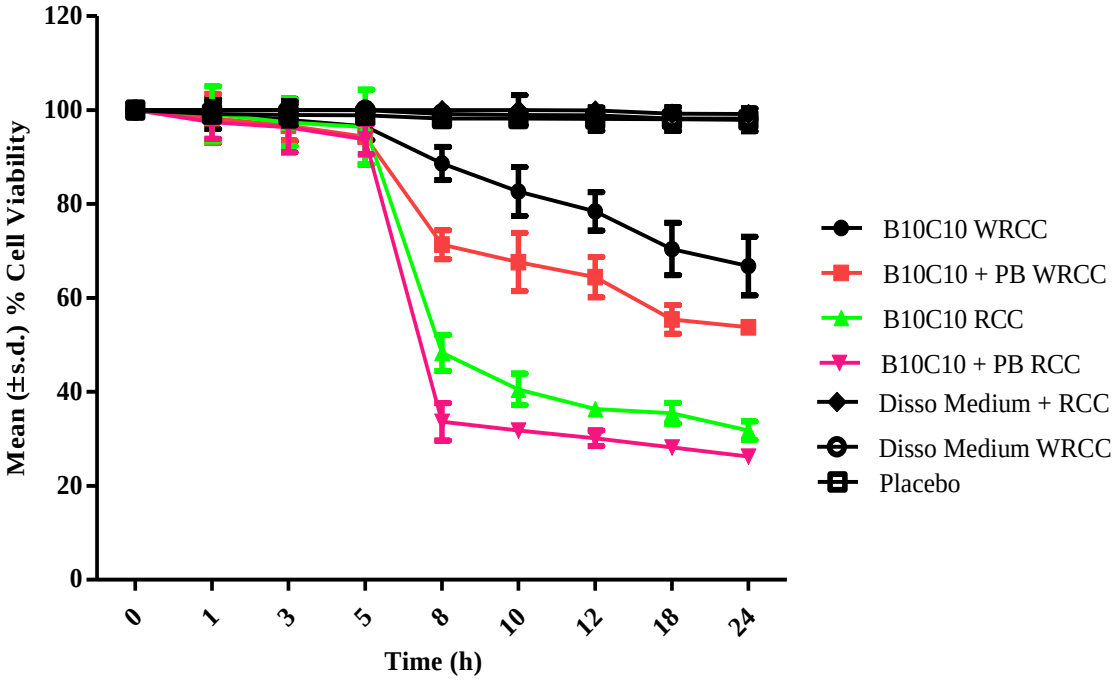


Figure 5-30: Overlay of % cell viability v/s time profile of 5-FU mini tablets.

Table 5-8: Cell viability corresponding to the % drug release from mini tablets.

Time (h)	B10C10 WRCC				B10C10 + PB WRCC				B10C10 RCC				B10C10 + PB RCC			
	% CV	% DR	Conc. (mg/900mL)	Conc. (µg/mL)	% CV	% DR	Conc. (mg/900mL)	Conc. (µg/mL)	% CV	% DR	Conc. (mg/L)	Conc. (µg/mL)	% CV	% DR	Conc. (mg/L)	Conc. (µg/mL)
5	96.50 ± 2.87	3.50 ± 0.36	0.35 ± 0.11	0.39 ± 0.11	94.35 ± 5.98	5.65 ± 1.11	0.56 ± 0.20	0.62 ± 0.20	96.40 ± 7.98	3.60 ± 0.32	0.36 ± 0.13	0.36 ± 0.13	93.78 ± 3.22	6.22 ± 0.36	0.62 ± 0.18	0.62 ± 0.18
8	88.63 ± 3.56	18.37 ± 2.12	1.84 ± 0.35	2.04 ± 0.35	71.35 ± 3.09	28.37 ± 2.10	2.84 ± 0.40	3.15 ± 0.40	42.33 ± 3.87	56.67 ± 4.32	5.76 ± 0.34	5.75 ± 0.34	38.65 ± 3.98	89.50 ± 3.46	8.95 ± 1.33	8.95 ± 1.33
10	82.66 ± 5.22	22.34 ± 2.43	2.23 ± 0.55	2.47 ± 0.55	67.66 ± 6.22	32.34 ± 3.19	3.23 ± 0.70	3.58 ± 0.70	40.35 ± 3.34	96.22 ± 5.66	9.62 ± 1.12	9.26 ± 1.12	32.78 ± 1.10	100.12 ± 3.29	10.01 ± 1.42	10.01 ± 1.42
12	78.44 ± 4.09	25.56 ± 2.17	2.55 ± 0.42	2.84 ± 0.42	64.44 ± 4.32	35.56 ± 3.87	3.55 ± 0.80	3.95 ± 0.80	36.36 ± 1.56	97.23 ± 6.18	9.72 ± 1.32	9.72 ± 1.32	30.15 ± 1.66	100.18 ± 4.37	10.02 ±1.59	10.02 ± 1.59
24	66.78 ± 6.22	36.22 ± 2.18	3.62 ± 0.65	4.02 ± 0.65	53.78 ± 1.10	46.22 ± 4.19	4.62 ± 0.90	5.13 ± 0.90	31.78 ± 1.98	99.87 ± 8.22	9.98 ± 1.12	9.99 ± 1.12	26.25 ± 0.36	100.98 ± 5.10	10.09 ± 1.53	10.09 ± 1.53

5.5. Pharmacokinetic studies

5.5.1. Bioanalytical method development and validation

The bioanalytical method was developed using the same chromatographic conditions as of in analytical method development (section 4.2.1). The chromatogram of blank plasma is shown in Figure 5-31. The retention time of 5-FU was found to be 7.595 min and 5-BU was 20.238 min (Figure 5-32). No additional matrix peaks of plasma contents were observed in the chromatogram.

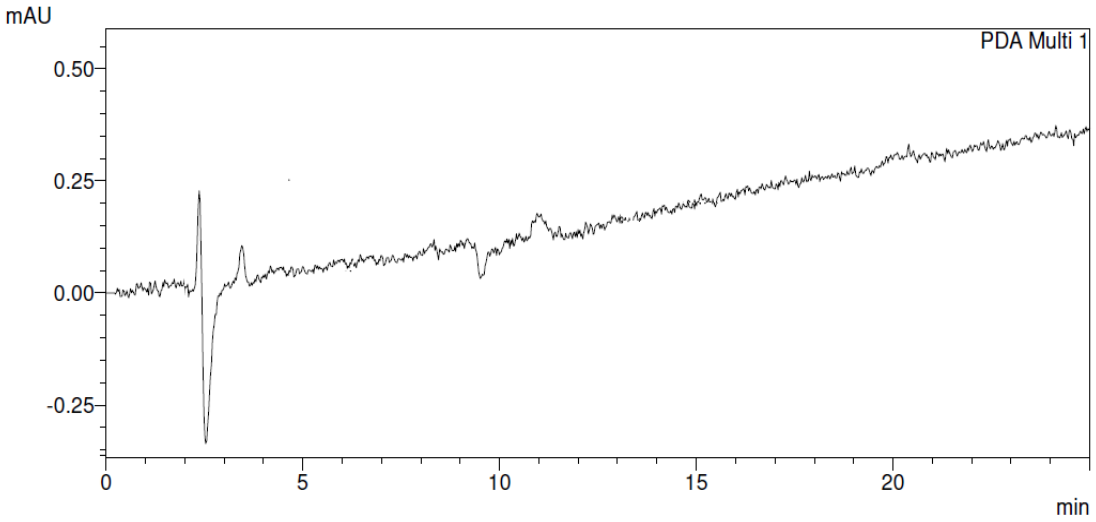


Figure 5-31: Blank chromatogram of rat plasma.

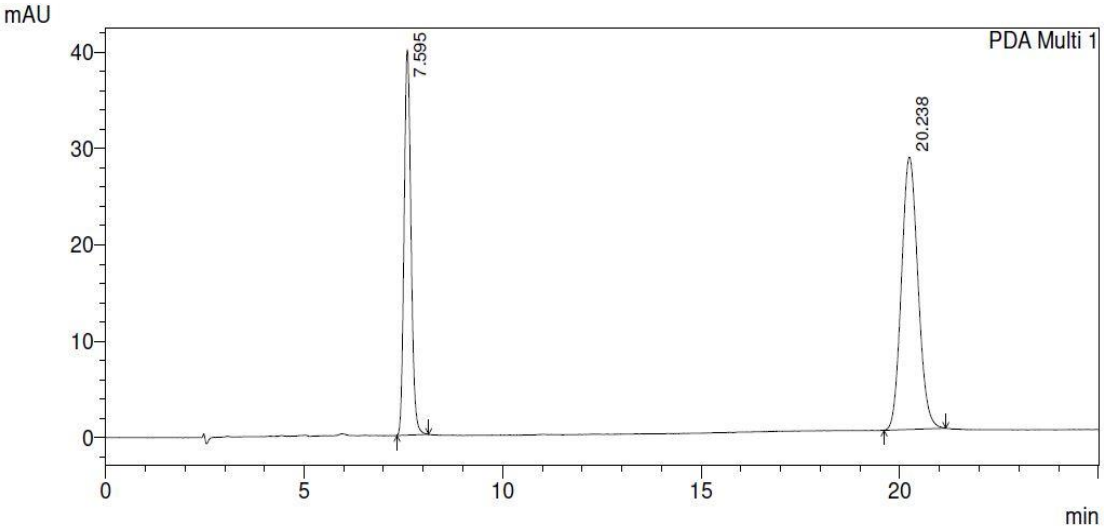


Figure 5-32: RP-HPLC chromatogram of 5-FU and 5-BU in rat plasma.

5.5.1.1. Linearity and Range

The calibration curves were developed according to procedure depicted in section 5.1.3. Both the obtained calibration curves in mobile phase as well as rate plasma were found to be linear in the range of 50-250 ng/mL with determination coefficient (r^2) of 0.998 for both the mobile phase and rat plasma (Figure 5-33 and Figure 5-34).

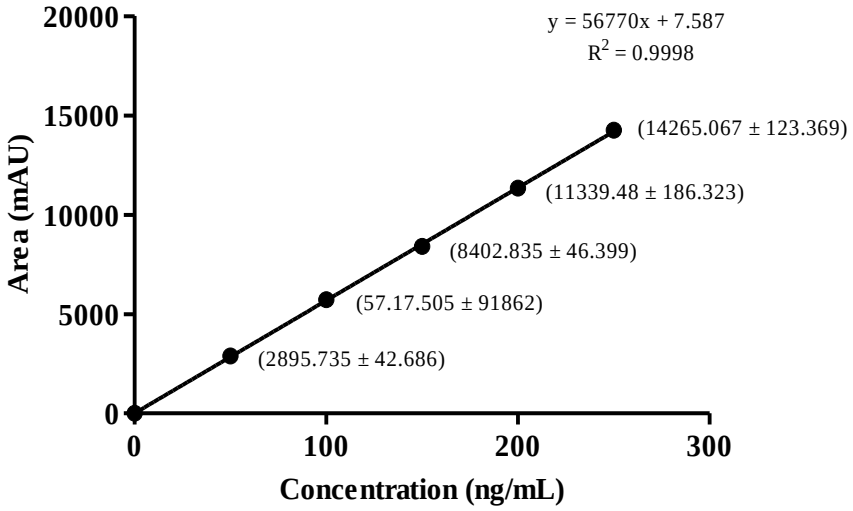


Figure 5-33: Calibration curve of 5-FU in mobile phase.

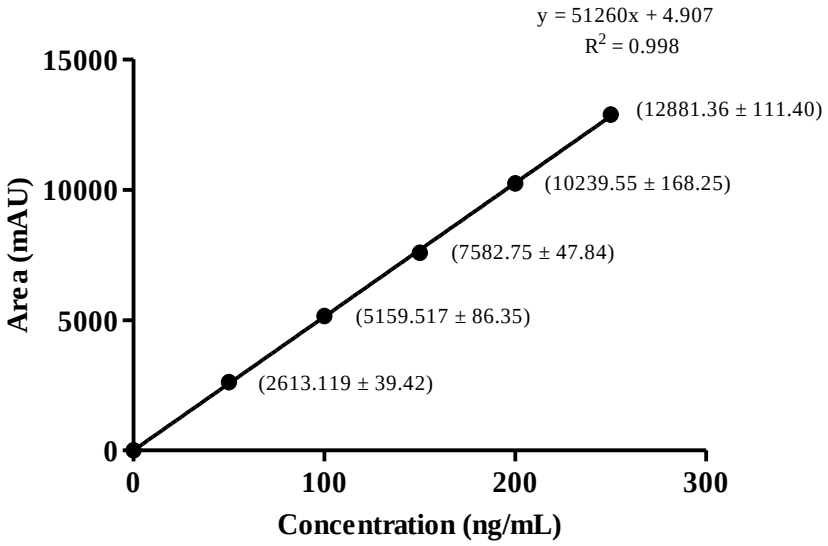


Figure 5-34: Calibration curve of 5-FU in mobile in rat plasma.

5.5.1.2. Method validation parameters

Method validation parameters, i.e., accuracy, precision, robustness, system suitability and, LOD and LOQ of the developed bio-analytical method were calculated as per the procedure mentioned in section 5.1.3. The developed bio-analytical method was found to be accurate and precise as the mean percentage recovery of all the 3 levels, i.e., LQC, MQC and HQC were found to be within the range of 95-105 % (Table 5-9) and, the percentage relative standard deviation for the 5 determinations of LQC, MQC and HQC solution at interday, intraday and interanalyst level under the same experimental condition were found to be less than 2 % (Table 5-10). The observed percentage relative deviation of the 5 determinations of MQC at deviated flow rate and the detection wavelength was found to be less than 2 %, hence indicates that the developed method was robust enough to be used for further studies (Table 5-11). System suitability parameters, i.e., HETP, theoretical plate, theoretical plate/meter and, tailing factor of peak and peak purity index were calculated for bio-analytical method (Table 5-12). The LOD and LOQ were found to be of the 0.44 ng/mL and 1.32 ng/mL respectively.

Table 5-9: Results of accuracy studies.

Levels	Concentration of standard solution (ng/mL)	Actual mean concentration (µg/mL)	% Recovery	SD	% RSD	Mean % recovey ± SD
LQC	120	117.73	98.11	1.00	1.02	98.73 ± 0.86
MQC	150	149.50	99.71	0.42	0.42	
HQC	180	177.01	98.35	0.77	0.78	

Table 5-10: Results of precision studies.

Parameters	Levels	Conc. (ng/mL)	Analytical response (area, injections) 1	2	3	4	5	Mean (N = 5)	SD	% RSD
Repeatability (Intraday precision)										
	LQC	120	6639.13	6832.19	6672.88	6722.21	6609.10	6695.10	87.39	1.30
	MQC	150	8498.42	8465.72	8366.92	8430.87	8352.22	8422.83	62.71	0.74
	HQC	180	10158.87	10131.38	10231.78	10089.13	9807.69	10083.77	162.84	1.61
Intermediate precision (Interday)										
Day 1	LQC	120	6595.08	6786.13	6678.93	6712.17	6921.34	6738.73	122.99	1.82
	MQC	150	8462.20	8552.32	8496.32	8316.02	8421.92	8449.75	88.74	1.05
	HQC	180	10078.11	10011.67	10011.07	10213.07	9878.87	10038.56	121.45	1.21
Day 2	LQC	120	6498.10	6603.08	6709.13	6688.70	6706.83	6641.17	90.98	1.37
	MQC	150	8579.98	8613.73	8575.71	8778.31	8576.88	8624.92	87.18	1.01
	HQC	180	10040.37	10117.05	10021.11	10017.03	10069.42	10053.00	41.36	0.41
Day 3	LQC	120	6639.13	6832.19	6672.88	6722.21	6609.10	6695.10	87.39	1.30
	MQC	150	8498.42	8465.72	8366.92	8430.87	8352.22	8422.83	62.71	0.74
	HQC	180	10158.87	10131.38	10231.78	10089.13	9807.69	10083.77	162.84	1.61
Intermediate precision (Intraday)										
Analyst 1	LQC	120	6512.12	6734.23	6631.57	6723.98	6854.37	6691.25	127.65	1.91
	MQC	150	8342.23	8424.21	8423.09	8316.08	8367.78	8374.67	48.30	0.57
	HQC	180	9975.32	10042.80	9957.08	10412.00	9753.87	10028.21	240.13	2.39
Analyst 2	LQC	120	6413.08	6683.06	6678.43	6637.87	6678.83	6618.25	116.16	1.75

	MQC	150	8532.67	8598.13	8513.41	8724.81	8534.09	8580.62	86.72	1.01
	HQC	180	9978.32	10009.80	9984.12	9987.93	10061.10	10004.25	33.94	0.34
Analyst 3	LQC	120	6628.78	6768.30	6645.71	6774.80	6703.12	6704.14	67.46	1.00
	MQC	150	8447.89	8412.45	8313.78	8389.18	8453.09	8403.27	56.50	0.67
	HQC	180	9897.90	10098.40	10170.30	10109.90	9987.70	10052.84	108.84	1.08

Table 5-11: Results of robustness studies of various parameters tested for 5-FU in rat plasma.

Variables	**Value**	**Conc. (ng/mL)**	**Mean peak area**	**SD**	**% RSD**	**Mean R_t (min)**	**SD**	**% RSD**
Flow rate (mL/min)	0.8	150	10066.20	50.65	0.50	8.97	0.10	1.14
	1.0	150	8422.83	62.71	0.74	7.64	0.08	1.08
	1.2	150	6553.88	45.43	0.69	6.03	0.04	0.72
Wavelength (nm)	264	150	8460.40	61.89	0.73	7.64	0.08	1.08
	266	150	8422.83	62.71	0.74	7.64	0.08	1.08
	268	150	8363.526	54.99	0.66	7.63	0.08	1.08

Table 5-12: Results of system suitability parameters.

Parameters	**Value**
HETP	19.79
Theoretical plate	7577.92
Tailing factor	1.14
Peak purity index	0.999

5.5.2. *In vivo* pharmacokinetic studies

The uncoated mini tablets (B10), coated mini tablets (B10C10), and coated mini tablets (B10C10) with probiotics (500 mg) were administered orally to rats of 3 different groups. The non-compartmental analysis was carried out and pharmacokinetic parameters were calculated. The results are shown in Table 5-13 and Figure 5-35. It is important to note that the rate of absorption of drug is determined by its T_{max} and extent of drug's absorption is decided by $AUC_{0-\infty}$, whereas, C_{max} indicates both of them (8, 181, 182). Hence, these parameters are useful to assess comparative bioavailability of drug. In order to analyse the level of significance paired "t" test was applied. The results indicated that T_{max} of uncoated tablet appeared at 3 h, whereas, it was found at 12 h for coated mini tablets and 9 h for mini tablets with probiotics. The results of pharmacokinetic parameters of 5-FU (uncoated tablets) are found similar to the previously published reports (183). This indicated that the uncoated tablet might have got disintegrated and appeared immediately in plasma because of faster absorption. The significant ($p < 0.05$) shift in T_{max} of 5-FU present in coated mini tablets with or without probiotics after 5 h indicated colon targeting efficiency of the developed formulation. It is important to note that the T_{max} of mini tablets administered with probiotics was found 3 h earlier than that of mini tablets administered alone. This indicated that probiotics have increased the release rate of drug by rapidly eating the polysaccharide coat and matrix thereby disintegrating the tablet. Furthermore, the elimination half-life ($t_{1/2}$) for uncoated 5-FU tablet was found to be 2 h, coated tablet without probiotic 6.42 h, and coated tablet with probiotic 5.72 h. This delay in $t_{1/2}$ for coated tablets as compared to uncoated one is also indicator of delayed absorption of drug and shorter $t_{1/2}$ for coated tablets administered with probiotics as that of tablets without probiotics is due to rapid digestion of polysaccharides. The $AUC_{0-\infty}$ was found maximum for uncoated tablets followed by coated tablets without probiotics then uncoated tablets co-administered with probiotics, indicating higher extent of absorption of drug from uncoated tablet. Higher AUC is the indication of higher bioavailability of drug in plasma as well as higher biodistribution of drugs to all the body parts. In case of 5-FU uncoated tablets, the release of drug and its absorption at first 3 h has indicated higher distribution of drug at the non-targeted site. Moreover, elimination of drug was observed after 5^{th} hour

that indicated poor site specificity of uncoated mini tablets. Smaller AUC and C_{max} of 5-FU from coated mini tablets with/without probiotics indicated poor absorption of drug from colon to the blood, indicating its local and restricted delivery as colonic site (184). The MRT was found maximum for 5-FU mini tablets co-administered with probiotics as that of the one administered alone. The longer MRT values indicated that 5-FU remained at the colonic site for longer time. The longer retention of 5-FU at the colonic site ensures prolonged action of drug at colonic site which could be helpful in providing longer duration of action of drug. The 'P value' was found to be less than 0.05 in the plasma concentration versus time profiles of coated min tablets co-administered with probiotics and without probiotics as compared to uncoated tablets, indicating significant difference in their pharmacokinetic profile. Moreover, a significant ($p < 0.05$) increase in plasma concentration of 5-FU was observed at 7^{th} hour in case of mini tablets co-administered with probiotics as compared to mini tablets administered without probiotics.

In a nutshell, the obtained results of dissolution, Caco-2 cell line and pharmacokinetic studies indicated that the developed formulation is targeting the drug to colon and better site specificity was obtained when the tablets were co-administered with probiotics.

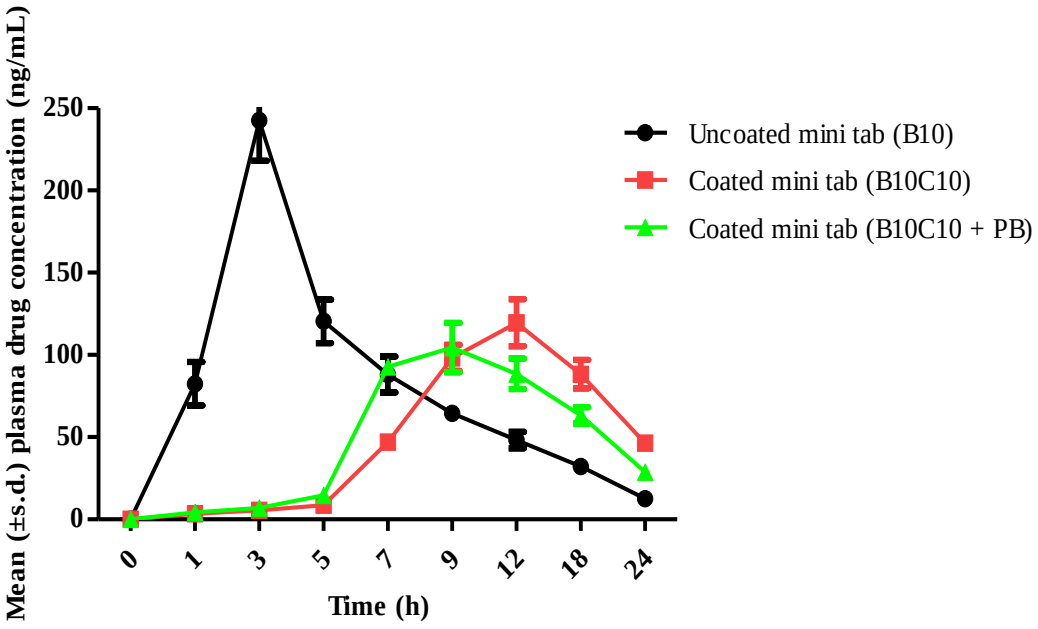


Figure 5-35: Mean (± s.d.) plasma concentration versus time profile of formulations.

Table 5-13: Results of pharmacokinetic parameters obtained for the formulations.

Parameters	Uncoated mini tablets (B10)	Coated mini tablets (B10C10)	Coated mini tablets (B10C10 + PB)
$t_{1/2}$ (h)	2.00 ± 0.13	6.42 ± 0.87	5.72 ± 0.56
T_{max} (h)	3.00 ± 0.23	12.00 ± 1.43	9.00 ± 1.12
C_{max} (ng/mL)	242.55 ± 24.56	119.56 ± 14.22	104.55 ± 14.98
AUC_{0-t} (ng/mL*h)	1494.70 ± 87.22	1299.80 ± 78.21	1104.99 ± 98.54
$AUC_{0-\infty}$ (ng/mL*h)	1609.70 ± 66.86	1437.67 ± 65.43	1235.31 ± 89.33
MRT (h)	9.84 ± 3.21	14.80 ± 2.12	17.60 ± 3.18

5.6. Stability studies

The 6 months stability studies of mini tablets at 2 different stability conditions, i.e., 25 ± 0.2°C/65 ± 5 % RH and 40 ± 0.2°C/75 ± 5 % RH, indicated that the tablets were found stable as no significant change ($p < 0.05$) was observed in parameters such as assay, hardness, friability and dissolution profiles of fresh and aged mini tablets. The f2 values between the dissolution profiles of fresh and aged tablets were found to be more than 50, indicating similar dissolution profile (Figure 5-36). The f2 value between fresh and aged tablets kept at 25 ± 0.2°C/65 ± 5 % RH was found to be 55.46 and 51.77 for tablets kept at 40 ± 0.2°C/75 ± 5 % RH.

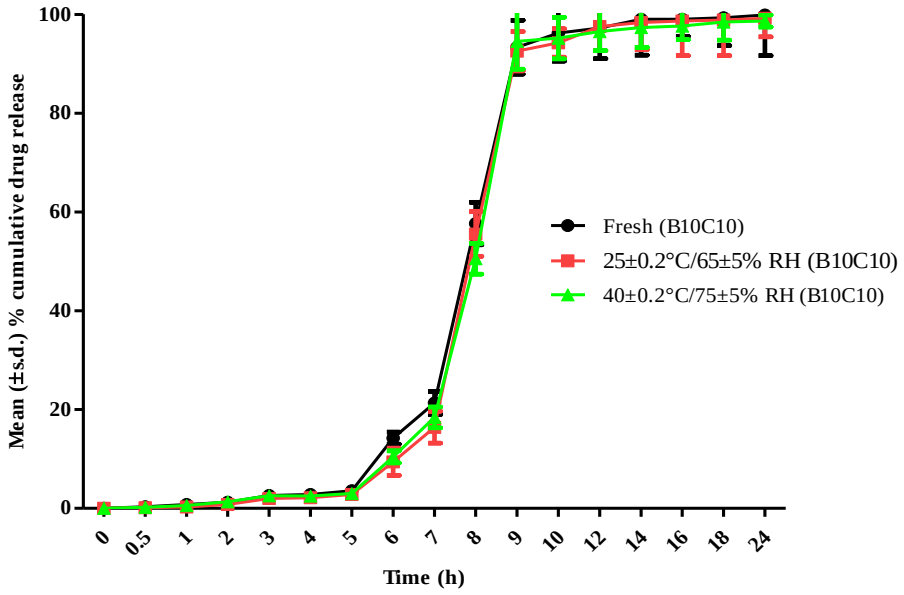


Figure 5-36: Mean (± s.d.) % cumulative drug release profile of aged and fresh samples kept at various storage conditions.

Chapter 6
Conclusion

This is the first report of the co-administration of colon targeted mini tablets of 5-FU with probiotics wherein natural polysaccharides serve dual purpose at 2 stages of its GI sojourn, i.e., providing impervious coating till its entry into colon and as prebiotic after its entry into colon. Colon targeted mini tablets were prepared using dual coating of pH sensitive polymer Eudragit® S100 and combination of 2 colon specific polysaccharides Guar gum and pectin. The prepared mini tablets were found to be compliant to the compendial Quality Control (QC) parameters. In dissolution studies, the mini tablets showed burst release after 5 h on addition of colonic contents indicating successful coating.

The pharmacokinetic studies showed a lower as well as delayed plasma concentration in the coated formulations endorsing the original hypothesis of reduction in drug exposure of the non-target sites. The C_{max} reached in plasma for coated mini tablets with and, without probiotics was 104.55 ± 14.98 ng/mL (0.104 µg/mL) and 119.56 ± 14.22 ng/mL (0.119 µg/mL) respectively. Interestingly, these concentrations were found to be non-toxic in the cell line studies (Table 5-8), indicating that the concentration of 5-FU reaching the non-target sites through systemic circulation will be non-toxic.

It is, therefore, concluded that mini tablets of 5-FU prepared using Eudragit® S100 and colon specific polysaccharides with concomitant administration of probiotics offer an efficient, scalable and cost-effective treatment of colon cancer. In fact, the dosage form developed seems to offer a platform technology for delivery of all drugs meant to be delivered specifically to the colon.

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APPENDICES

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

Division of Academic Affairs

LPU/DAA/EC/160204/040

Dated: 4th Feb 2016.

Ankit Kumar

Nagla Aman, Nepai

Kotla Road, Firozabad

Uttar Pardesh- 283203.

Subject: Letter of Candidacy for Ph.D.

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. degree on 12th Oct 2015 by accepting your thesis research proposal titled:

"Formulation and evaluation of colon targeted mini tablets of 5- Fluorouracil", supervised by Dr. Sachin Kumar Singh, Associate Professor, at Lovely Professional University, Phagwara, Punjab, and Co- supervised by Dr. Monica Gulati, Sr. Dean, at Lovely Professional University, Phagwara, Punjab.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. degree students of the University, and amendments, if any, made from time to time.

We wish you the very best in completing your thesis research requirements in the near future. Please do not hesitate to contact us in case you have questions about the rules and regulations of the University.

Signature

CENTRAL ANIMAL HOUSE FACILITY (CAHF)

Lovely School of Pharmaceutical Sciences, Lovely Professional University

Ludhiana- Jalandhar G.T. Road, Phagwara (Punjab), 144402

Registration Number -954/PO/Re/S/06/CPCSEA

CERTIFICATE

This is to certify that the project titled *"Formulation and evaluation of colon targated mini tablets of 5-Fluorouracil" has* been approved by the IAEC.

Name of Principal Investigator: Dr. Sachin Kumar Singh

IAEC approval number: LPU/IAEC/2019/ 48

Date of Approval: 16.2.2019

Animals approved: 24 SD Rats, Male

Remarks if any: -NA

Dr. Monica Gulati	**Dr. Navneet Khurana**	**Dr. Bimlesh Kumar**
Biological Scientist, Chairperson IAEC	Scientist, COD Pharmacology	Scientist In-Charge of Animal House, Member Secretary IAEC

Publications

- Singh SK, **Yadav AK**, Prudhviraj G, Gulati M, Kaur P, Vaidya Y. A novel dissolution method for evaluation of polysaccharide based colon specific delivery systems: a suitable alternative to animal sacrifice. European Journal of Pharmaceutical Sciences. 2015 Jun 20;73:72-80.
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Book chapters

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Presentations

- **Yadav AK**, Singh SK, Gulati M. Oral delivery of therapeutics to colon: Opportunities and limitations. Presented in Apsecrcon 2019 on the theme "Artificial intelligence: The New Era in Pharmaceutical Sciences", held at Abhilashi College of Pharmacy, Mandi, Himachal Pradesh, India. (13^{th} April 2019)
- **Yadav AK**, Singh SK, Gulati M. Role of colonic microflora in drug release of polysaccharide based oral colon targeted delivery systems. Presented in International Conference of Pharmacy (ICP-2017) held at Lovely Professional University, Punjab, India. (7^{th} to 8^{th} April 2017)

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A novel dissolution method for evaluation of polysaccharide based colon specific delivery systems: A suitable alternative to animal sacrifice

Sachin Kumar Singh, Ankit Kumar Yadav, G. Prudhviraj, Monica Gulati *, Puneet Kaur, Yogyata Vaidya

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144411, Punjab, India

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ABSTRACT

The most extensively used test for predicting in-vivo release kinetics of a drug from its orally administered dosage forms is dissolution testing. For polysaccharide based, colon targeted oral delivery systems, the entire path of the gut traversed by the dosage form needs to be simulated for assessing its in-vivo dissolution pattern. This includes the dissolution testing sequentially in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF). For SGF and SIF, simple and standardized composition is well-known. However, preparation of SCF requires addition of either the colonic contents of rodents or human faecal slurry. A method is proposed, wherein a mixture of five probiotics cultured in the presence of a prebiotic under anaerobic conditions is able to surrogate the colonic fluid. Release profiles of drug from colon targeted delivery systems in this medium were studied and compared to those generated in the conventionally used media containing rodent caecal contents and human faecal slurry. The results from the three studies were found to be quite similar. These findings suggest that the proposed medium may prove to be useful not only as a biorelevant and discriminatory method but may also help in achieving the 3Rs objective regarding the ethical use of animals.

1. Introduction

Dissolution testing is the most commonly employed in-vitro evaluation technique for predicting the in-vivo drug release kinetics from orally administered dosage forms including the modified release delivery systems (Yang, 2008; Kotla et al., 2014). There are two predominant challenges involved in designing dissolution methods for site specific delivery systems. First challenge involves creating a micro-environment that is a physiological replica of the biological milieu at the site of drug release. Second major challenge lies in simulating the conditions that the drug delivery system is likely to encounter during its transit from mouth to target site (Tomlinson, 1988). The more diverse the milieus encountered by the formulation, the more complex the dissolution method becomes. Due to the distal location of colon, the colon targeted oral delivery systems traverse the longest path, coming across the most varied milieus. Designing a suitable biorelevant medium, therefore, has proven to be a complex task. The situation is further complicated by the varied approaches that have been used for achieving colon targeting. The most commonly used strategies to formulate colon-specific drug delivery systems include timed release systems, prodrugs, pH sensitive polymer coating and colonic microflora activated delivery systems (Gulati et al., 2012; Rubinstein, 1995; Yang et al., 2002; Muraoka et al., 1998; Philip et al., 2008). Among these, the microflora activated delivery systems have been found to be quite promising. An abrupt increase in the number of microbiota and the resultant increase in enzymatic activities in the ascending colon represent an exclusive and non-continuous event independent of GI transit time and pH (Ibekwe et al., 2008; Yang et al., 2001; Prasad et al., 1998; Krishnaiah et al., 2002).

Human colon represents a dynamic and ecologically diverse environment, comprising over 400 distinct species of bacteria with a population of 10^{11}–10^{12} CFU/mL of colonic contents. These bacteria produce wide spectrum of reductive and hydrolytic enzymes, which are responsible for many biorelevant processes like carbohydrate and protein metabolism (Yang, 2008). Hence, in order to simulate the colonic milieu in vitro, the methodology should be designed in such a way that it involves the microbiota of colon. At the same time, the method should be convenient, inexpensive and reproducible.

The methods that have been reported for evaluation of colon targeted delivery systems include triggering by enzymes (Philip et al., 2008; Fetzner et al., 2004; Macfarlane et al., 1989; Maculotti et al., 2009; Maestrelli et al., 2008; Liu et al., 2012; Semde et al., 2000a,b Omar et al., 2007; Jain et al., 2007a,b; Lai

* Corresponding author at: Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144 401, India. Tel.: +91 9915020408; fax: +91 1824501900.
E-mail address: monicagulati14@gmail.com (M. Gulati).

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Research Paper

Effect of co-administration of probiotics with polysaccharide based colon targeted delivery systems to optimize site specific drug release

G. Prudhviraj [a], Yogyata Vaidya [a], Sachin Kumar Singh [a], Ankit Kumar Yadav [a], Puneet Kaur [a], Monica Gulati [a,*], K. Gowthamarajan [b]

[a] School of Pharmaceutical Sciences, Lovely Professional University, Punjab 144 401, India
[b] Department of Pharmaceutics, J.S.S. College of Pharmacy, Rocklands, Ooty, Tamil Nadu 643001, India

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ABSTRACT

Significant clinical success of colon targeted dosage forms has been limited by their inappropriate release profile at the target site. Their failure to release the drug completely in the colon may be attributed to changes in the colonic milieu because of pathological state, drug effect and psychological stress accompanying the diseased state or, a combination of these. Alteration in normal colonic pH and bacterial picture leads to incomplete release of drug from the designed delivery system. We report the effectiveness of a targeted delivery system wherein the constant replenishment of the colonic microbiota is achieved by concomitant administration of probiotics along with the polysaccharide based drug delivery system. Guar gum coated spheroids of sulfasalazine were prepared. In the dissolution studies, these spheroids showed markedly higher release in the simulated colonic fluid. *In vivo* experiments conducted in rats clearly demonstrated the therapeutic advantage of co-administration of probiotics with guar gum coated spheroids. Our results suggest that concomitant use of probiotics along with the polysaccharide based delivery systems can be a simple strategy to achieve satisfactory colon targeting of drugs.

1. Introduction

Earlier considered to be a disease of developed and industrialized countries, ulcerative colitis (UC) has seen a rampant rise in developing countries [1]. Past few decades have witnessed a sharp rise in the cases of Inflammatory Bowel Disease (IBD) in Asia [2]. Incidence rate of UC is reported to vary from 0.5 to 31.5 per 10^5 persons worldwide [3]. In Asia, however, it is reported as 5.3–53.6 per 10^5 [4,5], as compared to 37.5–238 per 10^5 persons in North America [6].

UC is a pathological condition characterized by chronic inflammation of large bowel. Patients suffer from bloody diarrhoea and generally the stool culture does not show the presence of bacteria or parasites. A number of factors contribute towards the etiopathogenesis of the disease. These include breakdown in patient's immune system [7], abnormal mucosal immune response against colonic microflora [8], environmental factors [9], genetic factors [10,11] and oxidative damage [12].

Human gut is host to a plethora of microorganisms, majority comprising of bacteria, though some fungi and protozoa also exist [13,14]. Commensal bacteria play an important role in maintaining the health of an individual. They prevent the predominance of pathogenic bacteria as they compete with them for space and food. They also produce certain molecules which have antibacterial properties and prevent the colonization by pathogenic bacteria [15].

UC is always accompanied by an imbalance between the beneficial and harmful bacteria in the colon. There is an overall decrease in *Bacteroides*, particularly *Bacteroides vulgates* [16], *Eubacterium*, *Lactobacillus* and *Bifidobacteria* and an increase in *E. coli* and *Clostridia* in IBD patients [17]. At some point, the immune tolerance to colonic microflora is broken and the host activates immune reaction against the colonic bacteria [18]. This causes inflammation of mucosa which is a characteristic feature of UC [13].

Conventional treatment of UC is aimed at reducing or eliminating the inflammation of colonic mucosa, as well as, combating other symptoms of the disease. Traditionally, aminosalicylates have played a central role in treatment of the disease [19]. Other alternatives are corticosteroids, immunomodulators such as azathioprine (AZA), 6-mercaptopurine (6MP), infliximab and cyclosporine [19,20]. Drugs such as steroids, AZA, 6 MP, and

* Corresponding author at: Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144401, India. Tel.: +91 9915020408; fax: +91 1824501900.
E-mail address: monicagulati14@gmail.com (M. Gulati).

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Polymorph control: Success so far and future expectations

Renuka, Sachin Kumar Singh*, Ankit Kumar Yadav, Monica Gulati, Amit Mittal, Rakesh Narang, Varun Garg

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, 144401, Punjab, India

Abstract: The phenomenon of the appearance and disappearance of polymorphs has been an enigma for the chemists and formulators alike. The ability to successfully and consistently produce the specific stable polymorphs directly affects the efficiency and speed of drug development, the robustness of manufacturing process, and quality and stability of APIs. Though a number of diverse methods have been utilized and reported for polymorph control, reliable techniques for polymorph control still remain far from perfect. This article discusses different methods for polymorph control like supersaturation, antisolvent addition, temperature and pH control and addition of additives. It also deliberates on their successful applications over the last two decades.
Keywords: Polymorphs, Stability, Supersaturation, Antisolvent, Temperature, pH control, Additives.

Introduction

Polymorphism is a solid-state phenomenon involving different forms of the same chemical compound which have distinctive properties[1, 2]. Need to control polymorph formation has been viewed as a critical issue by the pharmaceutical industry since a long time. Polymorphism was perceived as major problem in the development of azo pigments and copper phthalocyanide for the dye industry a century back in history also[3]. More recently, polymorphism of drugs, due its newly found applications has evoked an intense interest in the pharmaceutical industry[4]. The properties most often affected by polymorphism which are of utmost relevance in the pharmaceutical industry include stability, solubility, bioavailability and density. A distinguishing feature of polymorphs is their structural difference which can be observed as a difference in the spatial arrangement of the atoms of the molecule or as a difference in packing arrangements of the molecules in the unit cell[2]. The structural differences in turn, may, or may not affect the physiochemical properties of the compound in the solid state. Polymorphs can be prepared by normal traditional methods as well as by high throughput technologies. However, one of the most challenging properties of polymorphs is the difficulty of consistently preparing the desired polymorph in a pure form. Preparation of polymorphs can be accomplished by traditional methods such as crystallization from solutions and crystallization from melts[4], as well as modern methods such as supercritical fluid crystallization[5, 6], capillary crystallization[7, 8, 9], non-photochemical laser-induced nucleation[10], crystallization with polymer heteronuclei[11, 12], and template-assisted crystallization[13]. A potentiometric method for the crystallization of polymorphic forms of active pharmaceutical ingredients (APIs) has also been described[14]. Polymorphism has been extended to include zwitterions of acids containing amide groups able to accept protons[3]. The problems associated with polymorphism and the possible methods for isolating the desired polymorphs have been reported by Kitamura, 2009 and Llinas and Goodman[4, 15]. It has been suggested that the production of polymorphs in their pure form may be possible by applying thermodynamic and kinetic principles to control polymorph formation[16]. A number of reports exist about the quest for suitable solvents, or mixtures of

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Research Article

INFLUENCE OF FORMULATION PARAMETERS ON DISSOLUTION RATE ENHANCEMENT OF PIROXICAM USING LIQUISOLID TECHNIQUE

PEMBA LAHMO, SACHIN KUMAR SINGH*, VARUN GARG, PEDDI MAHARSHI, ANKIT KUMAR YADAV, MONICA GULATI, BIMLESH KUMAR, NARENDRA KUMAR PANDEY, PRABHJOT KAUR, RAKESH NARANG, AMIT MITTAL

Department of Pharmacy, School of Pharmaceutical Sciences, Lovely Professional University, Punjab, India.
Email: singhsachin23@gmail.com

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ABSTRACT

Objective: This study revealed formulation of a liquisolid system of poorly soluble piroxicam to enhance its dissolution rate. To formulate a liquisolid system loaded with piroxicam, solubility study was carried out in various non-volatile liquids.

Methods: In 1 ml of polyethylene glycol (PEG) 600, 100 mg piroxicam was added and stirred with gentle heating. To the above liquid medication, 1 g microcrystalline cellulose (MCC) 102 (as MCC has given better results), 1 g Syloid 244 FP, 2 g PEG 4000, 500 mg aerosil 200, and 0.255 g sodium starch glycolate (SSG) (5%) were added and mixed properly. The blend was compressed and subjected for quality control parameters.

Results: Among all the non-volatile liquids evaluated, piroxicam was most soluble in PEG 600. Using this as liquid medication, several liquisolid compacts were prepared by varying the ratios of MCC PH 102 as carrier and Syloid 244FP as coating material and evaluated for precompression studies. To further accelerate the release of drug, various additives were added in the formulation. Among them, PEG 4000 has shown better flow as well as compression properties. Hence, the final formulation (LS-16B) was prepared using a combination of MCC PH 102, Syloid 244 FP, PEG 4000 and SSG as superdisintegrant. The dissolution studies revealed that about 92.18% drug got released from liquisolid compacts in 120 minutes, whereas only 68.16% release was observed for pure piroxicam. X-ray diffraction and scanning electron microscopy images revealed the successful formation of liquisolid system.

Conclusion: It was concluded that dissolution rate of poorly soluble piroxicam could be enhanced using liquisolid technique.

Keywords: Piroxicam, Polyethylene glycol 600, Microcrystalline cellulose PH 102, Syloid 244 FP, Polyethylene glycol 4000.

INTRODUCTION

Dissolution plays an important role as a routine quality control test, for characterization of quality of dosage forms, for accepting product similarity under scale-up and post-approval changes related changes, for waiving bioequivalence requirements for lower strengths of a dosage form, and supports waivers for other bioequivalence requirements [1]. It involves mainly two steps: The liberation of the drug from the formulation matrix (disintegration) followed by solubilization of the drug particles in the liquid medium. Thus, the overall dissolution depends on the slower of these two steps. In the first step of dissolution, the cohesive properties of the formulated drug play a key role. Hence, if the first step of dissolution is rate-limiting, then the rate of dissolution is considered disintegration controlled.

In the second step of dissolution, the physicochemical properties of drug such as its chemical form (e.g., salt, free acid, free base) and physical form (e.g., amorphous or polymorph and primary particle size) plays an important role. If this latter step is rate-limiting, then the rate of dissolution is dissolution controlled [2,3]. This is the case for most poorly soluble compounds in immediate-release formulations.

Recent advanced technologies such as combinatorial chemistry and high-throughput screening have led to discovery of new drugs with good pharmacological activities [4,5]. About 35-40% of the drugs synthesized using these technologies have poor aqueous solubility [6]. The solubility of a drug not only determines the dissolution behavior of an active pharmaceutical ingredient (API) in the formulation, but it also affects the absorption as well as therapeutic efficacy of the drug. Some commonly used physical modifications to enhance the dissolution of API includes: (a) Reducing particle size to increase surface area, thus increasing dissolution rate of drug [7,8]; (b) solubilization in surfactant systems [9]; (c) formation of water soluble complexes [3]; (d) drug derivatization such as a strong electrolyte salt form that usually has higher dissolution rate, and (e) manipulation of solid state of drug substance to improve drug dissolution, i.e., by decreasing crystallinity of drug substance through formation of solid solutions [10-12]. The most common method is to increase surface area of the drug by micronization. However, in practice, the effect of micronization is often disappointing, especially when the drugs are encapsulated or tableted [5]. Micronized drugs also have the tendency to agglomerate as a result of their hydrophobicity, thus reducing their available surface area [5].

Although these multiple methods can overcome the problem of low solubility issue, they fail to provide cost effective technique due to the involvement of sophisticated machinery, advanced preparation techniques, and complicated technology [13].

Liquisolid technique is a recent approach that has emerged as a promising strategy for enhancing the release of poorly soluble drugs [14-20]. Liquisolid systems are composed of a non-volatile liquid vehicle having good solubility in water, drug, solid carrier, and coating materials [14-20]. The liquid portion in the formulation may be a liquid drug or a drug suspension or a drug solution in a suitable non-volatile liquid vehicle. The liquid vehicle is popularly called liquid medication. The liquid medication is adsorbed on the surface of a porous carrier (e.g., microcrystalline cellulose [MCC], hydroxy propyl methyl cellulose [HPMC], neusilin etc.). Once the carrier gets completely saturated with the non-volatile liquid, addition of coating material turns it into a dry, free flowing powder with good compressibility characteristics. The enhanced dissolution profile achieved by this technique can be

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Research Article

DISCRIMINATORY POTENTIAL OF BIPHASIC MEDIUM OVER COMPENDIAL AND BIORELEVANT MEDIUM FOR ASSESSMENT OF DISSOLUTION BEHAVIOR OF TABLETS CONTAINING MELOXICAM NANOPARTICLES

RANJITH ANISHETTY, SACHIN KUMAR SINGH*, VARUN GARG, ANKIT KUMAR YADAV, MONICA GULATI, BIMLESH KUMAR, NARENDRA KUMAR PANDEY, RAKESH NARANG, AMIT MITTAL

Department of Pharmacy, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.
Email: singhsachin23@gmail.com

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ABSTRACT

Objective: Dissolution test serves as a quality control tool for assessment of drug release from dosage form as well as a research tool to optimize new formulations. The existing guidelines by FDA, EMA, ICH, USP, etc., describe specifications for the dissolution of immediate release as well as modified release oral dosage form. However, none of them have discussed about the discriminatory potential of the medium to differentiate release profile of two or more products that are pharmaceutically equivalent. It is pertinent to add here that the pharmaceutical equivalents are not always bioequivalent. Hence, a discriminatory dissolution procedure is a must requirement to differentiate the release behavior of drug from a pharmaceutically equivalent product that contains different types and amount of excipient in the formulation. This also becomes more cumbersome when it is desirable for prediction of *in vivo* behavior of a drug when it is converted into a novel delivery system like nanoparticles. The reason could be the presence of excipients used to formulate drug nanoparticles into solid oral dosage form, may change the drug disintegration as well as dissolution behavior, which ultimately may lead to altered bioavailability.

Methods: In this study, the nanoparticles of meloxicam were prepared using wet media milling and the milled samples were dried using spray drier. The dried nanoparticles were converted into tablet dosage form by varying the type of diluent. To one batch lactose was used and another one was containing dicalcium phosphate (DCP). The assessment of release of meloxicam from these two batches was evaluated in various dissolution media.

Results: The study revealed that in all the cases the nanoparticulate tablets of Batch 1 have given increased dissolution profile as compared to marketed formulation (Muvera®), Batch 2 and controlled tablets of meloxicam. This proved that the excipients also play a major role in the release behavior of drug otherwise if it was not so, the nanoparticulate tablets of Batch 1 and Batch 2 would have given the same dissolution profile in all the tried media. Batch 1 containing lactose with a higher surface area provided more and rapid wetting of the drug by the dissolution media compared to Batch 2 that contained DCP as a major diluent.

Conclusion: Among all the dissolution media tried to evaluate the discriminatory power and simulation with a biorelevant medium, the biphasic medium of pH 1.8, 4.8 and 6.8 has promised to simulate with biorelevant media. However, the medium of pH 6.8 has shown the best dissolution profile.

Keywords: Solubility, Compendial media, Biphasic media, Dissolution, Meloxicam.

INTRODUCTION

Meloxicam is an antirheumatoid drug which falls in the category of non-steroidal anti-inflammatory drugs. "Previous studies on meloxicam pharmacokinetics shown that after oral administration, it has slow absorption with T_{max} that is longer than 5 hrs [1-5]. In comparison, intramuscular injection of meloxicam reached the maximum plasma concentration (C_{max}) within 1.5 hrs of administration and 90% of the C_{max} within 30-50 minutes over the dose range of 5-30 mg in humans [6,7]. Hence, intramuscular administration of meloxicam could shorten the onset of action since rapid pain relief is required in the case of acute and painful exacerbations of rheumatoid arthritis. However, due to the potential local tissue irritation and necrosis, intramuscular administration of meloxicam is not recommended for the chronic use and should be switched to oral formulation as soon as the rapid onset of action is achieved [7]. Hence, the development of an oral formulation of meloxicam with faster onset of action while maintaining the prolonged exposure could be a very good alternative." It belongs to BCS Class II and possesses poor solubility and thereby dissolution rate limited oral bioavailability. The details about physicochemical properties of meloxicam are shown in Table 1.

In last one decade, various approaches have been reported to improve the dissolution rate of meloxicam. Some of them include solubilization in surfactant solutions, the use of cosolvents, pH adjusted solutions, emulsions, liposomes, complexation with cyclodextrins, and solid dispersions [10-16].

However, above-mentioned techniques have some or the other limitations, like difficulty to scale up, clinical toxicity or stability, etc.

Nanosuspensions, on the other hand, have proven to be the cornerstone approach to overcome dissolution rate limited bioavailability of poorly soluble drugs [17].

There are several reports to overcome stability related problems, wherein nanosuspension have been prepared and been successful to get marketed [18]. Despite this the physiochemical stability related challenges such as sediments and Ostwald's ripening cannot be ignored. To overcome such problems, solidification of nanosuspensions through spray drying or lyophilization and the conversion into tablets, or capsules is now greatly practiced. Among, various oral dosage form systems, tablets are considered as the most common and convenient route due to their acceptable patient compliance, exposure of drugs to large surface area, rich blood supply, prolonged drug retention, advantage of scale up, and commercialization [18].

Dissolution test is utilized as either a research tool for optimizing new formulations or a quality control test to monitor the uniformity and

ORIGINAL ARTICLE

Systematic Development and Characterization of Liquisolid Compacts of Atorvastatin-Glipizide Binary Mixture to Achieve Enhanced Dissolution and Stability Profile

Jagadish Muthyala[1], Sachin Kumar Singh[1], Monica Gulati[1], Bimlesh Kumar[1], Harish Rathee[1], Deepak Ghai[1], Jasmine Kaur[1], Narendra Kumar Pandey[1], Ankit Kumar Yadav[1], Renuka[2]

[1]Department of Quality Assurance, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India. [2]Department of Pharmacy, Chandigarh University, Gharuan, Punjab, India

Abstract

Objective: The objective of present study is to evaluate the potential of liquisolid (LS) technology to enhance the dissolution characteristics of a combination of two poorly soluble drugs formulated as a single tablet. **Materials and Methods:** LS compacts have been prepared using compositions with varying ratios of propylene glycol (as water miscible non-volatile vehicle), lactose, and microcrystalline cellulose PH 102 (MCC PH 102) (as carriers), while Syloid® 244FP silica as a coating material. To evaluate the role of additives on various parameters such as loading factor, powder flow, disintegration, and dissolution profile of prepared LS formulations, number of additives such as Kollidon® 30, hydroxy propyl methyl cellulose-low viscosity, polyethylene glycol 4000, Kollidon® VA64, and low-substituted hydroxypropyl cellulose-LH-11 were added to the formulation. **Results and Discussion:** Among the tested carriers, MCC PH 102 was found to enhance drug release considerably. Addition of additives was found to further enhance powder flow and provide faster release of drugs from formulations. Among all the LS formulations prepared, LS - 18 demonstrated the fastest disintegration time (1.02 min) and fastest dissolution rate (84.6% in 10 min for glipizide and 44.6% for atorvastatin) with 100% drug release. **Conclusion:** The study demonstrated that LS technology can lead to suitable coformulation of two poorly soluble drugs for antidiabetic-antihyperlipidemic cotherapy of metabolic syndrome with enhanced dissolution.

Key words: Atorvastatin, glipizide, lactose, microcrystalline cellulose PH 102, Syloid® 244FP

INTRODUCTION

Advancement in the field of drug discovery, like combinatorial chemistry and high throughput screening have led to invention of a large number of active moieties with high therapeutic potential.[1] However, 40% of newly developed drugs are reported to be hydrophobic in nature.[2] Their low solubility becomes a major limiting factor in formulation development for their effective drug delivery. A number of approaches have, therefore, been explored to enhance the solubility of poorly soluble drugs. These approaches include increasing the surface area, formulation in a dissolved state,[3] particle size reduction,[4] cogrinding,[5] amorphization,[6] coamorphization,[7] liquisolid (LS) compacts,[8] preparation of inclusion complexes,[9] solid dispersions,[10] use of prodrugs,[11] generation of metastable polymorphs,[12] and lipid-based systems such as self - emulsifying drug delivery systems.[13] LS technique is a recent approach that has emerged as a promising strategy for enhancing the release of poorly soluble drugs.[14,15]

Address for correspondence:
Sachin Kumar Singh, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara - 144411, Punjab, India. Phone: +91-9888720835.
E-mail: singhsachin23@gmail.com

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Formulation and Evaluation of Oral Mucoadhesive Tablets of Cisplatin

Mohit Basotra[1,2], Ankit Kumar Yadav[1], Sachin Kumar Singh[1], Monica Gulati[*,1], Deepak Ghai[1], Harish Rathee[1], Bimlesh Kumar[1], Rakesh Narang[1]

[1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara - 144411, Punjab, India.

[2]Formulation and development, Immacule Lifesciences Pvt. Ltd. Village – Thanthewal, Ropar Road, Nalagarh, Solan, Himanchal Pradesh, India

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ABSTRACT

Background and Aim: Cisplatin, an anticancer drug is used to treat various types of cancer. It is available only in the form of injection to be administered as an i.v. infusion. For that, it needs to be administered under the supervision of an oncologist. The inconvenience and cost of hospitalization can be avoided by the development of oral formulation of cisplatin. There is no marketed tablet formulation of Cisplatin available till date. In the present study, an attempt was made to develop oral enteric coated, sustained release, mucoadhesive tablets of cisplatin in order to prevent upper GIT side effects like nausea and vomiting and to prolong its residence time in intestine. **Methods:** Eight formulations were developed by direct compression method using various proportions of mucoadhesive polymers, HPMC (400 cps) and carbopol 940. Prepared tablets were evaluated for physical parameters, swelling index, surface pH, mucoadhesive strength and *in vitro* drug release. **Results:** Among all the formulations, F4C was found to be the best as it showed high swelling index 125.51± 1.63 % at 12 h, optimum mucoadhesive strength of 13.11±0.65 g and superior *in vitro* drug release of 91.30% in 12 h respectively. Optimized F4C batch was further subjected to *ex-vivo* permeation study by everted gut sac technique and permeability co-efficient was found to be 0.0013 at 12 h. The prepared mucoadhesive sustained release tablets of optimized batch (F4C) were further enteric coated with Eudragit L 100 suspension up to 20 %. The final formulations were studied for *in vitro* drug release in 0.1N HCl for 2 h followed by that in 0.2M phosphate buffer pH 6.8 up to 16 h. Data showed no drug release in first 2 h in 0.1N HCl. However, sustained drug release of cisplatin could be observed in 0.2M phosphate buffer pH. A maximum of 82.52% drug release was observed at the end of 12 h. **Conclusion:** It was concluded that 20% Eudragit coated sustained release mucoadhesive tablets of cisplatin (F4C) containing 100 mg of carbopol has shown desired *in vitro* drug release as well as mucoadhesive strength and could be further explored for its *in vivo* performance.

KEY WORDS: Cisplatin, mucoadhesive tablet, direct compression method, enteric coated

1. INTRODUCTION

Cisplatin (Cis-diamminedichloroplatinum) is one of the most potent and effective anticancer drugs. It is a platinum-based chemotherapy drug used to treat various types of cancers like small cell lung cancer, urinary bladder cancer, ovarian cancer and germ cell tumors. It leads to formation of Platinum complexes in cells, which cause cross-linking of DNA, triggering apoptosis (programmed cell death). Cisplatin is administered intravenously as short-term infusion in physiological saline for treatment of solid malignancies[1]. The optimal dosage regimen of cisplatin with radiotherapy is not fully established, although a dose of 100 mg/m^2 on days 1, 22 and 43 is generally considered as the standard treatment[2]. However, a wider application of cisplatin is limited due to its toxic side effects. Its use leads to a very strong emetic effect. As is the case with many other chemotherapeutic agents, the pathophysiology of cisplatin-induced nausea and vomiting involves the direct stimulation of chemoreceptor trigger zone which may make the oral delivery of the drug impractical [3]. This is further complicated by the erratic absorption of the drug through the oral route [4]. Hence, the challenges in the development of the oral dosage form of Cisplatin include its delivery beyond stomach to prevent the reflux and to increase its absorption therein.

Coating of the tablets has been the most conventional way of modifying the release characteristic of a drug [5]. Enteric coating protects the formulation from stomach's acidic exposure, delivering the formulation to a relatively basic pH. It is a barrier applied to oral formulation that controls its location in the gastrointestinal tract where it releases the drug. Most enteric coatings work by presenting

***Corresponding author**
Dr. Monica Gulati
School of Pharmaceutical Sciences,
Lovely Professional University,
Phagwara - 144411, Punjab, India

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Research Article

FORMULATION OF CURCUMIN NANOSUSPENSION USING BOX-BEHNKEN DESIGN AND STUDY OF IMPACT OF DRYING TECHNIQUES ON ITS POWDER CHARACTERISTICS

JASMINE KAUR, PALAK BAWA, SARVI YADAV RAJESH, PARTH SHARMA, DEEPAK GHAI, JIVAN JYOTI, SANANDA SOM, SOUVIK MOHANTA, HARISH RATHEE, ADIL HUSSAIN MALIK, SACHIN KUMAR SINGH*, BIMLESH KUMAR, MONICA GULATI, NARENDRA KUMAR PANDEY, VARUN GARG, ANKIT KUMAR YADAV, RAKESH NARANG

Department of Quality Assurance, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.
Email: singhsachin23@gmail.com

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ABSTRACT

Objective: The objective of this study was to formulate curcumin nanosuspension (NS) using Box-Behnken design (BBD) and solvent-antisolvent technique to overcome the challenges related to its poor dissolution rate.

Methods: Sodium lauryl sulfate (SLS) and poly vinyl pyrrolidone K-60 (PVPK-60) have been used as a surfactant and polymer, respectively, to stabilize the NS. Ethanol was used as solvent to dissolve curcumin and water was used as antisolvent. The study revealed that SLS to curcumin ratio, PVPK-60 to curcumin ratio, solvent to antisolvent ratio and speed of mixing were the critical parameters that affected particle size and zeta potential of the formulation. Hence, based on Box- BBD, 25 formulations were prepared by varying these critical parameters. The optimized batch of CRM NS was further solidified using spray drying as well as rotary evaporation techniques to have a better insight for selection of solidification process in terms of retention of particle size, charge, flow, dissolution, and stability.

Results: About 39.47 folds decrease in particle size of raw CRM was observed after conversion into NS. Further, about 53.57 and 45.45 folds decrease in particle size was observed after spray drying and rotary evaporation. Both the dried nanoparticles have shown comparatively higher solubility, powder flow, and dissolution rate as that of raw CRM. Powder X-ray diffraction study revealed the formation of amorphous nanoparticles. Accelerated stability study revealed that nanoparticles dried by spray drying were able to retain the properties such as particle size, flow, and dissolution rate as compared to rotary evaporated powders.

Conclusion: It can be concluded that spray drying technique could offer many advantages while loading CRM nanoparticles into tablets for their oral administration.

Keywords: Curcumin, Spray drying, Rotary evaporation, Box-behnken design, Dissolution, Stability studies.

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INTRODUCTION

Curcumin (CRM) is a potent phytoconstituent derived from turmeric (*Curcuma longa,* Family Zingiberaceae) [1]. It has been mainly reported for its anticancer properties and found to be beneficial as antibacterial, antifungal, antiamoebic, antioxidant, antidiabetic, anti-HIV and in neuro-generative and respiratory diseases [2-9]. Despite having an array of medicinal properties, there are certain challenges with its physicochemical properties such as low aqueous solubility (3.12 g/L, 25°C), degradation at intestinal pH, fast hepatic metabolism and elimination, which reduces its bioavailability [10-12].

Poor aqueous solubility of CRM is a major challenge for its oral administration. Various methods have been reported earlier to enhance its solubility and dissolution rate. These include the formulation of solid dispersion, liposomes, complexation with phospholipids, and cyclodextrin [13-15]. Conjugation of bioavailability enhancers such as piperine, Bio-curcumin-95 (BCM-95) has been used as alternative strategies to enhance its bioavailability [16-19]. In the past decade, nanotechnology has emerged as a unique approach that has found enormous applications in delivering lipophilic drugs with better bioavailability [10,20]. In this approach, submicron particles are formed which get stabilized by use of steric and electrostatic stabilizers [21]. Among nanotechnology approaches that are used for the formulation of lipophilic drugs, nanosuspension (NS) has been reported as one of the most promising techniques to improve the dissolution rate limited bioavailability of such drugs [22]. In general, bottom up and top down techniques are used to prepare NS [22,23]. In bottom up process, drug is added in solvent that dissolves it completely and then added to antisolvent by aid of stirring to form precipitates. Different techniques such as solvent-antisolvent method and super critical fluid process fall under bottom up processes. In top down process, drug is suspended in the solvent and particle size reduction was carried out by ball milling, media milling, high-pressure homogenization, and microfluidization [22,23].

It is important to note that formulation of NS is greatly affected by technique used to prepare it, most importantly their physicochemical stability [24]. Top down method creates physical and chemical instability like crystal deformities and break down of heat labile drugs due to the generation of heat during milling process [24]. Whereas, by using bottom up technique, morphology and crystallinity of particles could be maintained. Supercritical fluid process under bottom up technique has been used to prepare NS on large scale [22,23]; however, it suffers from certain limitations such as operation at high pressure, temperature, and requirement of fine designed nozzles [23,24]. Alternatively, solvent-antisolvent method has been extensively reported to prepare NS due to its simplicity and ease of scale up. Solvent-antisolvent method is one of the simplest method which can be employed for small scale batches in laboratory and does not require already micronized particles.

Reduced particle size may lead to create high total surface energy which may lead to instability and particle agglomeration also known

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Solid self-nanoemulsifying drug delivery systems for oral delivery of polypeptide-k: Formulation, optimization, in-vitro and in-vivo antidiabetic evaluation

Varun Garg, Puneet Kaur, Sachin Kumar Singh*, Bimlesh Kumar, Palak Bawa, Monica Gulati, Ankit Kumar Yadav

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144411, Punjab, India

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Antidiabetic activity

ABSTRACT

Development of self-nanoemulsifying drug delivery systems (SNEDDS) of polypeptide-k (PPK) is reported with the aim to achieve its oral delivery. Box-Behnken design (BBD) was adopted to develop and optimize the composition of SNEDDS. Oleoyl polyoxyl-6 glycerides (A), Tween 80 (B), and diethylene glycol monoethyl ether (C) were used as oil, surfactant and co-surfactant, respectively as independent variables. The effect of variation in their composition was observed on the mean droplet size (y1), polydispersity index (PDI) (y2), % drug loading (y3) and zeta potential (y4). As per the optimal design, seventeen SNEDDS prototypes were prepared. The optimized composition of SNEDDS formulation was 25% v/v Oleoyl polyoxyl-6 glycerides, 37% *v*/v Tween 80, 38% *v*/v diethylene glycol monoethyl ether, and 3% w/v PPK. The optimized formulation revealed values of y1, y2, y3, and y4 as 31.89 nm, 0.16, 73.15%, and − 15.65 mV, respectively. Further the optimized liquid SNEDDS were solidified through spray drying using various hydrophilic and hydrophobic carriers. Among the various carriers, Aerosil 200 was found to provide desirable flow, compression, disintegration and dissolution properties. Both, liquid and solid-SNEDDS have shown release of > 90% within 10 min. The formulation was found stable with change in pH, dilution, temperature variation and freeze thaw cycles in terms of droplet size, zeta potential, drug precipitation and phase separation. Crystalline PPK was observed in amorphous state in solid SNEDDS when characterized through DSC and PXRD studies. The biochemical, hematological and histopathological results of streptozotocin induced diabetic rats shown promising antidiabetic potential of PPK loaded in SNEDDS at its both the doses (i.e. 400 mg/kg and 800 mg/kg) as compared to its naïve form at both the doses. The study revealed successful formulation of SNEDDS for oral delivery of PPK.

1. Introduction

Polypeptide-k is a peptide that is extracted from dried ripened seeds of *Momordica charantia* (MC). It has been well reported for its antidiabetic activity and this is available in market in the form of sublingual tablet under brand name Diabegard™, in powder form under brand name Sugard™ and in Organic Spirullina Atta noodles (http://polypeptide-k.blogspot.in/2011_06_01archive.html; Kaur et al., 2016; Lok et al., 2011). Furthermore, PPK has been reported to inhibit α-glucosidase and α-amylase up to 79.18% and 35.58% level.

Despite having this unique antidiabetic property, oral delivery of PPK remains a challenge. It suffers from poor aqueous solubility (50 mg/L) which causes dissolution rate limited bioavailability (Kaur et al., 2015a) and enzymatic degradation in the GI tract. Moreover, PPK being a protein; its oral delivery results in poor bioavailability due to its larger size and degradation in GIT (Rao and Shao, 2008). Previously, we have described the formulation of solid dispersion of PPK by spray drying, for enhancing its aqueous solubility and compared its results with its raw form (Kaur et al., 2015a). About 21 to 65% increase in solubility of PPK present in solid dispersion was observed over raw PPK in various aqueous buffers ranging from pH 1.2 to 9.6 (Kaur et al., 2015a). However, the developed formulation failed to provide promising diffusion of PPK through goat intestinal membrane during ex-vivo diffusion study.

There are a number of approaches that are reported to improve the dissolution rate limited bioavailability of poorly soluble drugs (Renuka et al., 2014). These approaches include increasing the surface area (Renuka et al., 2014), particle size reduction (Mahesh et al., 2014; Romero et al., 1999), formulation in a dissolved state (Brittain, 2007), liquisolid compacts (Singh et al., 2012), preparation of inclusion

* Corresponding author.
E-mail address: sachin.16030@lpu.co.in (S.K. Singh).

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ORIGINAL ARTICLE

Novel biorelevant dissolution medium as a prognostic tool for polysaccharide-based colon-targeted drug delivery system

Ankit Kumar Yadav, Manik Sadora, Sachin Kumar Singh, Monica Gulati, Peddi Maharshi, Abhinav Sharma, Bimlesh Kumar, Harish Rathee, Deepak Ghai, Adil Hussain Malik, Varun Garg, K. Gowthamrajan[1]

Department of Pharmaceutics, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, [1]Department of Pharmaceutics, J.S.S. College of Pharmacy, Ooty, Tamil Nadu, India

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ABSTRACT

To overcome the limitations of the conventionally used methods for evaluation of orally administered colon-targeted delivery systems, a novel dissolution method using probiotics has been recently reported. In the present study, universal suitability of this medium composed of five different probiotics is established. Different delivery systems – mini tablets, liquisolid compacts, and microspheres coated with different polysaccharides – were prepared and subjected to sequential dissolution testing in medium with and without microbiota. The results obtained from fluid thioglycollate medium (FTM)-based probiotic medium for all the polysaccharide-based formulations showed statistically similar dissolution profile to that in the rat and goat cecal content media. Hence, it can be concluded that the developed FTM-based probiotic medium, once established, may eliminate the need for further animal sacrifice in the dissolution testing of polysaccharide-based colon-targeted delivery system.

Key words: Fluid thioglycollate medium, liquisolid compacts, mini tablets, polysaccharides, probiotics

INTRODUCTION

Dissolution testing of polysaccharide-based oral colon-targeted delivery systems is a consistent challenge due to the varying pH, viscosity, presence of diverse microbiota, and motility conditions in the upper GI segments that the system needs to traverse before reaching the colon.[1] This challenge is further intensified by the diverse strategies used in the formulation of colon-targeted delivery systems, their release being designed to be facilitated by stimuli such as pH,[2,3] pH and time,[2] presence of enzymes,[4] time,[6-9] using prodrug based,[7] pressure controlled,[10] and microbially triggered delivery systems.[11-18] The most commonly reported medium to evaluate all these types of delivery systems is the one that utilizes colon contents of rodents.[5] However, this medium has some serious limitations including lack of reproducibility, high cost, complexity of procedure, and vivisection.[6,15] Other dissolution media include the use of human fecal slurries and goat cecal contents, for which the element of reproducibility is even less.

Recently, we reported a unique, simple, inexpensive, and animal-sparing dissolution medium using probiotic culture for establishing the release pattern of polysaccharide-based colon-specific delivery system.[15] Fluid thioglycollate medium (FTM) containing probiotic (BIOMIX-I) composed

Address for correspondence:
Dr. Monica Gulati,
Department of Pharmaceutics, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab - 144 402, India.
E-mail: monicagulati14@gmail.com

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RESEARCH ARTICLE

In-vitro and *In-vivo* Pharmacokinetic Evaluation of Guar Gum-Eudragit® S100 Based Colon-targeted Spheroids of Sulfasalazine Co-administered with Probiotics

Abhinav Sharma, Bimlesh Kumar, Sachin Kumar Singh*, Monica Gulati, Yogyata Vaidya, Manik, Harish Rathee, Deepak Ghai, Adil Hussain Malik, Ankit Kumar Yadav, Peddi Maharshi, Palak Bawa, Sarvi Yadav Rajesh, Parth Sharma, Narendra Kumar Pandey and Souvik Mohanta

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab-144 411, India

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Abstract: ***Background:*** Polysaccharide based delivery systems have been successfully used to target drugs to colon. In some recent reports, the superiority of concomitant administration of probiotics with such systems has been established. However, the pharmacokinetics of such symbiotic therapy remain unexplored hitherto.

Methods: This study deciphers the pharmacokinetic parameters of guar gum based colon targeted spheroids of sulfasalazine with co-administration of probiotics in experimental rats. Thirty rats were divided into five groups using Latin square design. These were subjected to treatment with delayed release formulation, uncoated spheroids, coated spheroid and coated spheroids along with probiotics.

Results: In case of delayed release formulation, negligible presence of sulfasalazine in plasma was observed in first 2h, followed by significant increase in sulfasalazine concentration after 3h. Higher plasma concentrations of sulfasalazine were detected for uncoated spheroids with and without probiotics. Negligible release of drug upto 5h and delayed T_{max} in case of guar-gum coated sulfasalazine spheroids with or without probiotics clearly indicated successful formulation of colon targeted spheroids. Further, for coated spheroids (both with and without probiotics), the value of T_{max} is found to be significantly higher than those with the other treatments.

Conclusion: Colon targeted spheroids were therefore, found to reduce absorption of drug which, in turn, is expected to reduce the side effects as only local action in colon is required for treatment of colitis. This is the first report on pharmacokinetic study of a colon targeted delivery system co-administered with probiotics.

Keywords: Sulfasalazine, spheroids, delayed release tablets, dissolution, probiotics, pharmacokinetics.

1. INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammation of colon which is characterized by bloody diarrhoea with absence of parasites in the stool culture. The etiological factors include, damage of patients' immune system [1], abnormal immunological response of colonic mucosa against colonic microflora [2], environmental factors [3], genetic factors [4, 5], and oxidative damage [6]. Commonly used treatments include corticosteroids and immunomodulators like azathioprine (AZA), 6-mercaptopurine (6-MP), infliximab and cyclosporine [7, 8]. These drugs are reported to cause limiting side effects on long term usage. Sulfasalazine (SFZ) is the cornerstone drug for the treatment of UC and is reported to be superior to corticosteroids, AZA, 6-MP and cyclosporine in terms of its safety and efficacy. Side effects associated with the use of Sulfasalazine include fever, arthralgia, rashes, Heinz body anaemia and agranulocytosis which are attributed to its metabolite, sulfapyridine [8, 9]. SFZ is poorly absorbed from colon, hence, formulation of colon targeted delivery system of SFZ is expected to reduce its side effects [10].

In past, a number of colon-targeted delivery systems have been reported that include time, pressure and osmotically controlled systems, pH and prodrug based and colonic microflora activated delivery systems [11]. Among them, microflora activated polysaccharide (e.g. guar gum, pectin, amylose, inulin *etc*.) based delivery systems have proven to be quite successful [11]. Different microflora activated polysaccharide based colon targeted drug delivery systems along with their merits and limitations have been extensively discussed in our previous reports [11, 12]. There are many formulations that have been developed in past such as compression coated matrix tablets, microspheres, beads, pellets, have been designed so far for colon targeting using polysaccha-

*Address correspondence to this author at Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara, 144411, India; Tel: +919888720835; Fax: +91 1824501900; E-mail: singhsachin23@gmail.com

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RESEARCH ARTICLE

A Novel Three-pronged Approach for Colon Delivery of Sulfasalazine: Concomitant Use of pH- Responsive, Microbially Triggered Polymers and Liquisolid Technology

Peddi Maharshi[a], Sachin Kumar Singh[a], Monica Gulati*[, a], Ankit Kumar Yadav[a], Varun Garg[a], Bimlesh Kumar[a] and K. Gowthamarajan[b]

[a]*School of Pharmaceutical Sciences, Lovely Professional University, Punjab-144 401, India;* [b]*Department of Pharmaceutics, J.S.S. College of Pharmacy, Rocklands, Ooty, Tamilnadu- 643 001, India*

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Abstract: ***Objective:*** A major challenge in targeting orally administered drugs to colon is their passage through the long gastrointestinal path comprising highly variant conditions in terms of pH, viscosity, gut motility and microbial flora. Approaches to pH controlled release and microbially triggered release have proved to be successful in achieving colon targeting only to a partial extent.

Methods: In an attempt to improve targeting, both these approaches have been combined together with the approach of liquisolid technology which, hitherto, remains unexplored for colon targeting. The combination of these three approaches is being reported for the first time to achieve colon targeting along with a burst release of a Biopharmaceutical Classification System (BCS) Class IV drug at the target site. pH controlled polymer, Eudragit® S-100 was used to prevent the release of sulfasalazine in the gastric region while microbially triggered polymers, pectin and guar gum were used to ferry the system through the intestinal region.

Results: Liquisolid formulation was designed to provide a burst release of sulfasalazine in colon on the digestion of polysaccharide coating.

Conclusion: The results support the premise that the combination of pH sensitive, microbially triggered polymers and liquisolid formulation technique appears to be a pragmatic approach for colonic delivery of orally administered drugs.

Keywords: Eudragit® S-100, pectin, guar gum, liquisolid technique, colon targeted delivery.

1. INTRODUCTION

Significant advances in drug delivery techniques through the design of breakthrough formulations have been able to achieve effective treatment of various diseases. Novel formulations, especially those based on nanotechnology have been developed to target all tissues through the fabrication of sustained, targeted stimuli-responsive dosage forms [1, 2]. These have made the treatment of hitherto considered insurmountable diseases a pragmatic target [3].

In last one and half decades, incidences of colonic diseases such as Crohn's disease, ulcerative colitis (UC), colorectal cancer and amoebiasis have increased drastically. Hence, there is a need to focus on local treatment of the diseases associated with the colon. Many approaches have been reported for oral colon targeted delivery. These include timed release systems, prodrugs, pH sensitive polymer coating and colonic microflora activated delivery systems [4-27]. However, all of them have faced some challenges in terms of failure to provide site specific release or, feasibility to scale up [9, 28, 29]. A major bottleneck in this direction is to develop a formulation that will not lose its intactness despite a strong variation in pH, viscosity, heterogeneity of fermentation activity and motility prevalent in various segments of gastrointestinal tract (GIT).

Among the reported strategies, microflora activated polysaccharide based delivery systems have shown promising results. These delivery systems remain unaffected in the upper part of GIT *i.e.* stomach and small intestine. However, when they reach the colon, they encounter an abrupt increase in concentrations of polysaccharide digesting enzymes due to presence of microbiota therein [29]. The colonic bacteria produce a wide spectrum of reductive and hydrolytic enzymes, such as β-D-glucosidase, β-D-galactosides, amylose, pectinase, xylamase, β-d-xylosidase, dextranase, which are responsible for carbohydrate metabolism [29-31].

A large number of polysaccharides have been explored for their potential as colon specific drug carriers. These include chitosan, pectin, chondroitin sulphate, cyclodextrin, dextrans, guar gum, inulin, amylose and locust bean gum [30, 32, 33]. This approach has been reported to be integrated with the pH – sensitive polymers to increase the efficiency of targeting [34]. To overcome the limitations of the individual gums *i.e.* low film forming capacity of pectin and

*Address correspondence to this author at Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara, 144401, India; Tel: +91 9915020408; Fax: +91 1824501900;
E-mail: monicagulati14@gmail.com

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Impact of solid carriers and spray drying on pre/post-compression properties, dissolution rate and bioavailability of solid self-nanoemulsifying drug delivery system loaded with simvastatin

Parth Sharma [a], Sachin Kumar Singh [a], Narendra Kumar Pandey [a,*], Sarvi Yadav Rajesh [a], Palak Bawa [a], Bimlesh Kumar [a], Monica Gulati [a], Saurabh Singh [b], Surajpal Verma [a], Ankit Kumar Yadav [a], Sheetu Wadhwa [a], Subheet Kumar Jain [c], Kuppusamy Gowthamarajan [d], Adil Hussain Malik [a], Suksham Gupta [a], Rubiya Khursheed [a]

[a] School of Pharmaceutical Sciences, Lovely Professional University, Punjab 144 401, India
[b] School of Pharmaceutical Science (Ayurvedic Pharmacy), Lovely Professional University, Punjab 144 401, India
[c] Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, India
[d] Department of Pharmaceutics, J.S.S. College of Pharmacy, Rocklands, Ooty, Tamilnadu, India

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ABSTRACT

In the present study, different solid self-nanoemulsifying drug delivery system (S-SNEDDS) was formulated by using porous hydrophilic and hydrophobic carriers to improve the dissolution rate and bioavailability of simvastatin (SIM). The prepared liquid-SNEDDS composed of Labrafil M 1944 CS/ Tween-80/ Ethanol (20% / 53.33%/ 26.67% *v*/v) with 0.1% SIM, resulted droplet size of 40.69 nm. The hydrophobic carriers used were Aerosil-200, Syloid 244FP, Syloid XDP 3150, Magnesium stearate, Micro Crystalline Cellulose PH102 and lactose and hydrophilic carriers used were Poly vinyl alcohol, Sodium carboxy methyl cellulose and hydroxypropyl-β-cyclodextrin. These S-SNEDDS were characterized through micromeritic, biopharmaceutical studies and stability studies. The spray dried S-SNEDDS prepared by using Aerosil 200 as hydrophobic carrier provided nanoemulsions with unchanged droplet size and drug release when subjected at different stress conditions such as thermodynamic stress and freeze thaw cycles. In vitro dissolution studies revealed that the L-SNEDDS and S-SNEDDS of Aerosil 200 were found to be remarkably superior over the unprocessed SIM and marketed SIM. Scanning electron microscope, Differential scanning calorimeter and Powder X-Ray Diffraction revealed crystalline SIM was present in a changed amorphous state in the SNEDDS formulations prepared with Aerosil 200 as carrier. Further, pharmacokinetic study carried out on rats revealed 0.5 h increase in time for maximal concentration (T_{max}), 3.75 folds increase in maximal concentration (C_{max}), 1.22 h increase in mean residence time, 1.54 folds increase in area under curve ($AUC_{0\text{-}t}$), 2.10 folds increase in $AUC_{0\text{-}\infty}$ and 3.28 folds increase in bioavailability confirms that the developed S-SNEDDS were superior than that of marketed formulation. Hence, it can be safely concluded that Aerosil 200 based S-SNEDDS were able to provide improvement in dissolution rate and oral bioavailability of SIM.

1. Introduction

The oral route is one of the most suitable routes for drug delivery for the patients and manufacturers of drugs. However deliverance of hydrophobic drugs through this route is almost hampered to half due to their poor solubility, poor bioavailability, lack of dosage proportionality, and high intra and inters subject variability. Therefore, a standout among the most imperative difficulties confronting the pharmaceutical sector today is planning for development of oral dosage form for new chemical substances that have poor aqueous solubility and bioavailability. Formulation scientists are breaking new grounds in the field of drug discovery to form new drug delivery systems for the number of chemical compounds that show aforementioned challenges [1].

Self-nanoemulsifying drug delivery system (SNEDDS) is currently being utilized for lipophilic medications that suffer from poor aqueous solubility and low oral bioavailability [2]. Self-emulsifying drug delivery systems are made out of at least two components, which give the self-emulsifying properties; a) more hydrophilic amphiphiles, b) more lipophilic amphiphile, if required, the co-solvents or a precipitation inhibitor. Upon gentle agitation and in the gastrointestinal liquids, these frameworks change into oil in water (O/W) emulsions (SEDDS), micro emulsions (SMEDDS) or nanoemulsions (SNEDDS) [3]. The formulation

* Corresponding author at: School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144401, India.
E-mail address: narendra.pandey@lpu.co.in (N.K. Pandey).

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Solidification of liquid Modified Apple Polysaccharide by its adsorption on solid porous carriers through spray drying and evaluation of its potential as binding agent for tablets

Souvik Mohanta, Sachin Kumar Singh *, Bimlesh Kumar, Monica Gulati, Jivan Jyoti, Sananda Som, Sakshi Panchal, Indu Melkani, Mayukh Banerjee, Shubham Kumar Sinha, Rubiya Khursheed, Ankit Kumar Yadav, Vishu Verma, Rajan Kumar, Deep Shikha Sharma, Adil Hussain Malik, Narendra Kumar Pandey, Sheetu Wadhwa

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144411, Punjab, India

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ABSTRACT

In the present study application of Modified Apple Polysaccharide (MAP) as tablet binder was evaluated. Liquid MAP was extracted from apple and solidified by adsorbing it on porous surface of Aerosil-200 and trehalose and this dispersion was dried using spray dryer. The concentration of excipients as well as spray drying conditions was optimised by using Box Behnken Design to achieve desirable powder characteristics. The optimised batch of solid MAP was characterized by DSC, PXRD, SEM, and FT-IR studies that confirmed complete adsorption of liquid MAP on the surface of Aerosil-200 and trehalose. This solid MAP was investigated for its binding efficacy for tablet formulation and its binding potential was compared with acacia and polyvinyl pyrrolidone K-30. Mesalamine (model drug) granules containing different concentration of binders were prepared by wet granulation. The granules were evaluated for micromeritic properties and results were found within the pharmacopoeial limits. The prepared tablets were subjected for post compression studies such as hardness, friability, disintegration, dissolution, physical stability, content uniformity and percentage elastic recovery and their results were found good. At 2.5% w/w concentration in tablet, the solid MAP has shown shorter disintegration time and faster dissolution profile as compared to other concentrations used including good physico-mechanical properties.

1. Introduction

Apple is very famous among all other fruits due to its high antioxidative property. Its daily consumption is directly linked with controlling the risk of colorectal diseases to a significant level. It has been proved that cloudy apple juice can decrease hyper proliferation, DNA damage and crypt foci development in the colon [1]. Intake of apple can prevent several diseases like asthma, different types of cancer, various pulmonary diseases, Alzheimer's disease, ageing, obesity and diabetes [1]. The main chemical constituents of apple are poly phenolic compounds including flavonoids and phenolic acids (alpha linoleic acid, ferulic acid, sinapic acid, caffeic acid, coumeric acid, urolic acid), vitamins (A, B1, B2 and B6), fibres (cellulose, lignin, pectin), a mixture of cholesterol free saturated, unsaturated and mono saturated fats, sweeteners (sucrose and fructose), thirst quenchers (tannins) and polysaccharides (galactose, galacturonic acid, arabinose, rhamnose, and other sugar residues) [2]. These above-mentioned polysaccharides present in the apple are altogether known as Modified Apple Polysaccharides (MAP). MAP has been well established for the prevention as well as treatment of colorectal diseases with well-defined mechanisms. The two most important regulators of ulcerative colitis and colorectal cancer are apoptosis and metastasis. It is reported that MAP up-regulates apoptosis and down regulates metastasis, hence, regulates colorectal ulcer (CU) and cancer [3].

Till date, researchers have administered MAP in its liquid dispersion form only for proving its prophylactic as well as curative action against CU. Despite having aforementioned benefits, MAP has not been placed in the market as nutraceutical to treat CU due to different issues related to its poor stability in its liquid form.

* Corresponding author.
E-mail address: sachin.16030@lpu.co.in (S.K. Singh).

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Research Article

INFLUENCE OF FORMULATION PARAMETERS ON DISSOLUTION RATE ENHANCEMENT OF ACYCLOVIR USING LIQUISOLID FORMULATION

KUBOTA MWAKA HAZEMBA, JIVAN JYOTI, SHEETU WADHWA*, SANANDA SOM, SOUVIK MOHANTA, ANKIT KUMAR YADAV, BIMLESH KUMAR, VARUN GARG, DEEP SHIKHA SHARMA, RUBIYA KHURSHEED, MONICA GULATI, SACHIN KUMAR SINGH

Department of Pharmacy, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.
E-mail: sheetupharma@gmail.com/sheetu.21001@lpu.co.in

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ABSTRACT

Objective: The objective of this research work is to explore the use of liquisolid technique in enhancement of acyclovir dissolution rate. This current study was planned to assess the impact of different formulation variables, such as non-volatile liquid type and concentrations of acyclovir on its dissolution rates profile.

Method: Acyclovir liquisolid tablets were prepared with Tween 60 (liquid vehicle), Microcrystalline cellulose PH 102 (acted as a carrier to turn liquid medication into free-flowing powder) and Syloid XDP (coating material). *In vitro,* drug dissolution rate of liquisolid formulations of acyclovir was performed and compared with pure acyclovir drug using USP dissolution apparatus (Type II) for 60 min at a paddle speed of 50 rpm and filled with 900 mL of distilled water.

Results: The dissolution study showed that 94.1% of the drug was released in 60 min of ratio 10 while only 66% of the pure drug acyclovir was released in 60 min. Hence, present work concluded that the acyclovir dissolution rate profile has been improved with the formation of liquisolid formulations.

Conclusion: From the present study, it may be ratified that the drug dissolution rate of acyclovir has been improved with the utilization of liquisolid formulations approach.

Keywords: Acyclovir, Dissolution, Non-volatile liquid, Liquisolid tablets.

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INTRODUCTION

Nowadays, low solubility is the principle issue related with new drug moieties which should be overcome. The vast majority of the procedures that have prevailing with regard to conquering this issue are spray drying, micronization approach, β-cyclodextrins inclusion complexes, solid dispersions, and eutectic mixtures. However, among all, the feasible and financially savvy is liquisolid system. In liquisolid strategy, the drug is held in a solubilized state and molecularly dispersed in a liquid which helps to achieve the improved drug dissolution [1].

To overcome the bioavailability issue of poorly soluble drugs due to insufficient dissolution rate, numerous techniques are being utilized. Hydrophilic polymers as solubility enhancers are used in various approaches which perform through many ways in the development of various techniques such as cosolvency and inclusion complexes which provide numerous advantages toward formulation development. However, most of the time, during storage, these techniques show stability issues, and low industrial viability and are not commercially successful. Moreover, these techniques suffered with limitations such as hygroscopic and sticky mass which lead to poor flow of powders [2].

Liquisolid technology emerged as a new drug delivery system, differentiated due to its features and potential to deliver numerous drugs. These systems have drawn the attention of pharmaceutics scientists and scholars in the area of poorly soluble drugs for their solubility enhancement and controlled dissolution profile as per the formulation requisite [2]. This technology is patented by Spireas *et al*., in 1999, and is a simple process of physical mixing with selected excipients which turns it into a free-flowing, dry powder. The main formulation components of liquisolid systems are non-volatile liquid vehicle, a carrier, and a coating material. Moreover, as per the objectives and need of the study, sometimes other excipients such as disintegrants or superdisintegrants are used [3-5].

One of the anticipated mechanisms for the enhance the dissolution rate of the drug from the liquisolid compact mass is the wettability of the latter in the dissolution media. The component that helps in the wetting of drug particles in liquisolid system is the non-volatile solvent [6]. These solvent systems reduce the interfacial tension which was exist between the tablet surface and selected dissolution medium which results in increase effective surface area and wettability for dissolution [7]. Due to this fact, these liquisolid compacts show improved dissolution profile and enhanced bioavailability of poorly soluble drugs. The release of drug from these liquisolid compacts is mainly depends on few parameters, such as drug characteristics, type of carrier, and the type of liquid vehicle used in the formulation. Therefore, these should be optimized, and its effect on dissolution rate should be evaluated [8,9].

The main components of liquisolid systems are carrier material, coating material, non-volatile solvent, and disintegrant. Carrier material such as various grades of sorbitol, cellulose, lactose, and starch holds sufficient adsorption property [10]. Coating materials are highly adsorptive and usually in very fine particle size range. These are various grades of colloidal silica such as Syloid

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Research Article

DESIGN AND PERFORMANCE VERIFICATION OF NEWLY DEVELOPED DISPOSABLE STATIC DIFFUSION CELL FOR DRUG DIFFUSION/PERMEABILITY STUDIES

ANKIT KUMAR YADAV[1], VARUN GARG[1], MONICA GULATI[1], PARIKSHIT BANSAL[2], KOMPAL BANSAL[2], PUNEET KAUR[3], SACHIN KUMAR SINGH[1]*, AMIT MITTAL[1], RAKESH NARANG[1], BIMLESH KUMAR[1], NARENDRA KUMAR PANDEY[1], SHEETU WADHWA[1], GOPAL LAL KHATIK[1], MAYUKH BANERJEE[1], SOUVIK MOHANTA[1], SHUBHAM SINHA[1], JIVAN JYOTI[1], SANANDA SOM[1], BHUPINDER KAPOOR[1], SAURABH SINGH[1]

[1]Department of Pharmacy, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara – 144411, Punjab, India. [2]Excellion Innovations and Inventions Pvt., Ltd., (EXII) Pvt. Ltd, 5568, Sector 38-West, Chandigarh – 160 014, India. [3]Washington State University, College of Pharmacy, Spokane, WA, USA
Email: singhsachin23@gmail.com

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ABSTRACT

Objectives: The present study describes a disposable static diffusion cell for *in vitro* diffusion studies to achieve better results as compared to well existing Franz diffusion cell (FDC) in terms of the absence of bubbles, variable receptor compartment, ease of handling, and faster results.

Materials and Methods: The cell consists of a cup-shaped donor compartment made of semi permeable that could be either cellophane membrane or, animal skin fitted to a rigid frame, which is supported on a plastic plate that contains a hole for the sample withdrawal. The receptor compartment is a separate unit, and it could be any container up to 500ml volume capacity. The most preferred receptor compartment is glass beaker. In the present study, goatskin was used as semi-permeable membrane and verification of its performance was carried out through diffusion studies using gel formulations of one each of the four-selected biopharmaceutical classification system (BCS) class drugs. Metronidazole, diclofenac sodium, fluconazole, and sulfadiazine were used as model drugs for BCS Class I, II, III, and IV, respectively.

Results: The newly developed diffusion cell (NDDC) was found to provide faster and more reproducible results as compared to FDC. At the time interval of 24 h, the cell was found to exhibit a higher diffusion of metronidazole, diclofenac sodium, fluconazole, and sulfadiazine by 0.65, 0.65, 0.32, and 0.81 folds, respectively. The faster release obtained with NDDC was attributed to a larger surface area of skin as compared to that in FDC.

Conclusion: It was concluded that better reproducibility of results could be achieved with NDDC.

Keywords: Newly developed diffusion cell, Franz diffusion cell, Biopharmaceutical classification system, Metronidazole, Diclofenac sodium, Fluconazole, Sulfadiazine.

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INTRODUCTION

Among the drug delivery systems, transdermal drug delivery offers avoidance of first-pass metabolism, decreased toxicity, fewer side effects, as well as greater patient compliance [1,2]. To evaluate the performance of prepared transdermal formulation *in vitro* static diffusion cells are used. This diffusion study helps the scientists to understand the relationship between skin, drug, and formulation [3,4]. This further helps to optimize the formulation and process variables for optimizing the formulation as well as help to assess the toxicity of the drug [5-8]. The most common technique for measuring dermal absorption *in vitro* is the application of the test substance in an appropriate formulation to the surface of a skin sample, which is mounted as a barrier between the donor compartment and receptor compartment of a diffusion cell [9]. Diffusion cells may be of static [3] or flow-through [10]. Static diffusion cells sample this chamber and replace with new perfusate at each time point. Flow-through cells use a pump to pass perfusate through the receptor chamber and collect flux by repeatedly collecting perfusate [9]. Static diffusion cells can be sub-divided on the basis of the skin orientation: The membrane can be placed horizontally or vertically. The majority of skin absorption studies are conducted using horizontal cells, with the skin surface open to the air. The use of vertical (or side-by-side) cells is more common when evaluating drug delivery systems such as sonophoresis, iontophoresis, or electroporation and requires immersion of both surfaces of the skin preparation, which may result in excessive hydration and possibly skin damage [11].

One of the most widely used static designs for studying *in vitro* permeation is the Franz diffusion cell (FDC) [12]. FDC comprises a donor chamber, an upper chamber through which the drug formulation to be studied/tested is placed into the device/cell. The chamber is attached to a flat ground glass joint. Another component is semi-permeable membrane which is the "functional" part of the device and comprises. In general, regenerated cellulose membrane is used, due to good permeability and resistance to various organic solvents/buffers. The membrane is placed horizontally between the donor chamber and receptor chamber and held in place by means of a clamp. The lower part of FDC is receptor chamber, which contains buffer/water, into which the drug diffuses. The chamber is surrounded by a "water jacket" through which heated water is circulated by means of a heater/circulator and helps to maintain the temperature of buffer/water inside the receptor chamber, constant simulating the body temperature. To measure the rate of drug diffusion across the semi-permeable membrane, from the "donor chamber" into the "receptor chamber," sampling of the solution inside the receptor chamber has to be carried out, at regular time intervals. A "sampling port" is provided in the form of an "arm" through which the "solution" can be simply withdrawn with the help of a needle/syringe [3]. Water/buffer inside the receptor chamber is stirred gently by means of a stirring arrangement, which comprises a magnetic bar placed inside the receptor chamber. The assembly is then placed over a magnetic stirrer so that the bar rotates and helps to stir the solution.

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Efficacy of *co*-administration of modified apple polysaccharide and probiotics in guar gum-Eudragit S100 based mesalamine mini tablets: A novel approach in treating ulcerative colitis

Souvik Mohanta [a], Sachin Kumar Singh [a,*], Bimlesh Kumar [a], Monica Gulati [a], Rajesh Kumar [a], Ankit Kumar Yadav [a], Sheetu Wadhwa [a], Jivan Jyoti [a], Sananda Som [a], Kamal Dua [b,c,d,e], Narendra Kumar Pandey [a]

[a] School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144411, Punjab, India.
[b] Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Australia
[c] School of Biomedical Sciences and Pharmacy, The University of Newcastle, Callaghan, NSW 2308, Australia
[d] Centre for Inflammation, Centenary Institute, Sydney, NSW 2050, Australia
[e] Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, Lot 1 Kookaburra Circuit, New Lambton Heights, Newcastle, NSW 2305, Australia

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ABSTRACT

Modified Apple Polysaccharide (MAP) has been reported to cure colorectal diseases by up-regulating apoptosis and down regulating metastasis. In the present study, mesalamine (MES) and MAP mini tablets have been prepared and co-administered with probiotics to provide site specific release of drug. Probiotics along with MAP, which acts as a prebiotic would replenish the colonic microflora that have been compromised due to colorectal pathology. MES mini tablets were prepared keeping guar gum in the core and coating them with Eudragit S100 and guar gum. The optimized batch was explored for its curative potential on acetic acid induced ulcerative colitis (UC) in rat model with and without administration of probiotic and MAP. The results revealed that the rats treated with the combination of MAP and MES mini tablets along with probiotics show maximum curative potential. It was also observed that MAP mini tablets show better curative potential as compared to probiotics. The results of disease activity index, macroscopic scoring, antioxidant studies, tumour alpha and histopathological examination suggested that the rats treated with combination of MES-MAP mini tablets and probiotics have maximum therapeutic effect followed by MES mini tablets alone, MAP mini tablets alone and probiotics.

1. Introduction

Ulcerative colitis (UC) is a type of chronic inflammatory disease of the colon but with unknown aetiology. The symptoms vary from abdominal pain, bloody diarrhoea and malnutrition. Colonic adenocarcinoma and dysplasia are the most potential threats to ulcer patients, and hence a continuous endoscopic surveillance is required for patient welfare [1]. Approximately 25–30% of the total affected patients require colectomy at certain points of their lives, if treatment with medications goes in vain. Colectomy is considered as the final and permanent cure for UC. General treatments may include one or more medications, balanced diet and intensive moral support to provide an improved quality of life to the patient [1]. Furthermore, people with UC suffer from bloody diarrhoea due to imbalance in gut microflora [2]. As per the available literature the reduction in bacterial count reaches to 30% and in case of lactic acid bacterial subspecies the count reduces from 32 to 18 [2]. The different drugs use for treating ulcerative colitis are mesalamine (MES), sulfasalazine, azathioprine, 6 mercaptopurine etc. MES is an anti-inflammatory agent which is grouped under salicylates and is considered as a first line drug for treating inflammatory bowel disease. It works by diminishing inflammation by the inhibition of prostaglandin production and blocking cyclooxygenase. However, alopecia, cramping, diarrhoea, interstitial nephritis, myalgia, flatulence and nausea are some of the side effects that are observed with long term use of MES [3–5]. Moreover, due to its multiple daily dosing, low adherence to treatment has been reported [6]. This causes frequent flares of colitis that ultimately increases medical costs hence, the strategies to improve drug's adherence are greatly in demand [7]. Hence, delivery of MES to colon, by passing the upper GI regions is expected to reduce the side effects.

In past few decades oral colon specific drug delivery systems (CSDDS) have attracted researchers as they help in delivering the drug at the targeted site. CSDDS works on the principle of protection of drugs' degradation in stomach and small intestine due to presence of acidic environment and other digestive enzymes and successfully delivering the drugs at colonic site [7]. Keeping this objective various delivery

* Corresponding author.
E-mail address: sachin.16030@lpu.co.in (S.K. Singh).

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Coadministration of Polypeptide-k and Curcumin Through Solid Self-Nanoemulsifying Drug Delivery System for Better Therapeutic Effect Against Diabetes Mellitus: Formulation, Optimization, Biopharmaceutical Characterization, and Pharmacodynamic Assessment

Varun Garg,[1] Puneet Kaur,[2] Monica Gulati,[1] Sachin Kumar Singh,[1] Bimlesh Kumar,[1] Narendra Kumar Pandey,[1] Ankit Kumar Yadav,[1] Rajesh Kumar,[1] Gowthamarajan Kuppusamy,[3] Anindita De,[3] Nethravathi Puttappa,[3] and Sheetu Wadhwa[1]

[1]*School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.*
[2]*Department of Pharmaceutical Sciences, Washington State University, College of Pharmacy, Spokane, Washington.*
[3]*Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research (Deemed To Be University), Ootacamund, Tamilnadu, India.*

ABSTRACT

An attempt has been made to prepare solid self-nanoemulsifying drug delivery system (SNEDDS) of polypeptide-k (PPK) and curcumin (CRM) using Labrafil M1944 CS as oil, Tween-80 as surfactant, Transcutol P as cosurfactant and Aerosil-200 (A-200) as porous hydrophobic carrier for improving their antidiabetic potential through oral delivery. Box–Behnken Design was used to optimize the liquid formulation based on the results of the mean droplet size, polydispersity index, percentage drug loading, and zeta potential. The formulation was adsorbed on Aerosil-200 through spray drying. The formulation showed desirable micromeritic, disintegration, and dissolution properties. About fivefold rise in the dissolution and permeation rate for drugs was observed from formulations vis a vis their unprocessed forms. The formulation was found to be stable with variation in pH, dilution, and temperature. The individual solid SNEDDS formulation of PPK and CRM and their combination were evaluated for antidiabetic potential and the results were compared with their naive forms on streptozotocin-induced diabetic rats. The results revealed better control of serum glucose level and other biochemical tests, such as liver parameters, lipid profiles, and antioxidant levels, as well as histological evaluation of pancreatic tissues in all the solid SNEDDS formulation as compared with their naive forms.

Keywords: solid SNEDDS, spray drying, diabetes, dissolution, diffusion, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder, which is caused either due to failure of insulin production by the body or, absence of response from cells to the produced insulin, and is characterized by increased blood glucose level.[1,2] According to the IDFs report of 2017, about 425 million people were reported to be suffering from DM and among them 90% cases were of T2DM. By 2045, this number is expected to reach 628.6 million.[3] The prevalence of DM is higher in India than in any other country in the world. India has a statistical figure of 72.9 million people suffering from DM. By 2045, the estimated number of people with DM in India will be 134.3 million, and that will take India to number one position.[3] The global economic burden reported for the treatment of DM was U.S. $1.3 trillion in 2015 and it is expected to rise to about U.S. $2.5 trillion by 2030.[4]

The two drugs, polypeptide-k (PPK) and curcumin (CRM), are well reported for their antidiabetic activities.[5–8] The dried ripened seeds of *Momordica charantia* are extracted to obtain PPK, while CRM is obtained from dried rhizomes of *Curcuma longa*.[6,8] Despite having very good antidiabetic potential, both the drugs suffer from poor aqueous solubility and

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ORIGINAL ARTICLE

A three-pronged formulation approach to improve oral bioavailability and therapeutic efficacy of two lipophilic drugs with gastric lability

Jivan Jyoti[1] · Nandha Kumar Anandhakrishnan[2] · Sachin Kumar Singh[1] · Bimlesh Kumar[1] · Monica Gulati[1] · K. Gowthamarajan[2] · Rajesh Kumar[1] · Ankit Kumar Yadav[1] · Bhupinder Kapoor[1] · Narendra Kumar Pandey[1] · Sananda Som[1] · Souvik Mohanta[1] · Indu Melkani[1] · Rubiya Khursheed[1] · Rakesh Narang[3]

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Abstract

The aim of present study was to co-administer curcumin (CRM) liquisolid pellets and coated duloxetine hydrochloride (DXH) pellets in rats to treat neuropathic pain (NP) associated with chronic constriction injury (CCI). To formulate liquisolid pellets of CRM, it was first dissolved in Tween-80 and then adsorbed on the porous surface of MCC PH102 and Syloid XDP that were used as carrier and coating materials, respectively. Central composite design was used to optimize the liquisolid formulation. The results of powder X-ray diffraction studies, differential scanning calorimetry, and scanning electron microscopy showed complete solubility of drug in Tween-80 followed by its complete adsorption on the porous surface of Syloid XDP and MCC PH102. Both DXH and liquisolid CRM powders were converted into pellets using extrusion-spheronization. DXH pellets were further coated with Eudragit S100 to bypass the gastric pH. About 32.31-fold increase in dissolution rate of CRM present in liquisolid formulation was observed as compared to its unprocessed form. Similarly, the dissolution profile in 0.1 N HCl for Eudragit S100-coated DXH showed complete protection of drug for 2 h and complete release after its introduction in buffer medium (0.2 M phosphate buffer pH 6.8). he pharmacokinetic studies carried out on rats revealed 7.3-fold increase in bioavailability of CRM present in liquisolid pellets and 4.1-fold increase in bioavailability of DXH present in coated pellets was observed as compared to their unprocessed pellets. This increase in bioavailability of drugs caused significant amelioration of CCI-induced pain in rats as compared to their unprocessed forms. The histological sections showed better improvement in regeneration of nerve fibers in rats.

Keywords Curcumin · Liquisolid pellets · Duloxetine hydrochloride · Chronic constriction injury · Oral bioavailability

Introduction

Neuropathic pain (NP) is a common neurological disorder of somatosensory system including peripheral fibers and central neurons in which nerves are damaged or destroyed and this leads to over-sensitization of nerves, causing severe pain from stimuli that are normally painless. About 7–10% of the general population worldwide is affected from NP. People can be affected by neuropathy at any age, but mostly it affects people over an age of 50 (8.9%) as compared to people below the age of 49 (5.6%). It is more frequent in women (8%) as compared to men (5.7%). Most commonly it affects lower and upper limbs, lower back, and neck [1]. It is important to note that all the factors that can cause loss of function within the sensory nervous system can cause NP. Some of these factors include Carpel tunnel syndrome, trauma, diabetes, vitamin deficiencies (most commonly vitamin B12 deficiency), human immunodeficiency virus infection, certain cancers, stroke, multiple sclerosis, cancer treatments, shingles, chronic alcohol intake, infections, and toxins [2]. As there is existence

✉ Sachin Kumar Singh
singhsachin23@gmail.com; sachin_pharma06@yahoo.co.in; sachin.16030@lpu.co.in

[1] School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab 144411, India

[2] Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research (Deemed to be University), Ootacamund, Tamilnadu, India

[3] Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, India

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Historical perspective

Gold nanoparticles: New routes across old boundaries

Yogita Kumari [a], Gurmandeep Kaur [a], Rajesh Kumar [a], Sachin Kumar Singh [a,*], Monica Gulati [a], Rubiya Khursheed [a], Ayinkamiye Clarisse [a], K. Gowthamarajan [b], V.V.S. Narayana Reddy Karri [b], Ravichandran Mahalingam [c], Dipanjoy Ghosh [a], Ankit Awasthi [a], Rajan Kumar [a], Ankit Kumar Yadav [a], Bhupinder Kapoor [a], Pankaj Kumar Singh [d], Kamal Dua [e], Omji Porwal [f]

[a] School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144411, Punjab, India
[b] Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research (Deemed to be University), Ootacamund, Tamilnadu, India
[c] Reech Pharma, LLC, 37200, Central Court Newark, CA 94560, United States
[d] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Hyderabad, India
[e] Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Australia
[f] Department of Pharmacognosy, Faculty of Pharmacy, Ishik University, Erbil, Kurdistan, Iraq

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ABSTRACT

In recent years, gold nanoparticles have emerged as unique non-invasive drug carriers for targeting drugs to their site of action. Their site specificity has helped in increasing drugs' efficacy at lower dose as well as reduction in their side effects. Moreover, their excellent optical properties and small size offer their utilization as diagnostic tools to diagnose tumors as well as other diseases. This review focuses on various approaches that have been used in last several years for preparation of gold nanoparticles, their characterization techniques and theranostic applications. Their toxicity related aspects are also highlighted. Gold nanoparticles are useful as theranostic agents, owing to their small size, biocompatible nature, size dependent physical, chemical and optical properties etc. However, the challenges associated with these nanoparticles such as scale up, cost, low drug payload, toxicity and stability have been the major impediments in their commercialization. The review looks into all these critical issues and identifies the possibilities to overcome these challenges for successful positioning of metallic nanoparticles in market.

Contents

* Corresponding author.
E-mail address: sachin.16030@lpu.co.in (S.K. Singh).

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Quality by Design-Based Crystallization of Curcumin Using Liquid Antisolvent Precipitation: Micromeritic, Biopharmaceutical, and Stability Aspects

Sananda Som,[1] Sachin Kumar Singh,[1] Gopal L. Khatik,[1] Bhupinder Kapoor,[1] Monica Gulati,[1] Gowthamarajan Kuppusamy,[2] Nandha Kumar Anandhakrishnan,[2] Bimlesh Kumar,[1] Ankit Kumar Yadav,[1] Rajesh Kumar,[1] Pankaj Kumar Singh,[3] Rubiya Khursheed,[1] Rajan Kumar,[1] Narendra Kumar Pandey,[1] Jivan Jyoti,[1] Souvik Mohanta,[1] and Omji Porwal[4]

[1]*School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, India.*
[2]*Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research (Deemed to be University), Ootacamund, Tamilnadu.*
[3]*Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Hyderabad, India.*
[4]*Department of Pharmacognosy, Faculty of Pharmacy, Ishik University, Kurdistan, Iraq.*

ABSTRACT

The aim of present study was to introduce the role of quality by design to produce curcumin crystals with enhanced dissolution rate and bioavailability. The liquid antisolvent method was used to produce crystals. The crystal growth was controlled using the Box–Behnken design. The variables used in the crystallization process included the ratio of pyrocatechol to polyethylene glycol (PEG) 1500, solvent addition rate, stirring time, and stirring speed. Combination of these variables was found to yield curcumin crystals of 2.45 ± 0.56 μm size and 0.321 polydispersity index that exhibited enhanced solubility, dissolution rate, product yield, and compressibility. The optimized curcumin crystals were characterized by Fourier-transform infrared spectrophotometer (FT-IR), nuclear magnetic resonance, differential scanning calorimetry, X-ray powder diffraction, and scanning electron microscopy. The dissolution rate and oral bioavailability of optimized curcumin crystals were found to be 2.66- and 7.08-folds higher than its unprocessed form. The optimized crystals were found stable for 6 months under accelerated temperature of 40°C and 75% relative humidity as there was no significant difference observed in the crystal size and dissolution profile.

Keywords: polymorph, Box–Behnken design, dissolution, bioavailability, crystallization

INTRODUCTION

Poor solubility of drugs poses a notable challenge in the development of new products leading to low and erratic bioavailability, resulting in both safety and efficacy concerns, particularly in case of oral administration.[1] Although numerous technologies exist for enhancing the bioavailability of drugs with low solubility, the success of these approaches greatly depends on the physical and chemical nature of the molecules being developed and a universal applicability of any approach has not yet been achieved.[1,2] Crystal engineering of drug substances provides a unique approach for improvement of physicochemical as well as biopharmaceutical properties such as solubility, bioavailability, absorption, metabolism, systematic elimination, stability, decomposition, and dissolution rate.[1] The approach is a combination of both crystal engineering and supramolecular chemistry, which allows researchers to design products with the desired physicochemical parameters.[3] This may be done by varying various intermolecular interactions, including hydrogen bonding and noncovalent interactions such as halogen bond, π-π, and columbic interaction. The desired characteristics can be achieved without altering the chemical composition of the active pharmaceutical ingredient (API).

A number of methods are reported to generate crystals of APIs such as the antisolvent addition method, supersaturation, additive mediated method, and grinding.[4] Among them, the antisolvent addition is the most preferred method for

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RESEARCH ARTICLE

Quality by Design-based Optimization of Formulation and Process Variables for Controlling Particle Size and Zeta Potential of Spray Dried Incinerated Copper Nanosuspension

Saurabh Singh[1], Sachin Kumar Singh[2,*], Malti G. Chauhan[3], Bimlesh Kumar[2], Narendra Kumar Pandey[2], Barinder Kaur[1], Arun Kumar[1], Souvik Mohanta[2], Monica Gulati[2], Sheetu Wadhwa[2], Ankit Kumar Yadav[2], Pankaj Kumar Singh[2,4], Yogita Kumari[2], Gurmandeep Kaur[2], Rubiya Khursheed[2] and A. Clarrisse[2]

[1]Department of Ayurvedic Pharmacy, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara - 144411, Punjab, India; [2]School of Pharmaceutical Sciences, Department of Ayurvedic Pharmacy, Lovely Professional University, Phagwara - 144411, Punjab, India; [3]Ayurvedic Pharmacopoeia Committee, Pharmacopoeia Commission for Indian Medicine & Homoeopathy, Ghaziabad, Government of India, Uttar Pradesh, India; [4]Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Hyderabad, India

Abstract: ***Background*:** In the present study copper nanosuspension was prepared from Incinerated Copper Powder (ICP) by top down media milling. *Glycyrrhiza glabra* (GG) and Gum Acacia (GA) were used as stabilizers in the formulation.

***Methods*:** Box Behnken Design was used to investigate the effect of formulation and process variables on particle size and zeta potential and optimize their ratio to get target product profile. The ratio of GA and GG to ICP was varied along with milling time and its speed. Further the prepared nanosuspensions were solidified using spray drying.

***Results*:** The particle size was found to be decreased with the increase in GG to ICP ratio, milling time and milling speed, whereas, reverse effect on particle size was observed with an increase in GA to ICP ratio. The zeta potential was found to be increased with the increase in GG to CB ratio and milling speed and it decreased with the increase in GA to ICP ratio and milling time. The obtained value for particle size was 117.9 nm and zeta potential were -9.46 mV which was in close agreement with the predicted values by the design which was, 121.86 nm for particle size and -8.07 mV for zeta potential respectively. This indicated the reliability of optimization procedure. The percentage drug loading of copper in the nanosuspension was 88.26%. The micromeritic evaluation of obtained spray dried nanoparticles revealed that the particles were having good flow and compactibility.

***Conclusion*:** It can be concluded that application of media milling, design of experiment and spray drying have offered very good copper nanosuspension that has the potential to be scaled up on industrial scale.

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*Address correspondence to this author at the School of Pharmaceutical Sciences, Lovely Professional University, Phagwara - 144411, Punjab, India; Tel: +919888720835; Fax: +91 1824501900; E-mails: singhsachin23@gmail.com; sachin_pharma06@yahoo.co.in

1. INTRODUCTION

Copper, a transition metal, has been reported to be effective in the treatment of type 2 diabetes mellitus by inhibition of α-amylase activity [1-3].

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Aquasomes: The Journey So Far and the Road Ahead

Monica Gulati[1*], Sachin Kumar Singh[1], Puneet Kaur[1], Ankit Yadav[1], Kirsten M. Pondman[2, 3] and Uday Kishore[3]

[1]*School of Pharmaceutical Sciences, Lovely Professional University, Punjab 144411, India*

[2]*Neuro Imaging, MIRA Institute, University of Twente, Enschede, The Netherlands*

[3]*Centre for Infection, Immunity and Disease Mechanisms, Biosciences, Brunel University, Uxbridge UB8 3PH, London, UK*

[*]*E-mail: monicagulati14@gmail.com*

Abstract: The development of aquasomes is one of the most remarkable milestones in the progress of ceramic nanoparticles. Though the use of ceramic nanoparticles in medicine has been documented since time immemorial in the form of Bhasmas, they have lately been recognized for their application in bone tissue engineering, dentistry, and later in drug delivery. In a recent advancement, ceramic nanoparticles have been designed with a coating of carbohydrates onto which the peptide drug molecules are adsorbed. This helps in preventing the denaturation of therapeutic biomolecules. These three layered structures have been christened "Aquasomes", which have been successfully tried for in vivo delivery of peptides, proteins, antigens and other drugs. They comprise a central solid nanocrystalline core, coated with polyhydroxy oligomers, upon which biologically active molecules are adsorbed. The solid core provides the structural stability whereas the carbohydrate coating protects against dehydration. This chapter is an update on the advancement in the development of aquasomes. We also discuss future trends and promises albeit with a clear understanding of the limitations of this delivery system.

Keywords: Aquasomes, ceramic, nanoparticles, bhasmas

INTRODUCTION

Though well-known in the ancient Indian traditional medical literature as Bhasmas, ceramic nanoparticles have gained wide popularity as drug delivery vehicles in the last two decades[1-3]. Apart from possessing the inherent advantages associated with nanoparticles such as large surface area-to-volume ratios, high payload, possibility of mass production and enhanced cellular uptake, ceramic nanoparticles offer added advantages as compared to polymeric nanoparticles. These include prolonged release and non-swellability leading to a largely immutable character[4]. After being successfully applied for anti-cancer drug delivery[5], photodynamic therapy[6] and bone tissue engineering[7], the second generation ceramic nanoparticles with various surface coatings have been introduced. The coating materials reported include PEG[8], metals like gold[9], peptide/ protein etc[10]. Three-layered, sugar coated ceramic nanoparticles have recently emerged as a new class of next-generation therapeutic vehicles[11]. Aquasomes are tri-layered self-assembled nanoparticles. They

Formulation, Solidification and *in vitro* Evaluation of Spray-Dried Powders Containing Meloxicam Nanoparticles to Enhance its Dissolution Rate

Ranjith Anishetty[1], Ankit Kumar Yadav[1*], Puneet Kaur[1], Dhananjay S Singare[2], Monica Gulati[1] and Sachin Kumar Singh[1]

[1]*School of Pharmaceutical Sciences, Lovely Professional University, Punjab 144411, India*

[2]*Piramal Pharmaceutical Development, Ahmedabad, Gujarat 382213, India*

[*]*E-mail: ankityadav208@gmail.com*

Abstract: In the present study an attempt has been made to formulate meloxicam nanosuspension in order to enhance its dissolution rate. The influence of excipients used for solidification of nanoparticles into tablet dosage form on dissolution rate of meloxicam was also evaluated. The nanosuspension of meloxicam was prepared using top down media milling method and solidified into dry, free flowing powder using spray drier. The formulated nanosuspension was compressed into tablet dosage form in two different compositions. Batch I contained lactose monohydrate and batch II contained dicalcium phosphate as major diluents. About 90% of drug release was observed in case of batch I in first 5 min, whereas batch II has shown only 25% release in first 5 min. However, only 40% drug release was observed in 75 min from the controlled batch of tablets. The dissolution profiles of fresh and aged nanosuspensions were found similar (F_2 = 53.25). Thus it can be concluded that the formulation procedure described in the present study can be a suitable method to prepare nanosuspension of poorly soluble drugs, with enhanced dissolution profile and stability.

Keywords: Meloxicam, Dissolution, Nanosuspension, Stability studies, Media milling

INTRODUCTION

According to Biopharmaceutical Classification System (BCS), due to low solubility and high permeability of Meloxicam, it is classified as class II compound[1]. The bioavailability of class II drugs can be increased by increasing their aqueous solubility. In view of this, various approaches used to improve the aqueous solubility of meloxicam include, use of co-solvents[2-4], solid dispersions, complexation and many other techniques[5,6]. These attempts appeared to be successful to increase the solubility as well as *in vitro* dissolution rate of meloxicam, however, they suffered with some limitations like poor *in vivo* performance or stability.

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ROLE OF COLONIC MICROFLORA IN THE DIGESTION OF POLYSACCHARIDES: A REVIEW

Prof. (Dr.) S. H. Ansari
President,
Society of Pharmaceutical Education
& Research, Gwalior [M.P.] India

Dr. Upendra Nagaich
Secretary,
Society of Pharmaceutical Education
& Research, Gwalior [M.P.] India

Prof. (Dr.) Monica Gulati
Chairperson-LOC
Sr. Dean, LFAMS,
Lovely Professional University,
Phagwara [Punjab] India

Mr. Bimlesh Kumar
Organizing Secretary,
Associate Professor, SPS
Lovely Professional University,
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