FORMULATION AND EVALUATION OF PREDNISOLONE RETENTION ENEMA AS DISPERSIBLE TABLET WITH VEHICLE

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Submitted by

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October 2017

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INTERNAL EXAMINER **EXTERNAL EXAMINER**

Date: Date:

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ACKNOWLEDGEMENT

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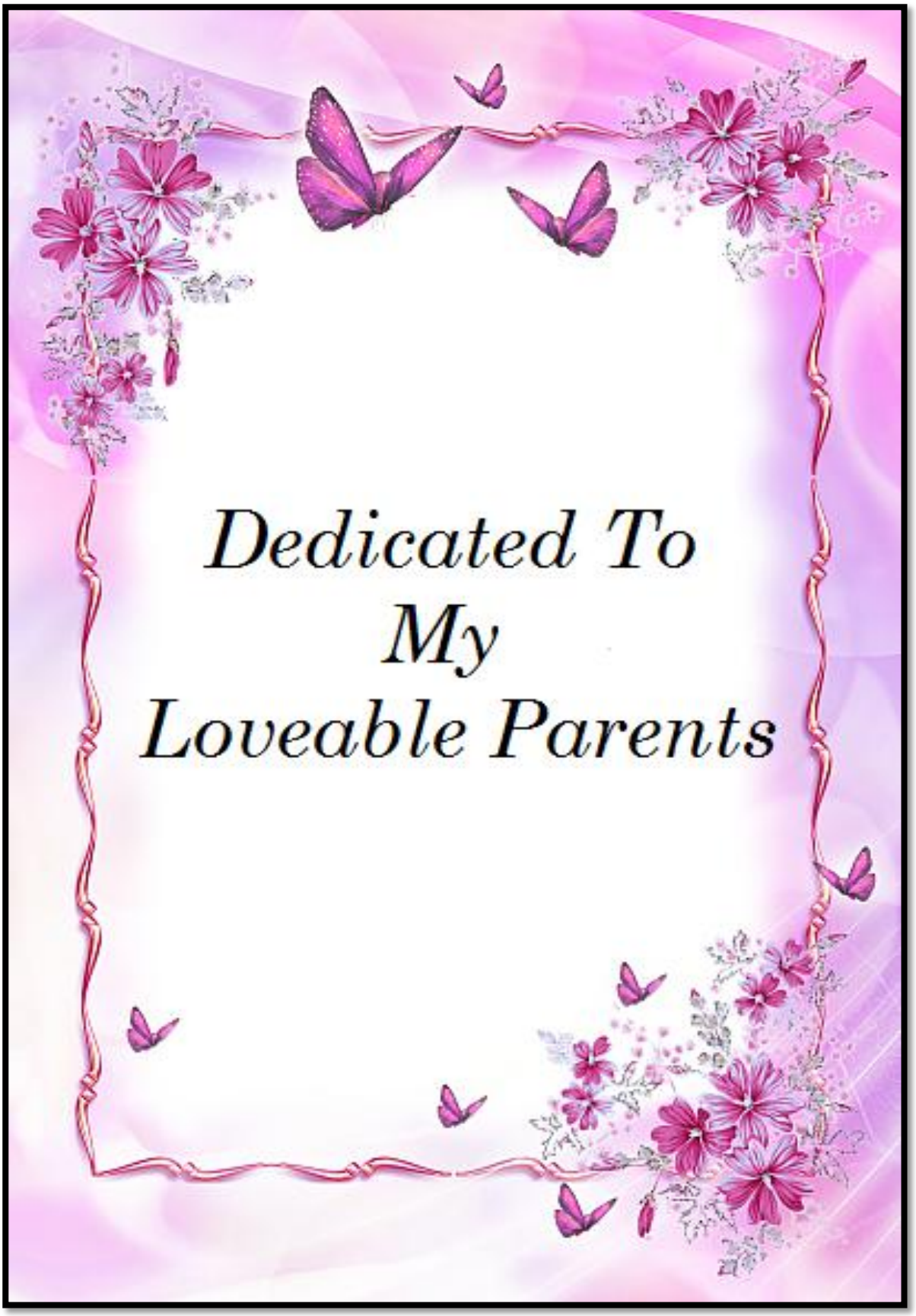
Dedicated To
My
Loveable Parents

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ABBREVIATIONS

IBD	Inflammatory Bowel Disease
CD	Crohn's Disease
UC	Ulcerative Colitis
GI	Gastro Intestinal
TLR	Toll – Like Receptor
HLA	Human Leukocyte Antigen
IL	Inter-Leukin
TNF	Tumour Necrosis Factor
NF – Kb	Nuclear Factor – κB
Th	T helper cell
NKT	Natural Killer T-cell
CXCL	Chemokine
T- reg	regulatory T cell
MAdCAM-1	Mucosal Address in Cell Adhesion Molecule 1
CT	Computed Tomography
FAD	Flavin Adenine Dinucleolate
FMN	Flavin Mono Nucleotide
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Response Element
nGRE	negative Glucocorticoid Response Element
DNA	Dinucleotide Aminoacid
DT	Dispersible Tablet
FDT	Fast Dissolving Tablet
UV	Ultra Violet
IR	Infra Red
FTIR	Fourier Transform Infrared Spectroscopy
DCL	Directly Compressible Lactose
I.P	Indian Pharmacopoeia
PhEUR	European Pharmacopoeia
J.P	Japanese Pharmacopoeia
U.S.P	United States Pharmacopoeia

NMT	Not More Than
SD	Standard Deviation
cfu/ ml	Colony Forming Units per milliliter
Rpm	Rotation Per Minute
RH	Relative Humidity
Nm	Nanometre
Cps	Centipoises
Hrs	Hours
Mins	Minutes
Secs	Seconds
Gm	Gram
Mg	Milligram
Ml	Millilitre
Mm	Millimetre
Cm	Centimetre
µm	Micrometre
µg/ml	Microgram per milliliter
gm/ml	Gram per milliliter
mg/ml	Milligram per milliliter
gm/cm^3	Gram per centimetre cube
Kg/cm^2	Kilogram per centimetre square
mPa.s	Millipoises
gm/mol	Gram per mole
w/w	Weight per weight
w/v	Weight per volume
i.e.	That is
e.g.	Example
%	Percentage
^oC	Degree Celsius
<	Less than
≥	Greater than equals
Θ	Theta
#	Mesh size

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a general term for a group of chronic inflammatory disorders of unknown etiology involving the gastrointestinal tract[1]. Crohn's disease (CD) and Ulcerative Colitis (UC) are inflammatory bowel diseases that cause chronic inflammation and damage in the gastrointestinal (GI) tract. Inflammation impairs the ability of affected GI organs to function properly, leading to symptoms such as persistent diarrhea, abdominal pain, rectal bleeding, weight loss and fatigue[2]. These are clinically characterized by recurrent inflammatory involvement of intestinal segments with several manifestations often resulting in an unpredictable course[1].

1.1.1. ULCERATIVE COLITIS (UC)

UC was described in the year of 1800 by Samuel Wilks. UC is characterized by continuous colonic mucosal inflammation that extends proximally from the rectum. It is a chronic disease that typically presents in the second or third decade of life with bloody diarrhoea and abdominal cramps[3]. In contrast with that of Crohn's disease, the inflammation of ulcerative colitis is limited to the colonic mucosa. The portion of the colon affected varies. Some patients have inflammation that is limited to the rectum (ulcerative proctitis), whereas others have more proximal disease. Pancolitis refers to ulcerative colitis that affects the entire colon[4]. The difference between normal colon and ulcerative colon was given in Figure 1[5].

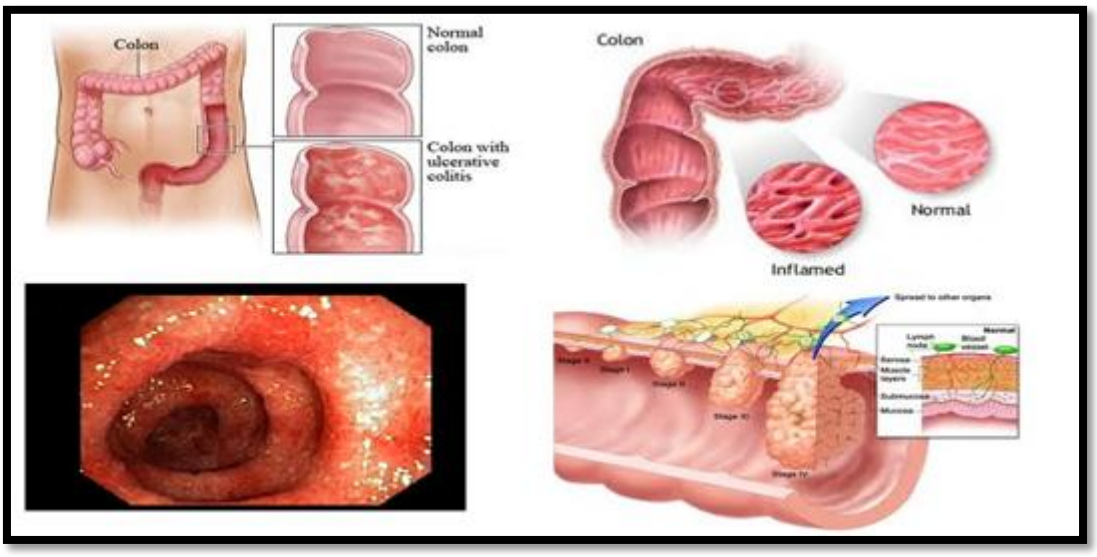


Figure 1: Difference between Normal Colon and Colon with UC

1.1.2. ETIOLOGY

The etiology is unknown. Risk factors include a history of recent infection with *Salmonella* or *Campylobactor*[4]. It is associated with an 8-10 times higher risk of developing ulcerative colitis in the following year[6]. UC has been hypothesized that it is an autoimmune disease in which the intestinal immune system attacks healthy intestinal cells and tissues. Susceptibility to this abnormal behavior of the intestinal immune system may be genetically inherited. People who have a first-degree relative (i.e. brother, sister, child, parent) with ulcerative colitis are more likely to develop the disease. In recent years approximately 30 genes that might increase susceptibility to the disease have been identified. Environmental factors may also play a role. Factors such as stress and eating certain foods do not cause ulcerative colitis but may worsen the symptoms[7].

1.1.3. EPIDEMIOLOGY

UC is more prevalent than Crohn's disease. North America and northern Europe have the highest incidence and prevalence rates of UC, with incidence varying from 9 to 20 cases per 1 lakh person-years and prevalence rates from 156 to 291 cases per 1 lakh people (Table:1). Rates are lowest in the southern hemisphere and eastern countries. Incidence has increased in countries that have adopted an industrialized lifestyle, which suggests that environmental factors might be crucial in the triggering of disease onset[8]. UC affects approximately 2.5 lakhs to 5 lakhs persons in the United States, with an annual incidence of 2-7 per 1 lakh persons. The overall incidence of the disease has remained constant over the past 5 decades[9]. UC has a bimodal pattern of incidence, with the main onset peak between ages 15 and 30 years, 12 and a second smaller peak between ages 50 and 70 years. Studies have noted either no preference regarding sex, 13 or a slight predilection for men[8]. The disease affects men and women at similar rates[9].

Table No.1: Incidence and Prevalence rates of UC from Selected Countries

Authors Name	Country	Study period	Incidence	Prevalence
Herrinton LJ *et al*.,	USA (California)	1996–2002	12	155·8
Loftus CG *et al*.,	USA (Olmsted County, MN)	1990–2000	8·8	214
Kappelman MD *et al*.,	US (33 states)	2003–2004	-	238
Bernstein CN, *et al*.,	Canada	1998–2000	9·9-19·5	162–24
Manninen P *et al*.,	Finland	1986–2000	19·6	291
Vind I *et al*.,	Denmark	2003–2005	13·4	-
Bjornsson S *et al*.,	Iceland	1990–1994	16·5	-
Stewenius J *et al*.,	Sweden	1958–1982	9·4	-
Rubin GP *et al*.,	England	-	13·9	243·4

However, 80% of the patients present with disease extending from the rectum to the splenic flexure and only 20% have pancolitis.

1.1.4. SYMPTOMS

UC may be insidious, with gradual onset of symptoms or the first attack may be acute and fulminate. More mild symptoms include a progressive loosening of the stool, abdominal cramping and diarrhoea. As the disease progresses from mild to more severe, the patient may also experience weight loss, fatigue, loss of appetite that may result in nutrient deficiencies, mucus in the stool, severe rectal bleeding, fever and anaemia[10]. In some cases, extra intestinal manifestations may be present as well. Extra intestinal symptoms can be an initial manifestation or can occur later in the course of the disease[1]. Initial symptoms of UC are given in Table No.2[1,10]. Extra intestinal manifestations of UC are given in Table No.3[9].

Table No.2: Symptoms and Effects of UC

Symptoms	Effects	Percentage
Area of intestinal tract affected	Any part of inner most lining of colon, continuous with no "patches" of normal tissue	-
Diarrhoea	Typically four episodes per day	96.4%
Abdominal pain/cramping	Mild tenderness, lower abdominal cramping	81.3%
Blood in stool	Present; amount depends on disease severity	89.3%
Fatigue	Result of excessive blood loss and anaemia	-
Physical examination	Rectal exam may show peri-anal irritation, fissures, haemorrhoids, fistulas and abscesses	40.2%
Weight loss/anorexia	Weight loss in more severe cases	38.4%
Loss of appetite	Often decreased during periods of disease exacerbation	15.2%
Fever	-	20.5%
Nausea	-	6.3%
Vomiting	-	4.5%
Skin changes	-	20.5%
Risk of colon cancer	Increased	-

Table No.3: Extra intestinal Manifestations of UC

Extra intestinal manifestations	Frequency (%)
Osteoporosis	15.0
Oral ulcerations	10.0
Arthritis	5.0 to 10.0
Primary sclerosing cholangitis	3.0
Uveitis	0.5 to 3.0
Pyoderma gangrenosum	0.5 to 2.0
Deep venous thrombosis	0.3
Pulmonary embolism	0.2

1.1.5. DISEASE SEVERITY AND LOCATION

The severity of UC can be characterized as mild, moderate, severe or fulminant mild disease consists of fewer than 4 stools per day (with or without blood) without systemic signs of toxic effects and normal inflammatory markers. Moderate disease is defined as 4 or more bloody stools per day with minimal signs of toxic effects. Severe disease is classified as more than 6 bloody stools per day with evidence of systemic toxic effects including fevers, tachycardia, anaemia or elevated inflammatory markers. Fulminant disease is characterized by having more than 10 bloody bowel movements and clinical signs of toxic effects including abdominal distension, blood transfusion requirements and colonic dilation on imaging[3]. The severity of UC is given in Figure 2[11].

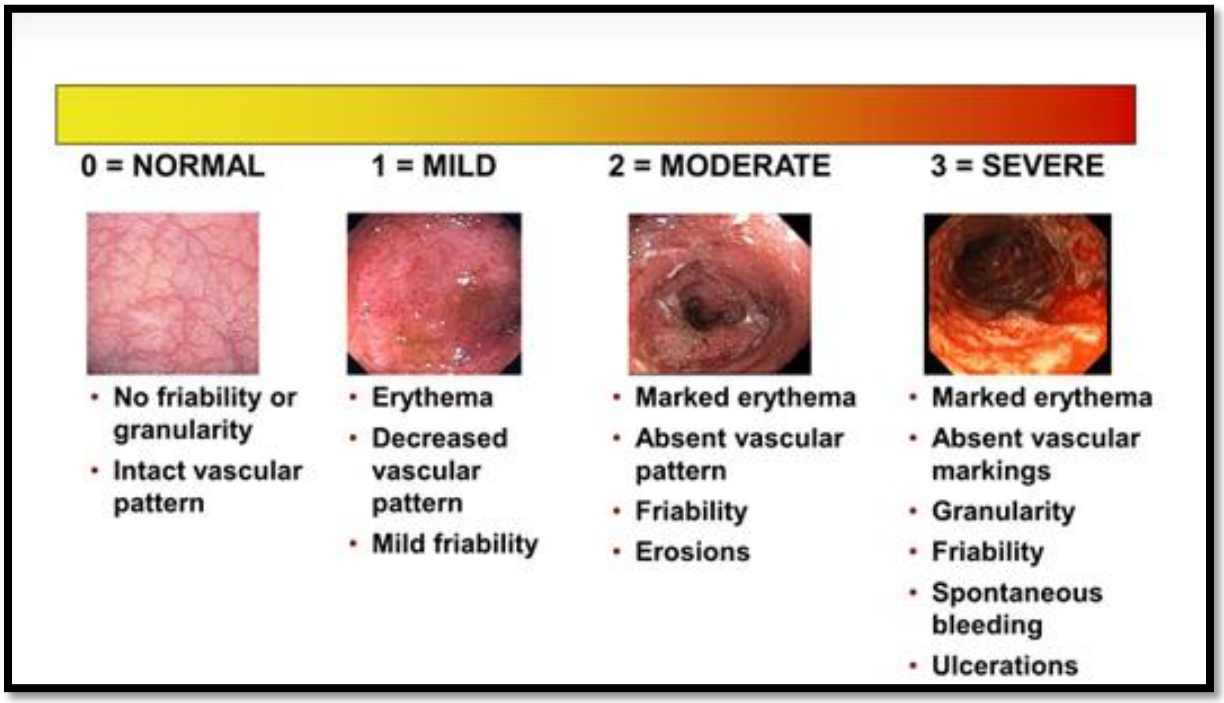


Figure 2: Severity of UC

1.1.6. TYPES OF ULCERATIVE COLITIS

UC can be categorized on the basis of the extent of the disease. The type of UC can be given in Figure 3[12].

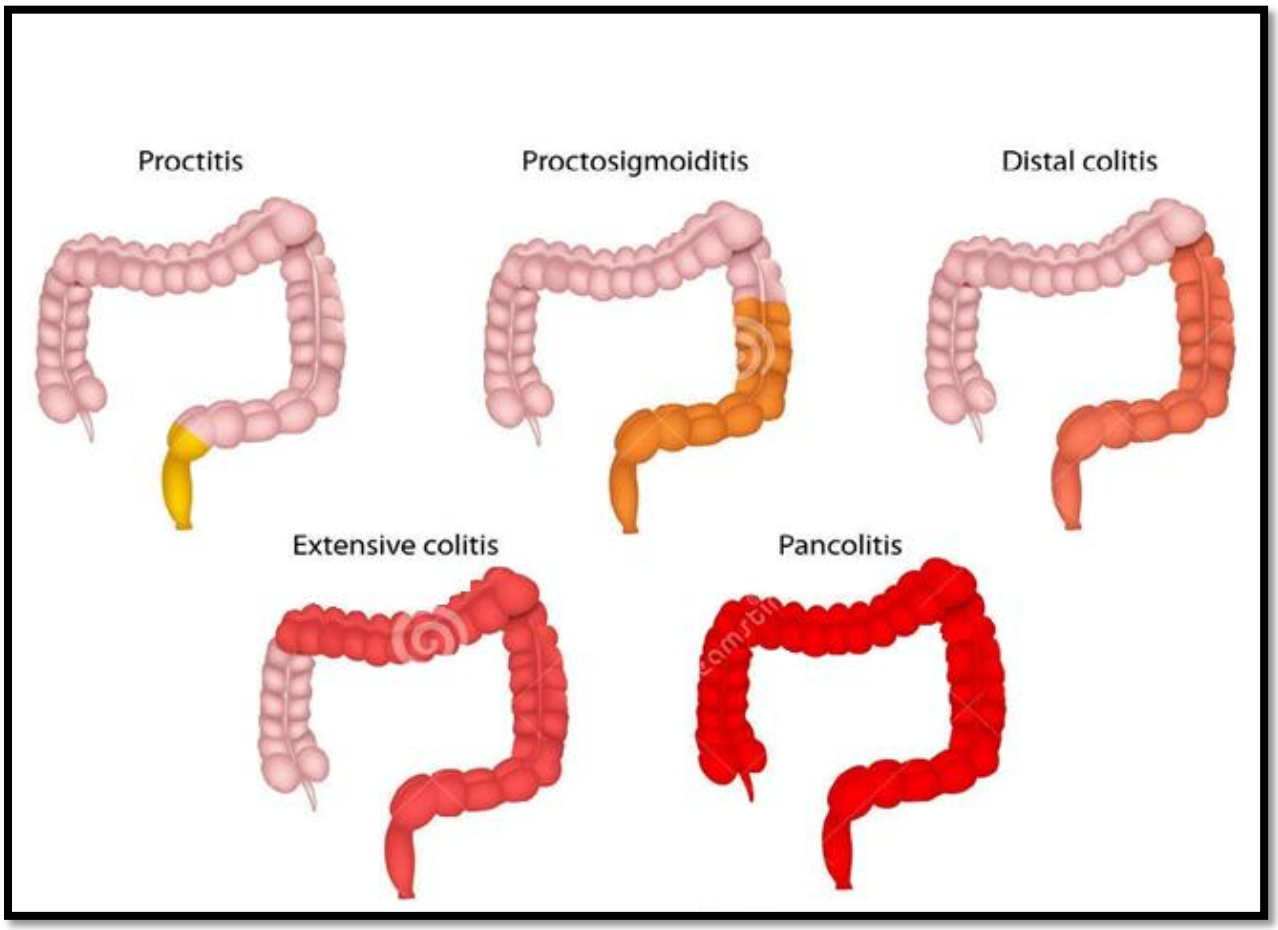


Figure 3: Types of UC

Types of UC[13]:

i. **Proctitis** : Limited to rectum.
ii. **Proctosigmoiditis** : Involves rectum and sigmoid colon (Lower segment).
iii. **Distal colitis** : Extends from rectum and entire left colon.
iv. **Extensive colitis** : Involves more than half the colon or the entire colon.
v. **Pancolitis** : Affects the entire large intestine.

1.1.7. PATHOPHYSIOLOGY

Disruption of tight junctions and the mucus film covering the epithelial layer causes increased permeability of the intestinal epithelium, resulting in increased uptake of luminal antigens. Macrophages and dendritic cells (innate immune cells) on recognition of non-

pathogenic bacteria (commensal microbiota) through molecular pattern recognition receptors (TLR) change their functional status from tolerogenic to an activated phenotype.

Activation of NF-kB pathways stimulates the transcription of pro inflammatory genes, resulting in increased production of pro-inflammatory cytokines (TNF-α, interleukins 12, 23, 6, and 1β). After processing of antigens, macrophages and dendritic cells present them to naive CD4 T-cells, promoting differentiation into Th2 effector cells, characterised by production of interleukin 4. Natural-killer T cells are the main source of interleukin 13, which has been associated with disruption of the epithelial cell barrier.

Circulating T cells bearing integrin-α4β7 bind to colonic endothelial cells of the microvasculature through the mucosal vascular address in-cell adhesion molecule 1, whose expression is enhanced in the inflamed intestine, leading to increased entry of gut-specific T cells into the lamina propria. Up-regulation of inflammatory chemokines such CXCL1, CXCL3 and CXCL8 leads to recruitment of circulating leucocytes which perpetuates the cycle of inflammation. The pathophysiology of UC is given in Figure 4[8].

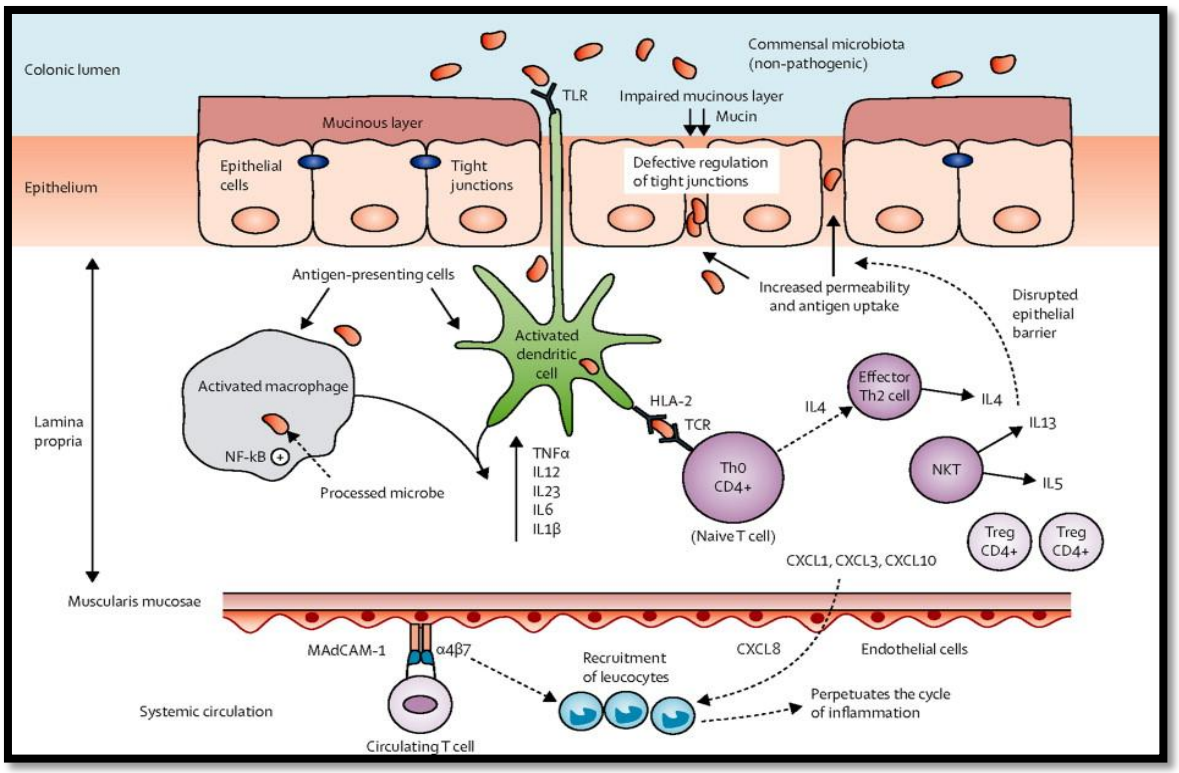


Figure 4: Pathophysiology of UC

Note: TLR– Toll-like receptor, HLA– human leucocyte antigen, IL – interleukin, TN– tumour necrosis factor, NF-κB–nuclear factor-κB, Th– T-helper, NKT– natural killer T-cell, CXCL– chemokine, Treg– regulatory T cell, MAdCAM-1– mucosal address in-cell adhesion molecule 1.

1.1.8. COMMON TESTS TO DIAGNOSE AND MAINTAIN UC[13]

1. Diagnostic testing
   - Fecal sample (Presence of bacteria, Parasites)
   - Blood sample (Infection, Anaemia, Inflammatory markers)
   - Biopsy of Intestinal lining
   - Liver and kidney function tests
2. Endoscopy
   - Gold standard for UC diagnosis
   - Flexible scope inserted into rectum
   - Sigmoidscope examines lower third of colon
   - Colonoscopy examines entire colon
3. Visual examination
   - Radiograph (Image shows constrictions)
   - Barium enema (Radio-opaque)
   - CT scan provides more detail than x-rays

1.1.9. TREATMENTS FOR UC

Treatment of UC was based on the types and severities of the disease[14].

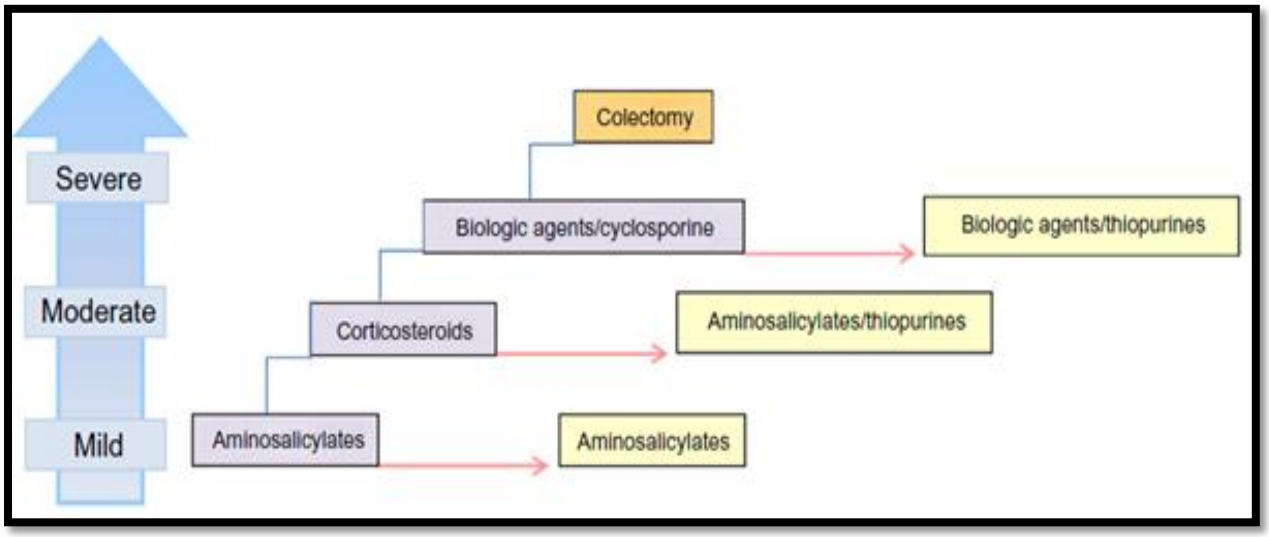


Figure 5: Treatment for UC

Several categories of drugs may be effective in the treating UC. The type of drugs taken will be depending on the severity of disease condition. Classifications of drugs are given in Table No.4.

Table No.4: Classification of UC Drugs[2-4, 15-21]

Name	Dose	Brand	Dosage form
ANTI-INFLAMMATORY DRUGS			
Sulfasalazine	500 mg	**Azulfidine**	Tablet
Mesalamine	0.375gm	**Aspiro**	Extended release capsules
Mesalamine	400 mg	**Asacol**	Delayed release tablet
Mesalamine	1.2 gm	**Lialda**	Delayed release tablet
Mesalamine	4 gm per 60 ml	**Rowasa**	Enema
Mesalamine	1000 mg	**Canasa**	Suppositories
Mesalamine	250 and 500 mg	**Pentasa**	Controlled release capsules
Mesalamine	1 gm in 100ml	**Pentasa**	Enema
Mesalamine	-	**Pentasa Enema 1%**	Enema
Balsalazide	750 mg	**Colazal**	Capsules
Olsalazine	250 mg	**Dipentum**	Capsules
CORTICOSTEROIDS			
Prednisolone	5 mg	**Reyos**	Tablet
Prednisolone	20 mg/100 ml	**Predsol**	Retension enema
Prednisolone	20 mg/100 ml	**Predenema**	Enema
Budesonide	3 mg	**Entocort**	Controlled release Capsules
Budesonide	0.02 mg/ ml	**Entocort**	Enema
Budesonide	0.2 mg/ ml	**Entocort Apulein**	Enema
IMMUNE SYSTEM SUPPRESORS			
Azathioprine	75 mg, 100 mg	**Azasan**	Scored tablets
Azathioprine	50 mg	**Imuran**	Scored tablets
Mercaptopurine	50 mg	**Purinethol**	Scored tablets
Mercaptopurine	50 mg	**Purixam**	Oral suspension
CALCINEURIN INHIBITORS			
Cyclosporine	25, 50, 100 mg	**Gengraf**	Capsules
Cyclosporine	-	**Neoral**	Capsules and Oral solutions
Cyclosporine	-	**Sandimmune**	Soft gelatin capsules, Oral solutions and Injection
ANTI-TNF AGENTS			
Infliximab	-	**Remicade**	Intravenous powder for Injection
Adalimumab	-	**Humira**	Subcutaneous injection
Golimumab	-	**Simponi**	Self Injection
Vedolizumab	-	**Entyvio**.	Intravenous infusion

1.1.10. OTHER MEDICATIONS[3]

1. Antibiotics
2. Anti-diarrheal agents
3. Pain relievers
4. Iron supplements

1.1.11. SURGERY [1, 3]

In UC, three situations are absolute indications for surgery: Exsanguinating haemorrhage, Frank perforation and Documented or strongly suspected carcinoma i.e. high grade dysplasia or low grade dysplasia in a mass lesion. Massive haemorrhage in UC is due to diffuse mucosal ulceration. If the hemorrhage is exsanguinating or even persisting despite maximal medical therapy, it is an indication for surgical treatment. Perforation (occurring in only 2-3 % of hospitalized UC patients at tertiary referral centers), is the most lethal complication of toxic megacolon. When colon cancer is identified, the need for surgery is obvious; similarly, the colonoscopic biopsy diagnosis of high grade dysplasia is often indicative of a concomitant or future cancer and is an indication for colectomy. Severe UC unresponsive to an intensive medical regimen or toxic megacolon are others indications for surgery.

1.1.11. ROUTE OF ADMINISTRATION

The UC drugs are given in following route of administration.

- ✓ Oral route
- ✓ Parentral route
- ✓ Rectal route

1.2. RECTAL DOSAGE FORMS[22]

Rectal administration is not often the first route of choice; but it becomes a good alternative when the oral route is inadvisable. Relatively low cost and lack of technical difficulties make rectal drug administration attractive when compared to parenteral therapy. The downside of rectal administration includes the aesthetics and stigma of violating the patient's dignity. This along with potential rectal irritation due to frequent administration and difficulty in titrate a correct dose due to limited strengths of commercial rectal dosage forms pose some challenges.

Psychologically, rectal dosage forms can provide a considerable placebo effect in the treatment of ano-rectal disorders. The user feels that something is really being done at the involved site and this can produce a positive attitude towards this mode of treatment of the disease or disorder. This may promote hope and the possibility of avoiding the embarrassment of telling the family and friends of what is happening in the private area.

Previously, the rectal pathway was reserved for the administration of locally active products such as those in the treatment of haemorrhoids, worms and constipation. In the treatment of haemorrhoids and anal fissures, a suggestion was made at one time that a suppository should be "hour glass" or "collar button" shaped so that the suppository would stay in the anal canal.

Now, it is well accepted that many active ingredients can be administered rectally and achieve therapeutic blood levels from any of several different dosage forms. Some medications are best administered by this route while others can be if needed.

1.2.1. ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS[22]

The rectum consists of the last few inches of the large intestine, terminating at the anus. The wall of the GI tract consists of several layers including the mucosa, submucosa, tunica muscularis and the visceral peritoneum. The mucous membrane of the rectum, where rectal dosage forms are generally administered, is made up of a layer of cylindrical epithelial cells differentiated from those of the intestine by the absence of villi.

The rectum contains three types of haemorrhoidal veins, namely the superior hemorrhoidal vein, middle haemorrhoidal vein and the inferior haemorrhoidal vein. These veins are act by transporting the active principle absorbed in the rectum to the blood system either directly by means of iliac veins and the vena cava (inferior and middle hemorrhoidal veins) or indirectly by means of the portal vein and the liver (superior hemorrhoidal vein).

The three hemorrhoidal veins are linked by an anastomosis network. Since it is not really possible to predict the position or exact location of the dosage form in the rectum, it is not really possible to predict exactly which way the active principle will be transported. It may be preferably by one pathway or another or a combination. However, it is generally accepted that at least 50% to 70% of the active ingredients administered rectally take the direct pathway thus bypassing the liver and avoiding the first-pass effect. There is also the possibility of absorption into the lymphatic vessels that should not be dismissed but may be minimal.

1.2.2. ADVANTAGES OF RECTAL ADMINISTRATION[24, 25]

The advantages of rectal administration include the following

1. **First pass effect**

Avoiding at least partially the first pass effect which may result in higher blood levels for those drugs subject to extensive first pass metabolism upon oral administration.

2. **Drug stability**

Avoiding the breakdown of certain drugs that is susceptible to gastric degradation.

3. **Large dose drugs**

Ability to administer somewhat larger doses of drugs than using oral administration.

4. **Irritating drugs**

Ability to administer drugs which may have an irritating effect on the oral or GI mucosa when administered orally.

5. **Unpleasant tasting or smelling drugs**

Ability to administer unpleasant tasting or smelling drugs whose oral administration is limited.

6. In children, the rectal route is especially useful. An ill child may refuse oral medication and may fear injections.

7. Rectal administration can be especially useful in terminal care.

Rectal administration provides for a rapid and in many cases extensive absorption of the active ingredient. The rapidity, intensity and duration of action are three parameters which must be considered during formulation for rectal administration and in many cases can be altered to meet the needs of the individual patient.

1.2.3. DISADVANTAGES[24, 25]

The disadvantages of rectal administration include the following,

1. Erratic absorption – drug absorption from a suppository is often incomplete and erratic.
2. Absorption from solutions used as an enema may be more reliable.
3. Not well accepted, may be some discomfort.
4. Self medication is not possible.

1.3. ENEMA

An enema is a solution or fluid suspension for rectal administration. There are two types: evacuant enemas and retention enemas. The volume given varies according to the type of enema. Retention enemas do not normally exceed 100 ml in volume; evacuant enemas may be as much as 2:1. Large volume enemas should be warmed to body temperature before administration[26]. Enemas are dosage forms designed to be administered rectally for clearing out the bowel or for administration of drugs or food. An enema is a method of administration and may involve solutions, suspensions, emulsions, foams and gels[24]. The medical name for an enema is "enteroclysis." However, enema is the more commonly used term. In the 17th century, clyster was used to describe the enema process. The process was performed using a "clyster syringe." The syringe consisted of a nozzle that entered the rectum and a plunger. During this time, an apothecary would administer the procedure. Women were often embarrassed if a male administered the enema. Most women were modest and did not enjoy their private parts being exposed. Now, individuals may administer the enema themselves without any assistance. In the 19th century, clyster syringes were replaced with enema bags and rectum nozzles[27].

1.3.1. RETENTION ENEMAS[24]

A number of solutions, suspensions and emulsions are administered rectally for the local effects of the medication (e.g. hydrocortisone) or for systemic absorption (e.g. aminophylline). In the case of aminophylline, the rectal route of administration minimizes the

undesirable gastrointestinal reactions associated with oral therapy. Clinically effective blood levels of the agents are usually obtained within 30 minutes following rectal instillation. Corticosteroids can be administered as retention enemas as adjunctive treatment of some patients with UC.

1.3.2. EVACUATION ENEMAS[24]

Rectal enemas are used to cleanse the bowel. Commercially, many enemas are available in disposable plastic squeeze bottles containing a premeasured amount of enema solution. The agents present are solutions of sodium phosphate, sodium biphosphate, glycerin, docusate potassium and light mineral oil. It may be prepared as solutions, suspensions, emulsions, powder and tablet for solution and suspensions.

1.3.3. SOLUTIONS[22]

Considerations in preparing solutions include solubility, solvent selection, pH, osmolality and stability of the drug. If the pH is too low or too high, it may be irritating to the mucosa. If the solution is hyperosmolar, it may pull fluids from the local area and initiate a defecation reflex.

1.3.4. SUSPENSIONS[22]

Suspensions are preparations containing finely divided drug particles distributed somewhat uniformly throughout a vehicle in which the drug exhibits a minimum degree of solubility. In most good pharmaceutical suspensions, the particle diameter is between 1 and 50 microns. The pharmacist may have to use a solid dosage form (e.g. tablet and capsule) of the drug and extemporaneously compound a liquid preparation or it can be made from the bulk powder. Typically, when formulating an extemporaneous suspension, the contents of a capsule, crushed tablets or bulk powder is placed in a mortar. The selected vehicle is then slowly added and mixed with the powder to create a paste and then diluted to the desired volume. To minimize stability problems of the extemporaneously prepared product, it should be placed in air-tight, light-resistant containers by the pharmacist and subsequently stored in the refrigerator by the patient. Because it is a suspension, the patient should be instructed to shake it well prior to use and on a daily basis watch for any color change or consistency change that might indicate a stability problem with the formulation. The following examples of rectal suspensions have frequently been compounded by pharmacists when not commercially available. Barium Sulfate for Suspension, U.S.P has been employed orally or

rectally for the diagnostic visualization of the gastrointestinal tract. Mesalamine (i.e. 5-aminosalicylic acid) suspension was introduced onto the market in 1988 as Rowasa® (Solvay) for treatment of Crohn's disease, distal UC, proctosigmoiditis and proctitis.

1.3.5. EMULSIONS[22]

An emulsion is a dispersion in which the dispersed phase is composed of small globules of a liquid distributed throughout a vehicle in which it is immiscible. Pharmaceutically, the process of emulsification enables the pharmacist to prepare relatively stable and homogeneous mixtures of two immiscible liquids. It permits the administration of a liquid drug in the form of minute globules rather than in bulk. The initial step in preparation of an emulsion is the selection of the emulsifier. Among the emulsifiers and stabilizers for pharmaceutical systems are some carbohydrate materials (acacia, tragacanth, agar, chondrus and pectin), protein substances (gelatin, egg yolk and casein), high molecular weight alcohols (stearyl alcohol, cetyl alcohol and glyceryl monostearate), wetting agents (which may be anionic, cationic or nonionic) and finely divided solids (colloidal clays including bentonite, magnesium hydroxide and aluminum hydroxide). Emulsions may be prepared by several methods, depending upon the nature of the emulsion components and the equipment available for use. On a small scale, as in the laboratory or pharmacy, emulsions may be prepared using a dry wedgewood or porcelain mortar and pestle, a mechanical blender or mixer such as a waring blender or a milk-shake mixer, a hand homogenizer, a bench-type homogenizer or sometimes a simple prescription bottle. On a large scale, large volume mixing tanks may be used to form the emulsion through the action of a high speed impeller. As desired, the product may be rendered finer by passage through a colloid mill, in which the particles are sheared between the small gap separating a high speed rotor and the stator or by passage through a large homogenizer, in which the liquid is forced under great pressure through a small valve opening.

1.3.6. POWDERS / TABLETS FOR RECTAL SOLUTIONS AND SUSPENSIONS[23]

Powders / tablets intended for the preparation of rectal solutions or suspensions are single-dose preparations that are dissolved or dispersed in water or other suitable solvents at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent aggregation of the particles. After dissolution or dispersion the preparation complies with the requirements for rectal solutions or rectal suspensions as appropriate.

1.4. TABLETS

Tablet is defined as a compressed solid dosage form containing medicaments with or without excipients[28]. According to the I.P Pharmaceutical tablets are solid flat or biconvex dishes unit dosage form prepared by compressing a drugs or a mixture of drugs with or without diluents. They vary in shape and differ greatly in size and weight, depending on amount of medicinal substances and the intended mode of administration. It is the most popular dosage form and 70% of the total medicines are dispensed in the form of tablet. All medicaments are available in the tablet form except where it is difficult to formulate or administer[29].

1.4.1. DISPERSIBLE TABLETS[30-32]

Dispersible tablets are uncoated or film-coated tablets that can be dispersed in liquid before administration giving a homogenous dispersion. Dispersible tablets usually disintegrate within three minutes when put in water.

1.4.1.1. ADVANTAGES OF DISPERSIBLE TABLETS[33]

1. More convenient for active pharmaceutical ingredients with insufficient stability in water.
2. More easily transportable and they generate less handling and transportation costs for the same amount of active ingredient (less volume and less weight).
3. Easier to produce and the production costs are less, which makes them more affordable than standard liquid formulations.

Dispersible tablets have less physical resistance than regular tablets; they are more sensitive to moisture and may degrade at higher humidity conditions. Each tablet must be protected from the ambient humidity. The dispersible tablets should not be divided or chewed. Dispersible tablets must be used immediately after removal from the blister packaging. Their stability outside of the blister cannot be guaranteed.

1.4.1.2. DISADVANTAGES OF DISPERSIBLE TABLETS[32]

1. It is hygroscopic in nature so must be keep in dry place.
2. It is also shows the fragile, effervescence granules property.
3. It requires special packaging for properly stabilization and safety of stable product.

1.4.1.2.MECHANISMS OF DISINTEGRATION[32]

Disintegrating tablets involve the following mechanisms to achieve the desired fast dissolving.

1. Water must quickly enter into the tablet matrix to cause rapid disintegration and instantaneous dissolution of the tablet.
2. Incorporation of an appropriate disintegrating agent or highly water soluble excipients in the tablet formulation.
3. There are some under mentioned mechanisms by which the tablet is broken down into the smaller particles and then subsequently result a solution or suspension of the drug.

The mechanisms are[34] -

- Swelling
- Porosity and Capillary Action (Wicking)
- Deformation
- Due to disintegrating particle/particle repulsive forces

1.4.1.4.STAGES OF DISINTEGRATION[35]

The different stages of disintegration of dispersible tablets were shown in Figure 6.

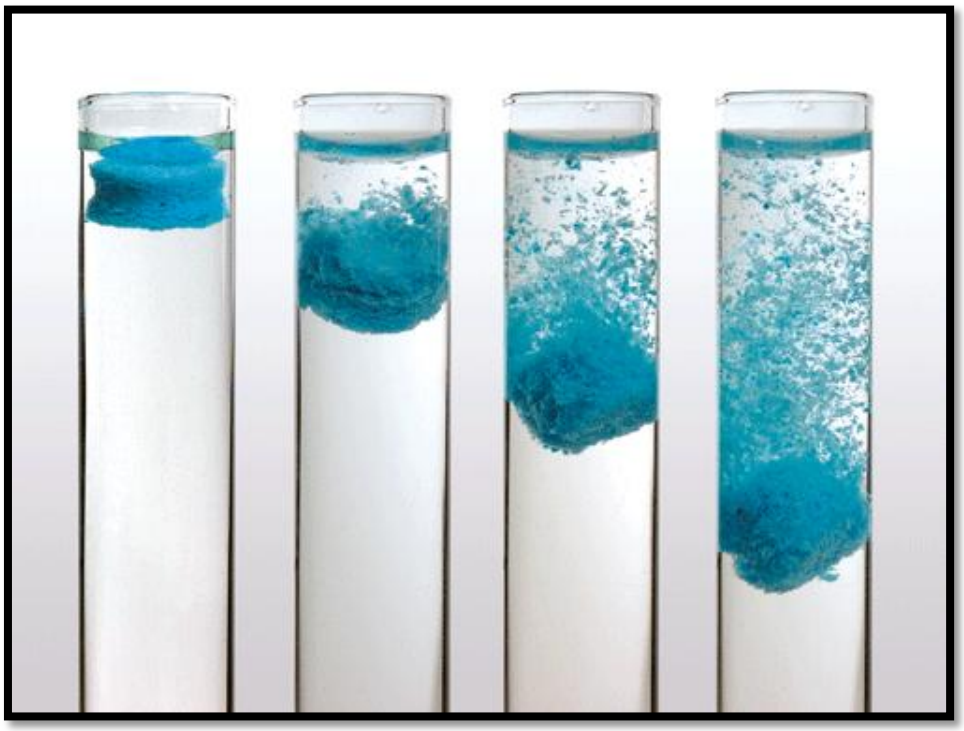


Figure 6: Disintegration Stages of Dispersible Tablets

1.4.1.5.FORMULATION OF DISPERSIBLE TABLETS

Selection of Excipients

Mainly seen excipients in dispersible tablets are as follows at least one disintegrant, a diluent, a lubricant and optionally a swelling agent, etc.

Ideal bulk excipients for disintegrating dosage forms should have the following properties

1. Disperses and dissolves within a few seconds without leaving any residue.
2. Enables sufficient drug loading and remains relatively unaffected by changes in humidity or temperature.

Excipients used for the formulation of dispersible tablet were given in Table No.5.

Table No.5: Details of Excipients used for the Formulation of Dispersible Tablets

Excipients	Function	Examples
Superdisintegrant	Increases the rate of disintegration and hence the dissolution. The presence of other formulation ingredients such as water-soluble excipients and effervescent agents further hastens the process of disintegration. For the success of fast dissolving tablet, the tablet having quick dissolving property, which is achieved by using the superdisintegrant.	Crospovidone, microcrystalline cellulose, sodium starch glycolate, sodium carboxy methyl cellulose, pregelatinzed starch, carboxy methyl cellulose and modified corn starch. Sodium starch glycolate has good flow ability than croscarmellose sodium. Crospovidone is fibrous nature and highly compactable.
Surface active Agents	Reduces interfacial tension and thus enhances solubilisation of FDT.	Sodium doecyl sulfate, sodium lauryl sulfate, polyoxyethylene sorbitan, fatty acid esters (Tweens), sorbitan fatty acid esters (Spans) and polyoxyethylene stearates.
Binders	Maintains integrity of dosage form prior to administration.	Polyvinylpyrrolidone (PVP), polyvinylalcohol (PVA) and hydroxy propyl methylcellulose (HPMC).
Lubricants	Lubricants help to reduce friction and wear by introducing a lubricating film between mechanical moving parts of tablet punching machine.	Stearic acid, magnesium stearates, zinc state, calcium state, talc, polyethylene glycol, liquid paraffin, magnesium lauryl sulphate and colloidal silicon dioxide.
Fillers	Enhances bulk of dosage Form.	Directly compressible spray dried mannitol, sorbitol, xylitol, calcium carbonate, magnesium carbonate, calcium phosphate, calcium sulfate, pregelatinzed starch, magnesium trisilicate and aluminium hydroxide.

1.4.1.6.PREPARATION OF DISPERSIBLE TABLETS[36, 37]

Tablet Processing

Pharmaceutical products are processed all over the world using the direct compressing, wet granulation or dry granulation methods. Method chosen depends on the ingredient's individual characteristics like flow property, compressibility etc. Right choice of method requires thorough investigation of each proposed ingredient in the formula for comprehensive approach for interactions and stability.

The process of tablet manufacturing was given in Figure 7.

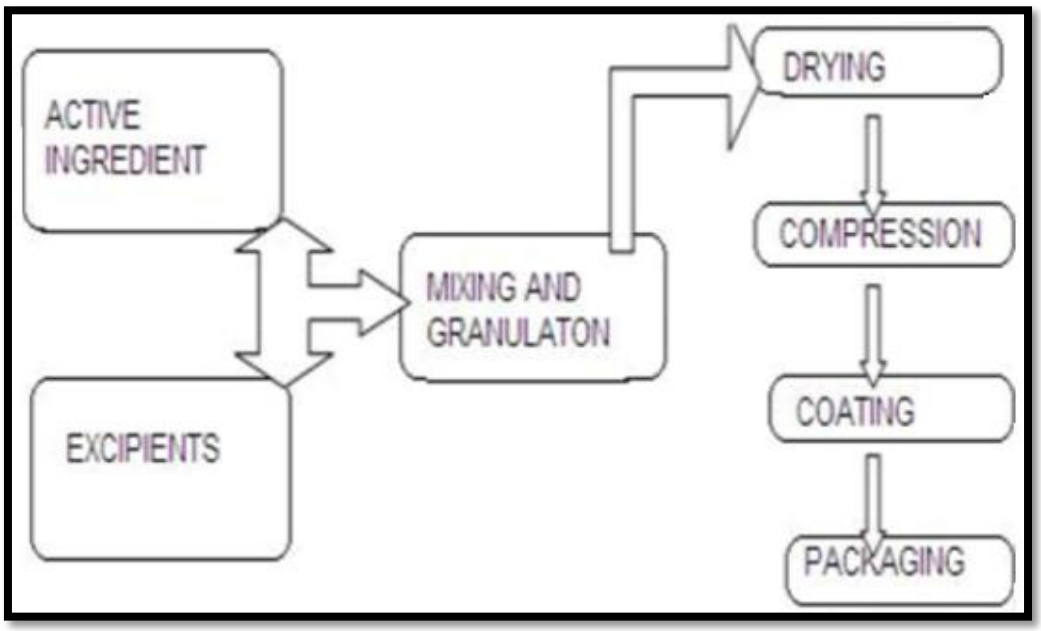


Figure 7: Process of Tablet Manufacturing

Direct Compression

The tablets are made by directly compressing the powdered materials without modifying the physical nature of the materials itself. Direct compression is generally done for the crystalline materials having good physical properties such as flow property, compressibility, etc. Main advantages of direct compression are time saving, safety of operations and low cost.

Wet Granulation

This is the most widely used method of tablet preparation. In this method the powders are bound by suitable binder by adhesion". The binder is added by diluting with suitable solvent prior to addition to the blended powders to form wet granules which is dried suitably

to expel the solvent forming dried granules. The surface tension forces and capillary pressure are primarily responsible for initial granules formation. The main advantage being it meets all the requirements for tablet formation though it is multi stage and time consuming.

Dry Granulation

The dry granulation process is used to form granules without using a liquid solution. This type of process is recommended for products, which are sensitive to moisture and heat. Forming granules without moisture requires compacting and densifying the powders. Dry granulation can be done on a tablet press using slugging tooling.

Slugging Method

Slugging method is a double compression method. Initially the powders are compressed in large size of punch. The compressed tablets were crushed into granules using mortar and pestle. Those granules were passed through initially by 16 # mesh then by 20 # mesh and finally passed through 30 # sieve mesh to get uniformed granules. Finally the granules are compressed to get uniform weight of tablet.

1.4.2. EVALUATION PARAMETERS[38, 39]

Tablets when formulated may undergo physical and chemical changes, which may alter their bioavailability. Therefore, the tablets are to be evaluated before dispensing to ensure their stability and bioavailability throughout their shelf life. Evaluation of tablets can be carried out by the following test.

Pre-compression Parameters

- ✓ Loss on drying
- ✓ Bulk density
- ✓ Tapped density
- ✓ Carr's index
- ✓ Hausner's ratio

Evaluation of Dispersible Tablets

- ✓ Tablet appearance
- ✓ Organoleptic parameters
- ✓ Identification markings on the tablets
- ✓ Hardness
- ✓ Thickness
- ✓ Weight variation test
- ✓ Friability
- ✓ *In-vitro* dispersion time
- ✓ Wetting time
- ✓ Water absorption ratio
- ✓ Uniformity of disintegration
- ✓ Disintegration test
- ✓ Drug content estimation
- ✓ *In-vitro* dissolution test

1.5. VEHICLE[40]

Vehicle solution is a homogenous mixture composed of two or more substances. In such a mixture, a solute is a substance dissolved in another substance, known as solvent. The mixing process of a solution happens at a scale where the effects of chemical polarity are involved, resulting in interactions that are specific to salvation. The solution assumes the characteristics of the solvent when the solvent is the larger function of the mixture, as is commonly the case. The concentration of a solute in a solution is the mass of that solute expressed as a percentage of the mass of the whole solution.

SOLUTE + SOLVENT ⟹ SOLUTION

1.5.1. CHARACTERISTICS OF VEHICLE

1. A solution is a homogenous mixture of two or more substances.
2. The particles of solute in a solution cannot be seen by the naked eye.
3. A solution does not allow beams of light to scatter.
4. A solution is should be stable.
5. The solute from a solution cannot be separated by filtration (or mechanically).
6. It is composed of only one phase.

1.5.2. INGREDIENTS IN THE VEHICLE

- ✓ Isotonic agent
- ✓ Co-solvents
- ✓ Preservatives
- ✓ Suspending agents
- ✓ Purified water

Isotonic agent[41]

Isotonic agent is a substance to maintain tonicity of the fluid to the body fluid. Sodium chloride is the example for isotonic agent

Co-solvent[42]

A second solvent added in small quantities to enhance the solvent power of the primary solvent.

Examples: Alcohol, poly ethylene glycol, propylene glycol and glycerin or glycerol etc.

Preservatives[43]

Preservatives are substances which are added to various pharmaceutical dosage forms and cosmetic preparations to prevent or inhibit microbial growth. An ideal preservative would be effective at low concentrations against all possible microorganisms be nontoxic and compatible with other constituent of the preparation and be stable for the shelf-life of the preparation.

Preservatives can be classified as

i) Acids: e.g. Benzoic acid, sorbic acids, boric acids,

ii) Esters: e.g. Methylparaben, ethylparaben, propylparaben, butylparaben, sodium benzoate, sodium propionate, potassium sorbate.

iii) Alcohols: e.g. Chlorobutanol, benzyl alcohol, phenyl ethyl alcohol.

iv) Phenols: e.g. Phenol, chlorocresol, O-phenyl phenol.

v) Mercurial compounds: e.g. Thiomersal, nitromersol, phenylmercuric nitrate, phenylmercuric acetate.

vi) Quaternary ammonium compounds: e.g. Benzalkonium chloride, cetyl pyridinium chloride.

Suspending agents[44]

Suspending agents are help to active pharmaceutical ingredients stay suspended in the formulation and prevent caking at the bottom of the container. One of the properties of a well-formulated suspension is that it can be easily resuspended by the use of moderate agitation or shaking.

Examples: Alginates, methyl cellulose, hydroxy methyl cellulose, carboxy ethyl cellulose, sodium carboxy methyl cellulose, microcrystalline cellulose, acacia, tragacanth, xanthan gum, bentonite, carbomer, carageenan, powdered cellulose and gelatin.

1.6. SUSPENSIONS[22]

Suspensions (Rectal) are liquid preparations intended for rectal application to obtain a local or systemic effect or they may be intended for diagnostic purposes.

Suspensions are preparations containing finely divided drug particles distributed somewhat uniformly throughout a vehicle in which the drug exhibits a minimum degree of solubility. In most good pharmaceutical suspensions, the particle diameter is between 1 and 50 microns. The pharmacist may have to use a solid dosage form (e.g. tablet and capsule) of the drug and extemporaneously compound a liquid preparation or it can be made from the bulk powder.

Typically, when formulating an extemporaneous suspension, the contents of a capsule, crushed tablets or bulk powder is placed in a mortar. The selected vehicle is then slowly added and mixed with the powder to create a paste and then diluted to the desired volume. To minimize stability problems of the extemporaneously prepared product, it should be placed in air-tight light-resistant containers by the pharmacist and subsequently stored in the refrigerator by the patient.

Because it is a suspension, the patient should be instructed to shake it well prior to use and on a daily basis watch for any color change or consistency change that might indicate a stability problem with the formulation. The following examples of rectal suspensions have frequently been compounded by pharmacists when not commercially available. Barium

Sulfate for suspension, U.S.P has been employed orally or rectally for the diagnostic visualization of the gastrointestinal tract.

Mesalamine (i.e. 5-aminosalicylic acid) suspension was introduced onto the market in 1988 as Rowasa® (Solvay) for treatment of Crohn's disease, distal ulcerative colitis, proctosigmoiditis and proctitis. Hydrocortizone rectal suspension was introduced from Ani pharmaceuticals, Baudette[45]. Mesalazine rectal suspension was introduced as Pentasa enema by Ferring Pharmaceuticals[46].

1.6.1. PREPARATION OF RECTAL SUSPENSION

One dispersible tablet was placed into a clear bottle containing vehicle solution for rectal suspension. Shake well until the tablet was completely dispersed.

TABLET + VEHICLE SOLUTION ⟹ SUSPENSION

1.6.2. EVALUATION OF SUSPENSIONS

- ✓ Sedimentation volume
- ✓ Degree of flocculation
- ✓ Re-dispersibility
- ✓ pH
- ✓ viscosity
- ✓ Microbiology test
- ✓ In-vitro drug release test
- ✓ Accelerated stability studies

CHAPTER 2

AIM AND PLAN OF WORK

2. AIM AND PLAN OF WORK

2.1. AIM AND OBJECTIVE

To formulate and evaluate Prednisolone retention enema as dispersible tablet with vehicle.

Prednisolone is a type of medicine called corticosteroid. It decreases immune system's response in various diseases to reduce symptoms such as pain, swelling and allergic type reactions. Prednisolone is 4 times more potent than hydrocortisone, also more selective glucocorticoid. It reduces inflammation by stopping cells from releasing chemicals that normally help to produce immune and allergic responses[47-49].

Riboflavin is an integral component of two enzymes FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide). FAD and FMN are involved in the activity of electron transport chain, an essential component of energy metabolism. Prednisolone decreases the inflammation by inhibiting the migration of polymorphonuclear leukocytes and reversal of increased capillary permeability. It suppresses the immune system by reducing the activity and protection of the lymphocytes and eosinopril. Riboflavin helps in this place to increase the energy metabolism in lymphocytes and eosinopril[50].

The objectives of this study are following

1. To formulate dispersible tablet and vehicle as enema formulation.
2. To select excipients and find any incompatibility between the drug and excipients.
3. To formulate Prednisolone dispersible tablet and vehicle.
4. To perform the precompression and tablets evaluation parameters.
5. To select the best formulation of dispersible tablet.
6. To prepare rectal suspension using best formulation of dispersible tablet.
7. To perform *in-vitro* drug release study for rectal suspension.
8. To conduct Microbiology studies for the rectal suspension.
9. To perform stability studies for the selected formulation of dispersible tablet.

2.2. PLAN OF WORK

The plan of this work can be outlined below,

- To carry out the preformulation studies for API
  - ✓ Description
  - ✓ Solubility
  - ✓ Melting point
  - ✓ Particle size distribution
  - ✓ Loss on drying
  - ✓ Flow properties
- Drug excipients compatibility studies
  - ✓ Physical observation
- Formulation of Prednisolone dispersible tablets by direct compression, wet granulation method and slugging method.
- Evaluation for precompression parameters
  - ✓ Angle of repose
  - ✓ Bulk density
  - ✓ Tapped density
  - ✓ Compressibility index
  - ✓ Hausner's ratio
  - ✓ Moisture content
- Evaluation of post compression parameters for compressed tablets
  - ✓ Hardness
  - ✓ Thickness
  - ✓ Weight variation
  - ✓ Friability
  - ✓ Disintegration test
  - ✓ Wetting time
  - ✓ Water absorption ratio
  - ✓ *In-vitro* dispersion time
  - ✓ Uniformity of dispersion
  - ✓ Drug content estimation
  - ✓ IR spectral analysis

- Formulation of vehicle solution for suspending Prednisolone dispersible tablet
- Formulation of rectal suspension using selected formulation of dispersible tablet.
- Evaluation of rectal suspension
    - ✓ Appearance
    - ✓ Colour
    - ✓ pH
    - ✓ Viscosity
    - ✓ *In-vitro* drug release study
- Microbiological evaluation.
- Accelerated stability studies.

CHAPTER 3

LITERATURE REVIEW

3. LITERATURE REVIEW

1. Enemacort Retention enema (2012)[51] is a dispersible tablet and solution for rectal suspension (2 mg budesonide/100 ml). Enemacort consists of two components, dispersible tablet (yellow circular tablet containing 2.3 mg budesonide) and vehicle 115 ml clear colourless solution. Tablet contains riboflavin 5-sodium phosphate, microcrystalline cellulose, croscarmellose sodium, maize starch, colloidal silicon dioxide, lactose anhydrous, magnesium stearate and purified talc. Vehicle contains sodium chloride, methyl paraben, propyl paraben, carboxy methyl cellulose sodium, propylene glycol and purified water.

2. Entocort (2014)[52] is a tablet and solution for rectal suspension. Total quantity of budesonide in a dose of prepared rectal suspension (115 ml) is 2.3 mg. Entocort rectal suspensions consists of two parts; a dispersible tablet, containing micronized budesonide and an isotonic solution. The rectal suspension is prepared before use. The tablet is round, faintly yellow, marked BAI on one side and 2.3 on the other. The solution is colourless. One tablet for rectal suspension contains Lactose anhydrous 263 mg, lactose monohydrate 1.3 mg, riboflavin sodium phosphate (E101), crospovidone, colloidal silica and magnesium stearate. 1ml solution contains: Sodium chloride 9 mg, methyl parahydroxybenzoate (E218), propyl parahydroxybenzoate (E216) and purified water.

3. Pentasa enema (2016)[53] Pentasa Enema is a rectal, 100 ml of a colourless to faint yellow suspension containing 1 gm Mesalazine is an active ingredient used for the treatment of UC. Other excipients are disodium edetate, sodium metabisulphate, sodium acetate, hydrochloric acid (concentrated) and purified water.

4. Predsol suppositories and enemas (2014)[54] The active ingredient in PREDSOL is prednisolone as sodium phosphate, which is a type of glucocorticoid belonging to a group of medicines called corticosteroids. Used in the treatment of UC and Crohn's disease. Each retension enema contains 20 mg/100 ml of prednisolone (as prednisolone sodium phosphate), sodium hydroxide, sodium phosphate-dibasic, sodium phosphate-monobasic, disodium edetate, Nipastat GL75 and water purified. Nipastat GL is a preservative which contains methyl hydroxybenzoate, ethyl hydroxybenzoate, propyl hydroxybenzoate, butyl hydroxybenzoate and isobutyl hydroxybenzoate.

5. Predsol retention enema (2015)[55] 20 mg/100 ml rectal solution contains 20 mg of the active substance prednisolone as the sodium phosphate ester. Predsol retention enema

belongs to a group of medicines called corticosteroids. Each 100 ml bottle contains 20 mg prednisolone as prednisolone sodium phosphate. The other ingredients are nipastat, disodium edetate, sodium acid phosphate, disodium phosphate anhydrous, sodium hydroxide and purified water.

6. Entocort Enema (2016)[56] It contains a medicine called budesonide. This belongs to a group of medicines called 'corticosteroids'. They are used to reduce inflammation and ulcers in the large intestine and rectum. Entocort enema contains 2 mg of budesonide at a concentration of 0.02 mg of budesonide per ml of solution. The other ingredients are lactose anhydrous, polyvidone, riboflavin sodium phosphate, lactose monohydrate, magnesium stearate, colloidal anhydrous silica, sodium chloride, methyl parahydroxy benzoate (E218), propyl parahydroxybenzoate (E216) and water purified.

7. Predonema Enema (2013)[57] contains prednisolone sodium phosphate 22 mg: the Japanese Pharmacopoeia (J.P), carboxy vinyl polymer, disodium hydrogen phosphate hydrate, ethyl parahydroxybenzoate, butyl parahydroxybenzoate, disodium edetate hydrate and sodium hydroxide. This product occurs as a slightly viscous, colourless transparent solution. Identification code of package material is KP-009.

8. Rowasa (2008)[58] The active ingredient in Rowasa (mesalamine) rectal suspension enema, a disposable (60 mL) unit is mesalamine, also known as 5-aminosalicylic acid (5-ASA). Chemically, mesalamine is 5-amino-2-hydroxybenzoic acid. Each rectal suspension enema unit contains 4 gms of mesalamine. In addition to mesalamine the preparation contains carbomer 934P, edetate disodium, potassium acetate, potassium metabisulfite, purified water and xanthum gum.

9. Predenema (2015)[59] is a single dose retention enema for application into the rectum. Each single dose, disposable enema contains a rectal solution of 20 mg prednisolone, as prednisolone sodium meta-sulphobenzoate in 100 ml. prednisolone belongs to a group of medicines called corticosteroids which are used to reduce inflammation. As well as the active ingredient also contains disodium edetate, parahydroxy benzoates (E214, E216, E218) and purified water.

10. Cortenema (2007)[60] is a convenient disposable single dose hydrocortisone, 100 mg in an aqueous solution containing carbomer 934P, polysorbate 80, purified water, sodium hydroxide and methyl paraben 0.18% as preservative.

11. Entocort, Apulein[61] is Budesonide 0.2 mg/ml Suspendol-S^{TM} rectal enema (Perrigo Paddock) containing Budesonide, propylene glycol and Suspendol- S^{TM} syrup. Suspendol-S^{TM} is a semi-clear viscous liquid contains purified water, carboxypolymethylene (as thickening and suspending agent), polysorbate 80, simethicone, sodium hydroxide, methyl paraben and propyl paraben.

CHAPTER 4

MATERIALS AND METHODS

LIST OF MATERIALS

4. MATERIALS AND METHODS

4.1. MATERIALS USED AND MANUFACTURERS

Table No.6: Materials Used and Manufacturers

S. No.	Material	Manufacturer
1.	Prednisolone	Tianjin Tianyao Pharma, China.
2.	Riboflavin – 5 sodium phosphate	Supriya Life Science, Mumbai
3.	Microcrystalline cellulose (PH101 and PH 112)	Wei Ming pharmaceuticals, Taiwan.
4.	Crospovidone	Nanhang industrial & co, China.
5.	Maize starch	Ridhi Sidhi Pharmaceyuical Pvt Ltd, New Delhi.
6.	Colloidal silicon dioxide	Wacker Silicones, Mumbai.
7.	Lactose (DCL 21)	Cabot sanmar Ltd, Chennai.
8.	Lactose monohydrate	DMV Fonterra excipients, Newzealand.
9.	Magnesium stearate	Amishi Drugs & Chemical, Gujarat.
10.	Purified talc	Gangotri inorganic (P) Ltd, Gujarat.
11.	Sodium chloride	Avantor Performance materials India ltd, Haryana.
12.	Methyl hydroxybenzoate	Rasula Pharmaceutical, Hyderabad.
13.	Propyl hydroxybenzoate	Alta Laboratories, Mumbai.
14.	Sodium carboxy methyl cellulose	Jalan cellulose Co Ltd, India.
15.	Propylene glycol	Manali Petrochemicals Ltd, Chennai.
16.	Purified water	Andavar &Co, Chennai.

DRUG PROFILE

4.2. DRUG PROFILE

4.2.1. PREDNISOLONE [62-65]

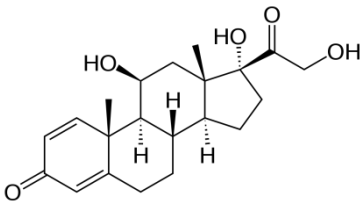
SYNONYMS : Metacortandralone.
Hydroretrocortine.
Deltacortril.
Meticortelone.

IUPAC NAME : (8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta [a] phenanthren-3-one.

MOLECULAR FORMULA : $C_{21}H_{28}O_5$

MOLECULAR WEIGHT : 360.45 gm/mol.

STRUCTURAL FORMULA :



PHYSICAL PROPERTIES : Solid, white to practically white crystalline powder, odourless.

SOLUBILITY : Practically insoluble in water.
Soluble 1 in 150 parts of ethanol.
Very slightly soluble in water.

MELTING POINT : 235^{o} C.

DOSAGE FORMS : Oral solution, Suspension, Tablets, Enema, Eye drops, Injections and Ointments.

DOSE : 20 mg.

DESCRIPTION : A glucocorticoid with the general properties of the corticosteroids. It is the drug of choice for all conditions in which routine systemic corticosteroid therapy is indicated, except adrenal deficiency states.

MECHANISM OF ACTION : Prednisolone decreases inflammation by inhibition of migration of polymorphonuclear leukocytes and reversal of increased capillary permeability. It suppresses the immune system by reducing the activity and production of the lymphocytes and eosinophils.

ABSORPTION : Readily absorbed from the GI tract with peak plasma concentrations after 1-2 hr (oral); initial absorption is affected by food.

DISTRIBUTION : Inactivated as it crosses the placenta; enters the breast milk.

METABOLISM : Prednisolone is a synthetic glucocorticoid that is used clinically for its anti-inflammatory properties. Prednisolone can diffuse passively across the cell membrane, where it binds to glucocorticoid receptors in the cytoplasm. Upon binding the glucocorticoid receptor (GR) dissociates from heat shock protein 90 and translocate into the nucleus. In the nucleus, GR dimers can bind to glucocorticoid response element (GRE) in the promoter region of anti-inflammatory genes, which activates their transcription of inflammatory mediators by binding to negative GRE (nGRE). GRs further interact with the transcription factors cAMP- responsive element binding protein and NF-kappa-B and inhibit their activation of inflammatory gene transcription. GRs also

recruit histone deacetylase 2 to inflammatory genes, which leads to DNA condensation at those loci, thus suppressing expression of those genes.

EXCRETION : Via urine (as free and conjugated metabolites).

PROTEIN BINDING : Very high (>90%).

PHARMACODYNAMICS : Prednisolone is a synthetic glucocorticoid used as anti-inflammatory or immunosuppressive agent. Prednisolone is indicated in the treatment of various conditions, including congenital adrenal hyperplasia, psoriatic arthritis, systemic lupus erythematosus, bullous dermatitis herpetiformis, seasonal or perennial allergic rhinitis, allergic corneal marginal ulcers, symptomatic sarcoidosis, idiopathic thrombocytopenic purpura in adults, leukemias and lymphomas in adults and UC. Glucocorticoids are adrenocortical steroids and it cause profound and varied metabolic effects. In addition, they modify the body's immune responses to diverse stimuli.

BIOLOGICAL HALF-LIFE : 2-3 hrs.

CLINICAL INDICATIONS : For the treatment of primary or secondary adrenocortical insufficiency, such as congenital adrenal hyperplasia, thyroiditis. Also used to treat psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, bursitis, acute gouty arthritis and epicondylitis. Also indicated for treatment of systemic lupus erythematosus, pemphigus and acute rhematic carditis. Can be used in the treatment of leukemias, lymphomas, thrombocytopenia purpura and autoimmune hemolytic anemia.

CONTRAINDICATIONS : Live vaccines; herpes simplex keratitis, systemic infections.

PRECAUTIONS : Patients with hypothyroidism, cirrhosis, UC, congestive heart failure, convulsive disorders, thrombo phlebitis, peptic ulcer elderly. Diabetes mellitus, hypertension, psychological disturbances, osteoporosis, pregnancy and lactation. Adrenal suppression and infection. May cause irreversible growth retardation, glaucoma, corneal perforation. Topical: Broken or infected skin. Not to be applied over large areas under occlusive dressings.

DRUG INTERACTIONS : Increased requirement of insulin and oral hypoglycaemics. Actions blunted by barbiturates, phenytoin, rifampicin. Increased bioavailability with estrogens and oral contraceptives. Increases plasma salicylate levels. Increased risk of convulsions when used with ciclosporin, increased clearance by carbimazole or carbamazepine. Increased risk of GI bleeding and ulceration when used with NSAIDs. May decrease methotrexate clearance.

PREGNANCY : There are no major human studies of prednisolone use in pregnant women, studies in several animals show that it may cause birth defects including increase cleft palate. Prednisolone should be used in pregnant women when benefits outweigh the risks and children born from mothers using prednisolone during pregnancy should be monitored for impaired adrenal function.

LACTATION : Prednisolone is found in breast milk of mothers, who are taking Prednisolone.

SIDE EFFECTS : Side effects with short term use include nausea and feeling tired. More severe side effects include psychiatric problems, which may occur in about 5% of people.

Common side effects with long term use include bone loss, weakness, yeast infections and easy bruising. While short term use in the later part of pregnancy is safe, use long term or early in early pregnancy is occasionally associated with harm to the baby.

OVERDOSE **:** Treatment of acute overdose includes immediate gastric lavage or emesis followed by supportive and symptomatic therapy. For chronic overdose, the dosage of prednisolone may be reduced temporarily or introduce alternate day treatment.

APPLICATIONS **:** It also suppresses the immune system. Prednisolone is used as an anti-inflammatory or an immunosuppressant medication. Prednisolone treats many different conditions such as allergic disorders, skin conditions, UC, arthritis, lupus, psoriasis and breathing disorders.

STORAGE **:** Stored at 20 – 25°C.

EXCIPIENTS PROFILE

4.3. EXCIPIENTS PROFILE

Table No. 7

4.3.1. RIBOFLAVIN-5-SODIUM PHOSPHATE[66]

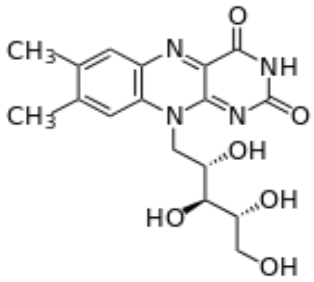
S. No.	Properties	Description
1	Non- proprietary name	BP: Riboflavin – 5 sodium phosphate.
2	Synonyms	Vitamin B2, Vactochrome, Lactoflavin, Vitamin G.
3	Chemical name	7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4-dione.
4	Molecular structure	
5	Molecular formula	$C_{17}H_{20}N_4O_6$
6	Molecular weight	376.37 gm/mol.
7	Melting point	>300°C.
8	Description	Nutritional factor found in milk, eggs, malted barley, liver, kidney, heart, and leafy vegetables. The richest natural source is yeast. It occurs in the free form only in the retina of the eye, in whey, and in urine; its principal forms in tissues and cells are as flavin mononucleotide and flavin-adenine dinucleotide.
9	Solubility	0.1 gm/mL, clear, orange-yellow.
10	Functional category	Coenzyme for a number of oxidative enzymes including NADH DEHYDROGENASE.
11	Application	Phototherapy, treatment of neonatal jaundice and a supplement of coenzyme.
12	Storage	2-8°C.
13	Stability	Stable, incompatible with strong oxidizing agents.

Table No. 8

4.3.2. MICROCRYSTALLINE CELLULOSE[67]

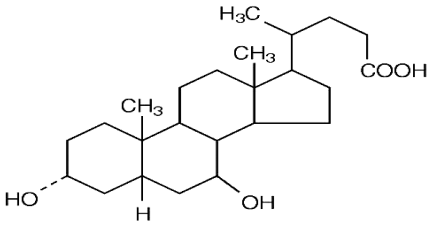
S. No.	Properties	Description
1	Non- proprietary name	BP: Microcrystalline cellulose. PhEur: cellulosum microcrystallinum.
2	Synonyms	Avicel PH, celex, cellulose gel, cellulosum microcrystallinum, emcocel, fibrocel, pharmacel, vivace, E46.
3	Chemical name	Cellulose.
4	Molecular structure	
5	Molecular weight	Approximately 36000.
6	Melting point	260 to 270^{o}C.
7	Density	1.512 to1.668 gm/cm^{3}.
8	Description	Microcrystalline cellulose is purified, white, odourless, tasteless, crystalline powder composed of porous particles.
9	Solubility	Insoluble in water, organic solvent, Slightly soluble in 5 % w/v NaOH solution.
10	Functional category	Adsorbent, suspending agent, tablet, capsule diluent and tablet disintegrant.
11	Application	Microcrystalline cellulose is used in pharmaceutical industries primarily as binder/diluents for tablets and capsules formulation where in both wet granulation and direct compression process and also used for lubricant, disintegrant properties. It is also used in cosmetics and food products.
12	Storage	It should be stored in well closed container.
13	Stability	Microcrystalline cellulose is a stable, though hygroscopic material.
14	Incompatibilities	Microcrystalline cellulose is incompatible with strong oxidizing agents.

Table No. 9

4.3.2.1. Properties of some commercially available grades of Microcrystalline Cellulose

Grade	Nominal mean particle size (µm)	Particle size analysis		Moisture content (%)
		Mesh size	Amount retained (%)	
Avicel PH-102	100	60	8.0	5.0
		200	45.0	
Avicel PH-112	100	60	8.0	1.5

Table No. 10

4.3.3. CROSPOVIDONE[67]

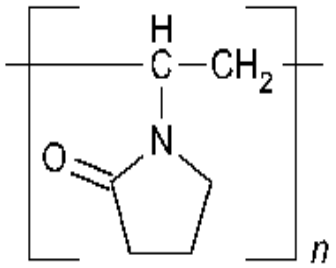
S. No.	Properties	Description
1	Non- proprietary name	BP : Crospovidone. PhEur: Crospovidon.
2	Synonyms	Cross linked polymer; kollidon CL; polyplasdone XL-10; polyvinylpolypyrrolidone.
3	Chemical name	1-ethenyl-2-pyrrolidinone Homopolymer.
4	Molecular structure	
5	Molecular weight	>1000000.
6	Moisture content	60% w/w.
7	Density	1.22 gm/cm^3
8	Description	Crospovidone is a white to creamy-white, finely divided, free-flowing, practically tasteless, odorless, hygroscopic powder.
9	Solubility	Partially Insoluble in water and most common organic solvents.
10	Functional category	Tablet disintegrant.
11	Application	Crospovidone is a water insoluble tablet disintegrate and dissolution agent used at 2-5% concentration in tablet prepared by direct compression or wet and dry granulation method.
12	Storage	It is a hygroscopic material. The bulk material should be stored in a well- closed container in a cool, dry place.

Table No. 11

4.3.4. MAIZE STARCH[67]

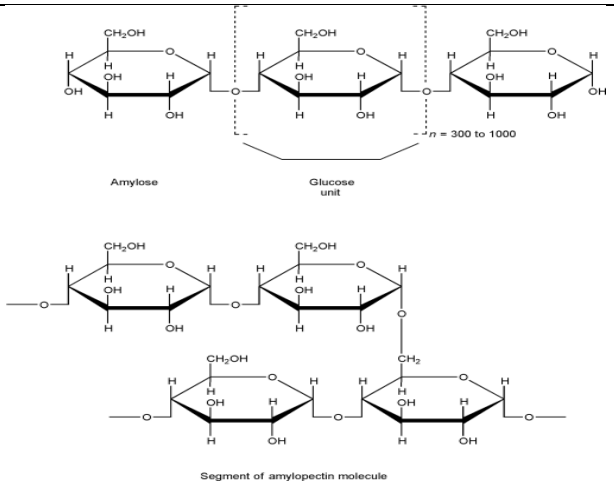
S. No.	Properties	Description
1	Non- proprietary name	Maize starch.
2	Synonyms	Amido, amidon, amilo, amylum, melojel.
3	Chemical name	Maize starch, potato starch, rice starch, tapioca starch, wheat starch.
4	Molecular structure	
5	Molecular formula	$(C_6H_{10}O_5)_n$ Where n = 300 – 1000
6	Molecular weight	50 000 – 160000
7	Description	Starch occurs as an odourless and tasteless, fine, white-colored powder comprising very small spherical or ovoid granules whose size and shape are characteristic for each botanical variety.
8	Solubility	Soluble in water when heated.
9	Functional category	Glidant; tablet and capsule diluent; tablet and capsule disintegrant; tablet binder.
10	Application	Starch is used as an excipient primarily in oral solid-dosage formulations where it is utilized as a binder, diluent, and disintegrant.
11	Storage	Store the corn starch in a sealed container and in a cool, dry place.

Table No. 12

4.3.5. COLLIODAL SILICON DIOXIDE[67,68]

S. No.	Properties	Description
1	Non-proprietary name	BP**:** Colloidal anhydrous silica. USPNF**:** colloidal silicon dioxide.
2	Synonyms	Aerosil, cab-o-sil, cab-o-sil M-5P, colloidal silica, fumed silica, light anhydrous silicic acid, silicic anhydride and silicon dioxide fumed.
3	Chemical name	Silica.
4	Molecular structure	SiO_2
5	Molecular weight	60.08 gm/mol.
6	Bulk Density	0.029–0.42 gm/cm^3
7	Tapped Density	0.05-0.12 gm/cm^3
8	Description	Colloidal silicon dioxide is sub microscopic fumed silica with a particle size of about 15nm. It is a light, loose, white coloured, odourless, tasteless, non-gritty amorphous powder.
9	Solubility	Practically insoluble in organic solvents, water and acids except hydrofluoric acid, soluble in hot solutions of alkali hydroxide forms a colloidal dispersion with water.
10	Functional category	Adsorbent, anticaking agent, glidant, suspending agent, tablet disintegrant and viscosity increasing agent.
11	Application	Colloidal silicon dioxide is widely used in pharmaceuticals, cosmetics and food products.
12	Storage	Colloidal silicon dioxide powder should be stored in a well-closed container.
13	Incompatibilities	Incompatible with diethylstilbestrol preparation.

Table No. 13

4.3.6. LACTOSE MONOHYDRATE[67, 69]

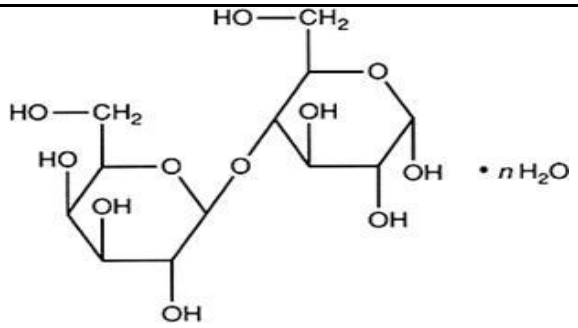
S. No.	Properties	Description
1	Non- proprietary name	BP : Lactose monohydrate. USPNF : Lactose monohydrate.
2	Synonyms	Fast-flo, (β-D-galactosido)-D-glucose, lactochem, microtose, milk sugar, pharmatose, saccharumlactis.
3	Chemical name	β-D-Galactopyranosyl-(1,4)-α-D-glucopyranose monohydrate.
4	Molecular structure	
5	Molecular weight	342.3 gm/mol.
6	Melting point	201-202^{o}C.
7	Density	1.545 gm/cm^{3}
8	Description	Lactose occurs as white to off-white crystalline particles powder.
9	Solubility	Chloroform, ethanol, ether are practically insoluble.
10	Functional category	Binding agent; diluents for dry-powder inhalers; tablet binder; tablet and capsule diluents.
11	Application	Lactose is widely used in tablets and capsules as filler. And also used in dry-powder inhalation as a diluents.
12	Storage	Lactose should be stored in a well closed container.
13	Incompatibilities	Lactose is incompatible with aminoacids, aminophylline and amphetamines.

Table No. 14

4.3.7. MAGNESIUM STEARATE[67]

S. No.	Properties	Description
1	Synonyms	Magnesium octadecanoate, stearic acid, magnesium salt.
2	Chemical name	Octadecanoate stearic acid magnesium salt.
3	Molecular structure	$[CH_3 (CH_2)_{16} COO]_2 Mg$.
4	Molecular weight	591.27 gm/mol.
5	Melting point	88^oC.
6	Description	It occurs as a fine, white precipitated or milled impalpable powder with a faint odour and a characteristic taste.
7	Solubility	Practically insoluble in ethanol, ether and water, slightly soluble in warm benzene and warm ethanol (95 %).
8	Functional category	Lubricant.
10	Application	It is widely used in cosmetics, food, pharmaceutical formulations. It is primarily used as lubricant in capsule and tablet manufacture at concentrations between 0.2-5.0%.
11	Stability	It is stable and should be stored in a well closed container in a cool and dry place.
12	Incompatibilities	It is incompatible with strong acids, alkalis and iron.

Table No. 15

4.3.8. PURIFIED TALC[67]

S.No.	Properties	Description
1	Synonyms	Altalc; E553; hydrous magnesium calcium silicate, luzenac pharma, magsilosmanthus, magsil Star, powdered talc, purified french chalk, soapstone, steatite superior.
2	Chemical name	Talc.
3	Molecular formula	$Mg_6 (Si_2O_5)_4(OH)_4$
4	Molecular weight	379.3 gm/mol.
5	Melting point	93^oC.
6	Description	Purified talc is a very fine, white to greyish-white, odourless, impalpable, unctuous, crystalline powder. It adheres readily to the skin and is soft to touch and free from grittiness.
7	Solubility	Practically insoluble in dilute acids and alkalis, organic solvents and water.
8	Functional category	Anti-caking agent, glidant, tablet and capsule diluent, tablet and capsule lubricant.
9	Application	It is widely used as a dissolution retardant in the development of controlled release products. Purified talc is also used as lubricant in tablet formulations, coating for pellets and as an adsorbent in topical preparations. Purified talc is used as a dusting powder and also used to clarify liquids and it is mainly used in food and cosmetic products as a lubricant.
10	Incompatibilities	Incompatible with quaternary ammonium compounds.
11	Storage	Stored at well closed container.

Table No. 16

4.3.12. SODIUM CHLORIDE[67]

S. No.	Properties	Description
1	Synonyms	Salt, Table salt, Halite, Saline.
2	Chemical name	Sodium chloride.
3	Molecular formula	NaCl
4	Molecular weight	58.44 gm/mol.
5	Boiling point	1465°C.
6	Density	1.199 gm/mL at 20°C
7	Melting point	800.7°C
8	pH	6.7 to 7.3; its aqueous solution is neutral.
9	Description	A white crystalline solid. Commercial grade usually contains some chlorides of calcium and magnesium which absorb moisture and cause caking.
10	Solubility	Soluble in water, slightly soluble in ethanol.
11	Functional category	Channeling agent, and as an osmotic agent, isotonic solutions.
12	Stability	Stable.
13	Incompatibility	Incompatible with strong oxidizing agents.
14	Storage	Keep container tightly closed.

Table No. 17

4.3.13. SODIUM CARBOXY METHYL CELLULOSE[67,70]

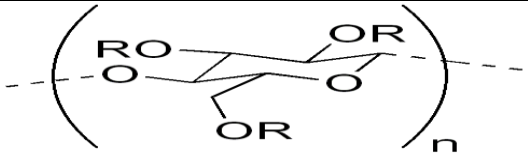
S. No	Properties	Description
1	Synonyms	Cellulose gum, carboxy methyl cellulose, sodium cellulose glycolate.
2	Chemical name	Carboxymethyl cellulose.
3	Molecular structure	 R = H or CH_2CO_2H
4	Molecular formula	Variable.
5	Molecular weight	Variable.
6	Viscosity	5mPa.s-8000mPa.s.
7	Density	1.6 gm/cm^3
8	Melting point	274°C.
9	Description	Sodium carboxylmethyl cellulose is odorless, tasteless and nontoxic white or yellowish powder.
10	Solubility	Favorable water solubility, insoluble in organic solvents such as methanol, ethanol, acetone, chloroform and benzene.
11	Functional category	Bulking agent, emulsifier, firming agent, gelling agent, glazing agent, humectant, stabilizer, thickener.
12	Applications	CMC is used in food under the E number E466 as a viscosity modifier or thickener, and to stabilize emulsions in various products including ice cream. It is also a constituent of many non-food products, such as toothpaste, laxatives, diet pills, water-based paints, detergents, textile sizing, and various paper products.
13	Stability	Stable.
14	Incompatibility	Incompatible with strong oxidizing agents.
15	Storage	Stored at room temperature.

Table No. 18

4.3.9. PROPYLENE GLYCOL[67]

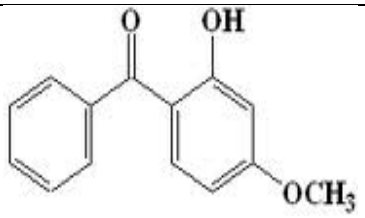
S. No.	Properties	Description
1	Synonyms	Dihydroxypropane, 2-hydroxypropanol, methylethylene glycol, methyl glycol, propane-1, 2-diol.
2	Chemical name	1, 2-Propanediol.
3	Molecular structure	
4	Molecular weight	76.09 gm/mol.
5	Boiling point	188^{o}C.
6	Description	Propylene glycol is a clear, colourless, viscous, odourless liquid with a sweet, slightly acrid taste resembling that of glycerin.
7	Solubility	Miscible with acetone, chloroform, ethanol (95%) glycerin and water; soluble at 1 in 6 parts of ether.
8	Functional category	Antimicrobial preservative, disinfectant, humectants plasticizer, solvent, stabilizer for vitamins, water-miscible co-solvent.
9	Application	Propylene glycol is widely used as a solvent, extractant and preservative in a variety of parenteral and nonparenteral pharmaceutical formulations.
10	Storage	Propylene glycol is hygroscopic and should be stored in a well-closed container, protected from light, in a cool, dry place.
11	Incompatibilities	Propylene glycol is incompatible with oxidizing reagent such as potassium permanganate.

Table No. 19

4.3.10. METHYL HYDROXYBENZOATE[67]

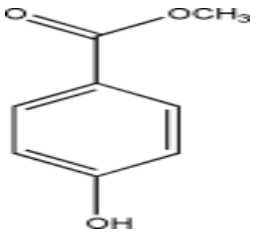
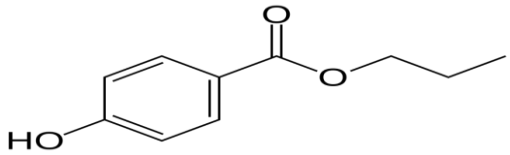
S. No.	Properties	Description
1	Synonyms	4-hydroxybenzoic acid methyl ester, methyl p-hydroxybenzoate.
2	Chemical name	Methyl-4-hydroxybenzoate.
3	Molecular structure	
4	Molecular formula	$C_8H_8O_3$
5	Molecular weight	152.15 gm/mol.
6	Description	Methyl hydroxybenzoate occurs as colourless crystals or a white crystalline powder. It is odourless or almost odourless and has a slight burning taste.
7	Solubility	Soluble in water when heated.
8	Functional category	Antimicrobial preservative.
9	Applications	Methyl hydroxybenzoate is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. It may be used either alone or in combination with other parabens or with other antimicrobial agents. In cosmetics, methyl hydroxybenzoate is the most frequently used antimicrobial preservative.
10	Storage	Stored at well closed container.

Table No. 20

4.3.11. PROPYL HYDROXYBENZOATE[67]

S. No.	Properties	Description
1	Synonyms	4-Hydroxybenzoesäurepropylester, propyl paraben, propyl *p*-hydroxybenzoate, propyl parahydroxybenzoate, nipasol, E216.
2	Chemical name	Propyl 4-hydroxybenzoate.
3	Molecular structure	
4	Molecular formula	$C_{10}H_{12}O_3$
5	Molecular weight	180.2 gm/mol.
6	Density	1.06 gm/cm³
7	Melting point	96 to 99°C
8	Description	Propyl hydroxybenzoate occurs as colourless crystals or a white crystalline powder. It is odourless or almost odourless and has a slight burning taste.
9	Solubility	Soluble in water when heated.
10	Functional category	Anti-fungal preservation agent.
11	Applications	It is a preservative typically found in many water-based cosmetics, such as creams, lotions, shampoos and bath products.
12	Storage	Stored at well closed container.

LIST OF EQUIPMENTS/ INSTRUMENTS

4.4. List of Equipments / Instruments

Table No. 21: List of Equipments / Instruments and Suppliers

S. No.	Instruments	Manufacturers/Suppliers
1	Electronic balance	Adventurer Mettler Toleda
2	Bulk density apparatus	Thermonik, Campbel Electronics
3	Electro Magnetic sieve shaker	Electro Pharma
4	Vernier calliper	Absolute digimatic, Mitutoyo
5	Monsanto Hardness Tester	Tab-Machines
6	Hot Plate	Pathak electrical works
7	Motor Blender	Remi Motor
8	Moisture balance	Citizen
9	pH Meter	Digisun Electronics
10	Melting point apparatus	Lab India
11	Ultra Sonicator	Lab man Scientific Instruments
12	Viscosity testing apparatus	Brook field Dv-E viscometer, Lab india
13	Hot air oven	Pathak electrical works
14	Friability test apparatus	Electro Lab
15	Disintegration tester	Electrolab,ED-21, India
16	Dissolution apparatus (Disso 2000)	Lab India dissolution test apparatus
17	UV Spectrophotometer	Shimadzu
18	FTIR Spectrophotometer 8300	Perkin Elmer
19	8-Station punching machine	Accura
20	Stability chamber	Thermo lab

METHODOLOGY

4.5. METHODOLOGY

4.5.1. PREFORMULATION STUDIES[71-73]

Preformulation may be described as the process of optimizing a drug through determination of those physical and chemical properties considered important in the formulation of a stable, effective and safe dosage form. The possible interactions with the various components intended for use in the final drug product are also considered. It is an effort that encompasses the study of such parameters as dissolution, polymorphic forms and crystal size and shape, pH profile of stability and drug – excipients interactions, which may have a profound effect on a drug's physiological availability and physical and chemical stability. Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system.

Before beginning the formal preformulation programs we must consider the following factors,

1. The amount of drug available.
2. The physicochemical properties of the drug already known.
3. Therapeutic category and anticipated dose of compound.
4. The nature of information, a formulation should have or would like to have.

Importance

There are critical differences between companies at the detailed level of knowledge and their ability to learn before doing.

1. Knowledge of the underlying variables and their relationship to performance.
2. Knowledge of the future manufacturing environment and the new variables introduced by that environment.
3. Part of the new drug development process.

Scope

Use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacy and stable product. At the same time provides the basis for optimization of drug product quality.

Some of the important parameters evaluated during preformulation studies are following

- **Physicochemical evaluation of drug molecule**
  - ✓ Description
  - ✓ Solubility
  - ✓ Melting point
  - ✓ Particle size determination
  - ✓ Loss on drying
  - ✓ Flow properties
- **Compatibility studies of the drug with excipients**

4.5.1.1. DESCRIPTION

It is the initial evaluation during preformulation studies which assess the colour and taste of the substance. This was only a descriptive test.

4.5.1.2. SOLUBILITY[74]

Aqueous solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy.

Table No. 22: Solubility Specifications

Descriptive terms	**Approximate volume of solvent in millilitres per gram of solute**
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble	More than 10,000

4.5.1.3. MELTING POINT[74]

The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle start to melt and last particle completely melts is regarded as melting range. Melting point of

Prednisolone was conducted by using melting point apparatus. One capillary tube was filled with pure Prednisolone and placed into the melting point apparatus. The temperature was noted at the time of the substance completely melts.

4.5.1.4. LOSS ON DRYING[74]

The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Loss on drying of prednisolone was measured by using moisture balance. Approximately weighed 1 gm of prednisolone and placed into a plate on the moisture balance. The temperature was set to be 60^0C. Then the moisture content present in the Prednisolone was measured in percentage.

4.5.1.5. FLOW PROPERTIES

1. Angle of Repose [75]

Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and horizontal plane. The angle of repose of the powder or granules was determined by fixed funnel method. To assess the flow property of the powder granules, the height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of the powder above a paper that was placed on a flat horizontal surface. Accurately weighed prednisolone was taken in a beaker. It was allowed to flow through the funnel freely on the surface of the paper to form a cone shaped pile. The diameter of the cone (d) and the height (h) of the pile was noted. From the diameter, radius (r) was calculated. The angle of repose (θ) was calculated using the following formula.

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

Where,

θ = Angle of repose

h = Height of the cone

r = Radius of the cone

Table No. 23: Angle of repose as an Indication of Powder Flow Property

S. No.	Type of flow	Angle of repose (degree)
1	Excellent	25– 30
2	Good	31 – 35
3	Fair	36 – 40
4	Passable	41 – 45
5	Poor	46 – 55
6	Very poor	56 – 65
7	Very very poor	>66

2. Density (gm/cm^3)[76]

a) Bulk Density (D_b)

Weighed quantity of prednisolone were transferred into a 50 ml measuring cylinder without tapping during transfer the volume occupied by the granules was measured. Bulk density (D_b) was measured by using formula.

$$D_b = m/V_o$$

Where,

D_b = bulk density

m = mass of the blend

V_o = untapped volume

b) Tapped Density (D_t)

Weighed quantity of Prednisolone was taken into a 50 ml measuring cylinder. Then cylinder was subjected to 50 taps in tapped density tester (Electro lab USP II). According to the U.S.P, the blend was subjected for 50 taps; the percentage volume variation was calculated by using the following formula.

$$D_t = m/V_t$$

Where,

D_t = tapped density

M = mass of the blend

V_t = Tapped volume

3. Measurement of powder compressibility[77]

a) Compressibility Index

The compressibility index is a measure of the propensity of a powder to consolidate. As such, it is a measure of the relative importance of inter-particular interaction. The compressibility index of the Prednisolone was determined by the Carr's compressibility index with the help of bulk density and tapped density value using the following formula.

$$\text{Compressibility index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

The values and its flow properties are given in the Table No.24.

b) Hausner's Ratio

The Hausner's ratio is a number that is correlated to the flowability of a powder or granular material. Hausner's ratio of the Prednisolone was determined by the ratio of tapped density and bulk density using the following formula.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

The values and its flow properties are given in the Table No.24.

Table No. 24: Compressibility Index, Hausner's Ratio and as an Indication of Powders/Granules Flow

S. No.	Compressibility Index (%)	Type of flow	Hausner's ratio
1	1 – 10	Excellent	1.00-1.11
2	11– 15	Good	1.12-1.18
3	16 – 20	Fair	1.19-1.25
4	21 – 25	Passable	1.26-1.34
5	26 – 31	Poor	1.35-1.45
6	32 – 37	Very poor	1.46-1.59
7	>38	Very very poor	>1.60

4.5.1.6. PARTICLE SIZE DISTRIBUTION[78]

In case of tablets, size influences the flow and the mixing efficiency of powders and granules. Size can also be a factor in stability. Fine materials are relatively more open to attack from atmospheric oxygen, the humidity and interacting excipients than coarse materials.

Particle size distribution of the Prednisolone was estimated by sieving method using magnetic sieve shaker. The sieves are stacked on top of one another in ascending degrees of coarseness. 10 gm of Prednisolone was placed on the top sieve. The nest of sieves was subjected to a standard period of agitation. The weight of material retained on each sieve was accurately determined. Percentage of powder retained on each sieve was calculated by using the following formula.

$$\text{Percentage retained} = \frac{\text{Mass retained on each seive}}{\text{Total weight}} \times 100$$

Table No. 25: Classification of Sample was based on the Percentage of Sample Retained or passed on Test Sieves

S. No.	Nature of sample	Result of determination
1	Coarse powder	NLT 95% of the sample mass pass through #14 and NMT 40% pass through #36
2	Moderately coarse powder	NLT 95% of the sample mass pass through #25 and NMT 40% pass through #60
3	Moderately fine powder	NLT 95% of the sample mass pass through #36 and NMT 40% pass through #100
4	Fine powder	NLT 95% of the sample mass pass through #100 and NMT 40% pass through #150
5	Very fine powder	NLT 95% of the sample mass pass through #150 and NMT 40% pass through #200
6	Super fine powder	NLT 90% by number of particles are less than 10µm

4.5.1.7. PHYSICAL DRUG-EXCIPIENTS COMPATIBILITY STUDIES[79]

In the tablet dosage form the drug is in intimate contact with one or more excipients, the latter could affect the stability of the drug. Knowledge of drug- excipients interactions therefore is very useful to the formulators in selecting appropriate excipients.

Compatibility studies were performed by preparing blend of different excipients with drug and stored at $40^{o}C \pm 2^{o}C/75 \pm 5\%$ RH for three weeks. The initial state of mixture was noted and further evaluation for the possible occurrence of any changes was performed every week up to 3rd week. The drug excipients compatibility in physical observation protocol is given in Table No. 26.

Table No. 26: Drug: Excipients Compatibility study Protocol

S. No.	Composition	Quantity (gm)	Ratio
1	Prednisolone	1.0	1:0
2	Prednisolone + Riboflavin-5- sodium phosphate	2.0	1:1
3	Prednisolone + Microcrystalline cellulose	2.0	1:1
4	Prednisolone + Crospovione	2.0	1:1
5	Prednisolone + Maize starch	2.0	1:1
6	Prednisolone + Colloidal anhydrous silica	1.5	1:0.5
7	Prednisolone + Lactose (DCL 21)	1.5	1:0.5
8	Prednisolone + Lactose monohydrate	1.5	1:0.5
9	Prednisolone + Methyl hydroxylbenzoate	1.5	1:0.5
10	Prednisolone + Propyl hydroxybenzoate	1.5	1:0.5
11	Prednisolone + Magnesium stearate	1.5	1:0.5
12	Prednisolone + Purified talc	1.5	1:0.5

4.5.2. FORMULATION OF PREDNISOLONE DISPERSIBLE TABLETS

4.5.2.1. SELECTION OF EXCIPIENTS

Excipients used in the present study were selected according to innovator excipient list. Excipients include Riboflavine-5- sodium phosphate, microcrystalline cellulose (PH102 and PH112), crospovidone, maize starch, colloidal anhydrous silica, lactose (DCL21), lactose monohydrate, methyl hydroxybenzoate, propyl hydroxybenzoate, magnesium stearate and purified talc in the tablet core. Methyl hydroxybenzoate, propyl hydroxybenzoate, propylene glycol, sodium chloride, sodium carboxy ethyl cellulose and purified water are used for the vehicle solution.

4.5.2.2. PROCEDURE:

Three methods were used for the formulation of Prednisolone dispersible tablets:

1. Direct compression method
2. Wet granulation method
3. Slugging method

Tablets were prepared by using direct compression for the formulations F1 – F3. Wet granulation is used for the formulation F4 and F5. Slugging method is used in formulation F6. The details of the formulation along with the excipients in quantities and in percentages used are mentioned in Table No.27 & 28.

Table No. 27: Formulations of Prednisolone Dispersible Tablets

S. No.	Ingredients (mg)	Quantity of ingredients (mg/tab)					
		Direct compression			Wet granulation		Slugging method
		F1	F2	F3	F4	F5	F6
1	Prednisolone	20	20	20	20	20	20
2	Riboflavine -5 sodium phosphate	3	3	3	3	3	3
3	Microcrystalline cellulose (PH112)	40	40	45	-	-	40
4	Microcrystalline cellulose (PH102)	-	-	-	40	40	-
5	Crospovidone	15	15	20	15	35	15
6	Maize starch	4	4	7	28	28	4
7	Maize starch Paste	-	-	-	2	2	-
8	Colloidal anhydrous silica	6	8	12	2	2	6
9	Lactose (DCL 21)	36	36	37	-	-	36
10	Lactose Monohydrate	-	-	-	34	34	-
11	Methyl hydroxybenzoate	-	-	-	0.4	0.4	-
12	Propyl hydroxybenzoate	-	-	-	0.1	0.1	-
13	Magnesium stearate	4	4	4	4	4	4
14	Purified talc	2	2	2	1.5	1.5	2
	Total weight (mg)	**130**	**132**	**150**	**150**	**170**	**130**

Table No. 28: Percentage of Ingredients used in Formulations

S. No.	Ingredients	Quantity of ingredients (%)					
		Direct compression			Wet granulation		Slugging method
		F1	F2	F3	F4	F5	F6
1	Prednisolone	15.38	15.15	13.33	13.33	11.76	15.38
2	Riboflavin -5 sodium phosphate	2.3	2.27	2	2	1.76	2.3
3	Microcrystalline cellulose (PH112)	30.77	30.3	30	-	-	30.77
4	Microcrystalline cellulose (PH102)	-	-	-	26.66	23.52	-
5	Crospovidone	11.53	11.36	13.33	10	20.58	11.53
6	Maize starch	3.07	3.03	4.66	18.66	16.47	3.07
7	Maize starch paste	-	-	-	1.33	1.17	-
8	Colloidal anhydrous silica	4.61	6.06	8	1.33	1.17	4.61
9	Lactose (DCL 21)	27.69	27.27	24.66	-	-	27.69
10	Lactose monohydrate	-	-	-	22.66	20	-
11	Methyl hydroxybenzoate	-	-	-	0.26	0.23	-
12	Propyl hydroxybenzoate	-	-	-	0.07	0.05	-
13	Magnesium stearate	3.07	3.03	2.66	2.66	2.35	3.07
14	Purified talc	1.53	1.51	1.33	1	0.88	1.53

DIRECT COMPRESSION METHOD

Weighing

The specified quantity of prednisolone and other all excipients were accurately weighed.

Sifting

Sift the following materials individually Prednisolone and micro crystalline cellulose (PH112) were sifted using 30 # mesh. Riboflavin -5- sodium phosphate, crospovidone, maize starch, and lactose (DCL21) were sifted through 40 # mesh. Colloidal anhydrous silica was passed through 60 # mesh sieve. The sifted powders were mixed in a polythene bag for ten minutes.

Lubrication

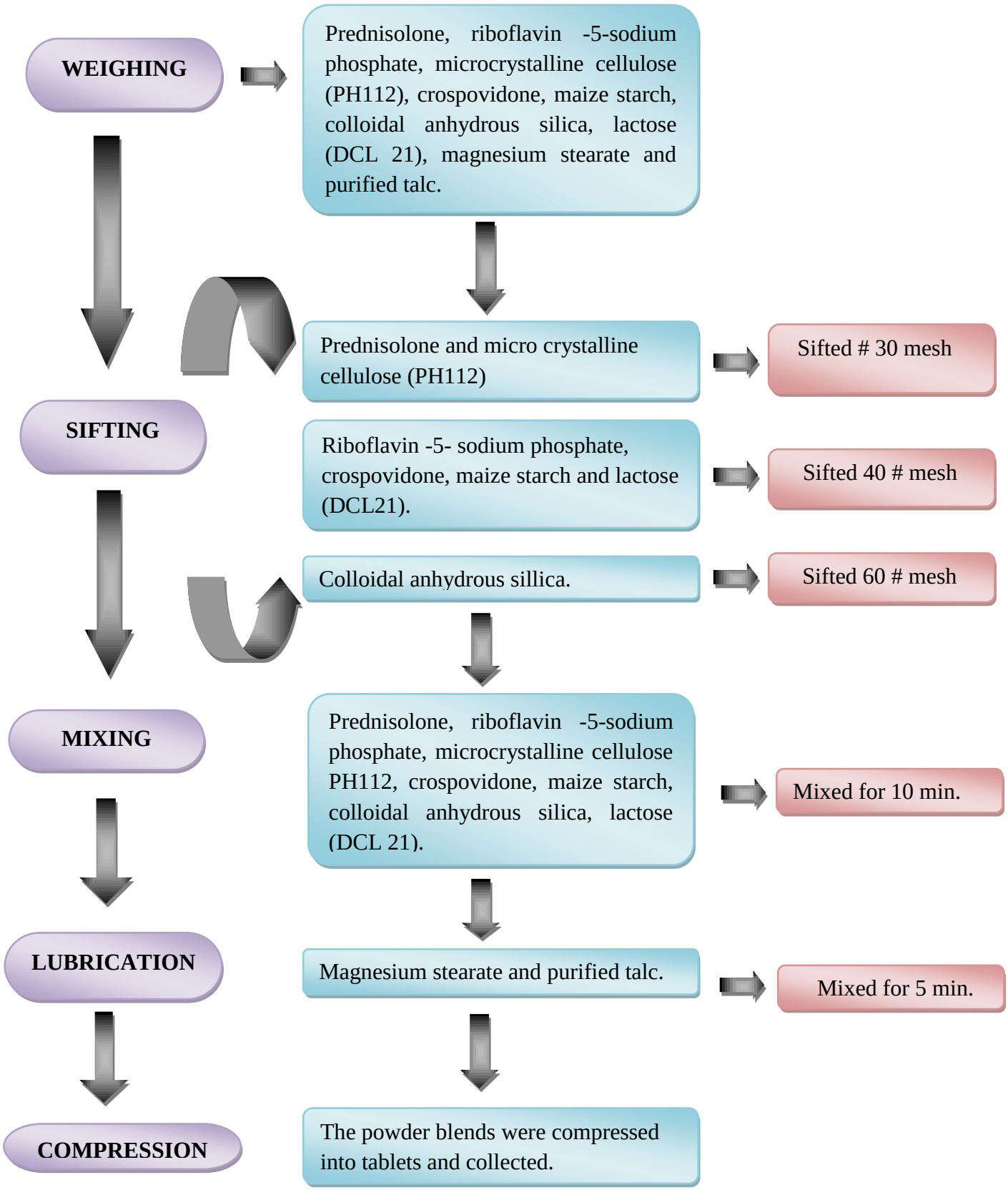
The above sifted granules were lubricated using magnesium stearate and purified talc, which was sifted through 60 # mesh size. Mix it for 5 minutes in a polythene bag.

Compression

Then final lubricated blend was compressed at an average weight of 130 mg, 132 mg and 150 mg using punch size 7.1 mm and 8 mm.

FORMULATION FLOWCHART OF DIRECT COMPRESSION METHOD

(F1-F3)



WET GRANULATION METHOD

Weighing

The specified quantity of prednisolone and other all excipients were accurately weighed.

Sifting

Individually prednisolone and maize starch were sifted using 60 # mesh. Riboflavin - 5- sodium phosphate, crospovidone, microcrystalline cellulose (PH 102) and lactose monohydrate were sifted through 40 # mesh. Colloidal anhydrous silica was passed through 60 # mesh sieve. The sifted powders were mixed in a polythene bag for ten minutes.

Binder preparation

The binder solution of starch mucilage paste was prepared by maize starch in purified water having methyl hydroxybenzoate and propyl hydroxybenzoate under stirring condition.

Granules preparation

The prepared mucilage was added slowly to the dry mixed powder and granulated using kneading method. The wet mass was dried at a temperature of $60^{o}C$ until the LOD (loss on drying) of granules is reached less than 1%. The dried granules were sifted through 20# mesh.

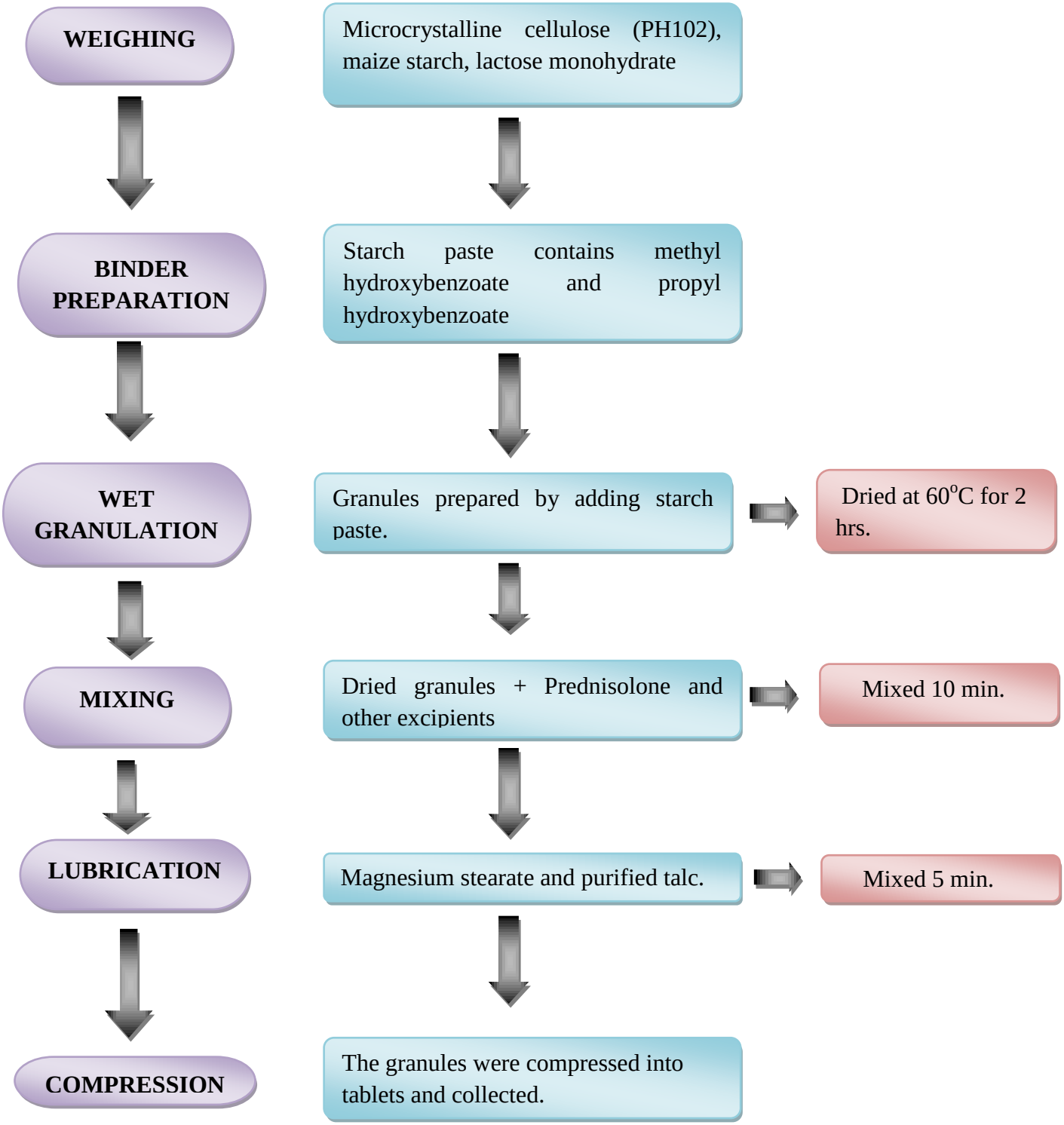
Lubrication

The above dried granules were lubricated using magnesium stearate and talc was sifted through 60 # mesh size. Mixed for 5 minutes in a polythene bag.

Compression

Then final lubricated blend was compressed at an average weight of 150 mg (F4) and 170 mg (F5) using the punch size of 8 mm.

FORMULATION FLOWCHART OF WET GRANULATION METHOD (F4 AND F5)



SLUGGING METHOD

Weighing

The specified quantity of prednisolone and other all excipients were accurately weighed.

Sifting

Individually Prednisolone and micro crystalline cellulose (PH112) were sifted using 30 # mesh. Riboflavine -5- sodium phosphate, crospovidone, maize starch, and lactose (DCL21) were sifted through 40 # mesh. Colloidal anhydrous silica was passed through 60 # mesh sieve. The sifted powders were mixed in a polythene bag for ten minutes.

Lubrication

The above sifted granules were lubricated using Magnesium stearate and purified talc which is sifted through 60 # mesh size mix for 5 minutes in a polythene bag.

Compression

Then final lubricated blend is compressed using the large punch size of 20 mm.

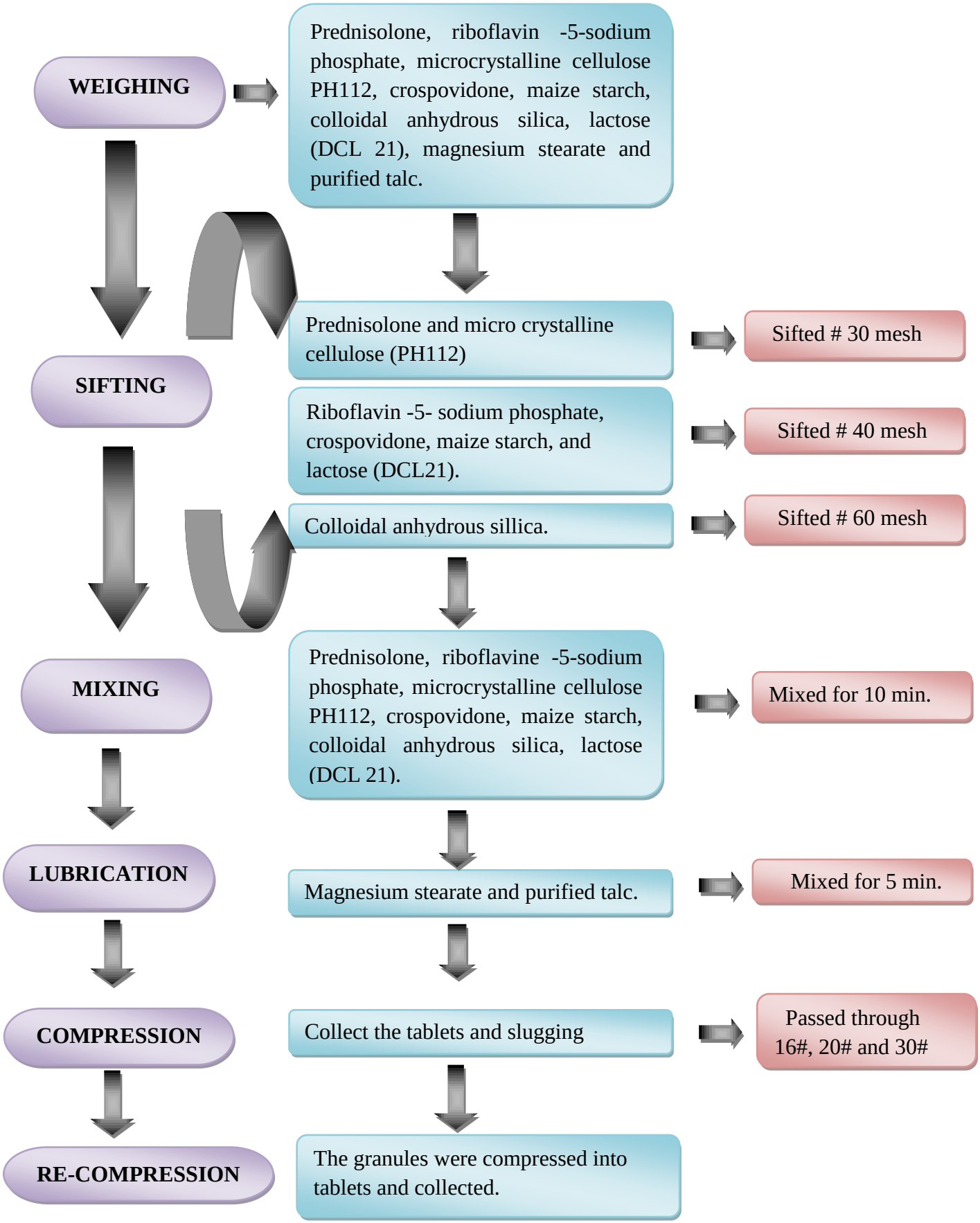
Slugging

The compressed tablets were crushed into granules using mortar and pestle. Those granules were passed through initially by 16 # mesh then by 20 # mesh and finally passed through 30# sieve mesh to get uniformed granules.

Re-compression

The collected granules were again compressed at an average weight of 130 mg using the punch size of 7.1 mm.

FORMULATION FLOWCHART OF SLUGGING METHOD (F6)



4.5.3. FORMULATION OF VEHICLE FOR SUSPENDING PREDNISOLONE DISPERSIBLE TABLET

1. Accurately weighed sodium chloride was dissolved in purified water.
2. Separately sodium carboxy methyl cellulose was dissolved in propylene glycol then poured into the sodium chloride mixture.
3. Methyl hydroxybenzoate and propyl hydroxybenzoate were dissolved in hot purified water. Then added into the above mixture.
4. Finally the volume of solution was made up to 100 ml with purified water.

FLOW CHAT FOR FORMULATION OF VEHICLE SOLUTION

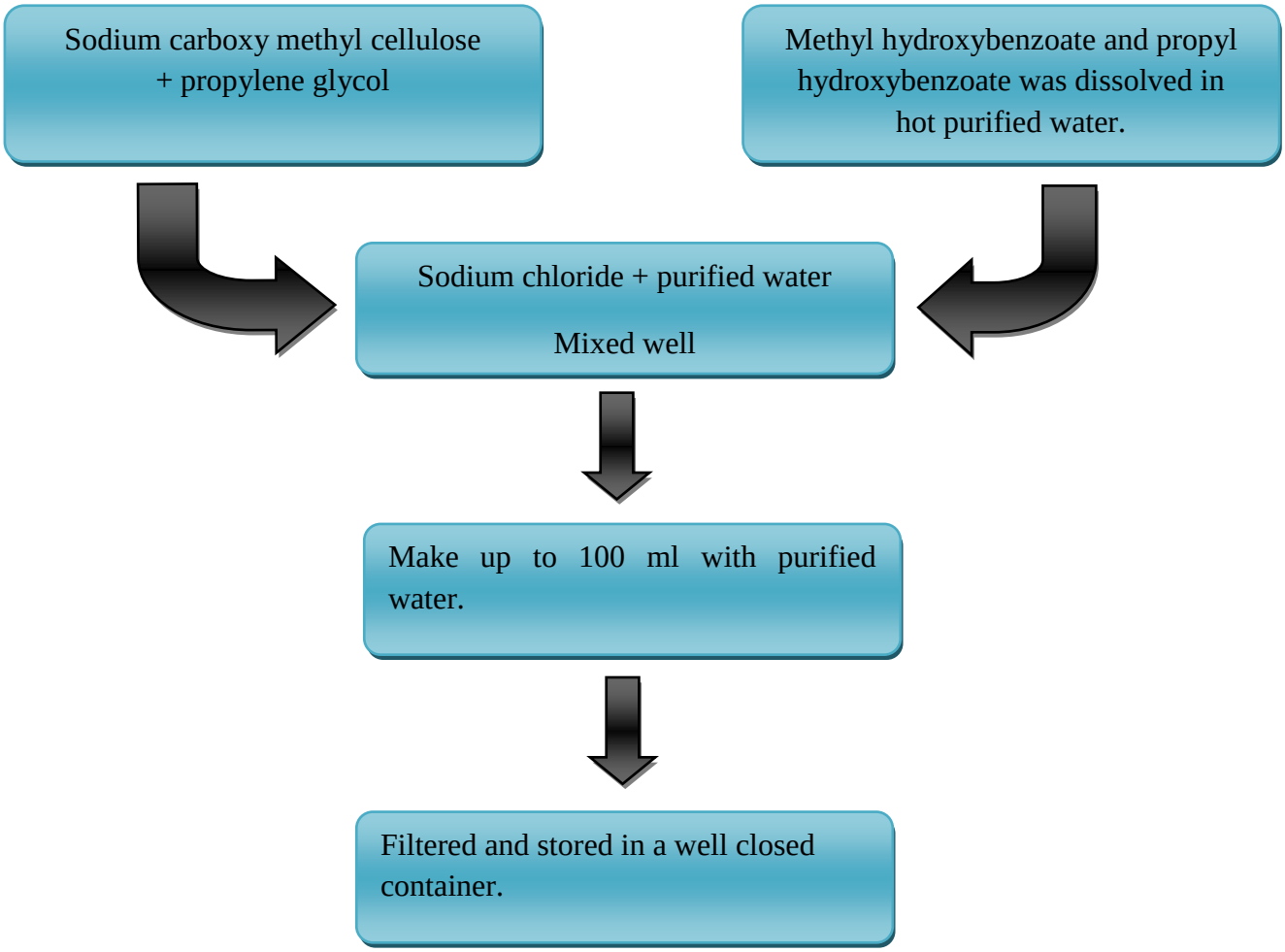


Table No. 29: Formulation of Vehicle for Suspending Prednisolone Dispersible Tablet

S. No.	Ingredients	Quantity (mg/100 ml) for 1 tablet
1	Sodium chloride	90
2	Methyl hydroxybenzoate	230
3	Propyl hydroxybenzoate	34
4	Sodium carboxy methyl cellulose	100
5	Propylene glycol	5000
6	Purified water	To 100 ml

4.5.4 EVALUATION OF PREDNISOLONE POWDER/GRANULES

The powders/granules were evaluated for the following parameters before compression into tablets.

1. Angle of repose
2. Bulk density
3. Tapped density
4. Compressibility index
5. Hausner's ratio
6. Moisture content

These procedures were discussed earlier in preformulation studies.

4.5.5. EVALUATION OF PREDNISOLONE DISPERSIBLE TABLETS[80-88]

The compressed tablets were evaluated for the following parameters.

General appearance

The tablets should be free from cracks, depression, pinholes etc. the colour and polish of the tablets should be uniform on whole surface. The tablets were examined externally under a biconvex lens for surface, cracks, depressions, pinholes, colour, polish, etc.

Hardness test or crushing strength

Hardness of the tablet is defined as the force required in breaking a tablet in a diametric compression test. In this test, a tablet was laced between two anvils, force was applied to the anvils and the crushing strength that just causes the tablet to break is recorded. Hence hardness is sometimes referred to as "Crushing Strength".

Tablets require certain amount of strength or hardness to withstand mechanical shocks of handling in manufacture, packaging and shipping. The hardness of a tablet, like its thickness, is a function of the die fill and compression force. At a constant die fill, the hardness value increases and thickness decreases as additional compression force is applied. At a constant compression force (fixed distance between upper and lower punches), hardness increases with increasing die fills and decreases with lower die fills.

It was measured using Monsanto tablet hardness tester. The values were expressed in kg/cm^2.

Thickness

Thickness mainly depends up on die filling, physical properties of material to be compressed under compression force. The thickness of the tablets was measured by using digital vernier callipers. The thickness was denoted in millimetre.

Weight variation test

20 tablets were individually weighed and the average weight was calculated. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation shown in Table No. 29. The percentage deviation of the tablets were calculated by using the following formula,

$$\text{Percentage deviation} = \frac{\text{weight of tablet (mg)} - \text{Average weight of tablets}}{\text{Average weight of tablets}} \times 100$$

Table No. 30: Weight variation of tablets and Percentage deviation

S. No.	Average weight of tablet (mg)	Percentage deviation
1	130 or less	± 10.0
2	130-324	± 7.5
3	More than 324	± 5.0

Friability

The friability of tablets was determined by using Roche friabilator. 20 tablets were weighed and placed into the friabilator and rotated at 25 rpm for 4 minutes. Then the tablets were taken out, de-dusted and reweighed. The percentage friability of the tablets was calculated by the formula.

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

Disintegration time

The test was carried out on six tablets using 900 ml distilled water at $37^oC \pm 2^oC$ was used as disintegration media and the time in seconds taken for complete disintegration of the tablets with no palpable mass remaining in the apparatus was measured in seconds.

Wetting time

A piece of tissue paper (10.75×12 mm) folded twice was placed in a culture dish (d=6.5 cm) containing 6 ml of water. A tablet was placed on the paper and the time for complete wetting was noted.

Water absorption ratio

A piece of tissue paper folded twice was placed in a small petri dish containing 6 ml of water. A tablet was placed on the tissue paper and allowed to completely wet. The wetted tablet was then weighed. The water absorption ratio (R) was calculated by using following formula.

$$R = \frac{W2 - W1}{W1} \times 100$$

Where,

R = Water absorption ratio

W2 = Weight after wetting

W1 = Weight before wetting

***In-vitro* dispersion time**

One tablet was placed into 10 ml of distilled water at 37°±0.5°C. Time required for complete dispersion of a tablet was noted.

Uniformity of dispersion

The fineness of dispersion test was done by two tablets in 100 ml of water and stir gently until completely dispersed. A smooth dispersion was obtained which passes through a sieve screen with a nominal mesh aperture of 710 mm (sieve no. 22).

Drug content estimation (By UV) [89,90]

Diluent Preparation

Methanol:Purified water (30:70). 300 ml methanol added into 700 ml of purified water to make 1000 ml of diluent.

Sample Preparation

Accurately weighed about 650 mg of crushed tablet power in a clean 100 ml volumetric flask and 50 ml of diluents was added. Mixed well and sonicated for 20 minutes. Then make up to 100 ml with diluents. Filtered and diluted 1 ml to 100 ml and from this, 5 ml was diluted to 10 ml.

Standard preparation

Accurately weighed 100 mg of Prednisolone working standard in a clean, 100 ml volumetric flask and dissolved into 50 ml of diluents. The volume was making up to 100 ml with diluents. 1 ml of this solution was diluted to 100 ml with diluents. From this, 5 ml of the resulting solution was diluted to 10 ml with diluents.

Procedure

The absorbance of both the standard and sample preparations was measured at 246 nm using diluents as a blank. The drug content of prednisolone present per tablet was calculated by using the following expression.

$$Drug\ content = \frac{absorbance\ of sample}{absorbance\ of\ standard} \times \frac{weight\ of\ standard}{100} \times \frac{1}{100} \times \frac{5}{10} \times \frac{100}{weight\ of\ sample} \times \frac{100}{1} \times \frac{10}{5} \times \frac{purity\ ot\ standard}{average\ weight} \times 100$$

$$Assay = \frac{drug\ content}{label\ claim} \times 100$$

IR spectral analysis

Compatibility studies were assured by FT-IR studies. The pure drug sample and the complete formula of formulation were chosen for the study. The FT-IR spectra's of the above samples were studied after a period of 30 days from preparation of the mixtures, to facilitate prompt detection of incompatibility. The spectra's were obtained by preparing potassium bromide (KBr) pellets under dry condition by using pellet press. The spectra of the crude drug sample and that of the drug-excipients mixtures were compared to check the incompatibility problems, if any.

4.5.6. FORMULATION OF RECTAL SUSPENSION[23]

One Prednisolone dispersible tablet from F6 formulation was placed into a container contains 100 ml of vehicle solution. Shaked well for 2-3 minutes for the tablet to disperse. A yellow colour suspension was obtained.

4.5.7. EVALUATION OF SUSPENSION

Determination of pH value[91]

The pH value conventionally represents the acidity or alkalinity of an aqueous solution. In the I.P. standards and limits of pH have been provided for those Pharmacopoeial substances in which pH as a measure of the hydrogen-ion activity is important from the standpoint stability or physiological suitability. The pH determination is carried out by using pH meter.

Determination of viscosity[91]

The determination of viscosity of newtonian liquids is carried out by means of a capillary viscometer, unless otherwise specified. For measurement of viscosity, the temperature of the substance being measured must be accurately controlled, since small temperature changes may lead to marked changes in viscosity. Viscosity was measured using Spindle 61, 50 RPM in Brook field DV- E viscometer.

In-vitro release study[92,93]

The release of Prednisolone from suspension was studied in 900 ml of phosphate buffer pH 7.4 as dissolution medium in a U.S.P type II dissolution (paddle) apparatus at 50 rpm and 37 ± 0.5^{o}C temperatures. 10 ml of suspension was placed in a bowl. 5 ml of sample was withdrawn at every 10 minutes interval up to one hour; the solution was filtered through membrane filter and make up to 10 ml with pH 7.4 phosphate buffer. The sample was analyzed in UV double beam spectrophotometer at 246 nm. pH 7.6 phosphate buffer as a blank. The percentage drug release was calculated by using following formula.

$$\%\ \text{of drug release} = \frac{\text{sample absorbance} \times \text{weight of std} \times \text{dilution factor} \times \text{purity of std} \times 100}{\text{std absorbance} \times \text{weight of sample} \times \text{label claim of drug}}$$

4.5.8. MICROBIOLOGICAL EVALUATION[94]

The formulated suspension was evaluated for microbial limit test. The following tests were performed:

1. Total aerobic microbial count.
2. Total combined yeast and mould count.
3. Test for specified microorganisms such as:
    - *Escherichia coli*
    - *Salmonella typhi*
    - *Staphylococcus aureus*
    - *Pseudomonas aeruginosa*

1. Total aerobic microbial count

Pre-treatment of the sample

10 ml of the suspension was diluted in buffered sodium chloride-peptone solution pH 7.0 and the volume made up to 100 ml with the same medium. The pH was adjusted to 7.

For bacteria

Using petri dishes 9 to 10 cm in diameter, added to each dish a mixture of 1 ml of the pre-treated preparation and about 15 ml of a liquefied Casein soya bean digest medium at not more than 45ºC. Alternatively, the pre-treated preparation was spreaded on the surface of the solidified medium in a petri dish of the same diameter. Two such petri dishes using the same dilution was prepared and incubated at 30 to 35°C for 4 days, unless a more reliable count is obtained in a shorter time. Formed colonies were counted.

For total combined yeast and mould

Proceed as described in the test for bacteria but Sabouraud dextrose agar medium was used and the plates were incubated at 20 to 25°C for 5 days. Then, the formed colonies were counted.

2. Test for specified microorganisms

Pre-treatment of samples

Proceed as described below the test for total aerobic microbial count but using lactose broth.

- ***Escherichia coli:***

The prescribed quantity of suspension was placed in a sterile screw-capped container. 50 ml of Casein soya bean digest broth was added. Shaked and allowed to stand for 1 hr (4 hrs for gelatin) and homogenised. Loosen the cap and incubated at 36-38°C for 18 to 24 hrs. Shake the container, 1 ml was transfered to 100 ml of MacConkey broth and incubate at 43-45ºC for 18-24 hrs. Subculture was on plates of MacConkey broth at 35-37º for 18-72 hrs. Growth of red, non-mucoid colonies of gram-negative rods indicates the possible presence of Escherichia coli.

- ***Salmonella typhi:***

A quantity of the pre-treated preparation was transferred under examination containing 1 ml of the product to 100 ml of Nutrient broth in a sterile screw-capped jar. Shakes well and allowed to stand for 4 hrs. Loosen the cap and incubated at 35 to 37ºC for 24 hrs. 1.0 ml of the enrichment culture was added to 10 ml of Selenite F broth and incubated at 36 to 38ºC for 48 hrs. From this culture, subculture on Brilliant green agar was prepared. The plates were incubated at 36 to 38ºC for 18 to 24 hrs. Upon examination, if none of the colonies formed confirms the sample for the absence for the genus Salmonella.

- ***Pseudomonas aeruginosa:***

100 ml of Casein soya bean digest broth with a quantity of the solution thus obtained containing 1 ml of sample suspension was inoculated. After mixing, it was incubated at 35 to 37ºC for 24 hrs. The medium for growth, streak a portion of the medium on the surface of Cetrimide agar, each plated on petri dishes was examined. Covered and incubated at 35 to 37ºC for 18 to 24 hrs.

- ***Staphylococcus aureus:***

Proceed as described under Pseudomonas aeruginosa. If, upon examination of the incubated plates, none of them contains colonies, the sample was absence of Staphylococcus aureus.

4.5.9. ACCELERATED STABILITY STUDIES[95,96]

Stability studies were aimed at determining the result of aging and storage under various conditions on the formulated dispersible tablet. It was carried out to evaluate the stability of F6 formulations after storing at different temperatures for a period of 3 months. The prepared tablets were kept at three different temperatures 4 ± 2°C, 28 ± 2°C and 45 ± 2°C for 3 months. Every month the tablets were evaluated for all the physical parameters. The *in vitro* drug release studies were determined by UV double beam spectrophotometer.

Evaluation parameters for stability studies

1. Colour and appearance
2. Hardness
3. Thickness
4. Weight variation
5. Friability
6. Disintegration time
7. Wetting time
8. Water absorption ratio
9. *In-vitro* dispersion time
10. Uniformity of dispersion
11. Drug content estimation
12. *In-vitro* drug release

CHAPTER 5

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

5.1. PREFORMULATION STUDIES FOR PREDNISOLONE PURE DRUG

5.1.1. DESCRIPTION

Table No. 31: Description of Prednisolone

Properties	As per I.P. Specification	Observation
Colour	White	White
Physical Nature	Crystalline powder	Crystalline powder
Taste	Bitter	Bitter
Odour	Odourless	Odourless

Corollary:

Colour, physical nature, taste and odour of Prednisolone were observed and their result shows same as the I.P. specification.

5.1.2. SOLUBILITY

Table No. 32: Solubility analysis of Prednisolone

Active ingredient	Aqueous solubility
Prednisolone	Insoluble

Corollary:

Based on the procedure, the solubility of Prednisolone was found to be insoluble in water.

5.1.3. PHYSICAL PROPERTIES

Table No. 33: Physical properties of Prednisolone

Properties	I.P. Ranges	Observed value
Melting point	230-235^{o}C	233.7^{o}C
Loss on drying	Not more than 1.0%	0.78 %

Corollary:

The observed value for melting point and loss on drying of Prednisolone was found to be within the I.P. ranges.

5.1.4. FLOW PROPERTIES

Table No. 34: Flow properties of Prednisolone

S. No.	Properties	Observation value	Types of flow
1	Angle of repose	40^{o}.35′	Very Poor
2	Bulk density	0.265 gm/ml	-
3	Tapped density	0.365 gm/ml	-
4	Compressibility Index	27.39	Poor
5	Hausner’s Ratio	1.37	Very Poor

Corollary:

According to the results showed in Table No. 34 the flow property of Prednisolone was found to be very poor. So it may require glidants to improve the flow property.

5.1.5. PARTICLE SIZE DISTRIBUTION

Table No. 35: Particle size distribution of Prednisolone

Sieve No.	Empty sieve weight (gm)	Quantity retained (gm)	Mass retained (gm)	Cumulative mass retained	Cumulative % retained	Percentage passing %
#20	367.80	368.20	0.40	0.40	4.00	96.00
#30	417.65	418.16	0.51	0.91	9.10	90.90
#40	358.05	359.97	1.92	2.83	28.30	71.70
#60	343.45	349.15	5.70	8.53	85.30	14.70
#80	340.75	341.72	0.97	9.50	95.00	5.00
#100	332.50	332.91	0.41	9.91	99.10	0.90
Base	540.45	540.54	0.09	10.00	100.00	0.00

Corollary:

From the particle size distribution analysis, Prednisolone particle size was found to be fine powder.

5.1.6. DRUG – EXCIPIENTS COMPATIBILITY STUDIES

Table No. 36: Physical Observation

S. No.	Composition	Initial	1st week	2nd week	3rd week
1	Prednisolone	White crystalline powder	NCC	NCC	NCC
2	Prednisolone + Riboflavin-5- sodium phosphate	Sunset yellow crystalline powder	NCC	NCC	NCC
3	Prednisolone + Microcrystalline cellulose	White to off white powder	NCC	NCC	NCC
4	Prednisolone + Crospovione	White to off white powder	NCC	NCC	NCC
5	Prednisolone + Maize starch	White to off white powder	NCC	NCC	NCC
6	Prednisolone + Colloidal anhydrous silica	White to off white powder	NCC	NCC	NCC
7	Prednisolone + Lactose (DCL 21)	White to off white powder	NCC	NCC	NCC
8	Prednisolone + Lactose monohydrate	White to off white powder	NCC	NCC	NCC
9	Prednisolone + Methyl hydroxybenzoate	White to off white powder	NCC	NCC	NCC
10	Prednisolone + Propyl hydroxybenzoate	White to off white powder	NCC	NCC	NCC
11	Prednisolone + Magnesium stearate	White to off white powder	NCC	NCC	NCC
12	Prednisolone + Purified talc	White to off white powder	NCC	NCC	NCC

***NCC** – No Characteristic change

Corollary:

From the drug excipients compatibility study, it was observed that there were no physical changes between drug and excipients. Thus it was concluded that the excipients selected for the formulations were compatible with Prednisolone.

5.2. EVALUATION OF FORMULATED PREDNISOLONE POWDER/GRANULES

Table No. 37: Evaluation of Formulated Prednisolone Powders/Granules

Formulation No.	Angle of repose (degree)	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's Index (%)	Hausner's ratio	Moisture content (%)
F1	$40^{o}.75'$	0.332	0.436	23.85	1.31	0.89
F2	$41^{o}.15'$	0.391	0.511	23.48	1.30	0.80
F3	$44^{o}.26'$	0.396	0.506	21.70	1.27	0.86
F4	$37^{o}.12'$	0.398	0.512	22.20	1.21	0.85
F5	$36^{o}.24'$	0.411	0.526	21.86	1.22	0.76
F6	$32^{o}.27'$	0.557	0.647	13.91	1.16	0.70

Corollary:

The Prednisolone powders/granules were evaluated for different parameters and the results are given in Table No. 37.

Angle of repose of the powder blend was found to be between $32^{o}.27'$ to $44^{o}.26'$. Angle of repose for F1 to F3 formulations was within the limit of 41^{o} to 45^{o} and their flow properties were passable. F4 and F5 formulations 36^{o} to 40^{o} and their flow properties were fair. In F6 formulation, the angle of repose is within the limit of 30^{o} to 35^{o}. This indicates the granules having good flow property.

Bulk density for F1 to F3 formulations was found to be between 0.332 and 0.396 (gm/ml), F4 and F5 formulations between 0.398 and 0.411 (gm/ml) and F6 formulation 0.557 (gm/ml). The values were found to be high for Slugging method than direct compression method and wet granulation method. This may be due to increase in void space observed in granules.

Tapped density was found to be between 0.436 and 0.511 (gm/ml) for F1 to F3 formulations, 0.512 and 0.526 (gm/ml) for F4 and F5 formulations and 0.647 (gm/ml) for F6 formulations. The values of the direct compression method and wet granulation method were found to be decrease when compared to slugging method since the granules size were larger than the powder blends. So it does not settle down easily like powders in case of direct compression method.

Compressibility index was found to be in the range of 21.70 to 23.85 % for F1 to F3 formulations, 22.20 and 21.86 % for F4 and F5 formulations, 13.91% for F6 formulation.

From the observed values the flow type is passable in direct compression and wet granulation method and good for slugging method.

Hausner's ratio was found to be between 1.27 to 1.31 for F1 to F3 formulations, 1.28 and 1.27 for F4 and F5 formulations and 1.16 for F6 formulation. From the observed values the flow type is passable for direct compression method and fair for wet granulation method. In slugging method, the flow property was good.

Moisture content for the F1 to F6 formulations was found to be between 0.7 to 1.0 % the moisture content of all formulations were within the limit (1%).

5.3. EVALUATION OF PREDNISOLONE DISPERSIBLE TABLETS

Description: Yellow coloured round shaped tablets

Table No. 38: Evaluation of Prednisolone Dispersible Tablets

Parameters	F1	F2	F3	F4	F5	F6
Hardness [a] (kg/cm^2)	2.95±0.37	3.40±0.46	2.55±0.69	3.80±0.42	4.35±0.41	2.30±0.42
Thickness [a] (mm)	2.93±0.03	2.82±0.04	3.42±0.02	3.01±0.05	3.62±0.06	2.97±0.01
Weight variation [b] (mg)	131.5±8.40	133 ±9.50	151±12.50	150±1.09	170±1.05	130±0.50
Friability [a*] (%)	0.23±0.03	0.24±0.05	0.27±0.04	0.26±0.06	0.25±0.07	0.11±0.01
Disintegration time [c*] (sec.)	53.80±2.59	67.00±2.45	72.20±2.86	180.60±1.95	187.40±1.95	51.00±2.07
Wetting time[*] (sec.)	50.0±1.00	57.6±1.14	55.2±1.48	61.0±1.00	63.0±1.58	46.20±0.84
Water absorption ratio[*] (%)	54.70±0.25	49.93±0.77	50.77±0.77	51.04±0.06	50.10±0.04	52.99±0.08
***In-vitro* dispersion time[*] (min.)**	2.06±0.03	2.15±0.19	2.33±0.43	2.49±0.37	2.55±0.06	1.32±0.01
Uniformity of dispersion	Pass	Pass	Pass	Pass	Pass	Pass
Drug content [d] (%)	98.50	99.20	99.43	99.86	99.95	99.99

Note: a = 10; b = 20; c = 6; d = 15

*** Values are expressed as mean ± SD, n = 5**

Corollary:

F1 formulation having high weight variation due to the poor flow property of powder. So the quantity of glidant was increased in the F2 formulation. Then also the weight variation was high due to the poor flow property of powder.

So the weight of the tablet was increased up to 150 mg by increasing the excipients in F3 formulation. This formulation also having high weight variation because the flow of powder was poor.

In F4 formulation, wet granulation method was chosen to avoid the weight variation. In this method the flow property was found to be fair and the weight variation was within the limits. But the disintegration time and *in-vitro* dispersion time was found to be high.

That's why the quantity of excipients was increased from 150 mg to 170 mg in F5 formulation. Here also the same problem occur, disintegration time and *in-vitro* dispersion time was somewhat more.

Then slugging method was preferred for F6 formulation. The average weight of one tablet was reduced to 130 mg. The granules obtained are of uniform in size. The flow property of the granules was found to be good. Disintegration time and *in vitro* dispersion time was reduced when compared to F5 formulation.

With the above data's F6 formulation was selected as a best formulation. Because its passes the all parameters. So this F6 formulation may suitable as a dispersible tablet formulation for rectal suspension formulation.

5.4. IR SPECTRAL ANALYSIS

The FTIR studies of pure Prednisolone and F6 formulation were carried out to study the interaction between the drug and excipients. The results are shown in Table No. 39 & 40 and Figure: 8 & 9.

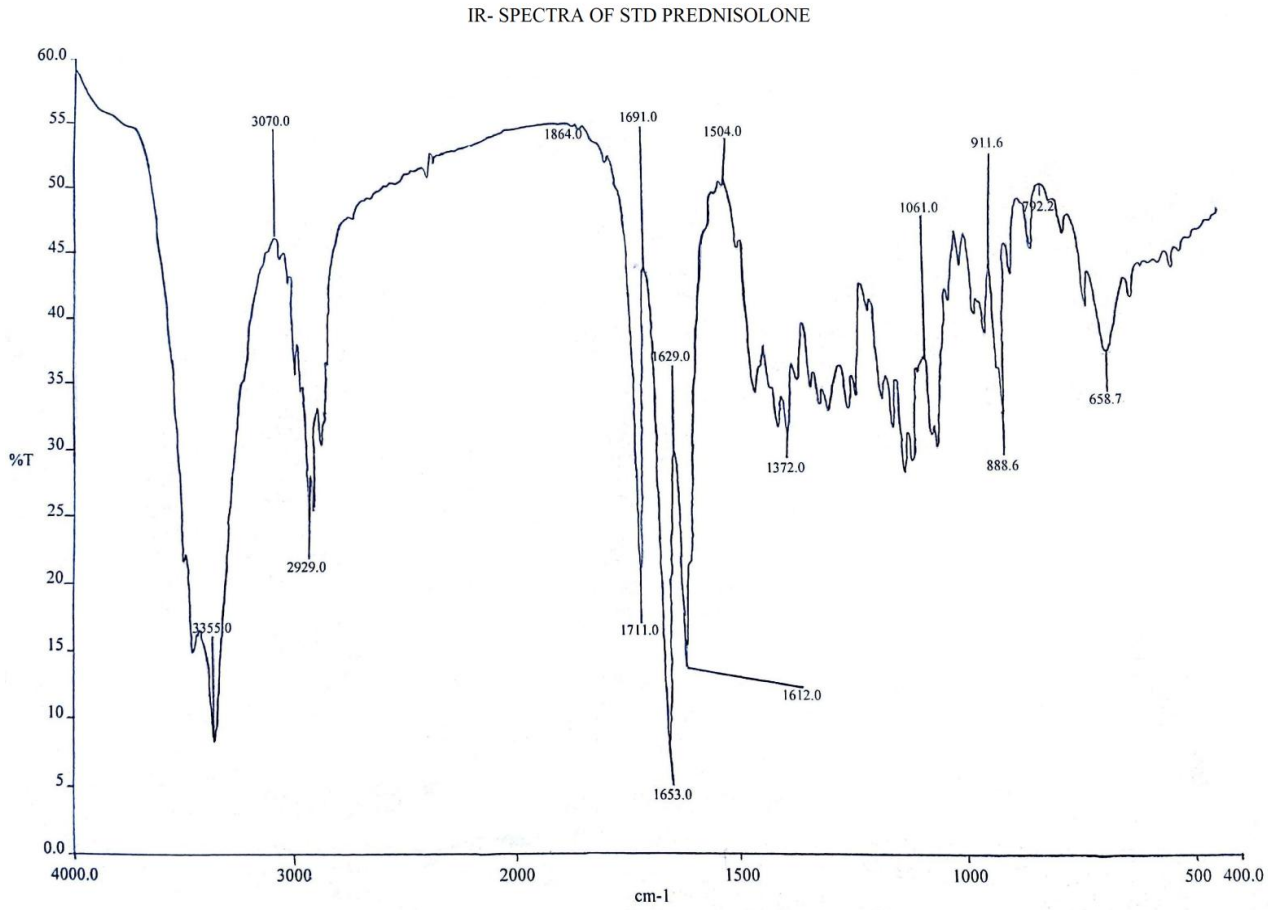


Figure 8: FT-IR Spectrum of Pure Prednisolone

Table No. 39: IR Spectrum of Prednisolone

S. No.	Wave number (cm^{-1})	Signal assignment
1	3355	OH stretching
2	2929	Alkane C-H stretching
3	1711	Acyclic stretching
4	1653	Aliphatic C-H stretching
5	1629	C=C stretching
6	1372	Alkane C-H bending
7	1061	C-O stretching

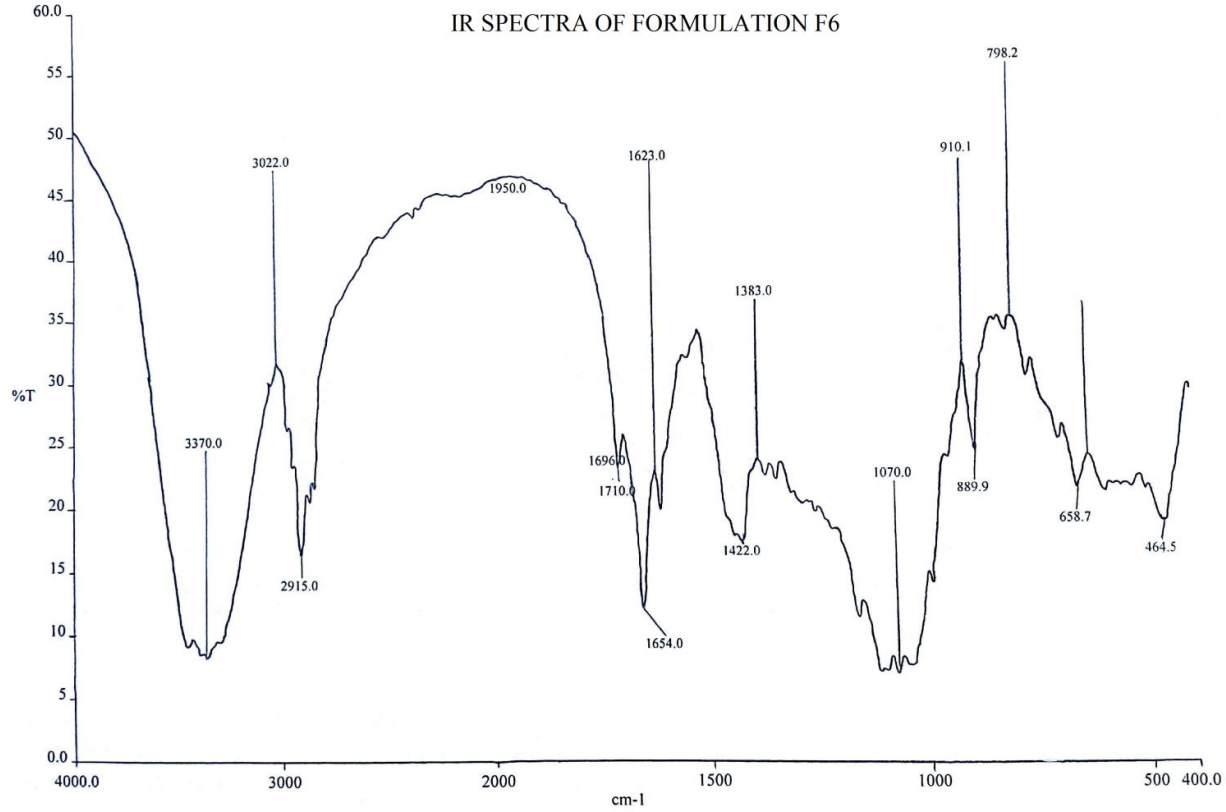


Figure 9: FT- IR Spectrum of F6 formulation

Table No. 40: IR Spectrum of F6 formulation

S. No.	Wave number (cm^{-1})	Signal assignment
1	3370	OH stretching
2	2915	Alkane C-H stretching
3	1710	Acyclic stretching
4	1654	Aliphatic C-H stretching
5	1623	C=C stretching
6	1383	Alkane C-H bending
7	1070	C-O stretching

Corollry:

From the IR spectral analysis, the result reveals that there was no interaction between the drug and excipients

5.5. EVALUATION STUDIES FOR RECTAL SUSPENSION

Table No. 41: Evaluation of F6 formulation in Rectal Suspension

S. No.	Parameter	F6
1	pH	6.2
2	Viscosity (cps)	55.9

Corollary:

From the above data the pH of F6 formulation was found to be 6.2 and the viscosity of F6 formulation was 55.9. High viscosity leads to high retention time.

IN-VITRO DRUG RELEASE STUDY

Table No. 42: *In-vitro* drug release of Prednisolone from F6 Formulation in Rectal Suspension*

S. No.	Time in minutes	% drug released
1	10	9.62 ± 0.09
2	20	21.54 ± 0.06
3	30	53.85 ± 0.03
4	40	78.17 ± 0.02
5	50	89.96 ± 0.02
6	60	99.07 ± 0.02

Note: * Values are expressed as mean ± SD, n = 5

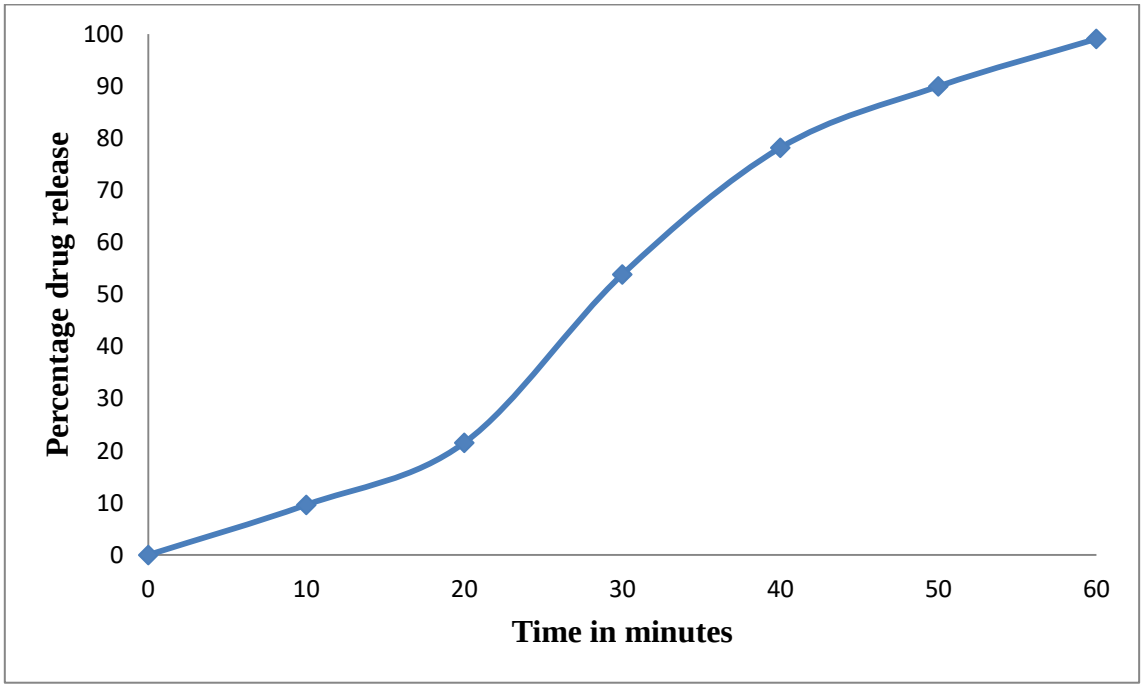


Figure 10: Graph for *in-vitro* drug release of F6 formulation in Rectal Suspension

Corollary:

The *in vitro* drug release of Prednisolone from F6 formulation in rectal suspension was found to be 99.07 ± 0.02 % at 60 min.

5.6. MICROBIOLOGICAL EVALUATION

The formulated suspension was evaluated microbiologically to determine the presence or absence of aerobic microbes, combined yeast and mould, *Escherichia Coli, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa.* The results were given in Table No. 43.

Table No. 43: Microbial Limit test for F6 formulation in Rectal Suspension

S. No.	Test/ specified microorganisms	Limits	Report
1	Total aerobic viable count	NMT 1000 cfu/ml	30 cfu/ml
2	Total combined yeast and mould count	NMT 100 cfu/ml	10 cfu/ml
3	*Escherichia coli*	Absent/ml	Absent
4	*Salmonella*	Absent/ml	Absent
5	*Pseudomonas aeruginosa*	Absent/ml	Absent
6	*Staphylococcus aureus*	Absent/ml	Absent

*cfu – Colony Forming Units

Corollary:

From the above data, the total aerobic viable count and total combined yeast count were observed that the cfu limits of formulated rectal suspension formulations were found to be within the limits. All the specified microorganisms were found to be absent. Thus the rectal suspension formulation was microbiologically stable.

5.7. ACCELERATED STABILITY STUDIES

Table No. 44: Stability studies for F6 formulation

Parameters	Initial period	4° ± 2° C			28° ± 2° C			45° ± 2° C		
		1st month	2nd month	3rd month	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Hardness[a] (kg/cm^2)	2.30±0.42	2.30±0.29	2.31±0.09	2.30±0.38	2.30 ± 0.31	2.32 ± 0.01	2.30 ± 0.45	2.31 ± 0.09	2.32 ± 0.09	2.30 ± 0.09
Thickness[a](mm)	2.97 ± 0.01	2.97 ± 0.04	2.96 ± 0.05	2.97 ± 0.10	2.97 ± 0.03	2.96 ± 0.28	2.96 ± 0.36	2.97 ± 0.05	2.96 ± 0.08	2.97 ± 0.02
Weight variation[b] (mg)	130.00 ± 0.50	129.00 ± 0.40	130.00 ± 0.10	131.00 ± 0.05	130.00 ± 0.60	130.00 ± 0.18	130.00 ± 0.10	129.00 ± 0.50	130.00 ± 0.20	131.00 ± 0.10
Friability[a*] (%)	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.11	0.11 ± 0.15	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.04	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.05
Disintegration time[c*] (sec)	51.00 ± 2.07	50.30 ± 1.20	49.80 ± 1.44	49.90 ± 1.12	50.10 ± 1.30	49.90 ±2.04	50.10 ±1.00	50.10 ±1.20	49.40 ± 3.44	49.90 ± 1.20
Wetting time[*] (sec.)	46.20 ± 0.84	46.30 ± 0.04	45.90 ± 0.13	46.10 ± 0.01	46.50 ± 0.04	44.80 ± 0.54	46.10 ± 0.61	46.50 ±0.14	45.80 ± 0.04	47.10 ± 0.01
Water absorption ratio[*] (%)	52.99 ± 0.08	51.99 ± 0.17	52.02 ± 0.05	52.05 ± 0.10	51.79 ± 0.07	51.54 ± 0.65	52.19 ± 0.10	51.99 ±0.07	52.54 ± 0.05	52.49 ± 0.10
***In-vitro* dispersion time[*] (min.)**	1.32 ± 0.01	1.31 ± 0.05	1.31 ± 0.03	1.31 ± 0.18	1.32 ± 0.10	1.32 ± 0.08	1.31 ± 0.23	1.32 ± 0.05	1.32 ± 0.03	1.31 ± 0.28
Uniformity of dispersion*	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Drug content[d] (%)	99.99	99.98	99.98	99.98	99.99	99.98	99.98	99.98	99.97	99.96

Note: a = 10; b = 20; c = 6; d = 15

*** Values are expressed as mean ± SD, n = 5**

Corollary:

According to the procedure the stability study of Prednisolone dispersible tablet was carried out at storage condition for $4^0 \pm 2^0$ C, $28^0 \pm 2^0$ C and $45^0 \pm 2^0$ C with a period of three months. The results revels that there was no change all physical parameters.

IN-VITRO DRUG RELEASE STUDY

Table No. 45: Stability study for *in-vitro* drug release of Prednisolone from F6 formulation in Rectal Suspension

S. No.	Time in minutes	Initial period	$4^\circ \pm 2^\circ$ C			$28^\circ \pm 2^\circ$ C			$45^\circ \pm 2^\circ$ C		
			1st month	2nd month	3rd month	1st month	2nd month	3rd month	1st month	2nd month	3rd month
1	10	9.63	9.62	9.61	9.59	9.64	9.62	9.60	9.65	9.60	9.58
2	20	21.56	21.25	21.49	21.58	22.15	21.78	21.54	22.05	21.69	21.48
3	30	53.87	54.05	53.78	53.84	54.06	54.25	53.94	54.15	53.95	53.74
4	40	78.19	78.44	78.38	78.15	78.74	78.54	78.32	78.94	78.68	78.25
5	50	89.95	88.81	89.23	89.99	88.78	89.73	89.92	88.9	89.65	89.78
6	60	99.06	99.06	99.06	99.05	99.06	99.05	99.05	99.05	99.05	99.04

Corollary:

The drug release of Prednisolone from the rectal suspension has no changes for a period of three months even storing at three different temperatures.

CHAPTER 6

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

SUMMARY

This work demonstrate that the formulation and evaluation of Prednisolone retention enema as the dispersible tablet with vehicle for the effective treatment of UC. It was carried out by performing the preformulation studies, formulation of Prednisolone dispersible tablet, formulation of vehicle, evaluation parameters, microbiology studies, *in-vitro* release studies and stability studies.

- ✓ For pure Prednisolone, preformulation studies such as angle of repose, bulk density, tapped density, compressibility index, Hausner's ratio, moisture content and particle size analysis were performed. The preformulation results revealed that the Prednisolone having poor flow properties, so it may requires glidants. The moisture content showed within 1% and the particle size was found to be fine powder.

- ✓ Drug – excipients incompatibility study was performed by physical observation. That there were no physical changes between drug and excipients. Thus it was concluded that the excipients selected for the formulation were compatible with Prednisolone.

- ✓ Prednisolone dispersible tablets were formulated by direct compression method, wet granulation method and slugging method using crospovidone as superdisintegrant.

- ✓ The formulated powder/granule blend was evaluated for precompression parameters like angle of repose, bulk density, tapped density, Hausner's ratio, compressibility index and moisture content. The results obtained indicate that F6 formulation formulated by slugging method has good flow property. The moisture content was within 1%.

- ✓ The formulated tablets were evaluated for hardness, thickness, weight variation, friability, disintegration time, wetting time, water absorption ratio, *in-vitro* dispersion time, uniformity of dispersion and drug content. All these parameters were found to be within the limits for F6 formulation.

- ✓ IR spectroscopic analysis was carried out to determine the compatibility of drug and excipients. The IR spectrum showed that the drug was compatible with excipients, which was used in the F6 formulation.

- ✓ From the data's obtained from precompression parameters and tablet evaluation F6 formulation was selected for further studies.

- ✓ The rectal suspension was prepared by using F6 formulation.

- ✓ pH and viscosity of the rectal suspension were carried out and the pH was found to be 6.2 and the viscosity was found to be 55.9 cps.

- ✓ *In-vitro* drug release of Prednisolone from F6 formulation in rectal suspension was 99.07 ± 0.02 % at 60 minutes.

- ✓ The microbiology studies for the rectal suspension with F6 formulation were carried out to determine the presence/absence of microorganisms in the formulation. The results showed that there was absence of microorganisms in F6 formulation in rectal suspension. So the formulation was microbiologically stable.

- ✓ The accelerated stability studies were performed for F6 formulation at three different temperatures such as $4^0 \pm 2^0$ C, $28^0 \pm 2^0$ C and $45^0 \pm 2^0$ C for a period of three months. In this storage condition for the three months period, there were no changes in all the tablets physical parameters.

- ✓ The accelerated stability studies of F6 formulation in rectal suspension (*in vitro* drug release) was also performed by stored in three different storage conditions for the period of three months. The results showed that there were no changes in percentage drug release.

CONCLUSION

Formulation and evaluation of Prednisolone retention enema as dispersible tablet with vehicle for the effective treatment of UC was successfully carried out.

Preformulation studies of powder/granules, formulation of Prednisolone dispersible tablets, formulation of vehicle, tablets evaluation parameters, *in-vitro* release study, microbiological evaluation and accelerated stability studies (three different temperatures) were performed. From all the above observations it was concluded that the formulation F6 by slugging method was better one compared to the other formulations.

Thus it can be concluded that the Prednisolone retention enema as dispersible tablet with vehicle possesses promising future delivery of rectal formulation of drugs in suspension form for the effective treatment of UC.

CHAPTER 7

FUTURE STUDY

7. FUTURE STUDY

Formulation F6 may be further investigated for following studies

- *In-vivo* study for drug release.
- Scale up techniques of the F6 formulation.
- The treatment of UC affected patients with the developed formulation.
- Stability study test for prolonged time period.
- Based on the reproducible results produced from batch to batch the company will decide to launch the product in future.

CHAPTER8

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8. BIBLIOGRAPHY

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