"FORMULATION, DEVELOPMENT AND OPTIMIZATION OF RECONSTITUTABLE ORAL SUSPENSION OF MYCOPHENOLATE MOFETIL"

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To Whom So Ever It May Concern

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During his tenure with us, he was found to be sincere and focused.

We wish him all the best for his career.

For, Intas Pharmaceuticals Ltd.,

Anuj Shah
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I hereby declare that the dissertation entitled "Formulation, Development and Optimization of Reconstitutable Oral Suspension of Mycophenolate Mofetil", is based on the original work carried out by me under the guidance **Mr. Manish Chauhan,** *Senior Group Leader, Formulation Development Department, Astron Division - Intas Pharmaceuticals Limited, and* **Dr. Renuka Mishra,** *Assistant Professor Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.*

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LIST OFABBREVIATIONS

Abbreviation	Full Form
ROS	Reconstitutable Oral Suspension
MHC	Major Histocompatibility study
HLA	Human Leukocyte Antigens
MMF	Mycophenolate Mofetil
MPA	Mycophenolic Acid
IMPDH	Ionosine Monophosphate Dehydrogenase
AUC	Area Under Curve
MPAG	Phenolic Glucuronide of Mycophenolic Acid
FTIR	Fourier Transforms Infrared Spectrophotometer
UV	Ultra Violate
µl	Micro Liter
CoA	Certificate of Analysis
API	Active Pharmaceutical Ingredient
LOD	Loss On Drying
NLT	Not Less Than
NMT	Not More Than
RH	Relative Humidity
ND	Not Detected
RS	Relative Substance
BQL	Below Quantified Limit
RSD	Relative Standard Deviation
% w/w	% weight by weight
DoE	Design of Experiment

Formulation, Development and Optimization of Reconstitutable Oral Suspension of Mycophenolate Mofetil

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ABSTRACT:

Mycophenolate Mofetil (MMF) is an immunomodulator drug used in prevention of rejection of organ during organ transplant. MMF requires daily dose of 3.5 or 4.0 g/day depending upon the patient and disease state being treated. Thus with conventional dosage form having dose of 200 mg in capsule and 500 mg in tablet and patient will require twelve units and six units respectively each day. As an alternative dosage form, respectively reconstitutable oral suspension (200 mg/ml) will provide ease of administration and convenience. Formulation of reconstitutable oral suspension was carried out by direct blending approach and wet granulation (using FBP) approach. Amongst them wet granulation (using FBP) was carried out for further study. Sorbitol (Neosorb P100T), citric acid (anhydrous), soybean lecithin, trisodium citrate, xanthan gum, methyl paraben, aspartame, mixed fruit flavor and colloidal silicon dioxide were used for formulation of reconstitutable oral suspension. During study, suspension was evaluated for various parameters like viscosity (600 -700 cps), pH (6 -7), deliverable volume (172 -178ml), % drug release in 20 min (81.5 -84.5%) and % assay of reconstituted suspension (98 -102%). Stability study of dry powder (40ºC/75%RH) and reconstituted suspension (30ºC/65%RH and 2-8ºC) was carried out for reproducible batch for one month and it was within preferred criteria. F2 value (88.53) indicated that reproducible batch was similar in terms of % drug release profile with compare to innovator product CellCept® (200mg/ml) oral suspension. Design space was identified using design expert software (Version 9.0) for selection of concentration of xanthan gum and soybean lecithin using 2^2 factorial designs with 3 center points.

Chapter - 1

Aim of Investigation

AIM OF INVESTIGATION

Immunosuppressant drugs decrease the ability of the body to reject a transplanted organ, such as a liver, heart or kidney. Immunosuppressant drugs are mostly used to prevent the rejection of transplanted organ. These drugs are also used to the cure or treat certain autoimmune diseases such as Rheumatoid arthritis and Lupus. Most of the immunosuppressant drugs are able to regulate or suppress the immune system by interfering in synthesis of DNA. Mycophenolate Mofetil is a class of immunosuppressant drug which is hydrolyzed and converted to active metabolite Mycophenolic acid (MPA). It is a selective, uncompetitive, potent, reversible inhibitor of Inosine Monophosphate Dehydrogenase (IMPDH) and inhibits de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Mycophenolate Mofetil requires daily dose of 3.5 or 4.0 gm/day depending upon the patient and the disease state being treated. Thus with conventional dosage form having 200 mg capsule and 500 mg tablet dose, the patient will require twelve units and six units respectively each day. As an alternative, oral suspension (200 mg/ml) will provide ease of administration and convenience. The objective of the present study is formulation of Reconstitutable Oral Suspension of Mycophenolate Mofetil which provides better patient compliance and convenience. The developed formulation will be compared with innovator product CellCept® 200 mg/ml oral suspension manufactured by Roche Laboratories, Inc., USA. Since, the % of drug in Reconstituted Oral Suspension is very high and drug being immune-suppressant in nature wet granulation approach will not be preferred. Instead direct blending approach or Fluid Bed Processor (FBP) will be selected for the study.

Compatibility studies of Mycophenolate Mofetil with different commonly used excipients will be carried out by accelerated thermal stress. Assay and Relative Substance will be evaluated. The Reconstituted Oral Suspension will be evaluated by following parameters namely pH (6-7), viscosity (600 – 700 cps), deliverable volume (172 – 178 ml), assay (98 – 102%w/w), sedimentation volume, redispersibility and in vitro drug release study (81.5 – 84.5%). Stability study of optimized batch of Reconstituted suspension will be carried out for 2 month at 30°C / 65% RH and 2-8ºC.

Chapter - 2

Introduction

2.1 INTRODUCTION TO PHARMACEUTICAL SUSPENSION [1 2]

A Suspension is a particular type of dispersed system or in which suspended phase is dispersed equally into the external phase. The internal phase is consisting homogenous solid compound having a particular range of particle size, is maintained equally in the suspending vehicle with using suspending agent. A suspension containing particles range 1 nm - 0.5 μm size is called colloidal suspension. When the particle size is between 1 - 100 μm is known as coarse suspension. Most of the pharmaceutical suspensions are coarse suspension

2.1.1 Pharmaceutical applications of suspensions:

Poorly soluble drugs are mostly preferred in oral liquid dosage forms particular for geriatric, pediatric and patients having difficulty in swallowing solids dosage form.

Rationale for Suspension

1. To reduce the instability of certain drugs in aqueous solution.
2. To mask unacceptable taste of drug, e.g. Paracetamol
3. Suspension can be used topical application: In case of calamine lotion evaporation of dispersing media will leave the active agent by light deposit.
4. It can be used for parenteral administration by intramuscular route. It controls rate of absorption of drug.

2.1.2 Characteristics of Ideal Suspension:

1. The dispersed particles of must not be settle down readily and the settled particle should redisperse easily on shaking. Since one cannot absolutely avoid the sedimentation, it is ideal that the particles should settle slowly.
2. The dispersed particle should not form cake upon settling.
3. The viscosity of the formulated suspension is easily pourable.
4. Formulated suspension should be chemically and physically stable.
5. It should have pleasant taste when administration through oral route.

2.2 RECONSTITUTABLE ORAL SUSPENSION [3]

Although conventional oral suspension can be administration without delay, there does an additional class of suspension exist called as dry powders or Reconstituted Oral Suspension (ROS) and it is reconstituted at the time of administration. ROS is preferred when drug stability is a major concern. After reconstitution, these suspensions have a short but satisfactory shelf life when stored at refrigerated temperature and must be used within 7 to 10 days except some ROS e.g. Mycophenolate Mofetil 200 mg/ml oral suspension which can be upto for 2 month after reconstitution.

2.2.1 Advantages of Reconstitutable Oral Suspension

1. The most common reason of formulation of ROS is inadequate chemical stability and physical stability of the drug in aqueous vehicles, as per chemical stability, conventional suspension has very short shelf life where as ROS has shelf life of at least 2 year in terms of physical stability like drug solubility is increased due to pH changes from chemical degradation, viscosity changes, incompatibility of excipients, conversion of polymorphic form caking and crystal growth.

2. Formulation of ROS reduces transportation expense as an aqueous vehicle is not present. It is least susceptible to temperature extremes as compared with conventional suspension.

2.2.2 Required Characteristic of Reconstitutable Oral Suspension

1. Satisfactory properties of ROS must be maintained before, during, and after reconstitution.
2. At the time of development, the dry blend or mixture should not get segregated.
3. At time of reconstitution the powder blend should disperse easily and homogeneous in the aqueous vehicle.
4. The reconstituted suspension must be easily redispersed and easy to pour which provide accurate & homogeneous dose.

5. The finished product must have an acceptable oraganoleptic property such as color, odor and taste.

2.2.3 Commonly used excipients:

The number of excipients was less compared to with conventional suspensions. The criteria for selecting excipients are based on the physical type of powder mixture preferred and suitability for reconstitution. Excipients should be kept at least number, because as more excipients are used, there are chances of problems like -

- The problem of compatibility with API or other excipients is increased.
- More processing is required for addition more excipients.
- More excipients will require more sampling and testing for quality control.

A general method of reducing the number of excipients is to use an excipient that performs more than one role, e.g. sucrose, sorbitol acts - as a sweetener and solid diluent.

Table 2.1 Commonly used excipients in ROS

Frequently Used	Examples
Suspending Agent	Sodium Alginate , Methylcellulose , HydroxyEthyl Cellulose , HydroxyPropyl Cellulose , Xanthan Gum , Acacia , Tragacanth.
Wetting Agent	Soybean Lecithin, Polysorbate 80, Sodium Lauryl Sulphate
Sweetener	Sucrose , Aspartame
Preservatives	Sorbic acid, Methyl Paraben , Propyl Paraben
Flavor	Cherry flavor, Vanilla flavor, Banana flavor, Mix Fruit Flavor
Buffer	Trisodium citrate dihydrate, Citric acid anhydrous
Color	FD&C Red No.3, FD&C Red No. 40, D&C Yellow No. 10
Anticaking Agent	Colloidal Silicon dioxide, Amorphous silica gel

2.2.3.1 Suspending agent:

Suspending agents are used as viscosity enhancer and hinder sedimentation rate. Suspending agent should be simply dispersed by vigorous hand shaking during reconstitution. Table 2.2 lists suspending agents suggested for utilize in formulation of ROS. Xanthan gum is a general suspending agent in suspension, because it is provides good batch-to-batch uniformity, few microbial problems and solution viscosity is practically independent of pH and temperature.

Table 2.2 List of suspending agent used in ROS

Suspending agents	Stability pH range	Concentrations
Sodium alginate	4-10	1–5 %
Methylcellulose	3-11	1–2 %
Hydroxypropyl cellulose	6-8	1-2%
Hydroxypropyl methylcellulose	3-11	1-2%
Carboxy methyl Cellulose	7-9	1-2%
Colloidal silicon dioxide	0-7.5	2-4 %

Other Suspending agents used include Acacia, Tragacanth , Sodium CMC , Xanthan gum.

2.2.3.2 Wetting agents

The wettbility depends on the affinity of drug for water. Many drugs in suspension are hydrophobic; they resist water and are not easily wetted and often float on the surface of water due to entrapped air. Wetting agent is generally used to aid in the dispersion of hydrophobic drugs. In formulation, selection of wetting agent is based on low concentration and maximum outcome of optimal dispersion. Wetting agent is used as surfactant to retard crystal growth in range of 0.05%w/w to 0.5 % w/w. Excess wetting can produces foaming and gives unpleasant taste. Another concern with wetting agents is increased risk of caking because coated particles oppose the aggregate formation, settle individually and may form dense or caked sediment.

Examples: Polysorbate 80, Soybean lecithin, Glycerin , Propylene glycol , Sodium Lauryl Sulphate.

2.2.3.3 Sweetener

Sweetener has significant role in reconstitute oral suspension. It is used for taste masking of drug. It can be concluded into 3 main groups -

(A) Bulk sweeteners: Sugars like xylose, glucose, dextrose and sucrose are used at concentration of 15% w/w - 70 % w/w of the total weight of the suspension. Sucrose is used as sweetener, suspending agent and bulking agent in the dry mixture. Combination of sweetening agent can also be used. Taste-masking composition consists of any one sweetening agent and one flavoring agent. The concentration of artificial sweetening agents is between 0 to 0.05 gm/ml.

(**B) Sugar alcohols:** Xylitol, Sorbitol, Mannitol and Glycerin

(C) Artificial sweetening agents: Sodium saccharin, Aspartame

2.2.3.4 Preservatives:

Chemical stability of excipient, safety and acceptability of the product is affected by microbial growth, so addition of preservatives is must required in most of the suspension and also in suspending agent and sweeteners added in formulation is which subject to microbial contamination. Microbial activity may cause of stability problem when suspension was not properly stored or not sufficient concentration of preservatives are added.

Table 2.3 List of preservatives used in ROS

Preservatives	Concentration
Benzalkonium chloride	0.01-0.02 %
Sodium benzoate	0.02-0.5 %
Sorbic acid	0.05-0.2 %
Methyl paraben	0.015-0.2 %

2.2.3.5 Flavoring agent

They are used to provide organoleptic preparation to the patient. They play important role in pediatric formulation. Both natural and artificial flavors are used including raspberry, pineapple, cherry, Banana & Mix Fruit flavor.

2.2.3.6 Buffering Agent

They are use to maintain pH and provide stability of the suspension. Buffers are used to keep the drug in insoluble form by the maintaining pH. Application of buffer is mention below.

- By change in pH, it prevent decomposition of active pharmaceutical excipients.
- Maintained the Physiological stability

Examples: Sodium Citrate, Citric acid

2.2.3.7 Coloring Agent

Coloring agents are use to keep for provide appealing visual appearance to the final product. As relatively large cations or anions, these agents may be chemically incompatible with other excipients. For example, FD&C Red No.3, FD&C Red No. 40 and FD &C Yellow No.6.

2.2.3.8 Anti - caking Agent

Anti-caking agents such as colloidal silicon dioxide, have essential function in ROS. As a desiccant, these agents avoid moisture from the dry mixture to facilitate good powder flow and prevent the caking. In addition, anti-caking agents divide the dry particles to inhibit fusion. They also give thermal insulation and insulate static charge conditions and are chemically inert.

2.2.4 Preparation of Dry Mixture

2.2.4.1 Powder Blend

Powder blend referred as powder mixture, is prepared by mixing the excipient in powder form. When excipients are in small quantities then blending of powder may be into two or more stage. Such excipient can be mixed uniformly with part of major excipient to aid their dispersion. Second stage involves mixing of other excipients.

Advantages

- Less Cost
- Easy to clean
- These is less chance of physical and chemical stability problem because no use of heat and solvent for preparation.
- Low moisture content can be achieved.

Disadvantages:

- It is chance for homogeneity problem.
- Poor flow can be the reason for demixing.
- The material lost throughout powder blending will have even greater importance if API is potent drug.

2.2.4.2 Granulated Product:

In the granulated products, most of all excipients are processed by granulation. Wet granulation is the common process for formulation of ROS. Granulating fluid is aqueous or non aqueous binder solution. There are two approach of incorporating the drug (1) Excipients and drug can be mix with other. (2) It can be dissolved in granulating fluid.

Wet granulation generally consists of the following steps.

The solid excipients are blended and massed with the granulating fluid. The wet mass is converted into dried granules and these granules are milled using vibratory sieve or oscillating granulator. For drugs susceptible of hydrolysis, non aqueous granulating fluids can be used.

.

The granulated product has some advantages over the powder product. These advantages are superior appearance; it enhanced flow property and reduces segregation problems.

Disadvantages:

- More capital requirement and energy.
- Difficult to remove traces of granulating fluid from interior of granules.
- The residual fluid may be produce instability.
- All other exicipient should be stable in granulating fluid.

2.2.4.3 Combination Product:

Granulated and Powdered excipients can be combining to resolve some disadvantages of granulated products. Less energy and equipment for granulation may be required if the majority of the diluents can be added after granulation. Heat sensitive excipients such as flavor should be added after drying of granulation.

The solid excipients are blended and massed with the granulating fluid. The wet mass is converted into dried granules and these granules are milled using vibratory sieve or oscillating granulator. Dried granules and other excipients are then blended. Usually API and other excipients of a fine particle size are granulated with or without a portion of the diluents. The presence of the diluents improves flow and reduces segregation and reduces dust formation.

Disadvantages:

- Enhanced possibility of non uniformity.
- To get the necessary degree of homogeneity, the particle sizes should be controlled.

2.2.4.4 Suggestion for processing the dry mixture:

- Use efficient mixer. Evaluate processing performance of batches on pilot scale up equipment.
- Determine duration of blending timing.
- Keep away from heat and moisture during mixing and finished batch should also be protected from moisture.
- For blend uniformity, sample should be taken from various place of the blender.

Poor flow ability or caking often occurs when individual particles fuse together. There are several reported causes, which include: Poor high temperature stability, Surface charges, Variation of relative humidity and crystallization.

2.2.5 Stability:

Chemical stability is frequently concern in reconstituted suspension than in conventional suspension because drug has poor stability in the presence of water. After reconstitution of dry powder separation may be observed. Physical stability of the suspension and also reconstitution is still a concern.

2.2.5.1 Chemical Stability

Chemical Stability should be evaluated for both dry mixture and reconstituted suspension at controlled temperature, room temperature and storage temperature. Stability evaluation of reconstituted oral suspension should be conducted in a container of the same material and size in which the product is marketed.

Degradation of the preservative is acceptable as long as satisfactory preservative is present to continue effectiveness. Testing at elevated temperatures causes large changes in physical property of viscosity; Higher Temperature can considerably change the solubility of the suspended drug.

2.2.5.2 Physical stability

Physical stability includes evaluation of pH, viscosity, sedimentation volume and ease of redispersion. Sedimentation volume is obtained by measuring the height of settled drug particles in undisturbed bottles at intervals of time. Sedimentation volume is the indication of the good suspendibility. Exposure to temperature cycle during which the suspension is frozen and thawed is another common method of evaluating physical stability method. After number of cycle, parameters such as distribution of particle size, crystal changes, viscosity and sedimentation volume can be measured. Crystallization can also be reported as problem.

2.2.6 Marketed Preparation of ROS

Table 2.4 Marketed Preparation of ROS

Marketed Product	Drug	Strength	Company
Penbritin Syrup 125 mg/5 ml and Penbritin Forte Syrup 250 mg/5 ml	Ampicillin	125, 250mg/ 5 mL	Chemidex Pharma
Co-amoxiclav 400/57mg/5ml	Amoxicillin trihydrate , amoxicillin. Potassium clavulanate	400/57 mg/5 ml	Sandoz
Suprax	Cefixim	100mg/5ml	Sanofi
Zithromax	Azithromycin	300/600/900/1200 mg/ml	Pfizer

2.3 OVERVIEW OF IMMUNOLOGY OF REJECTION [4]

Immunosuppression is reduction in efficacy of the immune system. Some portions of the immune system itself have immunosuppressive effects on other parts of the immune system

The most important functions, of the immune system are supervision for interfering micro-organisms that are potentially dangerous and must be neutralized. To achieve goal, the immune system has developed ways by which cells identify themselves as either self or foreign by examining its surface antigenic "fingerprint". These surface proteins recognized as antigens are also found on the surface of leukocytes and are referred to as Human Leukocyte Antigens (HLA). Expression of HLA antigens on leukocytes from a blood sample indirectly determines the type of antigens expressed on other tissues in the body, "HLA typing" is normally performed before organ transplants with a view to find a donor organ having close match to the recipients HLA proteins are responsible number of important roles in functioning of immune function. It includes antigen presenting proteins and components of the complement system.

When foreign antigens are recognized by the immune system, a series of events involving T cells and B cells occurs which results in tissue inflammation and death of the foreign cells, as summarized in Figure 2.1. Interleukin 2 (IL-2) plays a critical role in the activation of T cells. However, many other cytokines (e.g. IFN- γ, TNF-β, IL-4 & IL-5) are involved in later steps in the pathways resulting in activation of other cells (macrophages and B cells).

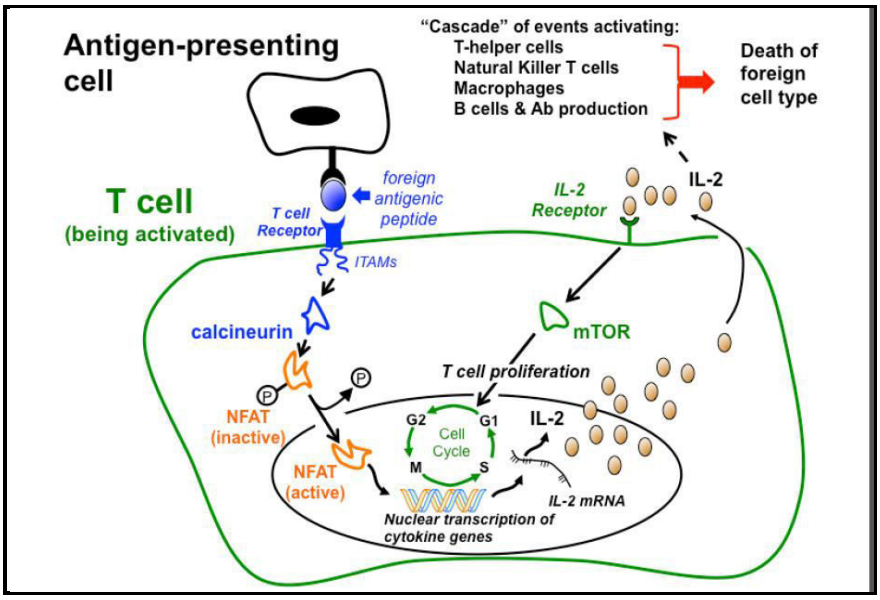


Figure 2.1 An outline of the essential events involved in activation of T cells when foreign antigen exists.

An outline of the essential events involves activation of T cells in response to a foreign antigen existing by antigen-presenting cells (which including dendritic cells, macrophages & B lymphocytes). This induces "Adaptive immune responses" related of multiple cell types that are concerned in cell-mediated immunity involving activated macrophages, natural killer T cells and cytotoxic T cells, as well as stimulation of humoral immunity involving B cells & plasma cells. This process will create antibodies binding to foreign antigens leading to phagocytosis and cellular toxicity of the foreign cells.

2.4 IMMUNOSUPPRESSANT DRUGS

2.4.1 Introduction:[4 5 6]

Immunosuppressant drugs are mainly used in patient's receiving organ transplants for preventing rejection of the transplanted organ. Immunosuppressant drugs are used to treat disease such as Rheumatoid arthritis, Psoriasis, Crohn's disease, etc. Most of the immunosuppressant's drugs are act through control the immune system by interfering in synthesis of DNA.

Role of Immunosuppressant Drug

Most of the patient who takes a treatment for an organ transplant requires administration of immunosuppressant drug. The body recognizes a transplanted organ as a foreign mass. By reducing the immune system, immunosuppressant drugs decrease the body's reaction to the foreign organ. Immunosuppressant drugs are playing a significant function in decreasing the risk of refusal, protecting the new transplanted organ and preserving vital function of the organ. Wide group of drugs are existing to reach the goal but mechanisms of action of all drugs are dissimilar for reducing the risk of rejection. When an organ is such transplanted from donor into recipient, the immune system of the recipient triggers the same response in opposition to the new organ it would have to any foreign material, leading to a chain of events that may damage the transplanted organ. It can be called rejection acute rejection occurs rapidly, where as in chronic rejection, it may take long time. Rejection of transplanted organ can occur even when there is close match of organ donated with the patient receiving transplant.

2.4.1 History of Immunosuppressant drug [4 7 8]

The first immunosuppressant, Azathioprine was made available in 1962. There after introduction of cyclosporine in 1983, gave extensively better outcome for non renal organs for example liver, heart, lung, and pancreas transplants.

The current therapy of Immunosuppression takes into account the organ individual transplanted (which have dissimilar pharmacologic requirements), characteristics of the recipient (e.g. whether or not they are presensitized or whether or not they have received a blood compatible organ) and different immunosuppressant which produce synergy while minimizing harmful side effects.

2.4.2 Description [9]

Immunosuppressant drugs are classified according to their mechanism of action. Three widely used immunosuppressant drugs currently in organ transplantations are as follow.

- Cyclosporins act by inhibiting T-cell activation and consequently preventing T-cells from attacking the transplanted organ.
- Azathioprines act by disruption of synthesis of DNA, RNA and cell division.
- Corticosteroids such as Prednisolone act by suppressing the inflammation related with rejection of transplant.

Mostly combination of drugs is prescribed after transplantation, one from each of the above mentioned groups; for example Azathioprine, Prednisolone and Cyclosporine. As the risk of rejection decrease, number of doses and the number of drugs taken may also be reduced However, most patients will require at least one immunosuppressive for the rest of their lives.

Immunosuppressant drugs can also be classified based on the particular transplant:

- Basiliximab and Daclizumab are used in combination with other drugs like Cyclosporin and Corticosteroids, in kidney transplants.

- Muromonab CD3 is used along with Cyclosporin in case of heart, kidney and liver transplant.
- Tacrolimus is used in case of the liver transplantation.
- Azathioprine is used in Rheumatoid arthritis as well as in kidney transplantation.
- Cyclosporin is used in heart, liver, kidney, pancreas and bone marrow transplantation.
- Mycopehnolate (CellCept) is used in combination with Cyclosporin in kidney, liver and heart transplants. It has also been used to prevent the kidney problems associated with lupus erythematosus.
- Sirolimus is used in combination with Cyclosporine and Corticosteroids in kidney transplantation.

2.4.3 Recommended dosage [7]

Immunosuppressant drugs are dispensed only with a physician's prescription. They are available in the form of tablet, capsule, liquid and parentral dosage form. The selection of dosage form mainly depends on the type and purpose of usage. A dose of the drug varies from patients to patient. Strict adhesion to the treatment is critical. The physician can only decide how much dose of drug will be required for treatment of each patient.

2.4.4 Different Phases of Immunosuppressive Therapy [4 10]

The level of Immunosuppression therapy is initially high to decrease the immune response to the allograft, but is gradually decreased over time as the risk of acute rejection decreases. Chronic over-Immunosuppression greatly increases the risk of opportunistic infection and malignancy, as well as other side effects that can develop with long term use. Immunosuppressive therapy protocols are commonly divided into two phases.

(A) Induction: T cells are the primary mediators of rejection. A short term therapy with a potent immunosuppressant drug is recommended to reduce the immune response of T cells. Induction agents cause depletion and disruption of T cell activation and proliferation. Because the risk of infections and malignancy correlates with the amount of Immunosuppression drugs, dose is decreased gradually to maintain required level up to 6 - 12 months post transplantation.

(B) Maintenance: Therapy with combination of low doses drugs with non-overlapping toxicities is used to avoid rejection of the allograft. The effectiveness and protection of different agents used for chronic therapy is regularly evaluated and when newer immunosuppressive agents enter to clinical market.

2.4.5 Three General Principles of Immunosuppressive therapy [11]

(A) Immune response of graft rejection are highest at the initial stage and decrease over time: Higher dose of Immunosuppressant drugs are proposed to give the highest concentration of Immunosuppression instantly after surgery followed by gradual decrease after several months of the heart, renal and lung transplants.

(B) Use low doses of several drugs. Combined regimens are more effective in treatment in organ transplant so keeping away use of higher (more toxic) doses of fewer drugs.

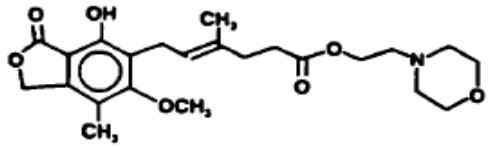
(C) Avoid over-Immunosuppression which increases weakness to infection and malignancy. Slowly tapering of drug regimens is required to decrease the possibility of infection and malignancy.

2.5 DRUG PROFILE: MYCOPHENOLATE MOFETIL [6 12 13 14 15]

Table 2.5 Pharmacopoeia status of Mycophenolate Mofetil

Monograph	IP	BP	USP	EP
Mycophenolate Mofetil	Yes	Yes	Yes	Yes

(A) Structure:



(B) IUPAC name: 12-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexonate

(C) Molecular formula: $C_{23}H_{31}NO_7$

(D) Molecular weight: 433.50 g/mol

(E) Description: A White or almost white crystalline powder.

(F) Category: Immunosuppressive Agent

(G) pKa: pKa1 = 5.6 (tertiary amine); pKa2 = 8.5 (phenol)

(H) Log p: 2.5

(I) Solubility: It is slightly soluble in water (43 µg/mL at pH 7.4); the solubility increases in acidic medium.

(J) Melting point: 93^0C to 96^0C

(K) BCS class: Class II (Low Solubility, High permeability)

(L) Indications: For the prophylaxis of organ rejection in patients receiving allogeneic renal, cardiac or hepatic transplants. Mycophenolate Mofetil (MMF) should be used concomitantly with cyclosporine and corticosteroids.

(M) Mechanism of action: Mycophenolate Mofetil (MMF) is hydrolyzed and transformed into Mycophenolic acid (MPA), which is active metabolite. MPA is a selective, uncompetitive, potent and reversible inhibitor of Inosine Monophosphate Dehydrogenase (IMPDH), and it inhibits de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. MPA also suppress antibody formation by B-lymphocytes. MPA prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Mycophenolate mofetil does blocks the coupling of these events to DNA synthesis and proliferation.

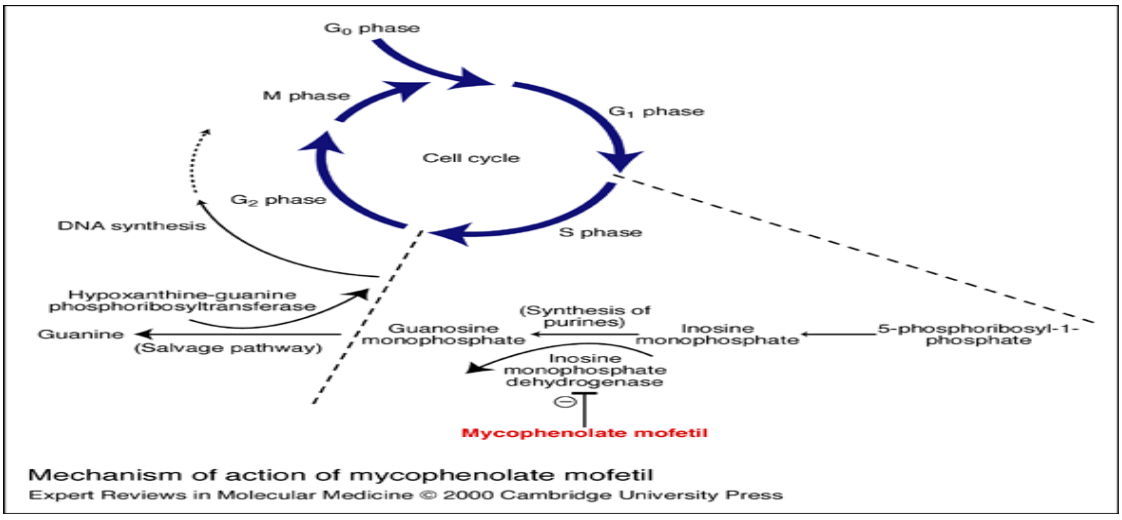


Figure 2.2 Mechanism of action of Mycophenolate Mofetil

(N) Pharmacokinetics:

Absorption: MMF undergoes rapid and extensive absorption and complete presystemic metabolism to the active metabolite Mycophenolic acid (MPA) after oral administration. The bioavailability of oral MMF is 94% relative to intravenous MMF. Food has no effect on the extent of absorption of MMF when administered at doses of 1.5g bid to renal transplant patients. However, MPA C_{max} is decreased by 40% in the presence of food.

Distribution: MMF is not quantifiable systemically in plasma following oral administration. MPA at clinically associated concentrations is 97% bound to plasma albumin.

Metabolism: MMF undergoes absolute metabolism to MPA and MPA is metabolized mainly by glucuronyl transferase to form the phenolic glucuronide of MPA (MPAG), which is not Pharmacology active.

Excretion: Orally administered radiolabelled MMF results in complete 93% of the administered dose recovered in the urine and 6% recovered in the feces. Most (about 87%) of the administered dose is excreted in the urine as MPAG. At clinically encountered concentrations, MPA and MPAG are not removed by haemodialysis. However, at high MPAG plasma concentrations (> 100µg/mL), small amounts of MPAG are removed.

Table 2.6 Pharmacokinetic parameter of Mycophenolate Mofetil

Pharmacokinetic parameter	Reported values
Tmax	Within an hour
Absolute bioavailability	~ 94 %
Hepatic first pass effect	Extensive
Food effect	Decreases C_{max} by 40 %,
Protein binding	Extensively protein bound (> 97 %)
Metabolites	One metabolite- MPA is active.
Route of elimination	Mainly through renal.
Elimination half life of active metabolite	~18 hours

(O) Toxicity: Adverse reaction includes diarrhea, leucopenia, sepsis, vomiting, and there is evidence of a higher frequency of certain types of infections.

(P) Adverse reactions: Depression, dizziness, tiredness, bradycardia, congestive heart failure, palpitations, peripheral, edema.

2.6 EXCIPIENT PROFILE [16]

2.6.1 Sorbitol

(A) Nonproprietary names: BP: Sorbitol

PhEur: Sorbitol

USP-NF: Sorbitol

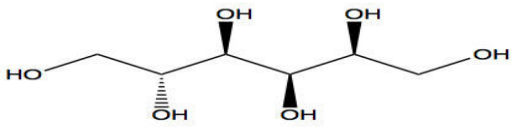
(B) Synonyms: Meritol; Neosorb; Sorbitab; sorbite; Dsorbitol; sorbitolum;

(C) Chemical name: D-Glucitol

(D) Empirical formula: $C_6H_{14}O_6$

(E) Molecular weight: 182.17 g/mol

(F) Structural formula:



(G) Functional category: Plasticizer, Stabilizing agent, Sweetening agent, Tablet and capsule diluent.

(I) Applications in pharmaceutical formulation: Sorbitol is used as diluent in formulation of tablet, capsule and dry suspension. In liquid preparations, sorbitol is used as a vehicle in sugar-free formulations and as a stabilizer in suspension. In formulation of syrups, sorbitol prevents crystallization in the region of the cap of bottles.

Table 2.7 Various grade of sorbitol

Grade	Mean particle size (mm)
Neosorb P100T	140
Neosorb P60 220	220
Neosorb P20/60 650	650
Neosorb P30/60 480	480
Neosorb P60W 260	260
Sorbitab SD 250 250	250
Sorbitab SD 500 500	500

Table 2.8 Application of sorbitol

Use	Concentration (%w/w)
IM injections	10–25
Oral solutions	20–35
Moisture control agent in tablets	3–10
Oral suspensions	70
Tablet binder and filler	25–90
Toothpastes	20–60

(J) Description: Sorbitol occur as white or nearly colorless, odorless, hygroscopic powder, crystalline. Sorbitol exists in grades and polymorphic form. They tend to cake less than the powdered form and have more attractive compression characteristics. Sorbitol has a sweet taste, pleasant cooling, and has approximately 50–60% of the sweetness of sucrose**.**

(K) Solubility: Sorbitol is soluble in water and slightly soluble in methanol. Practically it is insoluble in the chloroform and ether.

2.6.2 Citric Acid Anhydrous

(A) Nonproprietary names: BP, EP, Ph.Eur.: Citric Acid Anhydrous

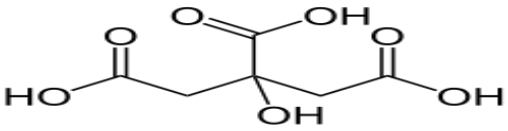
(B) Synonyms : Avicel PH, Celex, cellulose gel, Celphere, Ceolus KG, crystalline cellulose*,* Fibrocel*, Pharmacel,* Tabulose*, Vivapur.*

(C) Chemical name: 2-Hydroxy-1, 2, 3-propanetricarboxylic acid

(D) Empirical formula: $C_6H_8O_7$

(E) Molecular weight: 192.14 g/mol

(F) Structural formula:



(G) Functional Category: Acidifying agent, Antioxidant, Buffering agent, Chelating agent, Flavor enhancer, Preservative

(H) Applications in pharmaceutical formulation: Citric acid (as either the monohydrate or anhydrous material) is used to adjust the pH of solution. It has also been used to adjust the pH of tablet matrices in enteric-coated formulations for colon-specific drug delivery.

Table 2.9 Application of citric acid anhydrous

Use	Concentration (% w/w)
Buffer solution	0.1–2.0
Flavor enhancer for liquid formulations	0.3–2.0
Sequestering agent	0.3–2.0

(I) Description: Citric acid monohydrate occurs as colorless, or as a white crystalline, efflorescent powder. It is odorless and has a strong acidic taste. The crystal structure is orthorhombic.

(J) Solubility: Soluble 1 in 1.5 parts of ethanol (95%) and 1 in less than 1 part of water; sparingly soluble in ether.

2.6.3 Tri Sodium Citrate Dihydrate

(A) Nonproprietary names: BP: Sodium Citrate

USP: Sodium Citrate

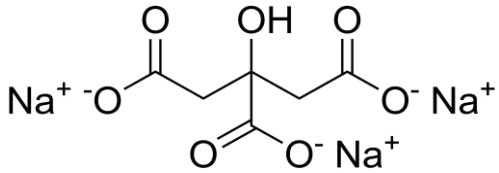
(B) Synonyms: Citric acid trisodium salt; sodium citrate tertiary; trisodium citrate.

(C) Chemical name: Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dehydrate

(D) Empirical formula: $C_6H_5Na_3O_7\_2H_2O$

(E) Molecular weight: 294.10 g/mol

(F) Structural formula:



(G) Functional category: Alkalizing agent, buffering agent, emulsifying agent; sequestering agent.

Table 2.10 Application of trisodium citrate dihydrate

Use	Concentration (% w/w)
Buffering agent	0.3–2.0
Injections	0.02–4.0
Ophthalmic solutions	0.1–2.0

(H) Applications in pharmaceutical formulation: It is used in products, mainly for adjust the pH of solutions. It is also used as a sequestering agent.

(I) Description: Trisodium citrate dihydrate consists of odorless, colorless, monoclinic crystals, or a white crystalline powder with a cooling, saline taste. It is slightly deliquescent in moist air, and in warm dry air it is efflorescent.

(J) Solubility: Soluble 1 in 1.5 of water, 1 in 0.6 of boiling water; practically insoluble in ethanol (95%).

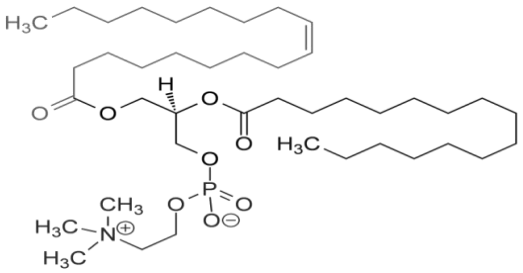
2.6.4 Soybean lecithin

(A) Nonproprietary names: USP-NF: Lecithin

(B) Synonyms :egg lecithin; mixed soybean phosphatides; ovolecithin; ProKote LSC; soybean lecithin; soybean phospholipids; Sternpur; vegetable lecithin.

(C) Chemical name: Lecithin

(D) Structural formula:



(E) Functional category: Emollient , Emulsifying agent , Solubilizing agent.

(F) Applications in pharmaceutical formulation: Lecithin is mostly used in pharmaceutical products as wetting, dispersing, emulsifying and stabilizing agents.

Table 2.11 Application of soybean lecithin

Use	Concentration (%)
Aerosol inhalation	0.1
Biorelevant dissolution media	0.059–0.295
IM injection	0.3–2.3
Oral suspensions	0.25–10.0

(G) Description: Lecithin varies significantly is physical form which is depending upon the free fatty acid content. Lecithin has practically no odor.

(H) Solubility : Lecithin is soluble in aliphatic and aromatic hydrocarbons. They are practically insoluble in cold vegetable and animal oils, polar solvents, and water. When mixed with water, lecithin hydrate to form emulsions.

2.6.5 Xanthan gum

(A) Nonproprietary names PhEur: Xanthan Gum

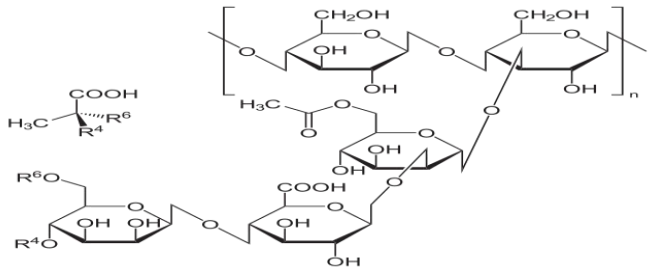
USP-NF: Xanthan Gum

(B) Synonyms: Corn sugar gum; E415; Grindsted; Keldent; Keltrol; Vanzan NF; xanthani gummi; Xantural.

(C) Empirical formula: $(C_{35}H_{49}O_{29})n$

(D) Molecular weight : approximately 1 X 106

(E) Structural formula:



(F) Functional category: Gelling agent, Stabilizing agent, Suspending agent, Sustained-release agent, Viscosity-increasing agent

(G) Applications in pharmaceutical formulations and technology: Xanthan gum is widely used as suspending agent and thickening agent suspension. It is nontoxic, compatible with most other pharmaceutical excipients, stability and viscosity properties over a wide range of pH and temperature.

(H) Description: Xanthan gum occurs as a cream or white colored, odorless, free flowing, and fine powder.

(I) Solubility: Practically insoluble in ethanol and ether; soluble in cold or warm water

2.5.7 Colloidal Silicon Dioxide

(A) Nonproprietary Names: BP: Colloidal anhydrous silica
PhEur: Silica colloidalis anhydrica
USPNF: Colloidal silicon dioxide

(B) Synonyms: Aerosil, Cab-O-Sil, colloidal silica, fumed silica, silicic anhydride.

(C) Chemical name: Silica

(D) Empirical formula: SiO_2

(E) Molecular weight : 60.08 g/mol

(F) Functional category : Anti-caking agent, emulsion stabilizer, glidant, suspending agent, tablet disintegrant, viscosity-increasing agent.

(G) Applications in Pharmaceutical Formulation or Technology: Colloidal silicon dioxide is widely used in pharmaceuticals, cosmetics, and food products. Its small particle size and large specific surface area improve the flow properties of dry powders in number of processes such as tableting.

Table 2.12 Application of colloidal silicon dioxide

Use	Concentration (%)
Aerosols	0.5 – 2
Emulsion stabilizer	1 – 5
Glidant	0.1 – 0.5
Suspending and thickening agent	2 – 10

(H) Description: Colloidal silicon dioxide is submicroscopic fumed silica. It is a light, loose, bluish-white-colored, odorless, tasteless, non-gritty amorphous powder.

(I) Solubility: Practically insoluble in organic solvents, water, and acids, except hydrofluoric acid, soluble in hot solutions of alkali hydroxide.

Chapter - 3

Review of Literature

3.1 LITERATURE REVIEW: RECONSTITUTABLE ORAL SUSPENSION

Lidgate, D. M. et al [17] prepared high dose oral suspension of MMF (200mg/ml). Dry suspension formulation contained MMF at 7.5-30% w/w concentration, suspending or viscosity enhancing agent 1-30 mg/ml , sweetener, flavor, buffer, and optionally containing flavor enhancer, wetting agent, antimicrobial agent and color. Xanthan gum, colloidal silicon dioxide, and sodium carboxymethyl cellulose were used as suspending agents. Suspending agents ranged preferably from 10-30 mg/ml in its dry suspension formulation. Combination of xanthan gum and colloidal silicon dioxide was used at 5.5 – 11.5 mg/ml. Soybean lecithin and poloxamer were used as wetting agent at 0-10 mg/ml. In this preparation, direct blending approach and wet granulation approach were used for preparation of dry suspension. Evaluation parameters like pH, sedimentation volume, deliverable volume, appearance, % homogeneity, viscosity, particle size, time to reconstitute were evaluated for reconstituted suspension.

Senapati M. et al [18] describe Pharmaceutical composition of MMF and process for its preparation. In this study, formulation of pharmaceutical comprised MMF in an amount of 75 to 200 mg/ml and a suspending agent(s) in an amount of less than 1 mg/ml, as a dry formulation, when constituted with water, for forms a suspension for oral administration.

Kotliar, E. M. et al [19] describe Azithromycin powder for oral suspension composition. Direct blending approach was used to formulate non cacking Azithromycin oral suspension. Excipient were sucrose as diluent (50% to 98% w/w) based on the total weight of the powder for oral suspension, sweeteners (50% to 98% w/w), binders (0.1% to 10% w/w), suspending agents (0.1% to 10% w/w), buffers, glidants, flavorants, dispersing agent (0.1 % to 4%,) colorants (0.005% w/w to 0.15%w/w) and wetting agents(0.1 %w/w to 4%w/w). Stability study of powder was conducted at room temperature 25°C/60% relative humidity and at accelerated conditions, i.e. about 40°C/75% RH for 6 month.

Scheler, S. et al [20] formulated powder mixtures for antibiotic dry syrup. In this formulation dry syrup of powder mixture of beta-lactam antibiotics as API and excipients were using direct blending approach of dry powder. It contained 80.0 to 95.0 %w/w powdered sugar, up to 1.0 %w/w preservative, up to 2.0 %w/w colloidal silicon dioxide.

Jain, D. et al [21] formulated and evaluated ROS of Ambroxol HCl and Azithromycin using direct blending approach. Xanthan gum and acacia were used as suspending agent. ROS showed sufficient chemical stability of the drug throughout shelf life. The prepared suspensions were evaluated by following parameter: flow properties, rheological and sedimentation behavior. The ROS of Azithromycin and Ambroxol HCl were provide to be stable over its proposed shelf life of 15 days after reconstitution. The reconstituted suspensions were stored at 4°C, 25°C and 45°C for 15 days. The reconstituted suspension stored at different temperature was evaluated after reconstitution and after 7th and 15th day of reconstitution.

Jafar, M. et al [22] studied readymix suspension of Ampicillin Trihydrate using direct blending approach. Physical characteristics like sedimentation volume, ease of redispersability and viscosity and chemical characteristic like content uniformity were evaluated. Stability studies were carried out at 25ºC/60% RH and 30ºC/60% RH for 90 days. After stability studies parameters like Sedimentation volume, viscosity, ease of redispersability, particle size distribution and in vitro dissolution were evaluated.

Shah, P. P. et al [23] formulated and evaluated taste masked oral reconstitutable suspension of Primaquine Phosphate (PRM). The purpose of this work was to mask the intensely bitter taste of PRM and to formulate suspension powder (sachets) of the taste masked drug. Taste masking was done using beta-cyclodextrin solid dispersion.

Wang, L. et al [24] prepared and evaluated taste masked oral suspension of Arbidol hydrochloride. Taste masking of bitter taste of Arbidol hydrochloride (ARB) with the combination of solid dispersion and flavors. Taste masking was effectively done by solid dispersion with octadecanol as the carrier by fusion method. Suspending agents, carriers and other excipients were selected.

Du, Y. et al [25] developed and evaluated taste-masked dry suspension of Cefuroxime Axetil (CA) for enhancement of oral bioavailability. CA is an ester prodrug of cefuroxime with a disagreeable taste when administrated orally. Dry suspensions were prepared using wet granulation method and solid dispersion method. Effect of different binder and suspending agent was evaluated on the drug release and sedimentation rate.

Provenza Bernal, N. et al [26] designed and performed physicochemical stability studies of pediatrics oral formulations of Sildenafil. Evaluation parameter included organoleptic properties, viscosity, pH, microbial studies. For oral treatments, organoleptic characteristics are important for children observance to therapeutic regimens. European Medicines Agency (EMA) pediatrics investigation plan guidelines indicate the particular significance of organoleptic testing in the development of oral treatment for children (EMA, 2006). Storage is accompanied by many changes including chemical reaction and physical and structural changes which affect both pharmacological and sensory qualities.

Sateesha, S. et al [27] **s**tudied the formulation and stability study of palatable Norfloxacin dry syrup for oral administration. The method involved coating of granules with special level of acrycoat E 100-40 and preparation of Norfloxacin microspheres using Eudragit E 100. The in-vitro studies showed satisfactory dissolution rate. Long term stability studies were carried out. Microspheres prepared with increased concentration of MCC and mannitol improved physical stability and were palatable with slight or no bitter after taste.

Singh V. et al [28] formulated and evaluated of Cephalexin monohydrate ROS with piperine. The aim behind this ROS system was to improve the chemical stability, increase the bioavailability, controlled the duration and onset of action of the drug. Evaluation parameter for the ROS were pH, viscosity, drug release, %drug content and sedimentation rate, studies of microbial test for efficacy for preservatives.

3.2 LITERATURE REVIEW: MYCOPHENOLATE MOFETIL

Dalal, P. et al [29] studied safety and efficacy of Mycophenolate Mofetil (MMF) in the prophylaxis of acute kidney transplantation rejection. MMF is a prodrug of mycophenolic acid (MPA) which is an inhibitor of inosine monophosphate dehydrogenase (IMPDH). It inhibits denovo pathway of guanosine nucleotide synthesis in T and B-lymphocytes and prevents their proliferation, thereby suppresses both cell mediated and humoral immune responses. Clinical trials in kidney transplant recipients showed efficacy of MMF in reducing the incidence and severity of acute rejection episodes. It also improved long term graft function as well as graft and patient survival in kidney transplant recipients.

Downing, H. J. et al [30] **s**ummarized Pediatric use of MMF. The study included pharmacokinetics, the clinical conditions for which it is used, the advantages compared with other immunosuppressant drug and the unresolved issues remaining with use in children. The review aims to focus on off- label use in children so as to identify areas that require further research and investigation.

Scheubel, E. et al [31] developed a simple dissolution technique to assess generic formulation differences of MMF. MMF is a BCS Class II drug that has a strongly pH-dependent solubility profile. Consequently, differences in solid-state properties, formulation, and manufacturing processes of MMF can lead to disparities in bioavailability between brands of the same drug. This study was conducted to compare the in vitro dissolution profile of the original MMF innovator brand (CellCept, Roche) with available generic products.

Chapter - 4

Experimental Work

4.1 LIST OF MATERIALS AND EQUIPMENTS

Various materials and equipments used to carry out the experimental work are listed below.

List of Materials

Table 4.1 List of Materials

Sr. no.	Name	Category	Suppliers of Material
1	Mycophenolate Mofetil	API	Biocon Limited, India
2	Aspartame	Sweetener	Neutrasweet CaOSA, USA
3	Citric acid (anhydrous)	Buffer	Merck KGoA, Germany
4	Colloidal silicon dioxide	Suspending Agent	Evonik Industries, Germany
5	Methyl Paraben	Preservatives	Gujarat Organics, India
6	Mixed Fruit Flavor	Flavor	Firmenich, Switzerland
7	TriSodium Dihydrate	Buffer	Canton Laboratories, India
8	Sorbitol (Neosorb P100T)	Sweetener	Roquette, France
9	Soybean Lecithin	Wetting Agent	Phospholipid GmbH
11	Xanthan Gum	Suspending Agent	CP Kelco, USA

List of Equipments:

Table 4.2 List of Equipments

Sr. no.	Equipment	Manufacturer	Model
1	Digital weighing balance	Mettler Toledo	PB602 – S
2	Fluid Bed Processor	Palm Glatt	GPGC 1.1
3	pH meter	Labindia	Pico pH meter
4	Viscometer	BrookField	Brookfield LV
5	Particle Size Measurement	Malvern	Malvern 20000
6	Electromagnetic sieve shaker	Alfa electronics	EMS 8
7	Loss on drying apparatus	Mettler Toledo	H843
8	Density tester	Electro lab	ETD1020
9	Flow meter	Erweka	GT 300 V 230
10	Dissolution apparatus	Electro lab	TDT-08L
11	HPLC apparatus	Perkin Elmer	LC2010C
12	Blender	Kalweka	VDM4
13	Stirrer	Remi motor	RQ-124A
14	Co – Mill	Quadro	US – 02111

4.2 PREFORMULATION STUDIES

Preformulation testing is the primary step in the formulation development process. The objective of preformulation studies is to study physical and chemical properties to generate information useful for development of therapeutically effective and pharmaceutically stable dosage forms.

4.2.1 Characterization of Mycophenolate Mofetil

4.2.1.1 Organoleptic Properties

The color of Mycophenolate Mofetil was observed by visual analysis in which sufficient quantity of sample was spread on a glass. Physical nature and other observations were recorded.

Table 4.3 Organoleptic properties specifications of API

USP Specification	Inference
A White or almost white crystalline powder	Almost white crystalline powder.

4.2.1.2 Solubility

Mycophenolate Mofetil was freely soluble in chloroform, tetra hydro furan and methylene chloride. It was also soluble in ethyl alcohol.

4.2.1.3 Identification of Mycophenolate Mofetil.

(A) By Fourier Transforms Infrared Spectrophotometer (FTIR) Spectra of Mycophenolate Mofetil: Mycophenolate Mofetil was examined by FTIR and comparison of the sample was carried out with reference standard. Working standard was prepared by Potassium bromide (For Infrared spectroscopy) discs or dispersion. The FTIR spectrum of the substance being examined should be match with the FTIR spectrum of Mycophenolate Mofetil working standard.

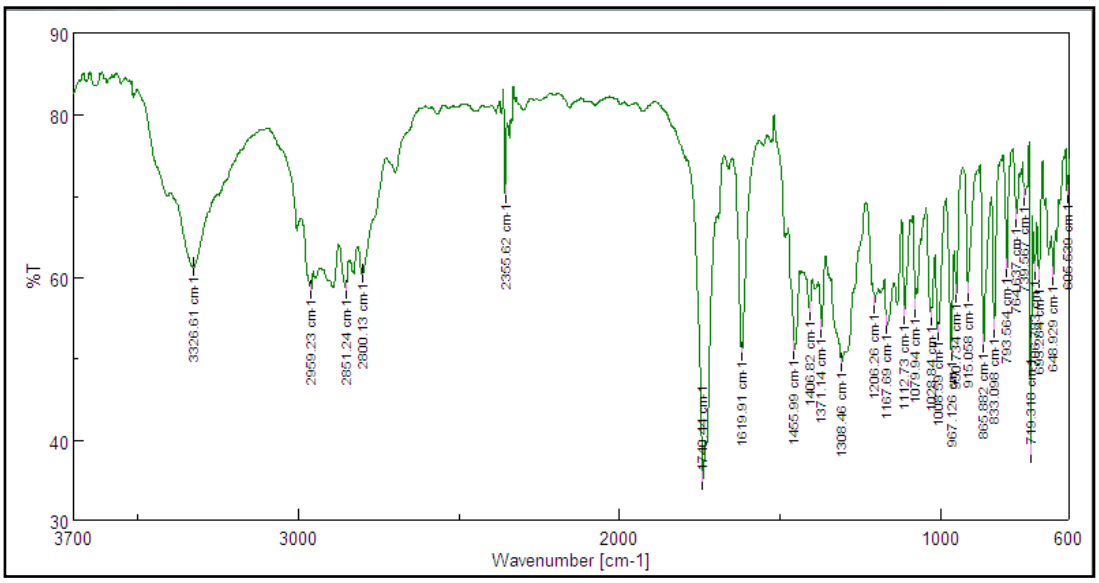


Figure 4.1 FTIR spectrum of Test sample of the Mycophenolate Mofetil

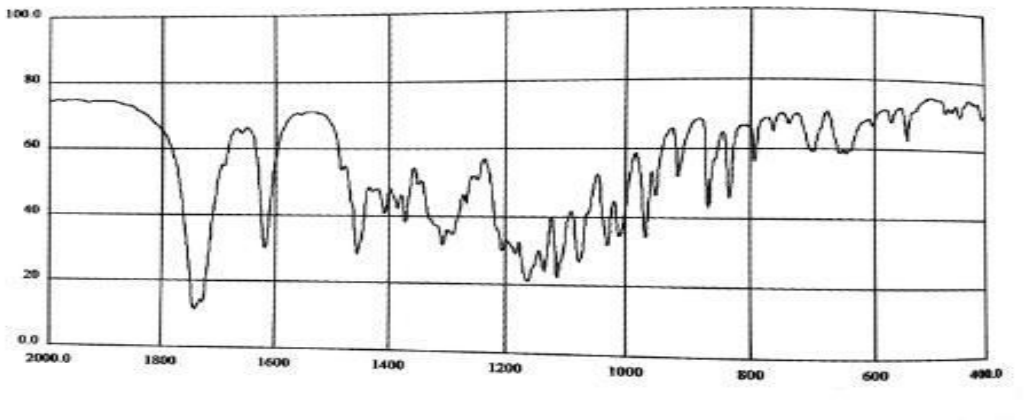


Figure 4.2 FTIR Spectrum of the Reference Spectrum of Mycophenolate Mofetil

Table 4. 4 FTIR Value

	Observed	Reported value
O - H stretch	3326 cm-1	3600 - 3200cm-1
N - H Bend	1619.19 cm-1	1650–1580 cm-1
C - N stretch	1133cm-1	1340 - 1020cm-1

Discussion:

The sample spectrum of Mycophenolate Mofetil was compared with reference standard and both spectra were found similar in peak values representing wave numbers.

(B) UV Absorbance Spectra of Mycophenolate Mofetil.

Standard Solution: 0.278 mg/ml of USP Mycophenolate Mofetil RS in 0.1N HCl

Mode: UV

Cell: 0.2 cm

Blank: 0.1 N HCl

Wavelength: λmax measured at 304 , 250 nm

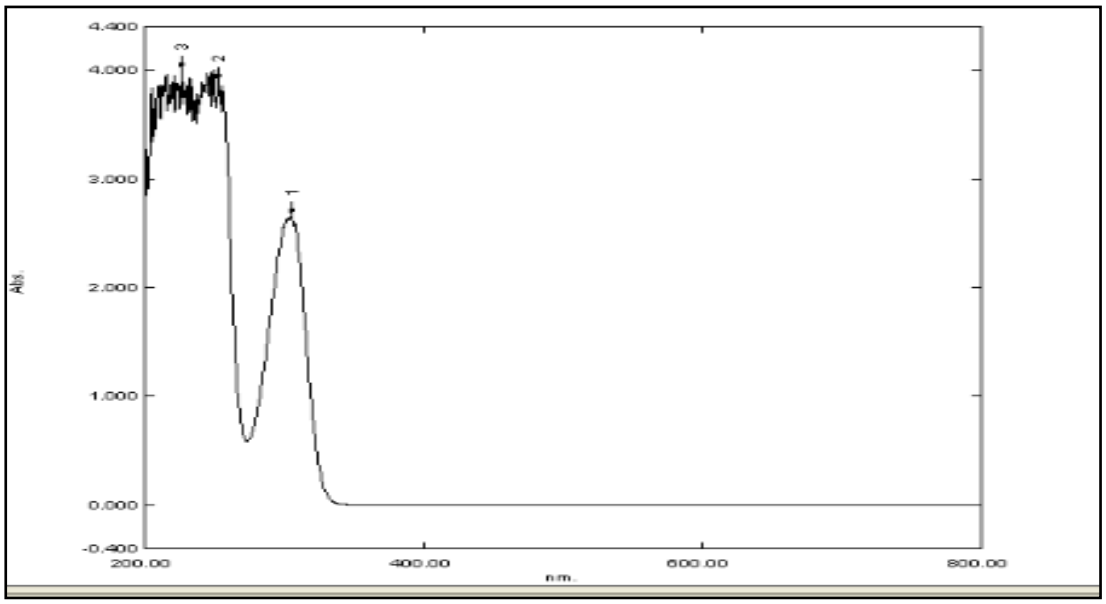


Figure 4.3 UV Spectrum of the Mycophenolate Mofetil

Discussion:

The above UV spectra of Mycophenolate Mofetil showed the λmax at 309 nm, 249 nm, which was constant after dilution and similar to the reported standard value.

(C) By High Performance Liquid Chromatography (HPLC) : Assay

Buffer: Triethylamine and water (1:325) adjusted with Phosphoric acid to a pH 5.3

Mobile Phase: Acetonitrile and Buffer (7:13)

Standard Solution: 0.4 mg/ml of USP Mycophenolate Mofetil RS in Acetonitrile

Sample Solution: 0.4 mg/ml of USP Mycophenolate Mofetil in Acetonitrile

Chromatographic System:

- Mode: LC
- Detector: UV 250 nm

- Column: 4.6 mm X 25 cm ; 5µm packing L7
- Column Temperature: 45 °C
- Flow Rate : 1.5ml/min
- Injection Volume: 10 µL

System Suitability:

- Sample : Standard Solution
- Suitability Requirement: Tailing Factor : NMT 2.0

  Relative Standard Deviation: NMT 1.0%

Analysis:

$$\frac{ru}{rs} X \frac{Cu}{Cs} X 100 \qquad \text{---------- (1)}$$

r_u : Peak Response from sample solution

r_s: Peak Response from Standard solution

C_s: Concentration of USP Mycophenolate Mofetil RS in standard solution (mg/ml)

C_u: Concentration of USP Mycophenolate Mofetil RS in Sample solution (mg/ml)

Table 4.5 Assay specification of Mycophenolate Mofetil (As per COA)

USP Specification	98.0- 102.0 %
Result	99.8 %

Discussion:

Assay of Mycophenolate Mofetil was compared with reference data, it was found that assay of the API was within the limit (~98% – 102% w/w).

4.2.1.4 Melting Point Range:

Table 4.6 Melting Point specification of Mycophenolate Mofetil (As per CoA)

USP Specification	Between 95.0°C to 97.5°C.
Observed Melting point	95.0°C and 96.2°C

4.2.1.5 Flow Properties

20 g powder was taken and placed in 100 ml of measuring cylinder. Volume occupied by the powder was noted down as V_0, without disturbing the cylinder. Then cylinder was fitted in instrument and 10 taps were preformed. After 10 taps, volume was noted down as Va. Again after 500 taps volume was noted down as Vb. The difference between Va and Vb was less than 2.0% so tapped volume was noted down without further processing. Bulk density and tapped density, Carr's index, Hausner ratio was calculated.

Table 4. 7 Flow Properties of Mycophenolate Mofetil

A. R. No	Batch /Lot No.	Source	Bulk Density	Tapped Density	Carr's Index	Hausner ratio	Flow Properties
RR0562	10143 1164	Biocon	0.35	0.58	39.65	1.65	Very Very Poor
			0.34	0.60	43.33	1.76	

Discussion:
From the above results it can be concluded that Mycophenolate Mofetil exhibits very very poor flow property.

4.2.1.6 Particle size analysis

Particle size of the drug was affected on physical and chemical properties. Malvern particle size analyzer using wet dispersion method was used to determine the particle size distribution of Mycophenolate Mofetil.

Table 4.8 Particle size distribution of Mycophenolate Mofetil (As per COA)

A.R No	Batch / Lot No.	Source	Particle size in μm (% of particles under size)		
			10 %	50 %	90 %
RR0562	101431164	Biocon	1	7	17

Discussion: From the results it can be concluded that the Mycophenolate Mofetil is a micronized powder which $d_{90} = 17\ \mu m$

4.2.1.7 Related Substances

HPLC system: HPLC with auto sampler, UV detector, pump and inbuilt column compartment (Make: Agilent 1100 series or equivalent)

Chromatographic Parameters:

- Detector : UV 250 nm
- Column: 4.6 mm X 25 cm ; 5µm packing L7
- Column Temperature: 45 °C
- Flow Rate : 1.5ml/min
- Injection Volume: 10 µL

Buffer: Triethylamine and water (1:325) adjusted with Phosphoric acid to a pH 5.3

Mobile Phase: Acetonitrile and Buffer (7:13)

Sample Solution: 2mg/ml of Mycophenolate Mofetil in Acetonitrile

System suitability:

- **Sample:** System Suitability Solution
- **Suitability Requirement:** Resolution: NLT 1.5 between Mycophenolate Mofetil related Compound A and Mycophenolate Mofetil Related Compound B

Analysis: Sample Solution

Calculate other percentage of each impurity in the portion of Mycophenolate Mofetil

$$\frac{Ru}{Rs} X100 \qquad \text{------ (2)}$$

Ru: Peak response of each impurity

Rt: Sum of all peak response

Table 4.9 Related Substance (As per CoA) of Mycophenolate Mofetil

Impurities	USP Specification	Result
Mycophenolic acid (Impurity F)	NMT 0.50%	0.06%
Mycophenolate Mofetil related compound A (Impurity A)	NMT 0.10%	Below limit
Mycophenolate Mofetil related compound B (Impurity H)	NMT 0.10%	ND
N-oxide analog (Impurity G)	NMT 0.10%	ND
1-Morpholinoethoxy analog (Impurity B)	NMT 0.10%	ND
O-Methyl analog (Impurity D)	NMT 0.10%	ND
Methyl mycophenolate (Impurity E)	NMT 0.10%	ND
Total unknown impurities	NMT 0.10%	ND
Total impurities:	NMT 0.70%	0.06%

*ND: Not Detected

Discussion:

The Related Substance determined was within the range of standard value, hence, it can be concluded that the drug sample had similar physical property as standard drug.

4.2.1.8 Hygroscopic Studies:

Moisture content of Mycophenolate Mofetil was determined using Moisture analyzer (Model - H843) instrument. Drug was directly placed in an open Petri dish to high humidity levels (40°C /75 % RH and 25°C / 60 % RH) and the moisture gain was monitored till equilibrium % loss on drying (LOD) was achieved. Loss on drying was measured by moisture analyzer.

Table 4.10 Hygroscopic Studies of Mycophenolate Mofetil

USP Specification	Not More Than 0.5%	
Exposure Time	**% Loss on drying (@75°C) at storage condition**	
	40°C / 75 % RH	**25°C / 60 % RH**
Initial	0.24 %	
1 Hr	0.17 %	0.18 %
8 Hr	0.36%	0.19 %
24 Hr	0.34 %	0.17 %

Discussion:

Since there was no change in the moisture content, it can be concluded that the drug was stable at high humidity condition and was non hygroscopic in nature.

4.2.1.9 pH – Solubility Profile

The solubility of Mycophenolate Mofetil in different pH range of the gastrointestinal tract was determined to aid in selecting suitable dissolution media for development purpose and to ensure that 'sink conditions' are maintained therein.

Table 4.11 pH solubility profile of Mycophenolate Mofetil

Sr. No.	Medium	Solubility (mg/ml)	Sink Condition possible?
1.	0.1 N HCl	46.3	Yes
2.	SGF pH 2.0	10.6	Yes
3.	Acetate buffer pH 4.5	0.84	No
4.	Acetate buffer pH 4.5 + 0.25% SLS	1.46	No
5.	Acetate buffer pH 4.5 + 0.5% SLS	3.09	Yes
6.	Phosphate buffer pH 6.0	0.15	No
7.	Phosphate buffer pH 6.0 + 0.25% SLS	1.37	No
8.	Phosphate buffer pH 6.0 + 0.5% SLS	2.49	Yes
9.	Phosphate buffer pH 7.5	0.14	No
10.	Phosphate buffer pH 7.5 + 0.25% SLS	0.83	No
11.	Phosphate buffer pH 7.5 + 0.5% SLS	1.61	No
12.	Purified water	0.16	No
13.	Purified water + 0.25% SLS	0.66	No
14.	Purified water + 0.5% SLS	1.64	No

Discussion:

Sink condition was possible if solubility is above 1.66 mg/ml. From the above solubility data it can be concluded that Mycophenolate Mofetil was having pH dependent solubility. The solubility is higher in the acidic media and reduces in the alkaline media.

4.2.2 Drug- Excipient Compatibility Study

Drug- Excipient compatibility study was carried out by placing drug alone or drug along with excipients in specific ratio in stopper vials at 2–8˚C, 40˚C/75%RH and 50˚C for 1 month and compatibility study was also carried out in open vial 40˚C/75%RH for 1 month for assay and related substances of the mixture were carried out at initial, 15 days and after 1 month.

Table 4.12 Time point and Condition of preformulation study of Mycophenolate Mofetil

Sr .No	Condition	Time Points
1.	Initial	On 0th day
2.	40°C & 75% RH (Open vials)	15days
		30 days
3.	40°C & 75% RH(Sealed vials)	30 days
4.	50°C (Sealed vials)	15days
		30 days
5.	2°-8°C (Control Samples)	30 days

4.2.2.1 Condition for Drug - Excipient compatibility study

Drug excipients compatibility study was carried out by placing drug alone and drug with excipients in different ratios. One month compatibility study was carried out at 40°C/ 75 % RH and 50°C. The results were evaluated on the basis on Assay value.

Table 4.13 Drug - Excipient compatibility study: Assay

Ingredients	Ratio	Assay	
		40ºC / 75%RH (Open)	50ºC seal
API (Mycophenolate Mofetil)	1:0	99.8	99.7
API + Sorbitol (Neosorb P 100T)	1:2	99.5	99.2
API + Citric acid (anhydrous)	1:0.05	99.7	99.9
API + TriSodium dihydrate	1:0.2	98.9	99.1
API + Soybean lecithin	1:0.1	99.2	98.9
API + Methyl paraben	1:0.1	99.0	99.0
API + Xanthan gum	1:0.1	99.1	98.9
API + Colloidal silicon dioxide	1:0.1	99.8	99.5
API + Aspartame	1:0.1	99.5	99.4
API + Mixed fruit flavor	1:0.1	99.4	99.2
API + Composite	1:3	99.7	99.5

Discussion:

Assay of Mycophenolate Mofetil with other excipients, was within the acceptable range value (~98 – 102 %w/w) at the condition 40ºC / 75%RH (Open) & 50ºC (seal). So it can be concluded that in presence of these excipients assay of Mycophenolate Mofetil was not affected. During study it was observed that there was no significant change in assay of blends due to thermal stress. Therefore, it can be concluded that selected excipients were compatible with Mycophenolate Mofetil**.**

4.2.2.2 Drug - Excipient compatibility study: Related Substance

Drug excipients compatibility study was carried out by placing drug alone and drug with excipients in different ratios. One month compatibility study was carried out at 40°C/ 75 % RH and 50°C. The chemical analysis results of the samples are shown in Table 4.14 and Table 4.15.

Table 4.14 Drug - Excipient compatibility study: Related Substance

(30 days 40°C & 75% RH (Open vials))

Ingredients	Ratio	Relative Substance(30 days 40°C & 75% RH (Open vials))								
		F	B	A	D	E	G	H	Other	Total
API	1:0	0.06	BQL	BQL	ND	ND	BQL	ND	ND	**0.10**
API + Sorbitol	1:2	0.07	ND	BQL	ND	ND	BQL	ND	BQL	**0.13**
API + Citric acid (anhydrous)	1:0.05	0.06	ND	ND	ND	ND	ND	ND	ND	**0.06**
API + TriSodium Dihydrate	1:0.2	0.08	BQL	BQL	ND	ND	BQL	ND	ND	**0.12**
API + Soybean Lecithin	1:0.1	0.13	BQL	BQL	ND	ND	BQL	ND	ND	**0.16**
API + Methyl Paraben	1:0.1	0.12	BQL	BQL	ND	ND	BQL	ND	ND	**0.15**
API + Xanthan gum	1:0.1	0.08	BQL	BQL	ND	ND	BQL	ND	ND	**0.10**
API + Colloidal silicon dioxide	1:0.1	0.10	BQL	BQL	ND	ND	BQL	ND	ND	**0.13**
API + Aspartame	1:0.1	0.10	BQL	BQL	ND	ND	BQL	ND	ND	**0.14**
API + Mixed Fruit Flavor	1:0.1	0.07	ND	ND	ND	ND	ND	ND	ND	**0.13**
API + Composite	1:3	0.06	BQL	BQL	ND	ND	BQL	ND	ND	**0.10**

*ND= Not Detected BQL: Below Quantified Limit

Table 4.15 Drug - Excipient compatibility study: Related Substance

(50°C (Sealed vials))

Ingredients	Ratio	Relative Substance(50°C (Sealed vials))								
		F	B	A	D	E	G	H	Other	Total
API	1:0	0.06	ND	ND	ND	ND	ND	ND	ND	0.15
API + Sorbitol	1:2	0.07	ND	BQL	ND	ND	BQL	ND	BQL	0.13
API + Citric acid (anhydrous)	1:0.05	0.06	BQL	BQL	ND	ND	BQL	ND	ND	0.04
API + Sodium CitrateDihydrate	1:0.2	0.08	BQL	BQL	ND	ND	BQL	ND	ND	0.18
API + Soybean Lecithin	1:0.1	0.13	BQL	BQL	ND	ND	BQL	ND	ND	0.12
API + Methyl Paraben	1:0.1	0.12	ND	BQL	ND	ND	ND	ND	ND	0.15
API + Xanthan gum	1:0.1	0.08	BQL	BQL	ND	ND	BQL	ND	ND	0.06
API + Colloidal Siilicon Dioxide	1:0.1	0.10	BQL	BQL	ND	ND	BQL	ND	ND	0.12
API + Aspartame	1:0.1	0.10	BQL	BQL	BQL	ND	BQL	ND	ND	0.20
API + Mixed Fruit Flavor	1:0.1	0.07	ND	ND	BQL	ND	ND	ND	BQL	0.23
API + Composite	1:3	0.06	BQL	BQL	BQL	ND	BQL	ND	ND	0.10

*ND = Not Detected, BQL= Below Quantified Limit

Discussion:

Based on the result, it was observed that there was no significant change in physical property of blend due to thermal stress. Besides, data related to relative substance also indicated that there was no significant change in levels of impurities in the above mentioned blends. Therefore, it can be concluded that the selected excipients were compatible with Mycophenolate Mofetil**.**

4.3 INNOVATOR CHARACTERIZATION

4.3.1 Physical Characterization of Innovator Product

The reference product CellCept® oral suspension (200 mg/ml) was manufactured by Roche Laboratories, Inc., USA. CellCept® Oral Suspension is available as 200 mg/ml in USA market. The physical characteristics of CellCept® Oral Suspension (200 mg/ml) are given in Table 4.16. The product details of CellCept® Oral Suspension are given in Table 4.17.

Table 4.16 Description of CellCept® Oral Suspension 200 mg / ml

Description	CellCept® Oral Suspension 200 mg / ml
Label claim	Each ml contains 200 mg Mycophenolate Mofetil after constitution
Excipients	Aspartame, Citric acid (anhydrous), Colloidal silicon dioxide, Methylparaben, Mixed fruit flavor, Sodium citrate dihydrate, Sorbitol, Soybean lecithin and Xanthan gum

Table 4.17 Product details of CellCept® Oral Suspension 200 mg / ml

No	Description	CellCept® Oral Suspension 200 mg / ml	
1	Name of product	CellCept® Oral Suspension 200 mg / ml	
2	Composition	Each ml contains 200 mg Mycophenolate Mofetil after constitution	
3	Manufactured by	Roche Laboratories, Inc., NJ	
4	Marketed by	Roche Laboratories Inc., USA	
Dosage form details			
5	B. No	Batch A	
6	Exp Date	June 16	
7	In use shelf life	60 days	
8	Powder Description	White Colored granular Powder	
9	Weight of Bottle	143.32gm	
10	Fill weight of Powder	110 gm	
11	Weight of Empty Bottle	33.32 gm	
12	Bulk Density of Powder	0.608 gm/ml	
13	Tapped Density of Powder	0.882 gm/ml	
14	Carr's Index	31.08%	
15	Hausner's ratio	1.451	
16	Sieve Analysis	**Sieve No**	**%Retain**
		40#	1%
		60#	16%
		80#	16%
		100#	7%
		Base	60%
17	% LOD @ 75° C	0.80%	
18	Dilution Recommendations as per Leaflet	Amount of Water to be added-94 ml and do as per procedure by Pharmacist	
19	Deliverable Volume (ml)	172 ml	
20	pH of Suspension	6.6	
21	Viscosity (cps)	650 (620 – 710 cps)	
22	**Storage Condition**	Dry Powder stored at 25°C (excursion permitted to 15 30°C).Constituted suspension stored at 25°C (excursion permitted to 15-30° C). Store in Refrigerator is at 2-8° C is acceptable. Do not freeze.	

4.3.2 Chemical Characterization of Innovator Product

The reference product CellCept® Oral Suspension was evaluated for chemical characteristics i.e., assay and related substances. The results are described in Table 4.18

Table 4.18 Chemical characteristics of CellCept® Oral Suspension 200 mg/ml

Tests	Results
Product Name	CellCept® Oral Suspension 200 mg/ml
Batch No.	Batch A
Expiry Date.	June 16
Assay after Reconstitution	99.6% (98.5% –100.05%)
Related Substances (%)	
Mycophenolic acid	0.03 %
Any other impurity	0.03 %
Total Impurities	0.06 %

4.3.3 Comparative Dissolution Profile of Cellcept® Oral Suspension 200mg/ml

Two batches of CellCept® Oral Suspension 200 mg/ml were studied for the dissolution profile and the results are described in Table 4.19 and graphically represented in Figure 4.4

- **Medium** : 0.1 N HCl
- **Volume** : 900 ml
- **Apparatus** : USP apparatus Type 2 (Paddle type)
- **RPM** : 40
- **Limit** **:** Not Less than 80% in 20 min

Table 4.19 Cumulative % drug release

S. No		Cumulative % drug dissolved at (time in minutes)				
		5	**10**	**15**	**20**	**30**
1	CellCept® Oral Suspension 200 mg/ml (B. No.: Batch A)					
	Average	42.75	53.79	66.34	83.01	98.50
2	CellCept® Oral Suspension 200 mg/ml (B. No.: Batch B)					
	Average	44.66	55.26	65.44	82.02	99.21

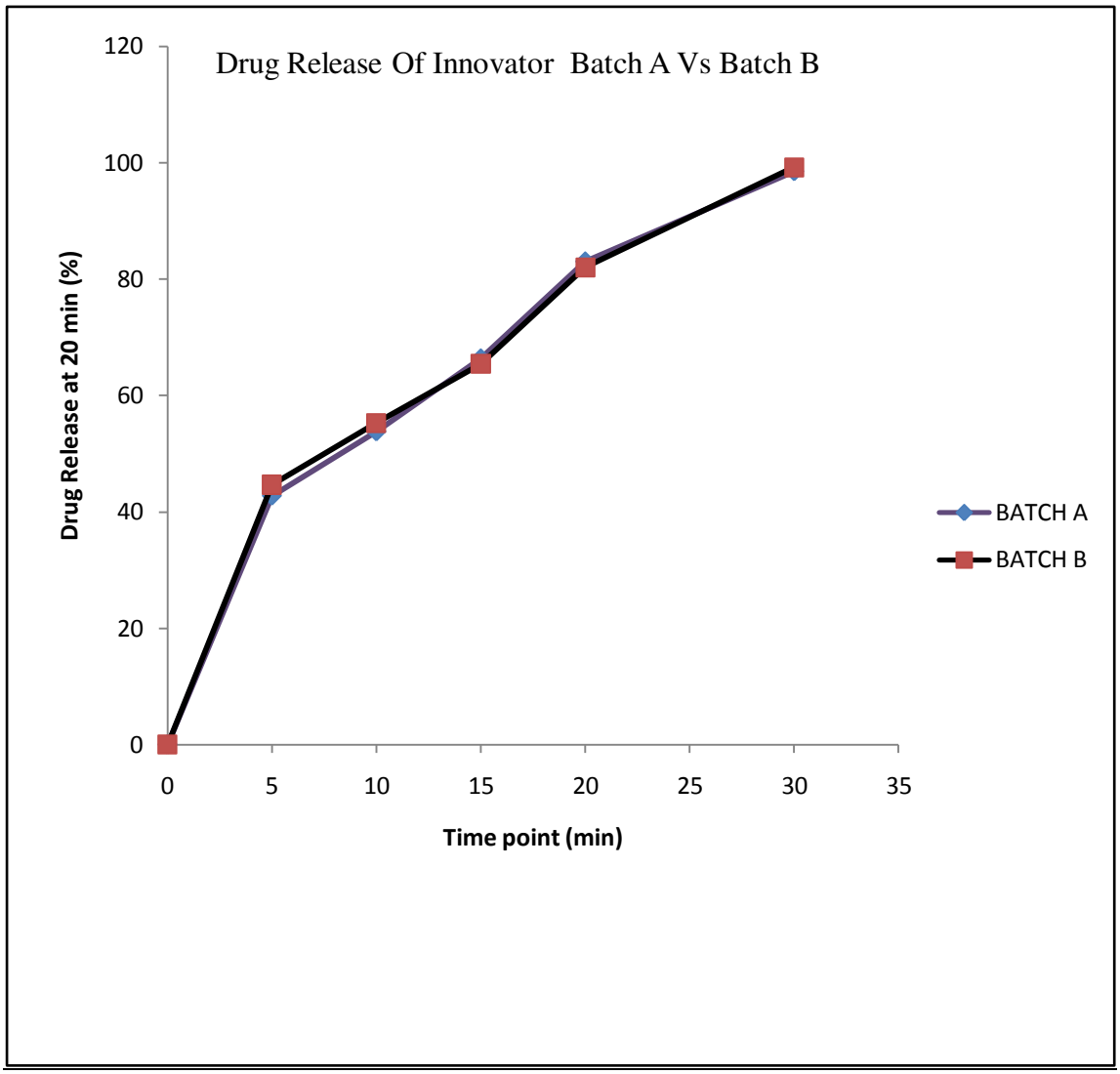


Figure 4.4 Drug release profile of CellCept® 200 mg/ml oral suspension

4.4 EVALUATION PARAMETER FOR RECONSTITUTABLE ORAL SUSPENSION

4.4.1 Procedure for Reconstitution

Labeled Claim: Each bottle contains 35g Mycophenolate Mofetil (MMF) in 110g Powder Blend. 5ml of the reconstituted suspension contains 1g of MMF.

Procedure:

- Tap the closed bottle several times to loosen the powder. Measure 94 ml of water in a graduated cylinder.
- Add approximately half quantity of water for constitution to the bottle and shake the closed bottle well for about 1 minute.
- Add remaining half quantity of water and shake the closed bottle well for about 1 minute.
- Remove the child-resistant cap and push bottle adapter into neck of bottle. Close bottle with child-resistant cap tightly. This will assure the proper fitting of the bottle adapter in the bottle and child-resistant status of the cap.

4.4.2 Evaluation Parameter

4.4.2.1 For Powder before Reconstitution:

(A) Flow Properties:

Bulk density It is used to describe a packing of particles or granules. The equation for determining bulk density is:

$$\textbf{Bulk Density} = \frac{\textbf{Weight of powder}}{\textbf{Bulk volume}} \qquad \text{------ (3)}$$

Tapped density: For measurement of tapped density, powder is filled in measuring cylinder. After that, mechanically tap on Taped density apparatus (Electrolab – ETD1020). After 10 taps, volume is measured and not more than 2% variation. If variation is more than 2%, it should be observed after 500 times tapping. If still variation is more than 2%, powder is tapped for 1250 times.

$$\textbf{Tapped Density} = \frac{\textbf{Weight of powder}}{\textbf{Tapped volume}} \qquad \textbf{---- (4)}$$

Compressibility Index (CI): Compressibility is indirectly related to the relative flow rate, cohesiveness and particle Size distribution of the powder. Tapped and apparent bulk density measurements can be used to estimate the compressibility of a material. The flow characters of blend are given in table 4.20.

$$\textbf{Carr's Index} = \frac{(\textbf{Tapped density} - \textbf{Bulk density})}{\textbf{Tapped density}} \textbf{ X 100} \quad \textbf{--(5)}$$

Hausner's Ratio: It is the ratio of bulk volume to tapped volume or tapped density to bulk density.

$$\textbf{Hausner's Ratio} = \frac{\textit{Tapped density}}{\textit{Bulk density}} \qquad \textbf{-------(6)}$$

Table 4. 20 Effect of Carr's Index and Hausner's Ratio on flow property

Carr's Index (%)	Flow character	Hausner's Ratio
< 10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
> 38	Very, very poor	> 1.60

(B) Particle size determination:

100 gm of moisture free dried powders was transferred to digital sieve shaker. Sieves (Electrolab) of different mesh size were used for analysis and amount of powder retained on sieves was calculated.

(C) Loss on Drying:

Loss on Drying (LOD) indicates % content of water present in sample. Samples weighing 1-2 gm were kept in Halogen moisture analyzer (Mettler Toledo) at 75ºC.

(D) Blend Uniformity: (Assay)

Standard Preparation: Transfer an accurately weighed quantity of about 100 mg of Mycophenolate Mofetil working standard in 100 ml of volumetric flask. Dissolve and dilute to make up the volume with methanol and mix. Final concentration of solution was 2µg/ml.

Assay Preparation: Transfer an accurately weighed quantity of powdered sample equivalent to 100 mg of Mycophenolate Mofetil in 100 ml volumetric flask. Add 70 ml of Methanol and sonicate for 15 minute taking care to maintain temperature of ultrasonic bath below 10ºC, Dilute to volume with methanol and Mix. Filter through 0.45µ Nylon filter discarding first 5 ml of the filtrate. Final concentration of solution was 2µg/ml.

System Suitability: Measure the absorbance of standard preparation six times at 250 nm using UV/VIS Spectrophotometer (Shimadzu UV-1800). Methanol was used as a blank and the results were recorded. Relative standard deviation of six absorbance observation should not be more than 2.00%.

Procedure:

Measure the absorbance of assay preparation at 250 nm using appropriate UV/VIS Spectrophotometer. Use methanol as a blank. Calculate the content of Mycophenolate Mofetil in % of label claim from the values of absorbance obtained from the standard preparation, Assay preparation and percentage potency of working standard used.

Calculation:

$$Assay = \frac{Au}{As} X \frac{W1}{100} X \frac{5}{50} X \frac{10}{50} X \frac{100}{W2} X \frac{50}{5} X \frac{50}{10} X \frac{100}{L.c} XP \quad \text{---- (7)}$$

Au: Absorbance of Assay preparation.

As: Mean absorbance of standard preparation.

W1: Weight of Mycophenolate Mofetil working standard in mg.

W2: Weight of sample taken in mg.

L.C.: Label Claim in %w/w.

P: Potency of Mycophenolate Mofetil working Standard in percentage on as is basis.

IN PROCESS SAMPLING PROTOCOL :
BLEND UNIFORMITY

Sampling Positions	Top	Bottom
Left Front	S1	S2
Left Back	S3	S4
Right Front	S5	S6
Right Back	S7	S8
Middle Centre	S9	S10

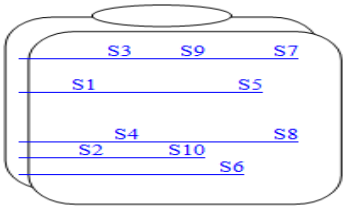


Figure 4.5 Sampling Point for the Blend Uniformity

4.4.2.2 Evaluation of Reconstituted oral suspension

(A) %Assay (Content Uniformity)

Buffer Phase I: Add 10 ml Triethylamine into a 1000 ml volumetric flask containing about 950 ml of water and mix. Adjust with phosphoric acid to pH 7.2 and dilute with water to volume.

Buffer Phase II: Add 10 ml Triethylamine into a 1000 ml volumetric flask containing about 950 ml of water and mix. Adjust with phosphoric acid to pH 3 and dilute with water to volume.

Solution A: Buffer I: Water = 4:9

Mobile Phase: Solution A: Acetonitrile = 7:3

Extraction Solvent: Buffer II: Water: Acetonitrile = 4:9:13

Diluents: Buffer II: Water: Acetonitrile = 4:9:7

Standard stock solution: 40 mg Mycophenolate Mofetil is added into 10 ml extraction solvent (4 mg/ml) and sonicated to aid dissolution.

Standard Solution: Take 2 ml stock solution and dilute up to 20 ml diluent.

Sample Stock Solution: Reconstitute 800 mg Mycophenolate Mofetil into 200 ml volumetric flask. Add 150 ml extraction solvent and Mix well. Sonicate for 10 minute by maintaining temperature of ultrasonic bath between 20ºC to 25ºC temperature. Dilute up to 200 ml volume with extraction solvent and mix well.

Sample Solution: Transfer 5.0 ml of sample stock solution to 50 ml volumetric flask and dilute with diluents to volume. Pass through filter 45µm pore size.

Chromatographic system:

- Mode: LC
- Detector: UV 249nm
- Column: Kromasil 100-5 phenyl (250 mm X 4.6 mm) (Make: Akzonobel)
- Flow Rate: 1.5 ml/min
- Column Temperature: 45ºC
- Auto sampler temperature: 5ºC

- Injection Volume: 25µL
- Retention time: 37 min
- Run time: 60 min

System suitability:

- Sample: Standard solution
- Tailing factor: NMT 2.0%
- Relative standard deviation: NMT 2.0% , standard solution

Analysis:

Sample: Standard Solution and Sample Solution

Calculate % of $C_{23}H_{31}NO_7$ in portion of Mycophenolate Mofetil for oral suspension taken

$$\boldsymbol{Assay = \frac{Au}{As}\ X\frac{W1}{10}X\frac{2}{20}X\frac{200}{W2}X\frac{50}{5}X\frac{W3}{L.C}X\frac{p}{100}X10} \quad \text{----------(8)}$$

Where,

Au: Mean peak area of sample solution

As: Mean peak area of standard solution

W1: Weight of standard mg

W2: Weight of sample taken mg

W3: Weight / ml of suspension in mg/ml

L.C.: Label Claim

P: Potency

(B) Dissolution:

Medium: 0.1N HCl, 900 ml deaerated

Apparatus: USP Type II (Paddle)

RPM: 40

Time: 20 min

Standard Solution: 28 mg Mycophenolate Mofetil is dissolved in 100 ml volumetric flask. Add 50 ml of 0.1 N HCl and sonicate. After sonication dilute up to 100 ml with 0.1 N HCl.

Sample Solution: Reconstitute Mycophenolate Mofetil oral suspension according to label instruction and shake well. Use a separate 3 ml syringe for each vessel. Withdraw 2 ml of suspension. Remove the air bubble from the syringe. Adjust the volume to 1.2 ml and precisely weigh the filled syringe. Operate the apparatus, holding the syringe above the surface of the medium, at a location that is halfway between the paddle shaft and vessel wall. Carefully introduce the sample to the vessel over a 5 – 10 sec period. Weigh the empty syringe and determine the weight of the sample (g). At the time specified, withdraw an aliquot and immediately pass through a suitable filter of 10µm pore size, discarding first few ml.

Spectrometric Condition:

- Mode: UV
- Analytical wavelength: 304 nm
- Cell: 0.2 cm
- Blank: Medium (0.1 N HCl)

$$\%\ \boldsymbol{Drug\ release} = \frac{Au}{As}\ X\frac{W1}{100}X\frac{900}{W2}X\frac{W3}{L.C}X\frac{p}{100}X10 \qquad \text{-----(9)}$$

Au: Absorbance of sample

As: Mean Absorbance of Standard.

W1: Weight of Mycophenolate Mofetil working standard in mg

W2: Weight of sample taken mg/ml (After weight – Before Weight)

L.C.: Suspension Label Claim of Mycophenolate Mofetil (mg/ml)

P: Potency of working standard is in %on as such basis.

Tolerance: NLT 80% (Q) of the labeled amount of Mycophenolate Mofetil is Dissolved.

(C) Particle Size:

Reference: In House

Instrument Used: Malvern Mastersizer 2000 with hydro 2000 S Sample handling Unit

Measuring Range: 0.02 to 2000µ

Model: General purpose

Measurement Time: 6 Seconds

Measurement snaps: 6000

Background Time: 6 Seconds

Background snaps: 6000

Stirrer Speed: 1900 RPM

Obscuration limits: 15% - 25%

Dispersant: Water

Sample Preparation: Weigh about 250 mg of substance being examined in 100 ml volumetric flask; add 25 ml of water and 2 drops of Nonidet P 40. Sonicate for about 2 minute and mix well.

Procedure: Ensure that laser intensity is more than 70%. Add the sample into Malvern Mastersizer 2000 to attain obscuration value between 15 to 25%. Calculate the particle size for two minute. Measure the sample and report the value.

(D) Related Substance

Buffer Phase I: Add 10 ml Triethylamine into a 1000 ml volumetric flask containing about 950 ml of water, and mix. Adjust with phosphoric acid to pH 7.2 and dilute with water to volume.

Buffer Phase II: Add 10 ml Triethylamine into a 1000 ml volumetric flask containing about 950 ml of water, and mix. Adjust with phosphoric acid to pH 3.0 and dilute with water to volume.

Solution A: Buffer I: Water = 4:9

Mobile Phase: Solution A: Acetonitrile = 7:3

Extraction Solvent: Buffer II: Water: Acetonitrile = 4:9:13

Diluents: Buffer II: Water: Acetonitrile = 4:9:7

Standard stock solution: 40 mg Mycophenolate Mofetil is added into 10 ml extraction solvent (4mg/ml). Sonicate to aid the dissolution.

Standard Solution: Taken 2 ml stock solution and dilute with up to 20 ml diluent.

Sample Stock Solution: Reconstitute 800 mg Mycophenolate Mofetil into 200 ml volumetric flask. Add 150 ml extraction solvent and Mix well. Sonicate for 10 minute. While taking care to maintain temperature of ultrasonic bath between 20ºC to 25ºC temperature. Dilute up to 200 ml volume with extraction solvent and mix well.

Sample Solution: Transfer 5.0 ml of sample stock solution to 50 ml volumetric flask, and dilute with diluents to volume. Pass through filter 45µm pore size.

Chromatographic system:

- Mode: LC
- Detector: UV 249nm
- Column: Kromasil 100-5 phenyl (250 mm X 4.6 mm) (Make: Akzonobel)
- Flow Rate: 1.5 ml/min
- Column Temperature: 45ºC
- Auto sampler temperature: 5ºC
- Injection Volume: 25µL

System Suitability Solution: 0.01 mg/ml of USP Mycophenolate Mofetil related compound A RS and 0.01 mg/ml of USP Mycophenolate Mofetil related compound B RS in diluents.

Sensitivity solution: 0.2µg/ml in diluents, from standard solution.

System Suitability: Sample: Standard solution, System Suitability solution, and sensitivity solution.

Suitability requirement: Resolution: Not Less Than 2.0 between Mycophenolate Mofetil related compound A, Mycophenolate Mofetil related compound B, system suitability solution.

- **Signal to noise ratio**: NLT 10 for Sensitivity solution.
- **Tailing Factor:** NMT 2.0 for Standard Solution
- **Relative Standard Deviation**: NMT 2.0% for standard solution

Analysis Sample: Sample solution and standard solution

Calculate % of each impurity in portion of Mycophenolate Mofetil for oral suspension taken.

$$\textbf{\textit{Relative Substance}} = \frac{Ru}{Rs} X \frac{Cs}{Cu} X \frac{1}{F} X 100 \qquad \text{---------(10)}$$

Where

Ru: Peak response of each individual impurity from sample solution.

Rs: Peak response of each individual impurity from standard solution.

Cs: Concentration of Mycophenolate Mofetil in standard solution.

Cu: Concentration of Mycophenolate Mofetil in sample solution.

F: Relative response factor

Table 4.21 Relative Substance profile for Mycophenolate Mofetil ROS

Name	Relative retention time	Relative response factor	Acceptance criteria NMT%
Mycophenolic Acid	0.12	1.4	3.3
Sorbitol ester of Mycophenolic Acid	0.24	0.77	0.2
Mycophenolate Mofetil	1.00	-	-
Any individual unspecified impurities	-	1.0	0.1
Total Impurities Not More Than 3.8%			

(E) Deliverable Volume:

The following test is give assurance that oral liquids when transferred from the original container, will deliver the volume of dosage form that is confirmed on the label of the article. These tests are applicable for less than 250 ml products, whether supplied as liquid preparations or reconstituted suspension. When content of the container was transfer to measuring cylinder , avoid the air bubble. Allow each container to drain for a period not to exceed 30 min for multiple unit containers and 5 sec for single unit containers, unless otherwise specified in the monograph. Measure the volume of each mixture when it is free from air bubble.

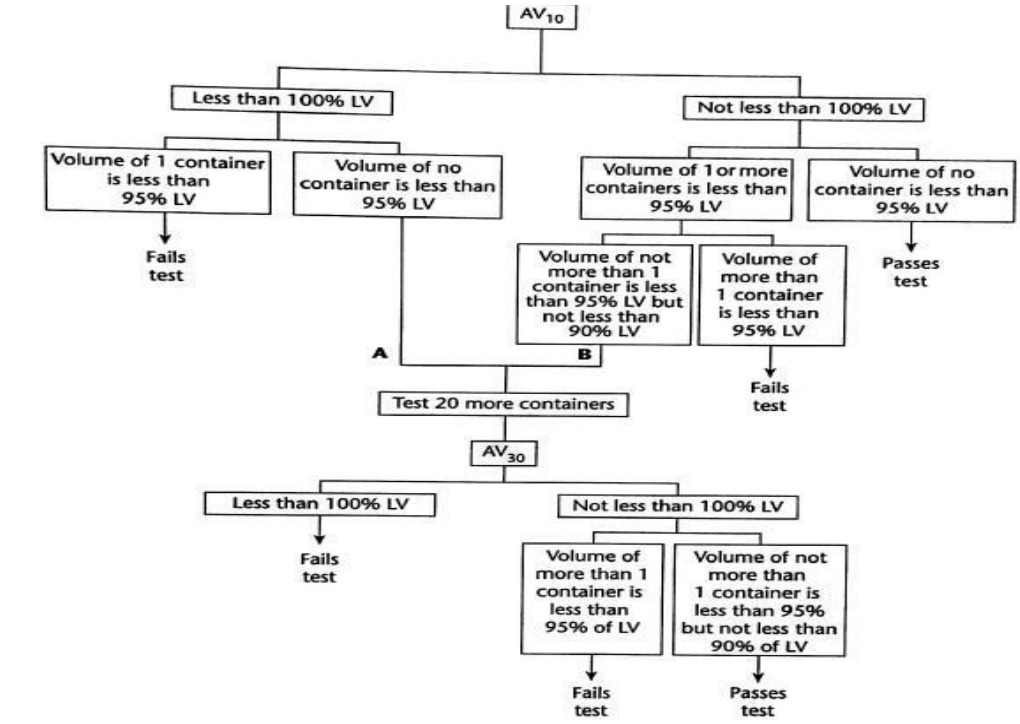


Figure 4.6 Procedure for measuring deliverable volume for multiple unit containers

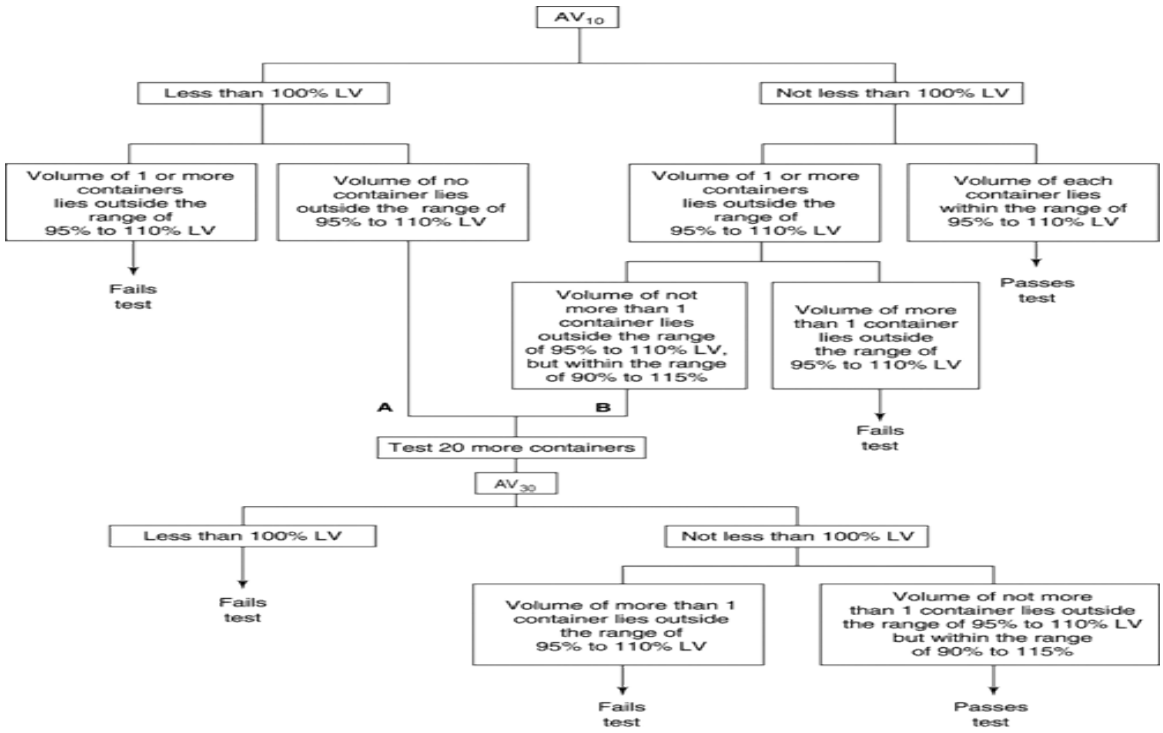


Figure 4.7 Procedure for measuring deliverable volume for single unit container

(F) pH measurement:

The pH value is representing the acidity or alkalinity of an aqueous solution. pH of suspension is often adjusted to ensure drug remains insoluble. Change in pH of the suspension followed by reconstitution was measured for all the formulations using a digital pH meter on day 1 and day 60 at 25ºC.

(G)Viscosity:

Brookfield viscometer (DV-II, Brookfield Eng. Lab. INC, USA) was used to measure viscosity and it is attached with spindle. Suspension (75 ml) was taken in 100 ml beaker. Viscosity should be high so that it hinders rapid sedimentation. Acceptable range of viscosity is 200 - 2500 cps but preferred range is 400 - 1000 cps.

(H) Sedimentation Volume (F):

The suspension was evaluated for physical stability by determining the sedimentation Volume. 50 ml of suspension was taken in 100 ml graduated measuring cylinder. The suspension was dispersed systematically by moving upside down for three times. After that, suspension was allowed to settle for three minutes and the volume of sediment was noted. This is the original volume of sediment (H_0).The cylinder was kept undisturbed for 14 days. The volume of sediment was read at 1 hr and volume on 14th day was considered as final volume of sediment (Hu).

$$\boldsymbol{Sedimentation\ Volume} = \frac{\boldsymbol{Hu}}{\boldsymbol{Ho}} \qquad \textbf{-------- (11)}$$

The sedimentation volume can have values ranging from 0 to1. The ultimate height of the solid phase after settling depends on the concentration of solid and particle size.

(I) Homogeneity:

After minimum shaking of the package containing suspension, the amount of drug substance present at top, bottom, middle of the packed suspension, will be equivalent within 10%.

(J) Percentage easy of redispersibility:

It is important parameter which reflects the quality of suspensions. It was checked after 1 week. The stored suspension in a measuring cylinder was inverted through 180 degree and number of inversions necessary to restore a homogeneous suspension was determined.

4.5 FORMULATION, DEVELOPMENT AND OPTIMIZATION USING DIRECT BLENDING APPROACH

Table 4.22 Composition of formulation trials using direct blending approach

Batch No	1001		1002		1003	
Ingredients	**%w/w**	**mg/ml**	**%w/w**	**mg/ml**	**%w/w**	**mg/ml**
Mycophenolate Mofetil	31.82	200.00	31.82	200.00	31.82	200.00
Sorbitol (Neosorb P 100 T)	61.10	384.06	60.30	379.03	60.35	379.34
Citric acid anhydrous	0.25	1.57	0.25	1.57	0.25	1.57
TriSodium citrate dehydrate	3.10	19.49	3.1	19.49	3.1	19.49
Methyl paraben	1.60	10.06	1.6	10.06	1.6	10.06
Xanthan gum	0.25	1.57	0.25	1.57	0.2	1.26
Colloidal silicon dioxide	0.80	5.03	1.6	10.06	1.6	10.06
Aspartame	0.25	1.57	0.25	1.57	0.25	1.57
Mix fruit flavor	0.83	5.22	0.83	5.22	0.83	5.22
Total	**100.00**	**628.57**	**100.00**	**628.57**	**100.00**	**628.57**

Procedure:

Step 1: Weight accurately citric acid (anhydrous), trisodium citrate dihydrate, methyl paraben, xanthan gum, colloidal silicon dioxide, aspartame and mixed fruit flavor.

Step 2: Weigh accurately MMF and sorbitol.

Step 3: Ingredient of step 1 were co-sifted with the 30 to 40 gm sorbitol through 40# Sieve.

Step 4: Ingredients of Step 2 were co-Sifted through 20# sieve.

Step 5: Ingredients in Step 1 and Step 2 were mixed in blender for the 15 min for 24 RPM

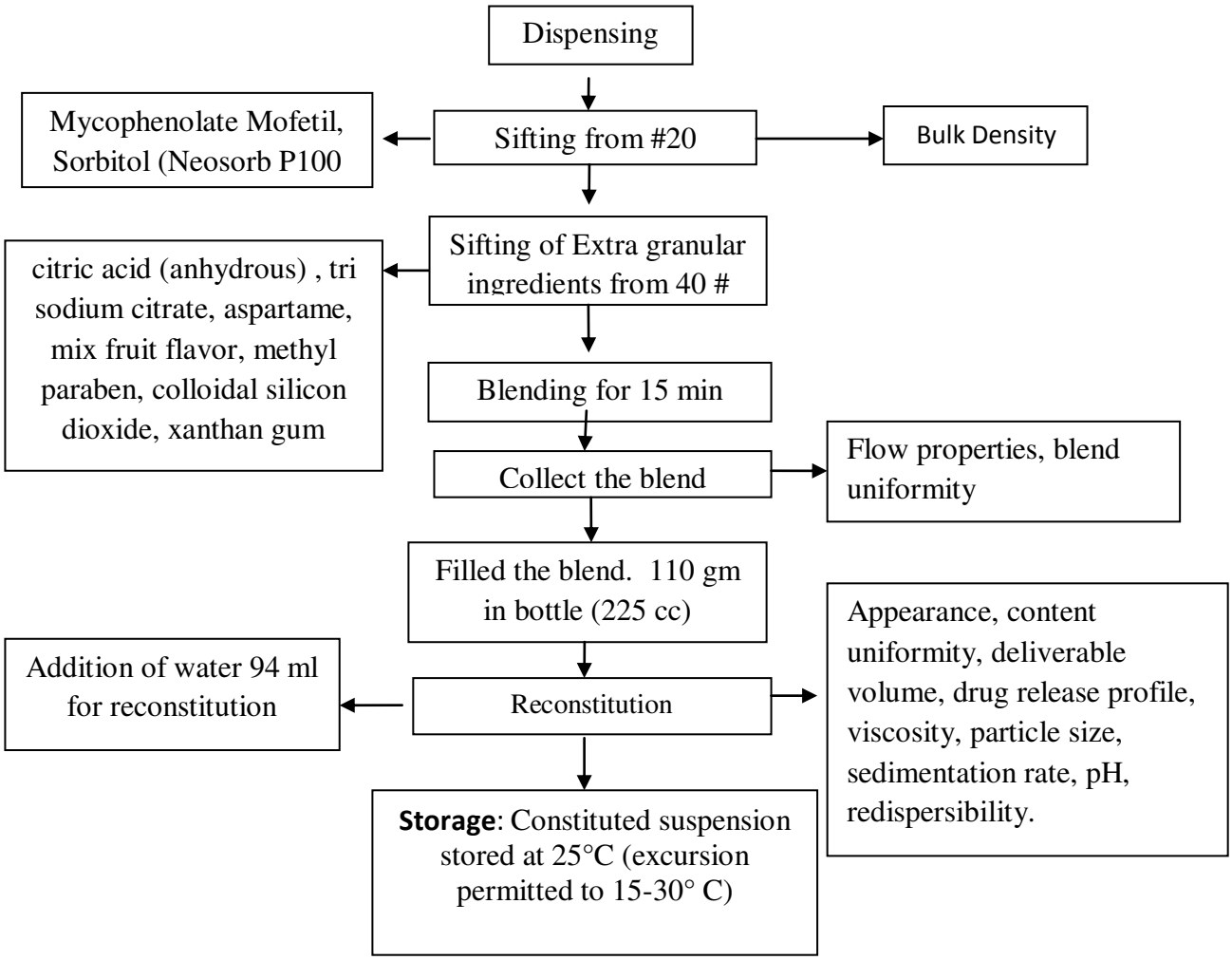
Step 6: Samples (n = 10) were withdrawn from blender as per sampling protocol for the blend uniformity.

Step 7: Filled 110 gm blend in 225cc bottle.

Step 8: For reconstituted with 94 ml of purified water and evaluated for appearance, content uniformity, deliverable volume, drug release profile, viscosity, particle size, sedimentation rate, pH and redispersibility.

Direct Blending

Ingredient ***Process*** ***Evaluation***



Result:

Table 4.23 Result of evaluation parameter of batches 1001 to 1003

Parameter		1001	1002	1003
Bulk Density (gm/ml)		0.411	0.532	0.556
Tapped Density (gm/ml)		0.742	0.781	0.806
Carr's Index		44.643 (Very Very Poor)	31.915 (Poor)	31.111 (Poor)
Hausner's Ratio		1.806 (Very Very Poor)	1.469 (Poor)	1.452 (Poor)
Deliverable Volume (ml)		Not evaluated	165	170
pH			5.2	5.3
Viscosity (cps)			2250	1200
Blend Uniformity (%)8	**Mean**		96.42	98.35
	Min – Max		90.30 – 101.1	91.3 – 102.9
	RSD		3.72	4.22
Content Uniformity (%)	**Mean**		94.43	95.97
	Min – Max		90.80 – 101.10	91.1 – 101.9
	RSD		3.42	3.59
Drug Release in 20 min (%)			Not evaluated	Not evaluated

Discussion

In batch 1001, it was observed that Carr's index was 44.643 and Hausner's ratio was 1.806. From this observation it was considered that lubricated blend has very very poor flow property and bottle could not be filled due to poor flow property and was not further evaluated. MMF is less dense material having very poor flow property. Colloidal silicon dioxide was used as glidant as increasing concentration of colloidal silicon dioxide should improve flow property of lubricated blend, however there was no significant improvement of flow property as concentration of colloidal silicon dioxide was increased from 0.8 %w/w to 1.6 %w/w in batch no 1002 and 1003. The viscosity of suspension was affected by concentration of xanthan gum. As concentration of xanthan gum was decrease from 0.25 %w/w to 0.2 %w/w in batch 1002 to batch 1003, viscosity decreased from 2250 cps to 1200 cps. From these results, it can be concluded that viscosity was considerably higher than expected value 400 cps -1000 cps. Therefore concentration of xanthan gum should be decrease in the further batches. Soybean lecithin is a waxy material which acts as wetting agent. As it is waxy material it cannot sifted from sieve and could not be added directly in formulation by direct blending approach.

Therefore, direct blending approach is not suitable for formulation of Reconstitutable Oral Suspension.

4.6 FORMULATION TRAILS USING WET GRANULATION APPROACH (RAPID MIXER GRANULATOR)

The aim was to formulate Mycophenolate Mofetil Reconstitutable Oral Suspension (200 mg/ml), which is robust, stable and bioequivalent to the innovator product. The product development work was initiated in line with innovator product CellCept® 200 mg/ml oral suspension. Since, the % of drug substance in reconstituted suspension was very high and Mycophenolate Mofetil being immuno-suppressant drug formulation, development was not initiated by wet granulation approach (using RMG). It was desirable to use closed assembly system for granulation e.g. Fluid bed granulator/drier. Trial details of initial feasibility batches are compiled in Table 4.24.

4.7 FORMULATION TRIALS USING WET GRANULATI METHOD (FLUID BED PROCESSOR (FBP) USING (TOP SPRAY))

4.7.1 Preliminary trials

Table 4.24 Composition for preliminary trials using FBP

Ingredient	1004		1005		1006	
	%w/w	mg/ml	%w/w	mg/ml	%w/w	mg/ml
Dry Mix						
Mycophenolate Mofetil	31.82	200.01	31.82	200.01	31.82	200.01
Sorbitol (Neosorb P 100 T)	31.82	200.01	31.82	200.01	31.82	200.01
Granulation						
Citric acid anhydrous	0.25	1.57	0.25	1.57	**0.20**	**1.26**
TriSodium citrate dihydrate	3.10	19.49	3.10	19.49	3.10	19.49
Soybean lecithin	**0.32**	**2.01**	**0.48**	**3.02**	**0.64**	**4.02**
Extra Granular						
Sorbitol (Neosorb P 100 T)	28.24	177.51	28.08	176.50	27.97	175.81
Methyl paraben	1.60	10.06	1.60	10.06	1.60	10.06
Xanthan gum	**0.17**	**1.07**	0.17	1.07	0.17	1.07
Colloidal silicon dioxide	1.60	10.06	1.60	10.06	1.60	10.06
Aspartame	0.25	1.57	0.25	1.57	0.25	1.57
Mix fruit flavor	0.83	5.22	0.83	5.22	0.83	5.22
Total	**100.00**	**628.57**	**100.00**	**628.57**	**100.00**	**628.57**

Procedure:

- Ingredients of dry mix were sifted **through 20 # sieve** and collected separately.
- Extra granular materials were sifted through **40 # sieve** and kept separately.
- Dry mix materials were loaded in PalmGlatt (GPGC1.1) and mixed for 3 min. **Soybean lecithin, citric acid (anhydrous), trisodium citrate dihydrate were dissolved in 80 gm purified water**.
- Dry mix was granulated with this binder solution by fluid bed granulation process. The granulation parameters are described in Table 4.25.

Table 4.25 Granulation Parameter

Base Plate	C
Inlet Temperature	45 - 55ºC
Product temperature	28 - 35ºC
Atomization pressure	1 - 1.2
Blower Speed	35 – 45
CFM	35 – 45
Spray Rate	**5 gm/min**

- Wet mass was dried till NMT 1%w/w loss on drying of granules at 75°C was obtained.
- Dried granules were milled through 0.5 mm screen.
- **Xanthan gum, sorbitol (Neosorb P100 T), methyl paraben, aspartame, colloidal silicon dioxide were added to the dried granules and mixed for 15 min in blender.**
- The blend was evaluated by blend uniformity and flow property.
- The 110 gm blend was filled into 225 cc bottle and reconstituted with 94 ml of purified water. Suspension was evaluated for appearance, content uniformity, deliverable volume, drug release profile, viscosity, particle size, sedimentation rate, pH and redispersibility.
- **Storage Condition**: Constituted suspension was stored at 25°C (excursion permitted to 15-30° C)

Result:

Table 4.26 Evaluation parameter of batches 1004 to 1006

Parameter		1004	1005	1006
Bulk Density (gm/ml)		0.635	0.596	0.497
Tapped Density (gm/ml)		0.840	0.773	0.654
Carr's Index		24.444 (Passable)	22.807 (Passable)	23.913(Passable)
Hausner's Ratio		1.324 (Passable)	1.295(Passable)	1.314(Passable)
Deliverable Volume (ml)		186 (0.32)	180 (0.48)	168 (0.64)
pH		5.1	5.3	5.9
Viscosity (cps)		850	890	900
Blend Uniformity (%)	**Mean**	95.58	96.67	95.8
	Min – Max	90.80 – 101.80	91.10 – 101.4	91.5 – 102.6
	RSD	3.96	3.57	3.52
Content Uniformity (%)	**Mean**	93.31	93.73	94.22
	Min – Max	91.30 – 97.50	90.80 – 97.40	90.30 – 97.20
	RSD	1.80	2.42	2.54

Discussion

Batches 1004 to 1006 were prepared by Fluid bed processor approach using different concentration of soybean lecithin, citric acid, xanthan gum and the effect on formulation was analyzed**.** Comparing the flow property of batches (1004 to 1006) was (1001 to 1003), it was concluded that flow property was better using Fluid Bed Processor compared to direct blending.

For proper distribution of citric acid (anhydrous), it was dissolved in water and sprinkled using top spray method on the dry mix. Soybean lecithin having waxy nature was dissolved in water with citric acid (anhydrous), trisodium citrate dihydrate and sprinkled on dry mix.

Deliverable volume should be between 174 to178 ml. Soybean lecithin has significant effect on deliverable volume. As concentration of soybean lecithin was increased from 0.32 %w/w to 0.64 %w/w in batch 1004 to batch 1006, deliverable volume decreased from 186 ml to 168 ml. In batch 1005, Deliverable volume 180 ml which was near to deliverable volume of innovator (176 ml), so concentration of soybean lecithin was fixed at 0.48 %w/w.

pH of suspension should be between 6 to7. From the result it was also observed that the pH of suspension was affected by concentration of citric acid (anhydrous). As concentration of citric acid (anhydrous) was decreased from 0.25 % to 0.2 % w/w in batch 1004 to batch 1006, pH increased from 5.1 to 5.9. Hence, in next trials concentration of citric acid (anhydrous) was reduced.

Viscosity of suspension should be between 600 cps to700 cps. The concentration of xanthan gum was kept at 0.17 %w/w for batches 1004 to 1006 which gave viscosity between 800 cps - 900 cps. As desired viscosity was lesser (600 cps -700 cps) than previous batch, it was decided to use with decreased concentration of the xanthan gum in next batches.

4.7.2 Optimization of Excipient concentration

Table 4.27 Composition for optimization of excipient concentration

Ingredient	1007		1008		1009		1010	
	%w/w	mg /ml	%w/w	mg /ml	%w/w	mg /ml	%w/w	mg/ ml
Dry Mix								
Mycophenolate Mofetil	31.82	200.01	31.82	200.01	31.82	200.01	31.82	200.01
Sorbitol (Neosorb P 100 T)	31.82	200.01	31.82	200.01	31.82	200.01	31.82	200.01
Granulation								
Citric acid (anhydrous)	0.20	1.26	0.15	0.94	0.15	0.94	**0.13**	**0.82**
Trisodium citrate dehydrate	**2.50**	**15.71**	2.50	15.71	2.50	15.71	2.50	15.71
Soybean lecithin	**0.48**	**3.02**	0.48	3.02	0.48	3.02	0.48	3.02
Extra Granular								
Sorbitol (Neosorb P100 T)	29.53	185.62	29.2	183.54	29.22	183.67	29.24	183.80
Methyl paraben	1.60	10.06	1.60	10.06	1.60	10.06	1.60	10.06
Xanthan gum	0.17	1.07	0.17	1.07	**0.15**	**0.94**	0.15	0.94
Colloidal silicon dioxide	**0.80**	**5.03**	**1.18**	**7.42**	1.18	7.42	1.18	7.42
Aspartame	0.25	1.57	0.25	1.57	0.25	1.57	0.25	1.57
Mix fruit flavor	0.83	5.22	0.83	5.22	0.83	5.22	0.83	5.22
Total	**100**	**628.57**	**100**	**628.57**	**100**	**628.57**	**100**	**628.57**

Procedure:

- Ingredients of **dry mix were sifted through 30# sieve** and collected separately.
- Dry mix materials were loaded in PalmGlatt and mixed for 3 min.
- **Soybean lecithin, citric acid (anhydrous), trisodium citrate dihydrate were dissolved in 80 gm purified water**. Dry mix was granulated with this binder solution by fluid bed granulation process. The granulation parameters are described in Table 4.25
- Wet mass was dried till NMT 1%w/w loss on drying of granules at 75°C was obtained.
- Dried granules were milled through 0.5 mm screen.
- In extra granular portion xanthan gum, mixed fruit flavor, methyl paraben, aspartames were sifted through 60# sieve. Sorbitols (Neosorb P100 T), colloidal silicon dioxide were shifted through 40# sieve and were added to the dried granules and mixed for 15 min in blender.
- The blend was evaluated by blend uniformity and flow property. 110 gm blend was filled into 225 cc bottle and reconstituted with 94 ml of purified water and evaluated for appearance, content uniformity, deliverable volume, drug release profile, viscosity, particle size, sedimentation rate, pH and redispersibility of the reconstituted suspension.
- **Storage Condition**: Constituted suspension store at 25°C (excursion permitted to 15-30° C)

Result:

Table 4.28 Result of evaluation parameter of batch 1007 to 1010

Batch no		1007	1008	1009	1010
Bulk Density (gm/ml)		0.590	0.608	0.608	0.595
Tapped Density (gm/ml)		0.810	0.781	0.792	0.770
Carr's Index		27.083 (Poor)	22.222 (Passable)	23.333 (Passable)	22.785 (Passable)
Hausner's Ratio		1.371 (Poor)	1.286 (Passable)	1.304 (Passable)	1.295 (Passable)
Particle Size Distribution	**40#**	Not Evaluated	Not Evaluated	Not Evaluated	4%
	60#				6%
	100#				27%
	Base				63%
Deliverable Volume (ml)		176	176	176	178
pH		5.75	6.5	6.52	6.76
Viscosity (cps)		790	870	620	640
Blend Uniformity (%)	**Mean**	98.1	98.51	98.3	98.8
	Min – Max	91.9- 101.9	92.2- 102.4	92.2 -101.9	94.2-102.6
	RSD	3.39	3.46	3.23	3.13
Content Uniformity (%)	**Mean**	95.69	95.53	96.10	96.33
	Min – Max	92.30-100.5	92.3 – 99.3	92.4 – 99.9	92.1 – 100.3
	RSD	3.26	2.90	2.85	2.98
Drug Release in 20 min (%)		Not Evaluated	81.6	83.5	83.08

Table 4.29 Physical observations at 30ºC/65%RH after 1 month for batch 1008 and 1010.

	1008	1010
Deliverable Volume (ml)	170	172
pH	6.28	6.69
Viscosity (cps)	850	700
Content Uniformity (%)	Not Evaluated	96.2
Drug Release in 20 min (%)	Not Evaluated	82.85

Table 4.30 Comparison of drug release for 1010 (Initial Vs 1 month stability)

Batch 1010	Initial	After 1 month at 30ºC/65%RH
5	41.36	38.51
10	48.73	46.84
15	63.12	61.53
20	83.08	82.85
30	99.73	99.85

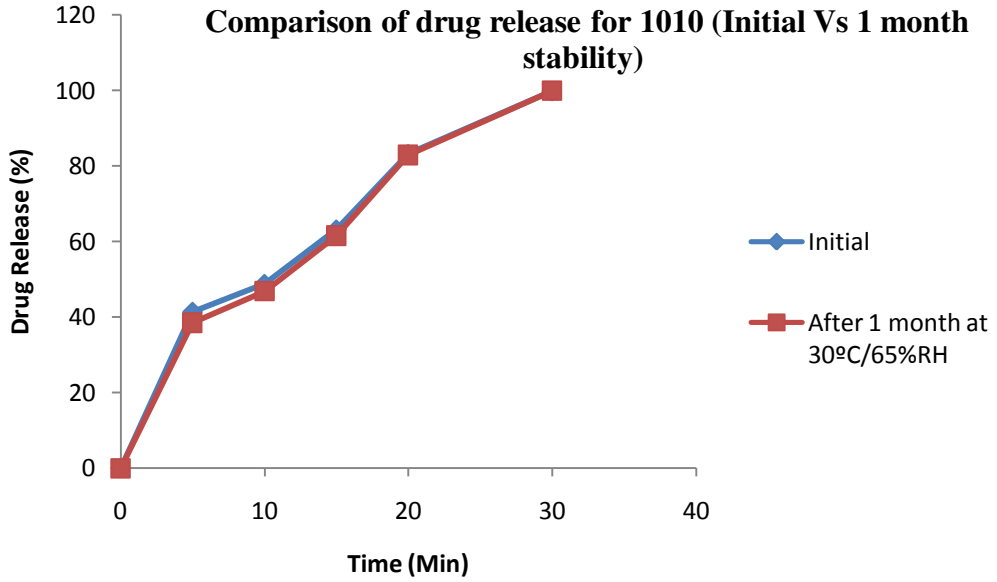


Figure 4.8 Comparative drug release of batch 1010 (Initial Vs after 1 month)

Discussion:

Batches 1007 to 1010 were prepared to select the excipient concentration. Batch 1007 was prepared with 2.50 %w/w of trisodium citrate dihydrate to study its impact on pH of suspension. Results revealed that concentration of trisodium citric dihyadte did not have any significant effect on pH of formulation so concentration of trisodium citric dihyadte was fixed at 2.50 %w/w.

It is desirable use minimum concentration of colloidal silicon dioxide, so batch 1007 was prepared with decreased amount of colloidal silicon dioxide. Problem of caking after 24 hrs of reconstitution was observed so in batch 1008 concentration of colloidal silicon dioxide was kept at 1.18 %w/w with a view to overcome caking. No caking was observed in case of batch 1008 and concentration of colloidal silicon dioxide was finalized at 1.18 %w/w for next batches.

Batch 1010 was prepared by decreasing the concentration of citric acid to 0.13 %w/w from 0.15 %w/w. No significant change in pH after 1 month stability study was observed (From 6.73 to 6.4) which was in desired range. The concentration of citric acid (anhydrous) was fixed at 0.13 %w/w for further batches. In case of batch 1008, Viscosity was 870 cps which was more than preferred range. So in batch 1010 concentration of xanthan gum was reduced from 0.17 % to 0.15 %w/w. The desired viscosity 600 -700 cps was observed. Additionally, for batch 1010 drug release profile was checked after stability was carried out and compared with that of initial drug release profile. Results revealed that there was no significant change in drug release profile. For batch 1010, deliverable volume after stability study was carried out and compared with that of initial deliverable volume. Deliverable Volume initially was 176 ml and after 1 month it was 170 ml and %RSD of blend uniformity was in acceptable range (<6%) but not in preferred range (<2%), which was addressed in the next stage of development. Thus optimization of process parameter was taken for further trials.

4.7.3 Optimization of the quantity of water in granulation

Table 4.31 Optimization of quantity of water in granulation

Quantity of Water	120 gm		160 gm	
Ingredient	1011		1012	
	%w/w	mg /ml	%w/w	mg/ ml
Dry Mix				
Mycophenolate Mofetil	31.82	200.01	31.82	200.01
Sorbitol (Neosorb P 100 T)	31.82	200.01	31.82	200.01
Granulation				
Citric acid anhydrous	0.13	0.82	0.13	0.82
TriSodium citrate dihydrate	2.50	15.71	2.50	15.71
Soybean lecithin	0.48	3.02	0.48	3.02
Extra Granular				
Sorbitol (Neosorb P 100 T)	29.24	183.80	29.24	183.80
Methyl paraben	1.60	10.06	1.60	10.06
Xanthan gum	0.15	0.94	0.15	0.94
Colloidal silicon dioxide	1.18	7.42	1.18	7.42
Aspartame	0.25	1.57	0.25	1.57
Mix fruit flavor	0.83	5.22	0.83	5.22
Total	**100**	**628.57**	**100**	**628.57**

Procedure:

- Ingredients of dry mix were sifted through 30 # sieve and collected separately.
- Dry mix materials were loaded in PalmGlatt and mixed for 3 min.
- **Soybean lecithin, citric acid (anhydrous), trisodium citrate dihydrate were dissolved in 120 gm and 160 gm purified water in 1011 and 1012 respectively.**
- Dry mix was granulated with binder solution by fluid bed granulation process. The granulation parameters are described in Table 4.25
- Wet mass was dried till NMT 1%w/w loss on drying of granules at 75°C was obtained.
- Dried granules were milled through 0.5 mm screen.

- In extra granular portion xanthan gum, mixed fruit flavor, methyl paraben, aspartames were sifted through 60# sieve. Sorbitol(Neosorb P100 T), colloidal silicon dioxide were shifted through 40# sieve and were added to the dried granules and **mixed for 15 min in blender**.
- The blend was evaluated by blend uniformity and flow property. 110 gm blend was filled into 225 cc bottle and reconstituted with 94 ml of purified water and evaluated for appearance, content uniformity, deliverable volume, drug release profile, viscosity, particle size, sedimentation rate, pH and redispersibility of the reconstituted suspension.
- **Storage Condition**: Constituted suspension store at at 25°C (excursion permitted to 15-30° C)

Result:

Table 4.32 Result of evaluation parameters of batches 1011 and 1012

Batch		**1011**	**1012**
Bulk Density (gm/ml)		0.671	0.747
Tapped Density (gm/ml)		0.815	0.878
Carr's Index		17.647 (Fair)	14.894(Good)
Hausner's Ratio		1.214 (Fair)	1.175 (Good)
Particle Size Distribution	**40#**	4%	8%
	60#	14%	16%
	100#	32%	29%
	Base	50%	43%
Deliverable Volume (ml)		178	178
pH		6.7	6.72
Viscosity (cps)		670	650
Blend Uniformity (%)	**Mean**	98.04	98.25
	Min – Max	93.20 – 101.9	92.3 – 101.3
	RSD	3.16	3.15
Content Uniformity (%)	**Mean**	96.02	98.03
	Min – Max	93.7 – 99.2	95.7 – 99.80
	RSD	1.73	1.33
Drug Release in 20 min (%)		82.6	81.2

Table 4.33 Physical observation of batch 1012 at 30ºC/65%RH after 1 month.

Batch no	1012	
	1 month	2 month
Deliverable Volume (ml)	170	166
pH	6.63	6.4
Viscosity (cps)	650	670
Content Uniformity (%)	96.1	97.1
Drug Release in 20 min (%)	82.8	83.1

Discussion:

In case of batches 1011 and 1012, quantity of water used for granulation was 120 gm and 160 gm respectively. Composition and process parameters were kept same as previous batches. It was observed that increasing the amount of water increased flow property. From the results of sieve analysis study for both batches, it was also observed that granules to fine ratio was increased which indicated less chances of segregation. The flow property of Batch 1012 was better than batch 2011. Hence it was decided to conduct stability study of batch 1012 at 30ºC/65%RH for 2 month. From the stability evaluation it was observed that all evaluated parameter were within the range except deliverable volume.

From the previous experience it was observed that deliverable volume decreased during stability study. This may be a result of decrease in the void spaces and liberation of entrapped air between particles. During blending process, particles are being rolled over each other in mixing process which may decrease air entrapment. So it was desired to optimize blending time.

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4.7.4 Optimization of blending time

Table 4.34 Optimization of blending time

Ingredient	1013*	
	%w/w	mg /ml
Dry Mix		
Mycophenolate Mofetil	31.82	200.01
Sorbitol (Neosorb P 100 T)	31.82	200.01
Granulation		
Citric acid anhydrous	0.13	0.82
TriSodium citrate dihydrate	2.50	15.71
Soybean lecithin	**0.48**	**3.02**
Extra Granular		
Sorbitol (Neosorb P 100 T)	29.24	183.80
Methyl paraben	1.60	10.06
Xanthan gum	0.15	0.94
Colloidal silicon dioxide	1.18	7.42
Aspartame	0.25	1.57
Mix fruit flavor	0.83	5.22
Total	**100**	**628.57**

*Sized granules were divided into **three equally** parts.

Batch no	**1013 A**	**1013 B**	**1013 C**
Blending time	15 min	30 min	45 min

Procedure:

- Ingredients of dry mix were sifted through sieve # 30 and collected separately.
- Dry mix materials were loaded in Palmglatt and mixed for 3 min.
- **Soybean lecithin, citric acid (anhydrous), trisodium citrate dihydrate were dissolved in 160 gm similar as batch 1012.**
- Dry mix was granulated with this binder solution by fluid bed granulation process. The granulation parameters are described in Table 4.25
- Wet mass was dried till NMT 1%w/w loss on drying of granules at 75°C was obtained.
- Dried granules were milled through 0.5 mm screen.
- Sized granules were divided into **three equally** parts.

- According to % yield of dry granules, extra granular portion consisting xanthan gum, mixed fruit flavor, methyl paraben, aspartame were sifted through 60# sieve, sorbitol (Neosorb P100 T), colloidal silicon dioxide were sifted through 40# **sieve.** Dry mix and extra granular portion were mixed for 15 min, 30 min, 45 min for respectively batch no 1013 A, 1013 B , 1013 C.
- The 110 gm blend was filled into 225 cc bottle and reconstituted with 94 ml of purified water. Suspension was evaluated the appearance, content uniformity, deliverable volume, drug release profile, viscosity, particle size, sedimentation rate, pH and redispersibility of the reconstituted suspension.
- **Storage Condition**: Constituted suspension was stored at 25°C (excursion permitted to 15-30° C)

Result:

Table 4.35 Results of effect of blending time

Batch no		**1013**		
		A	**B**	**C**
Blending time (min)		**15**	**30**	**45**
Bulk Density (gm/ml)		0.729	0.718	0.711
Tapped Density (gm/ml)		0.850	0.822	0.827
Carr's Index		14.286 (Good)	12.698 (Good)	14.00(Good)
Hausner's Ratio		1.167 (Good)	1.145 (Good)	1.163(Good)
Deliverable Volume (ml)		178.00	178	180
pH		6.60	6.68	6.72
Viscosity (cps)		620	640	650
Blend Uniformity (%)	**Mean**	98.38	99.01	99.13
	Min – Max	93.4 -101.6	96.2 -101.2	96.9-100.9
	RSD	3.13	1.47	1.37
Content Uniformity (%)	**Mean**	98.12	98.62	98.91
	Min – Max	96.1 – 100.1	96.5 – 100.3	96.8 – 100.6
	RSD	1.72	1.38	1.37
Drug Release in 20 min (%)		Not evaluated	84.1	84.2

Table 4.36 Physical observation of batch 1013 at 30ºC/65%RH after 2 month.

Batch	1013 A		1013 B		1013 C	
Month	**1**	**2**	**1**	**2**	**1**	**2**
Deliverable Volume (ml)	170	Not Evaluated	176	175	178	178
pH	6.3		6.55	6.50	6.63	6.57
Viscosity (cps)	600		620	630	630	630
Assay of Reconstituted Suspension (%)	97.8		98.2	98.3	98.5	98.7
Drug Release in 20 min (%)	Not Evaluated		83.4	82.9	83.2	83.5

Discussion:

Batches 1013 A to 1013 C were prepared to optimize blending time. In case of batch 1013 A the blending time was 15 min and %RSD of blend uniformity was observed to be more than 3%. Additionally after one month stability study at 30ºC/65%RH, it was observed that deliverable volume was decreased from 178 ml to 170 ml, which was not desirable.

Batch 1013 B & 1013 C were prepared with blending time of 30 min and 45 min respectively. % Relative Standard Deviation (RSD) of blend uniformity was less than 2% in both the batches. So based on results, both batches were selected for conducting the stability studies for 2 month at 30ºC/65%RH. The results of stability study revealed that deliverable volume of both the batches was near to desirable value 175 ml. So, blending time of 30 min was optimized.

As per theory Spray rate was effects on the evaluation parameter like % drug release and flow property so spray rate should be optimized.

4.7.5 Optimization of Spray Rate

Table 4.37 Optimization of spray rate in batch 1014 to 1016

Amount of Water in granulation	**160 gm**					
Blending time	**30 min**					
Ingredient	**1014**		**1015**		**1016**	
	%w/w	**mg/ml**	**%w/w**	**mg/ml**	**%w/w**	**mg/ml**
Dry Mix						
Mycophenolate Mofetil	31.82	200.01	31.82	200.01	31.82	200.01
Sorbitol (Neosorb P 100 T)	31.82	200.01	31.82	200.01	31.82	200.01
Granulation						
Citric acid anhydrous	0.13	0.82	0.13	0.82	0.13	0.82
TriSodium citrate dehydrate	2.50	15.71	2.50	15.71	2.50	15.71
Soybean lecithin	0.48	3.02	0.48	3.02	0.48	3.02
Extra Granular						
Sorbitol (Neosorb P 100 T)	29.24	183.80	29.24	183.80	29.24	183.80
Methyl paraben	1.60	10.06	1.60	10.06	1.60	10.06
Xanthan gum	0.15	0.94	0.15	0.94	0.15	0.94
Colloidal silicon dioxide	1.18	7.42	1.18	7.42	1.18	7.42
Aspartame	0.25	1.57	0.25	1.57	0.25	1.57
Mix fruit flavor	0.83	5.22	0.83	5.22	0.83	5.22
Total	**100.00**	**628.57**	**100.00**	**628.57**	**100.00**	**628.57**
Spray rate	**15 gm/min**		**10 gm/min**		**5 gm/min**	

Procedure:

- Ingredients of dry mix were sifted through 30# sieve and collected separately.
- Dry mix materials were loaded in PalmGlatt and mixed for 3 min.
- **Soybean lecithin, citric acid (anhydrous), trisodium citrate dihydrate were dissolved in 160 gm purified water**.
- Dry mix was granulated with this binder solution by fluid bed granulation process. The granulation parameters are described in Table 4.38.

Table 4.38 Granulation parameter for 1014 to 1016

Base Plate		C
Inlet Temperature		45 - 55ºC
Product temperature		28 - 35ºC
Atomization pressure		1 - 1.2
Blower Speed		35 – 45
CFM		35 – 45
Spray Rate	**1014**	**15 gm/min**
	1015	**10 gm/min**
	1016	**5 gm/min**

- Wet mass was dried till NMT 1%w/w loss on drying of granules at 75°C was obtained.
- Dried granules were milled through 0.5 mm screen.
- In extra granular portion xanthan gum, mixed fruit flavor, methyl paraben, aspartame were sifted through 60# sieve. Sorbitol (Neosorb P100 T), colloidal silicon dioxide were sifted through 40# sieve were added to the dried granules and mixed **for 30 min in blender.**
- The blend was evaluated for blend uniformity, and flow property.
- The 110 gm blend was filled into 225 cc bottle and reconstituted with 94 ml of purified water. Suspension was evaluated the appearance, content uniformity, deliverable volume, drug release profile, viscosity, particle size, sedimentation rate, pH and redispersibility of the reconstituted suspension.
- **Storage Condition**: Constituted suspension was stored at 25°C (excursion permitted to 15-30° C)

Result

Table 4.39 Results optimization of spray rate

Batch No		1014	1015	1016
Bulk Density (gm/ml)		0.723	0.714	0.718
Tapped Density (gm/ml)		0.850	0.849	0.846
Carr's Index		14.894	15.873	15.068
Hausner's Ratio		1.175	1.189	1.177
Deliverable Volume (ml)		178.00	178.00	178.00
pH		6.79	6.80	6.80
Viscosity (cps)		690	680.00	670.00
Blend Uniformity (%)	Mean	99.15	98.78	98.78
	Min – Max	96.20 – 100.7	95.90 – 100.20	96.90 -100.90
	RSD	1.42	1.39	1.51
Content Uniformity (%)	Mean	98.50	98.61	98.60
	Min – Max	96.60 – 100.5	97.1 – 100.9	96.8 – 100.2
	RSD	1.43	1.38	1.39
Drug Release in 20 min (%)		NA	84.70	83.20
Relative Substance				
Mycophenolic Acid (MPA)		Not Evaluated	Not Evaluated	0.08
Sorbitol ester of MPA				ND
Mycophenolate Mofetil				0.01
Any individual unspecified Impurities				ND
Total				0.09

Table 4.40 Physical observation at 30ºC/65%RH of 1015, 1016

Batch		1015	1016
		1 month	1 month
Deliverable Volume (ml)		178	176
pH		6.6	6.65
Viscosity (cps)		660	640
Assay of Reconstituted Suspension (%)		Not Evaluated	98.5
Drug Release in 20 min (%)			83.2
Relative Substance	Mycophenolic Acid (MPA)	Not Evaluated	0.10
	Sorbitol ester of MPA		ND
	Mycophenolate Mofetil		-
	Any individual unspecified Impurities		ND
	Total		0.10

Discussion

Batches 1014 to 1016 were prepared to optimize the spray rate. Spray rate was varied for all three batches 1014, 1015 and 1016 as mentioned in Table 4.37.

The spray rate for granulation batches 1014 to batches 1016 were 15 gm/min, 10 gm/min, 5gm/min respectively. From the evaluation of prepared batches, it was observed that all evaluated parameter like pH, Deliverable volume, viscosity, % assay of dry mix as well as reconstituted suspension were within limit, so it was concluded that spray rate during granulation process did not have any impact on the final formulation.

It was desirable to have proper distribution of soybean lecithin and citric acid (anhydrous). The spray rate 5gm/min was selected for further batches. Moreover the % related substance for selected batch 1016 was found (at initial and after one month stability study at 30ºC/65%RH) to be within specific limit i.e less than 3%

4.8 REPRODUCIBLE BATCH OF FORMULATION OF RECONSTITUTABLE ORAL SUSPENSION

Table 4.41 Composition of reproducible batch 1017

Quantity of Water	**160 gm**	
Blending Time	**30 min**	
Ingredient	**1017**	
	%w/w	**mg/ ml**
Dry Mix		
Mycophenolate Mofetil	31.82	200.01
Sorbitol (Neosorb P 100 T)	31.82	200.01
Granulation		
Citric acid anhydrous	0.13	0.82
TriSodium citrate dehydrate	2.50	15.71
Soybean lecithin	0.48	3.02
Extra Granular		
Sorbitol (Neosorb P 100 T)	29.24	183.80
Methyl paraben	1.60	10.06
Xanthan gum	0.15	0.94
Colloidal silicon dioxide	1.18	7.42
Aspartame	0.25	1.57
Mix fruit flavor	0.83	5.22
Total	**100**	**628.57**

Procedure:

- Ingredients of dry mix were sifted through 30# sieve and collected separately.
- Dry mix materials were loaded in Palm glatt and mixed for 3 min.
- **Soybean lecithin, citric acid (anhydrous), trisodium citrate dihydrate were dissolved in 160 gm purified water**
- Dry mix was granulated with binder solution by fluid bed granulation process. The granulation parameters are described in Table 4.42

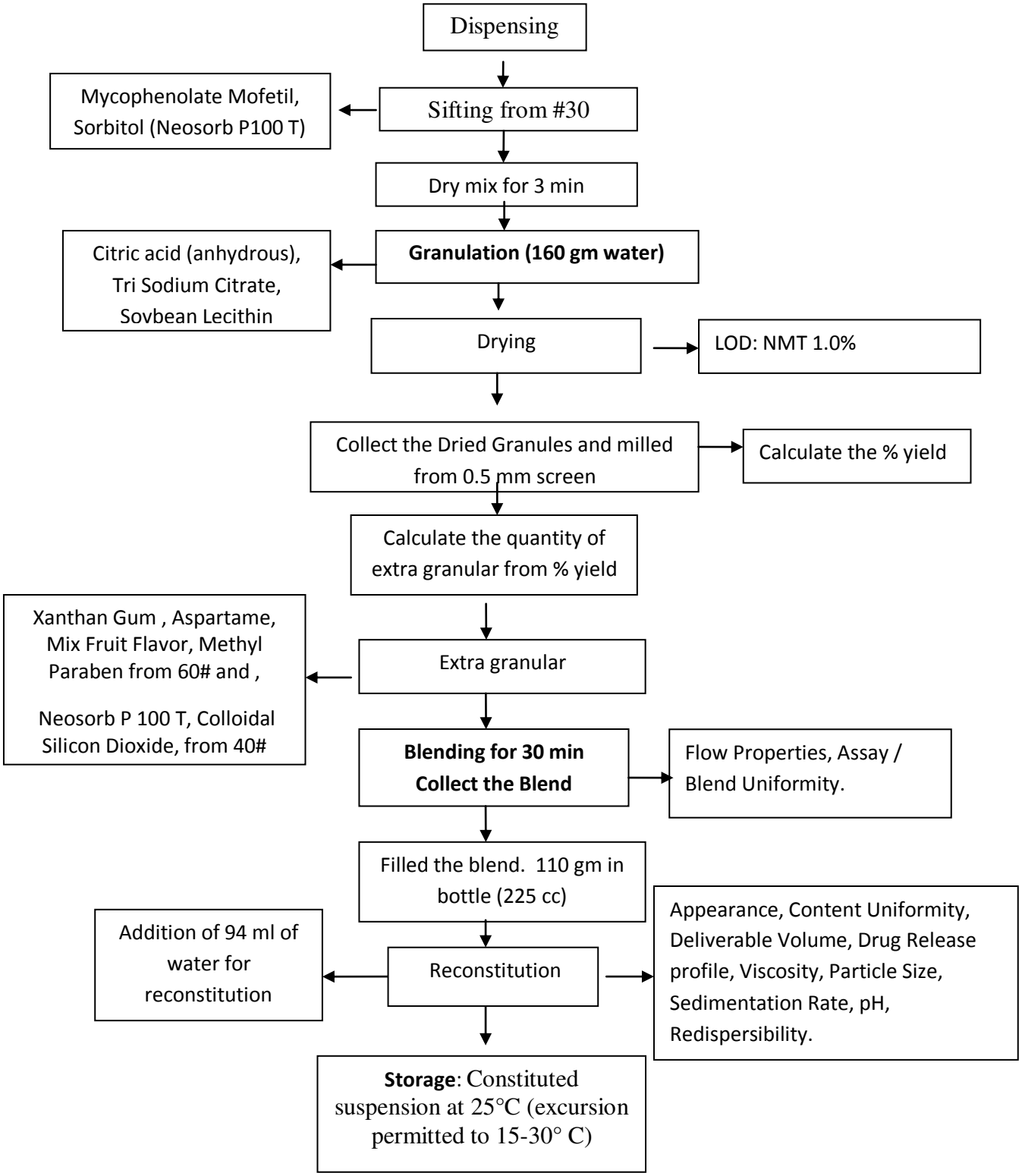
Table 4.42 Granulation Parameter of batch 1017

Base Plate	C
Inlet Temperature	45 - 55ºC
Product temperature	28 - 35ºC
Atomization pressure	1 - 1.2
Blower Speed	35 – 45
CFM	35 – 45
Spray Rate	**5 gm/min**

- Wet mass was dried till NMT 1%w/w loss on drying of granules at 75°C was obtained.
- Dried granules were milled through 0.5 mm screen.
- In extra granular portion xanthan gum, mixed fruit flavor, methyl paraben, aspartame was sifted through 60# sieve. Sorbitol(Neosorb P100 T), colloidal silicon dioxide were sifted through sieve 40# were added to the dried granules and mixed **for 30 min in blender.**
- The blend was evaluated for blend uniformity, and flow property.
- The 110 gm blend was filled into 225 cc bottle and reconstituted with 94 ml of purified water. Suspension was evaluated the appearance, content uniformity, deliverable volume, drug release profile, viscosity, particle size, sedimentation rate, pH and redispersibility of the reconstituted suspension.
- **Storage Condition**: Constituted suspension was stored at 25°C (excursion permitted to 15-30° C)

Flow Chart for formulation of Reconstitutable Oral Suspension

Ingredient ***Process*** ***Evaluation***



Result:

Table 4.43 Results of Reproducible Batch 1017

Batch no		1016	1017
Bulk Density (gm/ml)		0.718	0.718
Tapped Density (gm/ml)		0.846	0.824
Carr's Index		15.068	12.821
Hausner's Ratio		1.177	1.147
Particle Size Distribution	40#	Not Evaluated	4%
	60#		17%
	100#		33%
	Base		46%
Deliverable Volume (ml)		178	176
pH		6.80	6.7
Viscosity (cps)		670	670
Blend Uniformity (%)	Mean	98.78	99.15
	Min – Max	96.90 – 100.90	96.20 – 100.3
	RSD	1.51	1.45
Content Uniformity (%)	Mean	98.60	99.04
	Min – Max	96.8 – 100.2	97.1 – 100.9
	RSD	1.39	1.32
Sedimentation Volume		0.99	0.99
Redispersibility		NMT 20 sec	NMT 20 Sec
Drug Release in 20 min (%)		83.20	84.4
% Related Substance of Dry Powder			
Mycophenolic acid (Impurity F)		Not Evaluated	0.10
Mycophenolate Mofetil related compound A (Impurity A)			0.01
Mycophenolate Mofetil related compound B (Impurity H)			0.03
N-oxide analog (Impurity G)			ND
1-Morpholinoethoxy analog (Impurity B)			ND
O-Methyl analog (Impurity D)			ND
Methyl Mycophenolate (Impurity E)			ND
Total unknown impurities			0.01
Total impurities			0.15%
% Related Substance of Reconstituted suspension			

Related Substance	**Mycophenolic Acid**	0.08	0.09
	Sorbitol ester of Mycophenolic Acid	ND	0.01
	Mycophenolate Mofetil	0.01	-
	Any individual unspecified Impurities	ND	0.05
	Total	0.09	0.15

Table 4.44 Stability study of 1 month at 30ºC/65 %RH for batch 1016 and 1017

Stability	**1 Month**			
Condition	**30ºC/65%RH**		**2 – 8 ºC**	
Parameter	**1016**	**1017**	**1016**	**1017**
Deliverable Volume	176	174	176	173
pH	6.65	6.65	6.65	6.53
Viscosity	640	680	650	670
Assay	98.5	98.78	98.43	99.0
Drug Release in 20 min	83.2	83.1	82.5	83.1
Time to Reconstitute	10 Sec	15 Sec	10 Sec	15Sec
Related Substance				
Mycophenolic Acid	0.10	0.12	0.08	0.10
Sorbitol ester of Mycophenolic Acid	Not Detected	0.02	Not Detected	0.02
Mycophenolate Mofetil	--	--	--	--
Any individual unspecified Impurities	Not Detected	0.03	Not Detected	0.03
Total	0.10	0.12	0.09	0.15

Discussion:

Batch 1017 was prepared to check batch to batch reproducibility keeping composition and process parameters same as those of batch 1016 and results of both the batches were compared. Various parameters evaluated were flow property, % assay of dry mix and constituted suspension, pH, viscosity, % drug release, relative substances, sedimentation volume and redispersibility. Results are described in Table 4.43. From results it was concluded that all the evaluation parameters of dry mix and reconstituted suspension were similar to each other and also within the specified range Comparison between 1016 and 1017.

For both batches, all evaluation parameters were within preferred range. Stability study was conducted for both batches at 30ºC/65%RH and 2 - 8ºC of 1 month. Based on results of evaluation parameters, it was concluded that both batches were similar. It was concluded that above formulation of reconstituted oral suspension containing Mycophenolate Mofetil is reliable, robust and stable.

4.9 COMPARATIVES STUDY OF BATCH 1017 AND INNOVATOR BATCH A

Table 4.45 Comparative study of batch 1017 and innovator batch A

Parameter		Innovator (Batch A)	Batch 1017 (Reproducible batch)
Bulk Density (gm/ml)		0.690	0.718
Tapped Density (gm/ml)		0.811	0.824
Carr's Index		14.925	12.821
Hausner's Ratio		1.175	1.147
Particle Size Distribution (%)	**40#**	1	4
	60#	16	17
	100#	23	33
	Base	60	46
LOD of lubricated blend at 75°C		0.78	0.97
Deliverable Volume (ml)		173	176
Viscosity (cps)		650	670
pH		6.6	6.7
Assay of Reconstituted suspension		99.6	99.04
Particle size (D(0.9)) µm		9	11
% Drug release in min	**5**	44.66	44.58
	10	55.26	55.39
	15	65.44	67.09
	20	82.02	84.39
	30	99.21	98.20
Relative substance	**Mycophenolic Acid**	0.03%	0.09%
	Any other impurities	0.03%	0.06%
	Total impurities	0.06%	0.15%

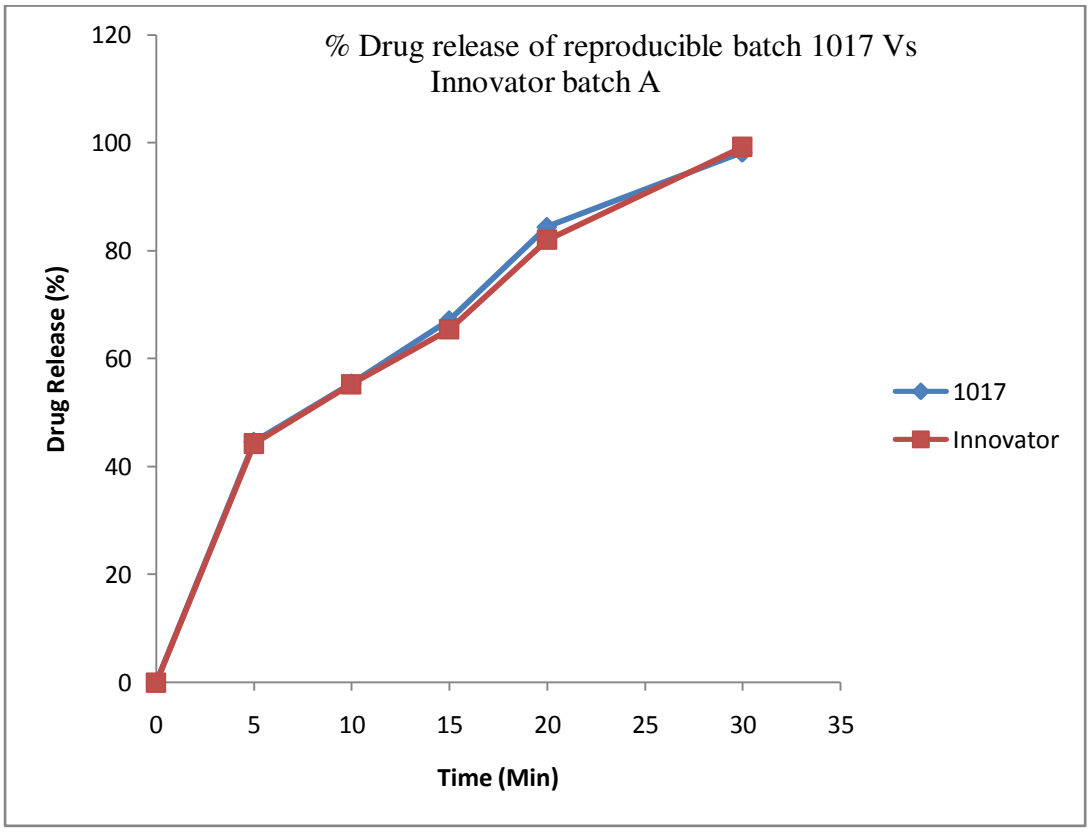


Figure 4.9 % Drug release of reproducible batch 1017 Vs Innovator batch A

Discussion:

Similarity factor (f2) is used for measures the closeness among the two profiles. F2 value is between 50-100 to indicate similarity between two dissolution profiles. It can be calculated from following formula

$$\mathbf{F2 = 50\ X\ log\{[1 + \frac{1}{n}\sum_{n}^{t=1}(Rt - Tt)^2]^{-0.5} \times 100\}\ ----(12)}$$

Where, n is the number of dissolution sample times, Rt and Tt are the individual or mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively. Based on calculation similarity factor was observed was 88.53

Based on Table 4.45, It can be concluded that innovator batch A and Reproducible batch 1017 were similar. From Table 4.45, The optimized batch 1017 was compared with marketed formulation (CellCept®200mg/ml for oral suspension), for %assay of dry mix as well as reconstituted suspension, pH, viscosity and %drug release. It was found that optimized batch showed similar result as marketed formulation (CellCept®200mg/ml for oral suspension). Similarity factor was 88.53 and it was concluded that reproducible batch 1017 and Innovator batch A were equivalent.

4.10 Concentration of xanthan gum and soybean lecithin using Design of Experiment

The design of an experiment can be clearly defined as the plan that governs the performance of an experiment. The conventional experiments need of more efforts and time, in particular where complex formulations are to be developed. Factorial designs are used when factors or conditions are significantly affected on the responses. Factors may be quantitative or qualitative. The levels of an each factor are the value or description assigned to arrangement of all levels of all factor. The full factorial design is selected by following nomenclature;

$$N=L^K \qquad \text{-----(13)}$$

Where; K = number of variables, L = number of variables levels, N = number of the experimental trials.

The purpose of the factorial design is to study the effect of change in levels of the factor or combination of factors on the response. Predictions based on the results of an undesired experiment will be more variable than those, which could be obtained in a designed experiment, in particular factorial design. The optimization procedure is facilitated by creation of an equation that describes the experimental results as a purpose of the factor levels. A factorial equation can be constructed, where the coefficients in the equation are interrelated to the effects and interaction of the factors. Equation for the 2^2 full factorial design is as per below.

$$Y = \beta 0 + AX1 + BX2 + ABX1X2 \qquad \text{------(14)}$$

Where,

β0= Intercept, Y: dependent variables, A is the predictable coefficient for the factor X1. B is the predictable coefficient for the factor X2. The main effects (X1 and X2) correspond to the standard result of changing one factor at a time from its low to high value. The interaction terms (X1X2) show how the response changes when two factors are concurrently changed. The magnitudes of the coefficients characterize the relative significance of each factor. Once equation has been recognized, an optimum

formulation can be found. With the use of computer a grid method can be used to recognize optimum regions and response surfaces may be depicted.

Advantages of factorial design:

1. Factorial designs have highest effectiveness in estimating main effects.
2. Lowest number of trials per independent variable is mandatory.
3. They form the basis for several other designs (like fractional factorial, composite etc.)
4. More information is obtained with less work.
5. They can be used as building block to characterize a large response surface.
6. The special effects are measured with maximum accuracy
7. Both quantitative and qualitative variables can be examined and results can be simply interpreted.

Applications:

1. To facilitate and interpret the mechanism of an experimental system.
2. To suggest or implement, a practical process or a set of condition, in an industrial manufacturing process.

4.10.1 2^2 Full Factorial Designs

The two factors, two level design is noted down as a 2^2 factorial design. It means that 2 factors are considered, each at 2 levels which are frequently referred to as low and high. These levels are coded value as -1 and +1. It is a simplest two level design. Based on reproducible batch and optimized batch. DoE is applied to provide design space for the robust formulation. In this study, concentration of xanthan gum and concentration of soybean lecithin were chosen as the independent variables. The dependent variables included viscosity, deliverable volume and drug release in 20 min. A 2^2 factorial design with 3 center point was employed to study the effect of independent variable, on dependent variables.

Table 4.46 2^2 factorial design with 3 center point layout

Factor				
Independent Variable	Coded Value		Actual Value	
X1: Conc. Xanthan Gum	-1		0.1%	
	+1		0.2%	
X2: Conc. Of Soybean Lecithin	-1		0.32%	
	+1		0.64%	
Response				
Dependent Variable	Y1 : Viscosity			
	Y2 : Deliverable Volume			
	Y3 : Drug release in 20 min			
Optimization Batches using DOE				
Batch No	Transformed Factors		Actual value	
	X1	X2	X1	X2
1018	-1	-1	0.1	0.32
1019	-1	+1	0.1	0.64
1020	1	-1	0.2	0.32
1021	1	+1	0.2	0.64
1022	0	0	0.15	0.48
1023	0	0	0.15	0.48
1024	0	0	0.15	0.48

4.10.2 Responses generated by 2^2 Factorial design with 3 center point

Table 4.47 Responses generated by 2^2 Factorial design with 3 center point

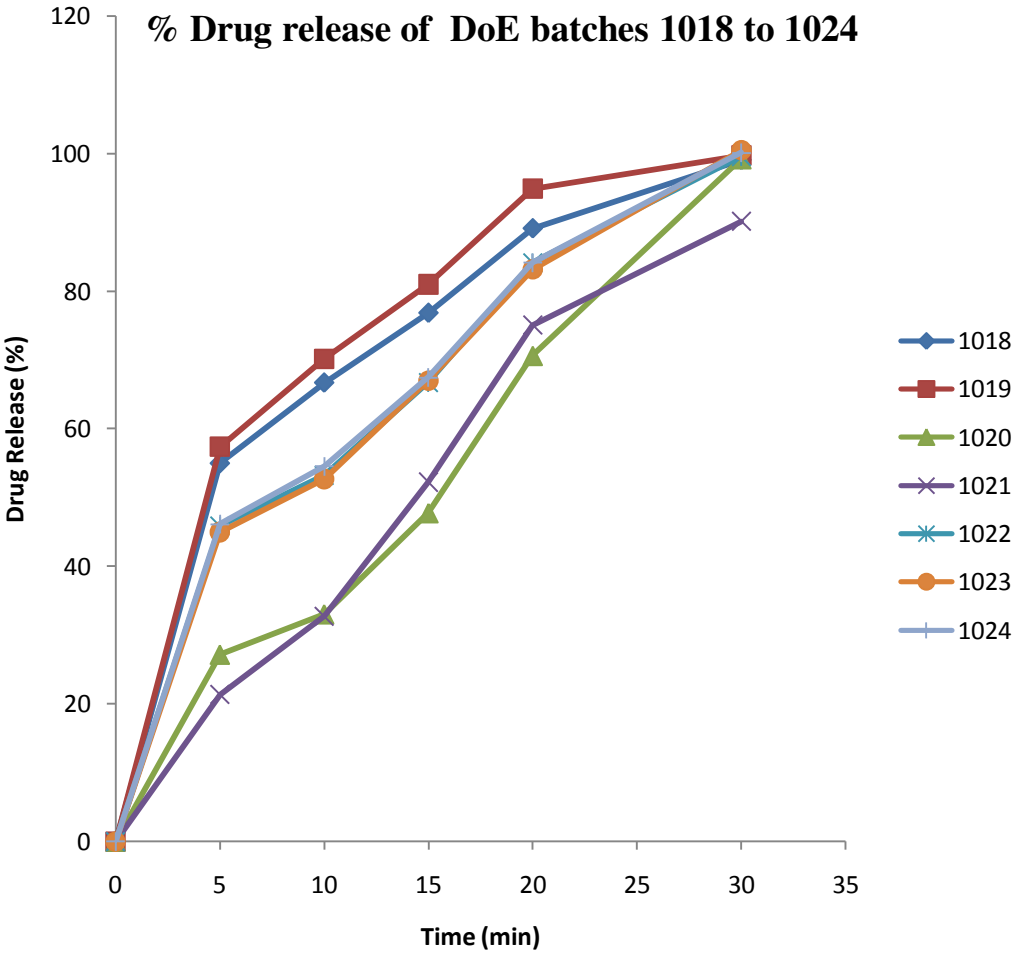
Batch no	**X1 : Conc. of Xanthan Gum**	**X2 : Conc. of Soybean Lecithin**	**Y1: Viscosity**	**Y2: Deliverable Volume**	**Y3: Drug release in 20 min**
1018	0.1	0.32	350	190	89.12
1019	0.1	0.64	240	168	94.84
1020	0.2	0.32	1250	185	70.6
1021	0.2	0.64	1100	164	75.1
1022	0.15	0.48	670	178	84.09
1023	0.15	0.48	650	176	83.18
1024	0.15	0.48	690	177	84.15

4.10.3 Dissolution Profile of batches 1018 to 1024:

Table 4.48 Dissolution Profile of DoE batches 1018 to 1024

Time	1018	1019	1020	1021	1022	1023	1024
5	54.98	57.35	27.18	21.37	45.85	44.97	46.15
10	66.70	70.14	33.04	32.78	53.22	52.69	54.55
15	76.84	80.95	47.74	52.24	66.69	67.01	67.44
20	89.12	94.84	70.60	75.10	84.09	83.18	84.15
30	99.06	99.69	99.19	90.15	99.56	100.46	100.12

Figure 4.10 %Drug release of batches 1018 to 1024



4.10.4 Comparison of evaluation parameter of factorial design batches 1018 to 1012 DoE batches

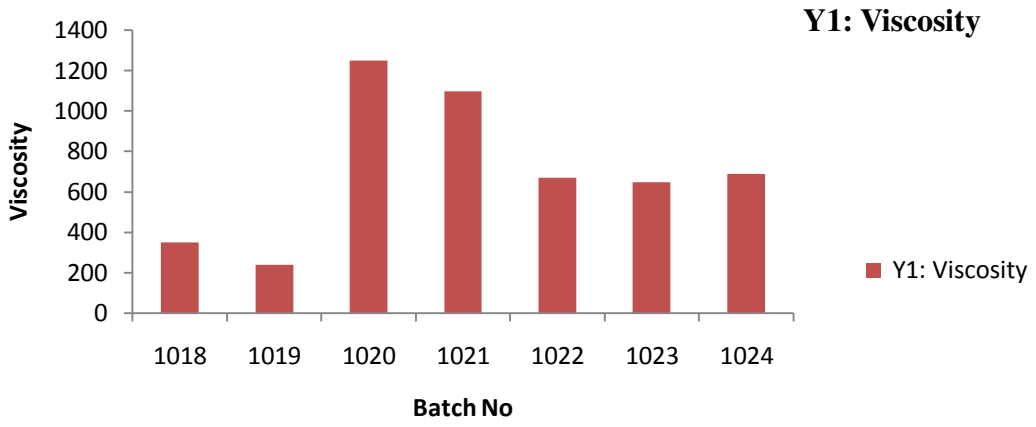


Figure 4.11 Comparison of viscosity for batches 1018 to 1024

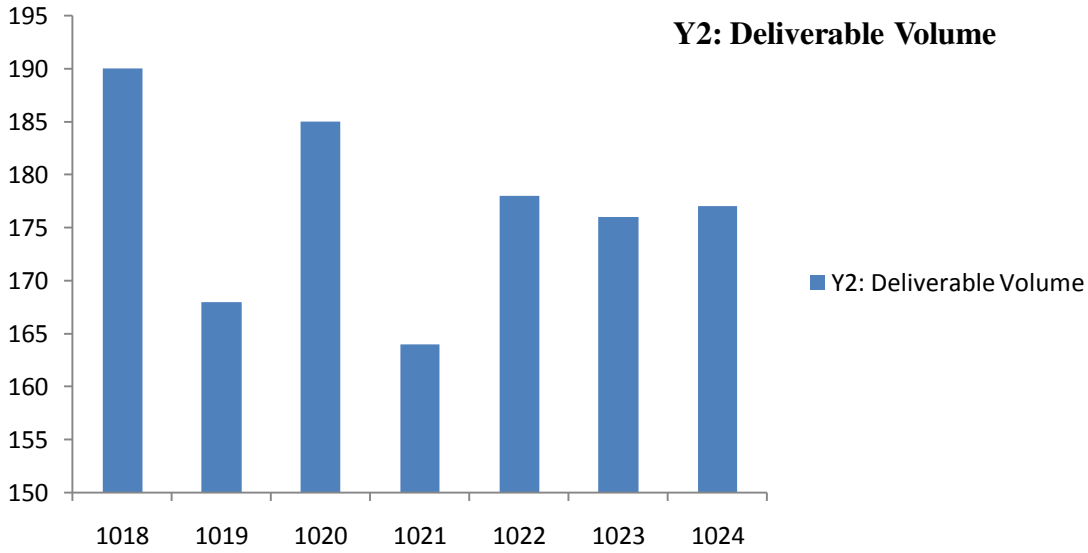


Figure 4.12 Comparison of deliverable volume for batches 1018 to 1024

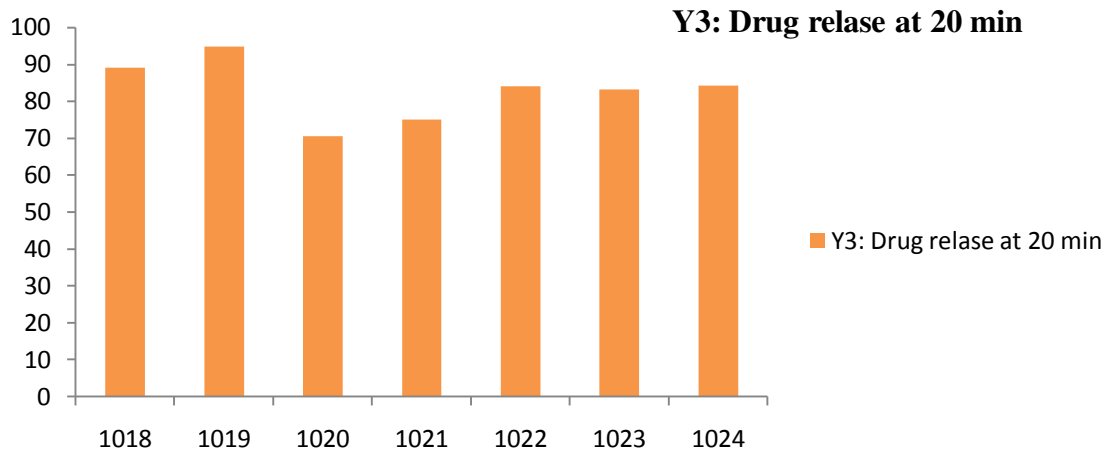


Figure 4.13 Comparison of %drug release at 20 min for batches 1018 to 1024

4.10.5 Interpretation

DoE is applied to provide design space for the robust formulation. In this study, concentration of xanthan gum and concentration of soybean lecithin were chosen as the independent variables. The dependent variables included viscosity, deliverable volume and drug release in 20 min. Using one-way ANOVA , studied the effect of formulation variables on the response variables with help of Design-Expert® 9.0.4 Stat Ease, USA. The design was evaluated using a 2FI model, which has following equation.

$$Y = b0 + A\,X1 + B\,X2 + AB\,X1\,X2 \qquad \text{-----(15)}$$

Where Y is the response variable, b0 the constant and A, B, AB is the regression coefficient. X1 and X2 stand for the main effect; X1X2 are the interaction terms that shows how the response changes when two factors are simultaneously changed.

(A) Viscosity:

The Viscosity of individual batches of prepared formulations is presented in Table 4.47. The values of Viscosity were ranged between 240 cps to 1250 cps.

Table 4.49 Regression analysis of effect of X1 and X2 on viscosity

Mean	707.14	
R-square	0.9899	
Adj.Rsquare	0.9799	
Pred R-square	0.6535	
Adeq Precision	25.805	
C.V.	7.32	
Co-efficient		
Coefficient	**Coefficient value**	**P value**
Model	+707.14	0.0017
X1 : Conc of xanthan gum	+40.00	0.0004
X2: Conc of soybean lecithin	-65.00	0.0869
X1X2	-10	0.7251
Coded Equation	Y1:707.14 + 40.00 X1 – 65.00 X2 – 10.00 X1X2	

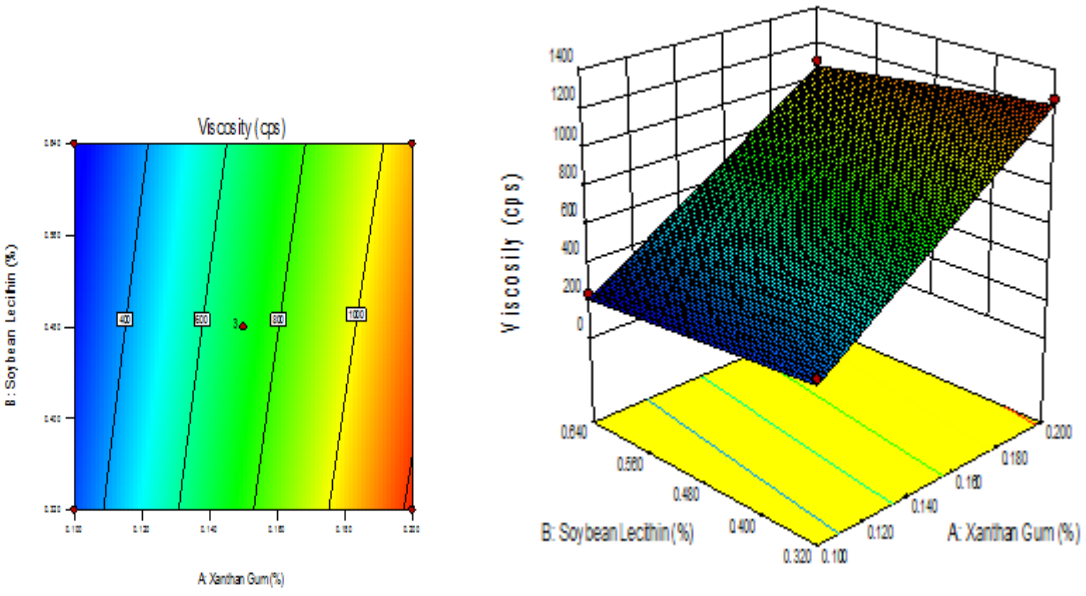


Figure 4.14 Contour plot and 3D surface plot of the effect of xanthan gum and soybean lecithin on viscosity

Viscosity for all 7 batches 1018 to 1024 showed good correlation co efficient 0.9899 near to 1.0. From Table 4.49, it was observed that p value was 0.0017. Concentration of xanthan gum (X1) has p value 0.0004 which is less than 0.05 and concentration of soybean lecithin (X2) has p value 0.0869 and interaction term X1X2 has p value 0.7251.Thus concentration of xanthan gum (X1) has more significant effect compare to concentration of soybean lecithin., there was no interaction between X1X2. So it was concluded that concentration of soybean lecithin (X2) and interaction of variables X1X2 did not have significant effect on viscosity. From equation in Table 4.49, relationship between concentrations of xanthan gum (X1), concentration of soybean lecithin (X2) can be established. As the concentration of xanthan gum (X1) increase, Viscosity also increased.

(B) Deliverable Volume:

The Deliverable Volume of individual batches of prepared formulations is presented in Table 4.47. The values of Deliverable Volume were ranged between 164 ml to 190 ml.

Table 4.50 Regression analysis of effect of X1 and X2 on deliverable volume

Mean	176.86	
R-square	0.9957	
Adj.Rsquare	0.9913	
Pred R-square	0.9860	
Adeq Precision	41.040	
C.V.	0.47	
Co-efficient		
Coefficient	**Coefficient value**	**P value**
Model	+176.86	0.0005
X1 : Conc of xanthan gum	-2.25	0.0126
X2: Conc of Soybean lecithin	-10.75	0.0001
X1X2	+0.25	0.5928
Coded Equation	Y2:176.86 – 2.25 X1 – 10.75 X2 + 0.25X1X2	

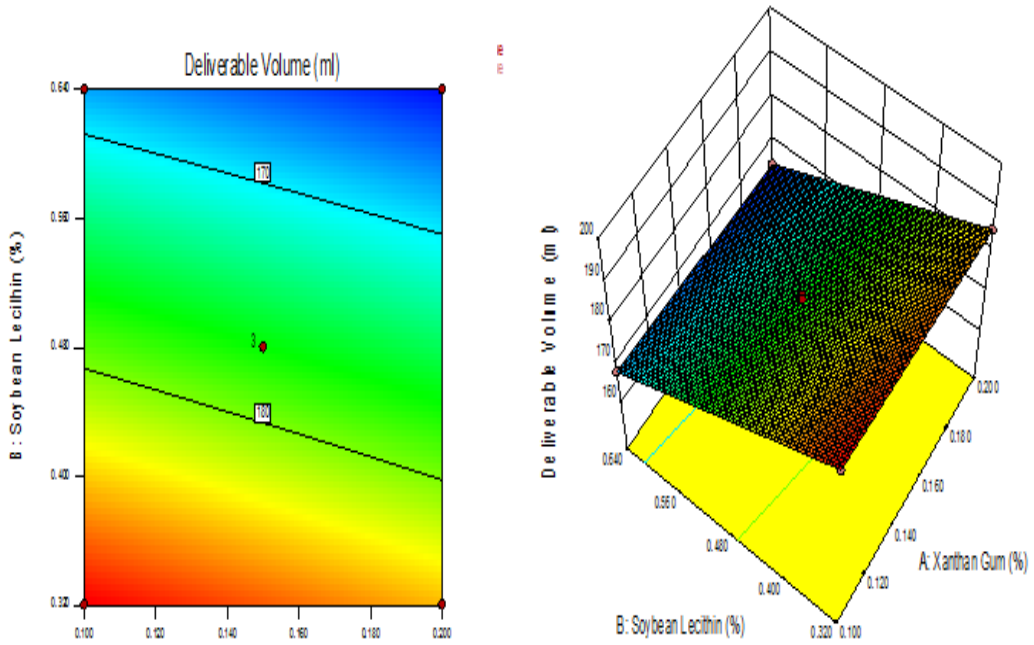


Figure 4.15 Contour plot and 3D surface plot of the effect of xanthan gum and soybean lecithin on Deliverable volume.

Deliverable volume for all 7 batches 1018 to 1024 showed good correlation co efficient 0.9957 near to 1.0. From Table 4.50, it was observed that p value was 0.0005. Concentration of xanthan gum (X1) has p value 0.0126 which is less than 0.05 and concentration of soybean lecithin (X2) has p value 0.0001 and interaction term X1X2 has p value 0.5928.Thus concentration of soybean lecithin (X2) has more significant effect compare to concentration of xanthan gum (X1) as p value was >0.05, there was no interaction between X1X2. So it was concluded that concentration of xanthan gum (X1), concentration of soybean lecithin (X2) have significant effect on deliverable volume and interaction of variables X1X2 did not have significant effect on deliverable volume From equation in Table 4.50, relationship between concentration of xanthan gum (X1), concentration of soybean lecithin (X2) can be established. As the concentration of xanthan gum (X1) and Concentration of soybean lecithin (X2) decreases, deliverable volume is increased.

(C) Drug Release at 20 min:

The Drug release at 20 min of individual batches of prepared formulations is presented in Table 4.47. The values of drug release at 20 min were ranged between 70.64% to 94.5% .

Table 4.51 Regression analysis of effect of X1 and X2 %Drug Release in 20 Min

Mean	83.01	
R-square	0.9901	
Adj.Rsquare	0.9803	
Pred R-square	0.6787	
Adeq Precision	28.085	
C.V.	1.38	
Co-efficient		
Coefficient	**Coefficient value**	**P value**
Model	83.01	0.0017
X1 : Conc of xanthan gum	-9.57	0.0005
X2: Conc of soybean lecithin	2.55	0.0208
X1X2	-0.30	0.6302
Coded Equation	Y3:83.01 - 9.57 X1 + 2.55 X2 – 0.30 X1X2	

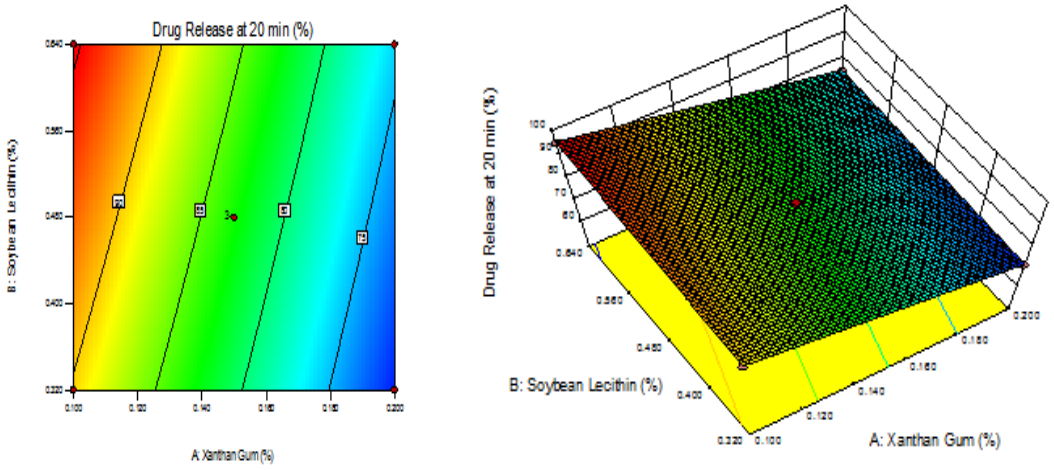


Figure 4.16 Coutour plot and 3D surface plot :the effect of xanthan gum and soybean lecithin on Drug release at 20 min.

% drug release in 20 min for all 7 batches 1018 to 1024 showed good correlation co efficient 0.9901 near to 1.0. From Table 4.51, it was observed that p value was 0.0017. Concentration of xanthan gum (X1) has p value 0.0005, concentration of soybean lecithin (X2) has p value 0.0208 which is less than 0.05 and interaction term X1X2 has p value 0.6302. Thus concentration of xanthan gum (X1) has more significant effect compare to concentration of soybean lecithin (X2) as p value was <0.05, there was no interaction between X1X2. So it was concluded that concentration of xanthan gum (X1), concentration of soybean lecithin (X2) have significant effect on % drug release and interaction of variables X1X2 did not have significant effect on % drug release. From equation in Table 4.51, relationship between concentration of xanthan gum (X1), concentration of soybean lecithin (X2) can be established. As the concentration of xanthan gum (X1) decreases, Concentration of soybean lecithin (X2) increases, % drug release is increased.

4.10.6 Selection of design space for concentration of xanthan gum and concentration of soybean lecithin:

The percentage of Xanthan gum and soybean lecithin had a significant impact on % drug release, viscosity and deliverable volume. Increasing the percentage of xanthan gum increased viscosity but decreased % drug release and effected deliverable volume. The percentage of soybean lecithin had significant impact on % drug release, viscosity and deliverable volume. Increasing the percentage of soybean lecithin increase % drug release and deliverable volume. To obtain desired response, concentration of xanthan gum and concentration of soybean lecithin was selected for optimization. The 2^2 facorial design with 3 center point were used to establish acceptable ranges for formulation variables. Figure 4.20 showed the overlay plot of all of the responses. The yellow region indicates that desired response can be obtained in this colored region. For the provided design space, range of the response was as per below: Xanthan Gum: 0.1 – 0.2%w/w

Soybean Lecithin: 0.32 – 0.64%w/w

Deliverable Volume: 172 – 180 ml

Viscosity: 600 – 700 cps

Drug release at 20 min: 81.5 – 84.5%

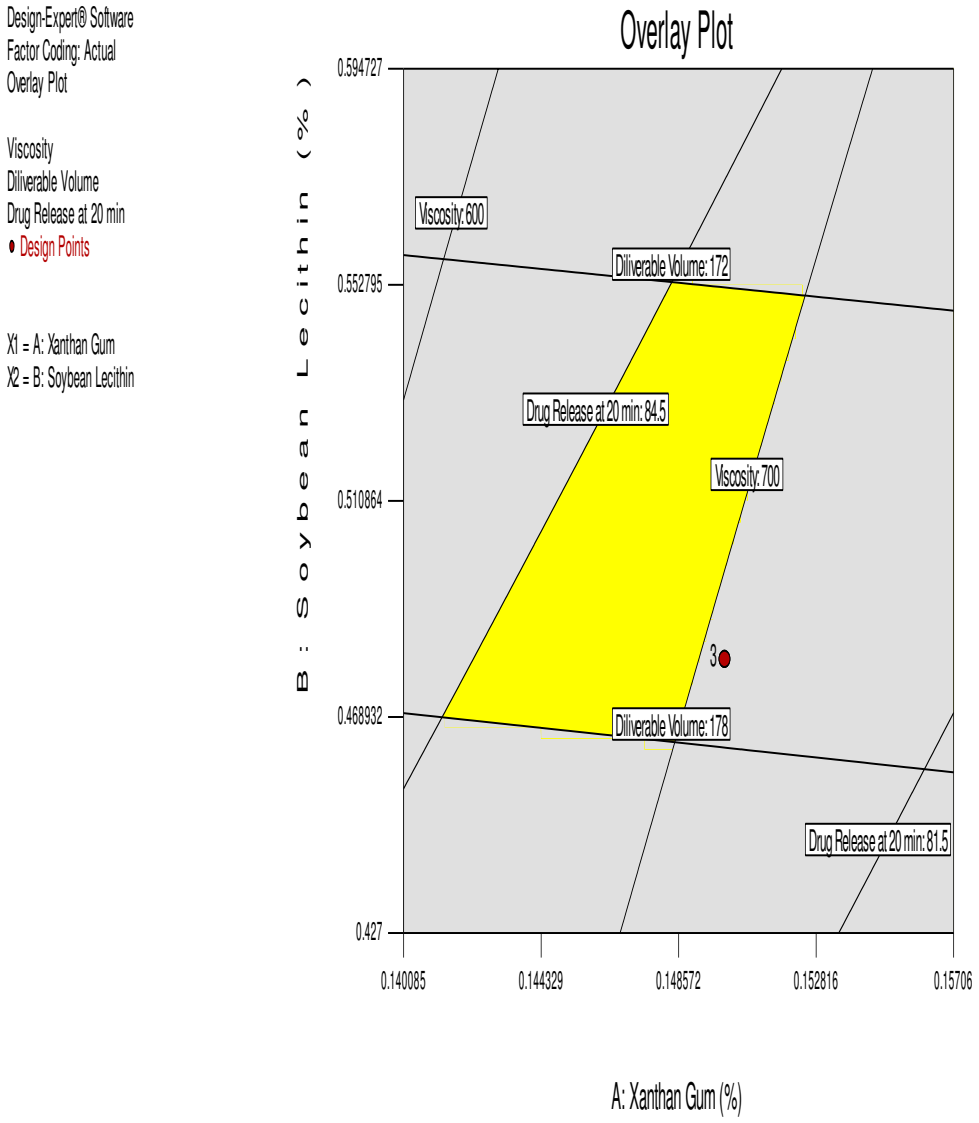


Figure 4.17 Overlay plot for Design Space

Discussion:

Based on preferred criteria of response namely viscosity (600 – 700 cps), deliverable volume (172 – 178 ml), and % drug release in 20 min (81.5 – 84.5%). Design space for **Xanthan Gum was 0.143 – 0.150%w/w Soybean Lecithin was 0.472 – 0.552%w/w.** In design space region, all formulation of Reconstitutable Oral Suspension will provide desired result.

Chapter - 5

Summary

SUMMARY

Mycophenolate Mofetil, an Immunosuppressant drug requires daily dose of 3.5 or 4.0 gm/day depending upon the patient and the disease state being treated. Conventional dosage form such as tablet and capsule contain 200 mg and 500 mg dose, thus the patient will require twelve units and six units respectively each day, giving rise to patient inconvenience and non-compliance. As an alternative oral reconstitutable suspension (200 mg/ml) will provide ease of administration and convenience**.** As the % of drug substance in reconstitutable oral suspension was very high and drug being immuno-suppressant in nature, all trials were taken in closed assembly system by direct blending approach or Wet granulation using Fluid Bed Processor (FBP) process. Feasibility of formulation through direct blending was checked and it was found that direct blending cannot be used as soybean lecithin, a waxy excipient was used in the formulation. Hence granulation in FBP was selected as final process for manufacturing formulation.

Effect of Excipients:

- Concentration of soybean lecithin at 0.48 %w/w was optimized. Deliverable volume and % drug release were found satisfactory at this concentration.
- Concentration of xanthan gum was optimized at 0.15% w/w concentration deliverable volume, % drug release and viscosity were found satisfactory.
- Citric acid anhydrous played a significantly role in maintenance of pH, where trisodium citrate dihydrate did not have significant effect on pH. Citric acid anhydrous and trisodium citrate dihydrate were optimized at 0.13%w/w and 2.50%w/w concentration respectively.
- Colloidal silicon dioxide at 1.18 %w/w concentration was necessary for the formulation of stable suspension.

Effect of Process Parameter:

- Quantity of water in granulation: Upon addition 160 gm of water in granulation, flow property was found satisfactory.
- Blending Time: Blending time was optimized at 30 min, % RSD of blend uniformity and deliverable volume were found satisfactory after stability study.

F2 value of reproducible batch 1017, with respect to innovator batch A was 88.53 which indicated that reproducible batch was similar with innovator for batch A in terms of % drug release profile. All other parameters like pH, viscosity, % assay and deliverable volume were matching with innovator product CellCept® 200 mg/ml oral suspension.

Design of Experiment approach:

To obtain desired response, concentration of xanthan gum and soybean lecithin was selected for optimization. The 2^2 factorial design with 3 center point, models were used to establish acceptable ranges for formulation variables for desired response ranges, viscosity (600 – 700 cps), deliverable volume (172 – 178 ml) and % drug release in 20 min (81.5 – 84.5%). Design Expert software provides design space of significant factor xanthan gum at 0.143 – 0.150 %w/w and soybean Lecithin at 0.472 – 0.552 %w/w.

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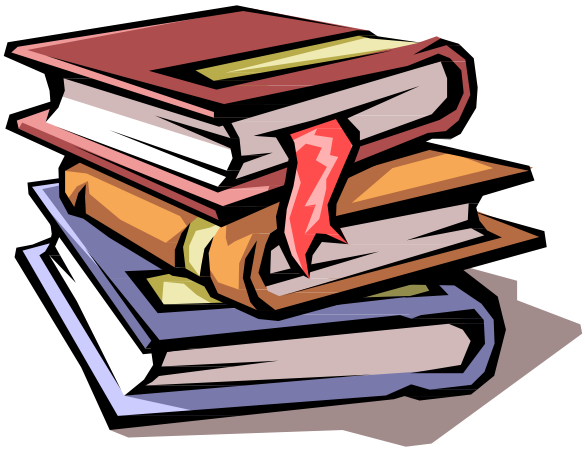
Chapter - 6



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