"FORMULATION AND EVALUATION OF RIBAVIRIN DRY POWDER INHALATION FOR PULMONARY DELIVERY BY LYOPHILLIZATION"

[1*]Dattatray J. Patil, [2]Dr. Kiran A. Wadkar, [3]Dr. Manish S. Kondawar
[1]Research Student, [2]HOD Pharmacognosy, [3]HOD Quality Assurance
Quality Assurance
Appasaheb Birnale College of pharmacy Sangli, Maharashtra.

Abstract:

The basic aim of this study deals with formulation and evaluation of Ribavirin DPI for pulmonary delivery by Lyophillization. Ribavirin is an antiviral medication used to treat RSV infection, hepatitis C and viral hemorrhagic fever. Ribavirin formulation available in the form of tablet, capsule and inhalation solution. For inhalation solutions mechanical instruments are required, and they can be overcome by use liposomes, dry powder inhalation meter dose inhalation etc. Total 6 batches were prepared and subjected for evaluation of flow properties. All the parameters were within limits as per Indian pharmacopeia which shows good flow properties, Particle size, Zeta potential, DSC, XRD. Drug and excipient compatibility was carried out by FTIR spectroscopy. In-vitro dissolution study was performed which shows batch B as a optimized batch because of low concentration of polymers in formulation. The developed formulation was found to be stable during the stability studies of 3 months.

Key words: Ribavirin, DPI, Particle Size, In vitro Aerosolization, In vitro dissolution.

1. INTRODUCTION

1.1. GENERAL:

The respiratory tract is one of the route for drug delivery. The potential advantages of delivering a drug to the lung by inhalation have been well know. The drug shows biological effect in the lung, It include rapid action at low dose and minimum side effects than other drug delivered.(1)

For this delivery of therapeutic agents has known, which is poorly understood, for many years. But the pulmonary tract considered as favorable and best route for the administration of substances. (e.g., asthma, COPD, microbial infections).(2)

The various agents has been administered to the lung via oral inhalation, in the treatment of diverse disease status. The frequent use of inhalation therapy is in the treatment of COPD using drugs like short & long acting β sympathomimetic, corticosteroids, and anticholinergic agents.(3)

1.2. PULMONARY DRUG DELIVERY:

The human lung containing 5 lobules as well as 10 bronchopulmonary sections, and they are arranged in adjacent to each other. The section lung lobules collected as 3–5 terminal bronchioles. Bronchiole are the smallest structural unit of the lung, It contains alveolar duct, alveolar sac as well as alveoli .The function of Alveolar epithelial type I cells represent lining the surface of the alveoli. The main function of cells are to provide a surface gas exchange as well as to act as a permeability barrier. Type II cells has a smallest surface area/ cell. They play a important role in synthesis, secretion and recycling of surfactant (lung surfactant).

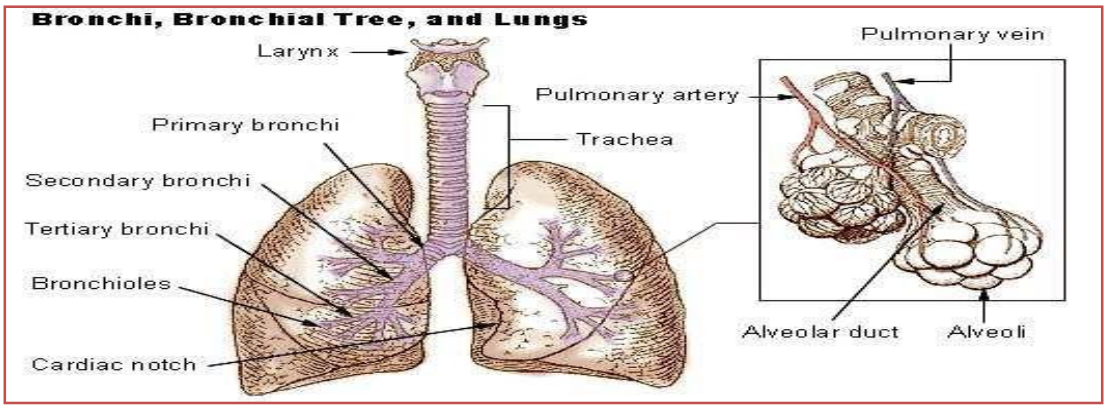

Figure 1: Bronchi, bronchial tree and lungs.

The alveolar blood barrier is a simplest form of epithelial cell & a basement membrane. Before entering the systemic circulation, solutes must cross a thin layer of fluid, the epithelial lining fluid. This layer tends to collect at the corners of the alveoli as well as covered by an adjucent layer of surfactant. Dislike the larger airways, the alveolar region is lined with a surface active layer containing phosphor-lipids. The surface acting material fluid plays main character in maintaining alveolar fluid homeostasis. The airways from the upper airways to the bronchioles are viscoelastic, gel-like mucus layer i.e 0.5–5.0 mm thick. The secretion inside layer consists of 2 layers: a low viscosity fluid layer, which surrounds the cilia, and a more viscous layer at top the mucus. The mucus is a defensive layer that consists of a complex mixture of glycoprotein released primarily by the goblet cells and native glands. The mucus absolute removes inhaled particles from the airways by entrapment and mucociliary transport which is depends upon viscosity and elasticity. The lung tissue is highly pulmonary targeting problematic because of fast absorption of drugs.(4)

For local administration, Small molecules in the lungs, are successful treat in respiratory disease such as asthma, rhinitis and COPD.

A lung is an good-looking environment for biomolecules, which are extremely susceptible to enzymatic degraded in the GI tract (ventricle and guts) as well as hepatic degradation (first pass metabolism). But, respiratory system itself restricts the entrance of particulate matter by various means: e.g. geometry of the airways and clearance mechanisms of the lungs. Thus inhalation particles have to be aerodynamically improved to reach absorbed in the alveolar epithelium.(5)

1.3 Advantages:

1. Provides local action within the respiratory tract
2. Provides rapid drug action
3. Provides reduced dose
4. Allows for a reduction in systemic side-effects; It can be employed as an alternative route to drug interaction when two or more medications are used concurrently
5. Reduces extracellular enzyme levels compared to GI tract due to the large alveolar surface area
6. Reduces evasion of first pass hepatic metabolism by absorbed drug
7. Offers the potential for pulmonary administration of systemically active materials

1.4 Disadvantages:

1. The duration of activity is often short-lived due to the rapid removal of drug from the lungs or due to drug metabolism.
2. Require frequent dosing.(6)

1.5 DRY POWDER FOR INHALATION (DPI):

Three main delivery systems have been invented namely, pressurized metered-dose inhaler (MDI), Nebulizer and dry powder inhaler (DPI). The effectiveness of inhalation therapy is not tall, subsequently only about 10% of the inhaled dose of the drug reaches the alveoli. To a certain extent, it may be possible to increase the fraction of dose deposited in the lungs by training the patient in 'correct' inhalation techniques. But, the therapeutic effectiveness of the inhaled drug is administered by the aerosol characteristics, inter-patient variability and the technique by which the patient uses the inhaler.

The MDI is still the mostly prescribed inhalation system. But, it has several disadvantages:

1. Droplets leaving the actuator orifice can be too big also very high velocity resulting in wide oropharyngeal deposition.

2. The productivity of the MDI is delivered in the course of vital capacity man works slightly than tidal breathing and hence it is important to coordinate the aerosol discharge with inspiration.

3. Dysrhythmias and paradoxical broncho-constriction with MDI have given increase in some disagreement about the safety of propellants/ surfactants.

4. The use of chlorofluorocarbon (CFC) propellants is to be limited in forthcoming due to their consequence in the ozone exhaustion.

Pulmonary drug delivery by DPI, because of its propellant permitted nature, high patient obedience, high dose transport capacity as well as drug stability and patent defense. It has been freshly become subject of active research to realize full potential of lungs for local and systemic treatment of diseases such as asthma and COPD. Dry powder inhalers (DPI) is a device carry a dry powder formulation of an active drug is delivered for local or systemic effect via the pulmonary route.[7] While DPI are difficult in nature and their performance relies on many aspects., as well as the design of inhaler, the powder formulation also the airflow generated by the patient.

Advantages and disadvantages of DPI are mentioned as follows,[8]

1.6 Advantages:

1. No propellants
2. Offers local action within the respiratory tract and non-invasive
3. Avoids hepatic first-pass metabolism
4. Allows for a reduction in systemic side-effects
5. Provides quick drug action
6. Optimum particle size of drug for shallow lung delivery
7. High drug dose carrying capacities, reproducibility (Mono-disperse)
8. Breath actuated hence no hand-mouth bringing together required
9. Slight extra-pulmonary loss of drug due to low oropharyngeal deposition, low device 10.Retention and low inhaled loss
11. Reduces extracellular enzyme levels compared to GI tract due to the greater alveolar surface area
12. Enhanced patient compliance, easy to use and suitable to carry and do not require spacers

1.7 Disadvantages:

1. Respirable dose dependent on inspiratory flow rate
2. Humidity may affects on powder to aggregate and capsules to soften
3. Dose missing if patient mistakably exhales into the DPI

DPI contains the drug and carrier particles either mixed or co-precipitated together into dry powder form. Dry powders for inhalation are formulated moreover as loose agglomerates of micronized drug particles through aerodynamic particle sizes of less than 5μm or else as carrier-based collaborate mixtures with micronized drug particles followed by compared to the surface of large lactose, mannitol carriers. Lactose/ Mannitol are the most commonly used coarse carrier for inhalation .

Size of drug/dry powder should be near spherical in shape. Monodispersed with aerodynamic diameter range of 0.5 to 5μm.[8] A particle size of 2–5 μm yield optimum used, whereas for systemic effects particle size of less than 2μm is needed for drug deposition in the small peripheral airways. Particles greater than 5μm may also result in systemic effects due to impaction in the throat (i.e. oropharyngeal delivery) and oral absorption. The dry powder formulation is aerosolized through a DPI device, where the drug particles are separated from the carrier (from drug– carrier mixtures) or de-agglomerates drug particles, and the dose is delivered into the patient's deep lungs. In these systems, particle size and flow property, formulation, drug–carrier a However powders in this size range (1-5 μm) exhibit strong inter-particulate cohesion, leading to poor powder flow properties .Furthermore, factors known to influence the aerosolization properties of dry powders (e.g. particle morphology, density and surface composition cannot be controlled effectively during the micronization process.

Researchers in the field have investigated a number of approaches to improve powder aerosolization,[9] such as mixing the micronized drug with inert carrier particles or modification of particle morphology particle surface roughness, particle porosity or powder density. Whereas micronization is a destructive technique, spray drying and freeze drying is a one-step constructive process that provides greater control over particle size, particle morphology and powder density

1.5.1 Uptake of inhaled drug after inhalation therapy:

There are several advantages in delivering drugs, to the lungs including a noninvasive method of delivery; the surface area of the lung is between 80 m^2 and 140 m^2. In addition, in most pulmonary regions, the thickness of the alveolar epithelium is only between 0.1μm and 0.2μm. The total distance between epithelial surface and blood in the alveolar area is between 0.5μm and 1.0μm which are much less than in the bronchial

system (distance between mucus surface and blood: 30µm – 40 µm). Thus, it appears that pharmaceuticals after deep inhalation and deposition in the peripheral (i.e. alveolar) region of the lung can be rapidly absorbed. Pulmonary delivery therefore has the advantage, compared to nasal delivery, that it is possible to obtain a sufficiently high absorption without the need of enhancers *(WO/2003/086516).* Another advantage is that these drugs are not subject of a hepatic first pass effect after their absorption as shown in figure.

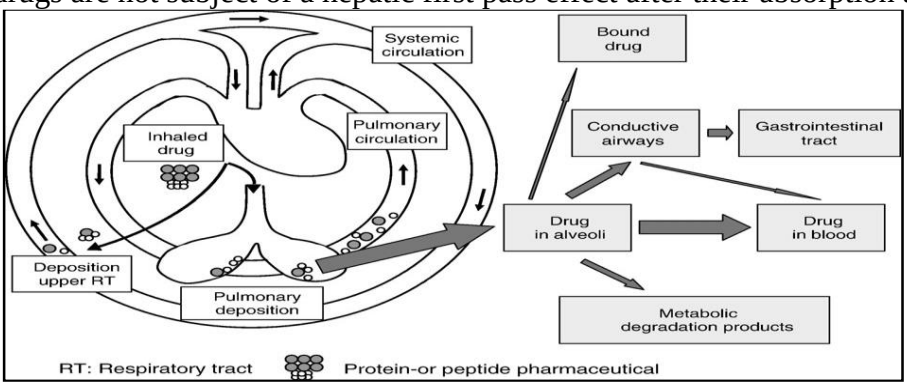

figure 2: uptake of inhaled drug after peripheral/ alveolar deposition.

On the other hand, the human lung has different defense mechanisms to prevent aerosol particles penetrating into the deep lung. Primarily, the oropharyngeal region and the bronchial tree are excellent filters to eliminate aerosol particles from the inhaled air and particles deposited on ciliated epithelium are subject to mucociliary transport to the GI tract. However, after deposition in the alveolar region of the lung, a number of mechanisms inhibit the absorption of inhaled pharmaceuticals. There are a number of absorption barriers (i.e. mucus layer, alveolar lining fluid layer, macrophages and other cells, alveolar epithelium and basement membrane) which act to varying extents by inhibiting drug permeation into the circulation, there exists competing cellular uptake pathways (e.g. particle phagocytosis by macrophages), and of course proteolytic degradation can limit the amount of intact drug available for absorption. The function of these barriers can be impaired by very different substances and consequently the absorption of drugs can be increased, for example, by the use of absorbance enhancers (e.g. cyclo-dextrins, detergents and bile acids). Furthermore, proteolytic degradation can be inhibited by protease inhibitors and phagocytosis by macrophages reduced by packaging of substances into porous particles. In principle, absorption kinetics of inhaled substances depend on their molecular weight (small molecules are more rapidly absorbed than larger ones), pH-value, electrical charge, Solubility and stability of the inhaled substance.

The other target regions within the lung for inhalable drugs are the large and small bronchial airways. Different pulmonary diseases are located in these parts of the respiratory tract. The most relevant are: asthma, chronic obstructive pulmonary disease (COPD) and bronchial tumors. To treat these diseases locally, one has to deliver the drugs specifically to this region. However, a minor proportion of drugs can also be absorbed into systemic circulation after such a tracheobronchial deposition. In contrast to the inhalation of drugs for systemic treatment, the inhalation therapy of asthma and COPD by means of Nebulizers and metered dose inhalers (MDI) has been clinically established for many years and the treatments involve generally low molecular weight molecules in formulations free of Stabilizers and absorption enhancers.[(10)]

2. INTRODUCTION OF LYOPHILLIZATION

In general, Lyophillization is a stabilizing process in which the sample is frozen followed by a reduction of the water content by sublimation and then by desorption to values that will no longer allow biological growth or chemical reactions. In 1890, it was reported the first time that biological specimens can be preserved under vacuum conditions and at temperature below 0 °C. Several years later, the interest in freeze-drying, now often called Lyophillization, significantly increased with the growing numbers of antibiotics and other sensitive pharmaceuticals. At the end of the past century, Lyophillization has evolved to a well-established technology for preservation of biopharmaceuticals products. During that time, fundamental concepts with regard to process design and formulation development have been established.[(11)]

Fig 3: Lab scale Freeze dryer (Delvac)

2.1 Lyophillization:

The term "Lyophillization," which means "to make solvent loving," is less descriptive than the alternative definition "freeze-drying." Several alternative definitions have been used to describe freeze-drying. Operationally we could define freeze-drying as a controllable method of dehydrating labile products by vacuum desiccation.

Technically, freeze-drying may be defined as:

- Cooling of the liquid sample, followed by the conversion of freezable solution water into ice; crystallization of crystallizable solutes and the formation of an amorphous matrix comprising non-crystallizing solutes associated with unfrozen moisture.
- Sublimation of ice under vacuum.
- "Evaporation" of water from the amorphous matrix.
- Desorption of chemical absorbed moisture resident in the apparently dried cake.[12]

Lyophilization or freeze drying is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying). Freeze drying is a process of drying in which water is sublimed from the product after it is frozen. It is a drying process applicable to manufacture of certain pharmaceuticals and biologicals that are thermolabile or otherwise unstable in aqueous solutions for prolonged storage periods, but that are stable in the dry state. The term "Lyophilization" describes a process to produce a product that "loves the dry state." Lyophilization is performed at temperature and pressure conditions below the triple point of water, to enable sublimation of ice.[13]

2.2 Characteristics of a lyophilized product

1. Long-term stability.
2. Short reconstitution time.
3. Elegant cake appearance.
4. Maintenance of the characteristics of the original dosage form upon reconstitution, including solution properties; structure or conformation of proteins; and particle-size distribution of suspensions.
5. Isotonicity upon Reconstitution.

2.3 Advantages

1. Chemical decomposition is minimized.
2. Removal of water without excessive heating.
3. Enhanced product stability in a dry state.
4. Ease of processing a liquid, simplifies aseptic handling.
5. More compatible with sterile operations than dry powder filling.
6. Rapid and easy dissolution of reconstituted product.
7. Water can be removed at low temperature, avoiding damage to heat-sensitive materials.
8. If freeze-drying is done properly, the dried product has a high specific surface area, which facilitates rapid, complete rehydration (or "reconstitution") of the solid.

9. Sample solubility, shrinkage, unacceptable appearance, or loss of activity may all be improved when freeze-drying is used rather than an alternative technique.
10. Particulate contamination is often reduced when samples are freeze-dried rather than spray or air-dried.

2.4 Disadvantages

1. Increased handling and processing time.
2. Volatile compounds may be removed by vacuum.
3. Need for sterile diluents upon reconstitution.
4. Cost and complexity of equipment.
5. Some drugs, particularly biological systems such as proteins, liposomal systems, and vaccines, are damaged by freezing, freeze-drying, or both.
6. Often the stability of a drug in the solid state depends on its physical state.

2.5 Principles of freeze-drying:-

Freeze-drying or Lyophilization is an effective way of drying materials without harming them. It makes use of the physical phenomenon of sublimation, which involves the direct transition between the solid state and the gaseous state without passing through the liquid phase.

2.6 Fundamental process step in Lyophillization

1) Freezing: The product is frozen. This provides a necessary condition for low temperature drying.

2) Vaccum: After freezing, a product is placed under vaccum. This enables the frozen solvent in the product to vapourize without passing the liquid phase, a process know as Sublimation.

3) Heat: Heat is applied to frozen product to accelerate sublimation.

4) Condensation: Low temperature condenser plates removed the vaporized solvent from the vaccum chamber by converting it back to a solid. This completes the process. Resulting process has a very large surface area thus promoting rapid dissolution of dried product.

2.5.1 The principle involved in Lyophillization as follows:

1) Heat transfer :

Heat supplies the energy necessary for sublimation of the water. An ice crystal is composed of pure water that is crystal lattice. The molecules have natural vibrations, so that extra thermal energy increases and probability of water molecules breaking free. When the water molecules breaks free, it diffuses through the dried surface of the solid and sublime, the thickness of dry outer surface of the specimen increases, and thus more energy is required to transport the molecules through the dry shell. Heat transfer to the product can be divided into three components: direct conduction, gas conduction and radiation. The pathways for transfer of energy through these three mechanisms are illustrated in Fig 4.

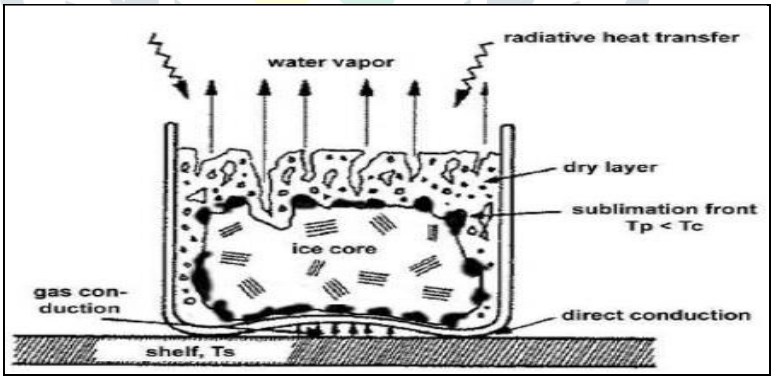

figure 4. type of heat transfer to the products.

Conduction is the main contributor to the heat transfer. It represents the heat energy transmitted from the shelf to the vial at the area where both are in direct contact. This area depends on the container type used, is especially low for well plates or molded vials, and only covers a fraction of the total vial bottom even for tubing vials designed for lyophilization. The amount of heat conveyed is proportional to the temperature difference between the cold vial and the warmer shelf. The driving force in conduction is the temperature gradient between different solids. Conduction can be modeled by Fourier's law:

$$\frac{dQ}{dt} = A\lambda\frac{dT}{dz} \quad \text{(Eq.1.1)}$$

dQ/dt - Heat flow,
A - Area of the surface,
λ - Thermal conductivity of the material and
dT - Temperature gradient across the thickness of the material.

For solids in series, the heat transfer rate, dQ/dt, can be thought of as the temperature gradient divided by the sum of the resistances. Heat is supplied to the interior of the shelf, either through electric coils or by a heated flowing liquid. The first resistance is the shelf, with a temperature difference from the interior to the surface. The next resistance is the tray or pan upon which the vials are placed, with a temperature difference from the shelf surface to the top of the tray. The third resistance is the glass vial, with a temperature difference between the tray surface and the bottom of the product in the vial. The fourth resistance is the frozen product inside the vial, with a temperature gradient between the ice at the bottom of the vial and the ice at the sublimation interface.

Radiation heat transfer must also be taken into account in lyophilization. Heat transfer by radiation takes place between two surfaces with different temperatures, i.e. the cold vial and the shelf, the top shelf, as well as chamber door and walls. The warmer surface radiates electromagnetic energy which is absorbed by the colder surface. Although this pathway also depends on the distance between the surfaces, the most important parameter is the temperature difference. Radiative heat transfer can be described by the Stefan Boltzmann equation

$$\frac{dQr}{dt} = Av\bar{e}\sigma(T_2^4 - T_1^4) \quad \text{.....(Eq.1.2)}$$

dQr/dt - Represents the amount of energy per time transmitted by radiation,

Av - Vial area (top or bottom5),

ē - Effective emissivity for exchange of radiation (between 0 and 1),

σ - Boltzmann constant, and

$(T_2^4 - T_1^4)$ - Difference between the temp. of the two surfaces to the 4th power.

The effective emissivity is an important parameter for surface materials used in the construction of a freeze dryer. While acrylic glass shows especially high emissivity (0.95), the radiation of polished stainless steel is much lower (0.4). This difference needs to be regarded during transfer and scale-up of lyophilization cycles between freeze-dryers with different radiation characteristics.

2) Mass transfer :

The mass transfer of water vapor from the product to the condenser is determined by several resistances to vapor flow that limit the flow rate. The most important factor is the resistance of the already dried layer to mass transfer, the so called product resistance (Rp). The water vapor which sublimes at the sublimation front needs to diffuse through a network of small pores in the dried matrix. These pores are created when ice crystals are removed by sublimation, and their size, shape and interconnection are influenced by the freezing process. Rp values depend on the thickness of the already dried cake layer, and change during the course of the drying process.

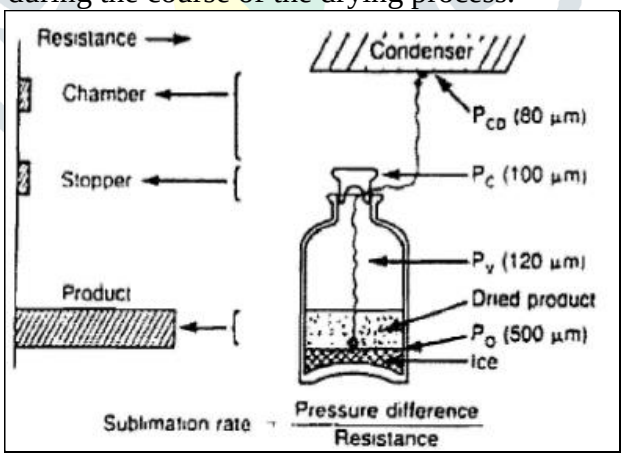

figure 5. resistances and their relative contributions in mass transfer

In modeling, the product can be thought of a porous solid, with Knudson flow. The stopper can be modeled as a solid with transition flow through small tubes. The chamber can be modeled as a gas with viscous flow. The resistance associated with the product, Rp, depends on the cross sectional area of the product, Ap by However, this really becomes a moving boundary problem, as Rp increases with time as the ice moves out of the product cake and must be solved through numerical methods.

2.5.2.1 Coupling between heat and mass transfer

During the steady state of primary drying, the heat removed by sublimation of ice is in equilibrium with the amount of heat introduced into the product. Heat and mass transfer during freeze-drying are coupled which can be described by:

$$\frac{dQ}{dt} = \left(dm/_{dt}\right).\Delta HS + ms.\,cv\left(\frac{dT}{dt}\right) \quad \text{.....(Eq.1.3)}$$

dQ/dt - Heat flow to the product,
dm/dt - Mass removal by sublimation,
ΔHS - Temperature-dependent heat of sublimation of ice (cal/g),
ms - Sample mass (g),
cv - Specific heat of the sample (cal/K*g) and
dT/dt - Change of product temperature (K/s).

The first term describes the rate of heat removal by sublimation, the second term signifies the rate of heat removal through a change in product temperature which is mainly the case during the early stage of primary drying. Since the second specific heat term is usually small compared to the sublimation term, the heat transfer during steady state primary drying can be described with the simplified equation:

$$\frac{dQ}{dt} = \left(dm/_{dt}\right).\Delta HS \quad \text{..... (Eq.1.4)}$$

This implies that essentially all heat introduced into the product is used to convert ice into water vapor by sublimation, and the product temperature is assumed to remain constant. This simplified model is the basis for numerous modeling approaches of the freeze-drying process.[14]

Lyophillization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase.[15] Lyophilization is performed at temperature and pressure conditions below the triple point, to enable sublimation of ice. The entire process is performed at low temperature and pressure, hence is suited for drying of thermolabile compounds. Steps involved in lyophilization start from sample preparation followed by freezing, primary drying and secondary drying, to obtain the final dried product with desired moisture content.[16] The concentration gradient of water vapor between the drying front and condenser is the driving force for removal of water during lyophilization. The vapor pressure of water increases with an increase in temperature during the primary drying. Therefore, primary drying temperature should be kept as high as possible, but below the critical process temperature, to avoid a loss of cake structure. This critical process temperature is the collapse temperature for amorphous substance, or eutectic melt for the crystalline substance. During freezing, ice crystals start separating out until the solution becomes maximally concentrated. On further cooling, phase separation of the solute and ice takes place.[17]

2.7 Lyophillization cycle

The freeze drying process consists of three stages.

1. Freezing
2. Primary drying
3. Secondary drying

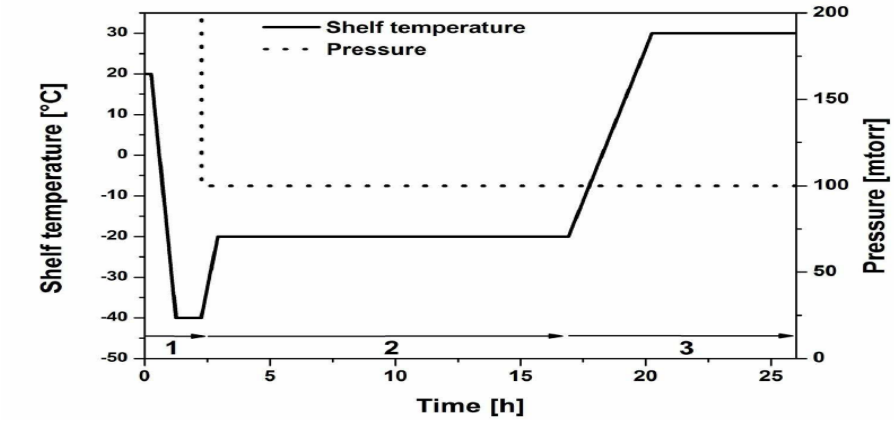

figure 6. schematic illustration of a typical lyophilization cycle.

1. Freezing
2. Primary drying, and
3. Secondary drying.

2.7.1 Freezing

Freezing is a critical step, since the microstructure established by the freezing process usually represents the microstructure of the dried product. The product must be frozen to a low enough temperature to be completely solidify. The material to be freeze-dried must be adequately pre-frozen. The method of pre-freezing and the final temperature of the frozen product can affect the ability to successfully freeze dry the material.[18] Rapid cooling results in small ice crystals, useful in preserving structures to be examined microscopically, but resulting in a product that is more difficult to freeze dry. Slower cooling results in large ice crystals and less restrictive channel in the matrix during the drying process. Products freeze in two ways, the majority of products that are subjected to freeze-drying consists primarily of water, the solvent and materials dissolved or suspended in the water, the solute.

The mixtures of substances that freeze at lower temperature than the surrounding water. This is called the eutectic temperature. Eutectic point is the point where all the three phases' i.e. solid, liquid and gaseous phases coexist. It is very important in freeze-drying to pre freeze the product to below the eutectic temperature before beginning the freeze-drying process. The second type of frozen product is a suspension that undergoes glass formation during the freezing process. Instead of forming eutectics, the entire suspension becomes increasingly viscous as the temperature is lowered. Finally the products freeze at the glass transition point forming a vitreous solid. This type of product is extremely difficult to freeze dry. [19,20]

2.7.2 Primary drying

After pre-freezing the product, conditions must be established in which ice can be removed from the frozen product via sublimation, resulting in a dry, structurally intact product. This requires very carefully control of the two parameters. i) Temperature and ii) Pressure involved in freeze-drying system. The rate of sublimation of ice from a frozen product depends upon the difference in vapor pressure of the product compared to the vapor pressure of the ice collector. Molecules migrate from the high pressure sample to a lower pressure area. Since vapor pressure is related to temperature, it is necessary that the product temperature is warmer than the cold trap (ice collector) temperature. It is extremely important that the temperature at which a product is freeze dried is balanced between the temperature that maintains the frozen integrity of the product and the temperature that maximizes the vapor pressure of the product. This is the balance is key to optimum drying.

A third component essential in freeze-drying system is energy. Energy is essential in the form of heat. Almost ten times, much energy is required to sublime a gram of water from the frozen to the gaseous state as is required to freeze a gram of water, (2700 joules per gram of ice). Heat must be applied to the product to encourage the removal of water in the form of vapor from the frozen product. The heat must be very carefully controlled, as applying more heat than the evaporative cooling in the system can warm the product above its eutectic or collapse temperature. Heat can be applied by several means one method is to apply heat directly through a thermal conductor shelf such as is used in tray drying. Another method is to use ambient heat as in manifold drying[21].

2.7.3 Secondary drying

After primary freeze-drying is complete, and all ice has sublimed, bound moisture is still present in the product. The product appears dry, but the residual moisture content may be as high as 7-8% continued drying is necessary at warmer temperature to reduce the residual moisture content to optimum values. This process is called 'Isothermal Desorption' as the bound water is desorbed from the product[12].Secondary drying is normally continued at a product temperature higher than ambient but compatible with the sensitivity of the product. In contrast to processing conditions for primary drying which use low shelf temperature and a moderate vacuum, desorption drying is facilitated by raising shelf temperature and reducing chamber pressure to a minimum. Care should be exercised in raising shelf temperature too highly; since, protein polymerization or biodegradation may result from using high processing temperature during secondary drying. Secondary drying is usually carried out for approximately 1/3 or 1/2 the time required for primary drying.

The general practice in freeze-drying is to increase the shelf temperature during secondary drying and to decrease chamber pressure to the lowest attainable level. The practice is based on the ice is no longer present and there is no concern about "melt track" the product can withstand higher heat input.[13] Also, the water remaining during secondary drying is more strongly bound, thus requiring more energy for its removal. Decreasing the chamber pressure to the maximum attainable vacuum has traditionally been thought to favor desorption of water.

2.8 Application:-

1) Pharmaceutical and biotechnology:

Pharmaceutical companies often use freeze drying to increase the shelf life of products, such as vaccines and injectable.

2) Food industry:

Freeze drying is used to preserve food and make it very lightweight .This process has been popularized in the form of freeze dried ice cream, an example of astronaut food.

3) Other uses:

Organization such as the document conservation laboratory at the united states have studies on freeze drying as recovery method of water damaged books and documents .In bacteriology freeze drying is used to conserve special strains.

2. PLAN OF WORK:

1. LITERATURE SURVEY
2. SELECTION OF DRUG
3. PROCUREMENT OF DRUG AND EXCIPIENTS
4. EXPERIMENTAL
   - A. PREFORMULATION STUDIES
     - ❖ Authentication of Drug and Excipient
     - ❖ Solubility Study
     - ❖ Calibration Curve
     - ❖ Compatibility study
   - B. FORMULATION AND CHARACTERIZATION OF LYOPHILLIZED POWDER
     - ❖ Particle size analysis
     - ❖ Drying yield and Drug content
     - ❖ DSC and FTIR analysis
     - ❖ Crystalline nature - X-RD
     - ❖ Powder density measurement
     - ❖ Formulation development and In vitro powder aerosolization
     - ❖ In vitro drug release by dissolution study
5. COMPILATION AND ANALYSIS OF DATA.
6. RESULT AND DISCUSSION
7. CONCLUSION
8. REFERENCES.

3. MATERIALS AND INSTRUMENTS:

3.1 MATERIALS:

Below Table shows the list of material obtained as gift sample as well as purchased from commercial sources for the experimental work of this study.

table 1: list of materials and chemicals

Name of Material/ Chemical	Obtained/Purchased
Ribavirin	Gift sample
Poloxamer 188	Research Lab., Mumbai
D-mannitol	S. D. fine chemicals, Mumbai
Pvpk-30	Poona chemical Lab., Pune
Potassium dihydrogen phosphate (KH_2PO_4)	Finar chemicals Ltd., Ahmadabad
SLS	Finar chem. Ltd., Ahmadabad
Potassium bromide	Loba chemicals, Mumbai

3.2 INSTRUMENTS AND EQUIPMENTS:

table 2: list of instruments and equipments used

Name of the Instrument/Equipment	Make and Model
Digital Analytical Balance	SHIMADZU AUX 220, Japan
Bath Sonicator	Lab-Hosp Corporation LHC-670
Lab stirrer	Remi Motors
Magnetic stirrer	Whirlmatic Mega, SPECTRALAB
Freeze Dryer	Delvac, Mumbai
UV-Visible double beam spectrophotometer	JASCO, V-550, JAPAN
FTIR spectrophotometer	JASCO FTIR-410, JAPAN
KBr press	Techno search instrument M-15
Particle size analyzer	Malvern analyzer
Differential scanning calorimetry (DSC)	DuPont 2000 USA
X-Ray diffraction apparatus	Philips PW- 3710 BASED
Andersen cascade impactor (ACI)	Copley Scientific Limited, Nottingham, UK
Rota haler®	Glaxo, UK
Dissolution test apparatus	Lab India Disso 2000

4. EXPERIMENTAL WORK-

- All the chemicals other than listed in materials used were are of Analytical grade. Distilled water used was further filtered through 0.45 µm membrane filter.
- From this point forward, D-mannitol, Ribavirin, Pvpk-30 and Poloxamer 188 will be referred to as mannitol, Rb, Pvpk-30 and F-68.

4.1. CHARACTERIZATION OF STANDARD DRUGS:-

a) Description-

The sample of amlodipine and atorvastatin was visually evaluated for physical state and odour.

b) Melting point-

Melting point of the pure drug and excipient was determined by using melting point apparatus. The thermometer used was previously calibrated. The temperature at which sample starts melting is considered as lower limit and at which completely melt is considered as upper limit.[22,23]

4.2 PREFORMULATION STUDY:

4.2.1 Drug excipient authentication:

4.2.1.1 Fourier transform infrared spectrometry (FTIR):

Fourier transform infrared spectrometry (FTIR) of pure drugs: Rb; excipients: SLS, Mannitol, PVPK-30 and Poloxamer 188 was carried out using a JASCO FTIR-410, JAPAN FTIR Spectrophotometry .The spectra were scanned over wavelength region of 400 to 4000 cm^{-1}, resolution of 4 cm^{-1} and accumulation of 20 scans were used in order to obtain good quality spectra by making a pellet of the sample with KBr.

The procedure consisted of grinding the sample with KBr in an agate mortar and pestle and compressing the sample in an evacuable KBr die by applying a pressure of 5 tons for 5 min in a hydraulic press, Techno search instrument M-15 KBr press (KBr pellet method). The pellet was placed in the light path and the spectrum was obtained. The resultant spectra were then compared with standard reference and observed for any type of deviation from the standard or RS spectra. [24]

4.2.1.2 Selection of solvent for Solubility study -

For spectral analysis the selection of proper solvent was needed in which the drug should be completely soluble. The solubility of drugs were checked by using different solvents, with the help of the reported data drug solubility obtained as follows.

Table no.3 - solubility of drug in different solvents

Sr. No	Solvent
1	Water
2	Ethanol
3	Ethyl Acetate
4	Chloroform
5	Phosphate buffer pH=7.4

4.2.1.3 Determination of Calibration curve:

- **Selection of dissolution medium:**

Various dissolution Media were used. For the sake of solubility of drugs in phosphate buffer (pH-7.4) was used according to the solubility characteristics of drugs, dissolve the drugs completely.[25]

- **Preparation of working standard stock solution:**

Individual standard stock solutions were prepared by dissolving 5 mg of drug in 50 ml volumetric flask separately. The contents were dissolved with the aid of shaking and sonication for about 15 minutes. The resultant individual stock solution was of concentration 100µg/ml.

- **Preparation of sample solution:**

From the above stock solution of concentration of 100 µg/ml, serial dilutions were done so as to get sample solution of concentration range from 1 µg/ml to 15 µg/ml for drugs individually.

- **Determination of absorption maxima:**

From the standard stock solutions of Rb (100 µg/ml) pipette out 1 ml of each in two separate 10 ml volumetric flask and make up the volume to get a concentration of 10 µg/ml each. Both the solutions were scanned in the spectrum mode over the range of 200-400 nm.

- **Preparation of standard calibration curve:**

The respective absorbance of serial dilutions were recorded at 207 nm and calibration curves were plotted.

4.2.2 Compatibility study:

The proper design and formulation of a dosage form requires consideration of physical, chemical and biological characteristics of all the drug substances and excipients to be used in fabricating the product. The drug and the excipients must be compatible with one another to produce a product that is stable, effective, attractive, easy to administer and safe.[26]

4.2.2.1 Compatibility study by FTIR-spectrometry:

The possible interaction between drug and excipient was studied by FTIR spectrometry. The spectra were recorded in the wavelength region of 400 to 4000 cm^{-1} using a FTIR spectrophotometer (JASCO FTIR-410, JAPAN). Compatibility studies were carried out at initial, first, second and third week and analyzed for its physical appearance and drug stability.

4.3 FORMULATION AND OPTIMIZATION:

4.3.1 Preparation of Nano suspension by Emulsion Diffusion Method =

Nano suspension were prepared by solvent evaporation technique. The drug (Rb 20 mg) was dissolved in ethyl acetate (organic Phase)in the ratio of 2:1 in 1st beaker at room mg)temperature. This was poured into 40 ml water containing Different ratio of SLS, PVPK-30, Poloxamer 188 mixed in 2nd beaker at room temperature. Add 40 ml water (aqueous phase) and stir well. Pour 1st beaker into 2nd beaker (at a rate of 0.5ml/min).The solution was stirred for 5-6 min. and then sonication was done for 10min.(by using ULTRASONIC HOMOGENISER).

The suspension was diluted with 60 ml D.W. and stir for 1 hrs.to induce diffusion of organic solvent in to the aq. phase. The prepared Nano suspension was left stirring at room temperature to evaporate the organic solvent.[27]

Table 4: formulation components for batches =

Batch No.	Drug : Ethyl Acetate	SLS:PVPK-30:POLOXAMER 188
A	**2:1**	**1:1:1**
B	**2:1**	**1:0:1**
C	**2:1**	**2:2:1**
D	**2:1**	**2:2:0**
E	**2:1**	**2:2:2**
F	**2:1**	**0:1:2**

Mannitol (50 mg) was used as diluent for each batch.

4.3.2 Conversion of dispersion (suspension by emulsion diffusion method) into Dry powder for inhalation by Lyophillization:

Freeze drying using a ,DELVAC Lab Freeze Dryer with a co-current 0.7 mm, 7 fluid nozzle equipped with autojet deblocking system, was applied in order to retrieve respirable powders in dried state from suspension described above. The following conditions were used during Freeze drying as mentioned in the table 5. The resultant dry powder was blown in container. Powders were kept in glass vials and stored in glass vials and stored in desiccator at ambient temperature before use.

table 5: freeze drying parameters

Parameter	Optimized conditions
Inlet Temperature	-65^{0}C
Outlet Temperature	37^{0}C
Atomization pressure	20-30 psi
Feed Pump Speed	10 ml
Vacuum	0.340 mbar

4.4 CHARACTERIZATION OF DRY POWDER FOR INHALATION (DPI):

4.4.1 Particle size analysis:

The particle size and polydispersity of the particles in dried state were determined by (Malvern particle size analyzer). Samples were dispersed in water as dispersant medium. Average size and polydispersity index (PDI) is determined.

4.4.2 Percentage yield (%) and drug content:

The yields of preparation were determined by the weight of the products, Lyophilized powders, with respect to the weight of the initial drugs and excipients used.

Yield (%) = 100 × (Recovery of weight of Lyophilized powders / weight of the Drugs + weight of excipients)

The drug content of Lyophillized powders was determined using UV spectrophotometry. Samples from each batch of Lyophillized formulation were dissolved in phosphate buffer (pH-7.4) and the actual drug content was determined by first-derivative UV spectrophotometric method (JASCO model V-550, JAPAN UV-visible double beam spectrophotometer). Drug content was calculated from the ratio of actual drug content to total weight of Lyophillized powders taken for analysis and expressed as a percentage.[28]

4.4.3 Fourier transform infrared spectrometry (FTIR):

Fourier transform infrared spectrometry (FTIR) spectra were recorded with a JASCO FTIR-410, JAPAN FTIR Spectrophotometer to evaluate the molecular states of pure drugs: Rb; excipients: Mannitol, Sls, Pvpk and Poloxamer 188; physical mixture and all dried formulations. The spectra were scanned over wavelength region of 400 to 4000 cm^{-1}, resolution of 4 cm^{-1} and accumulation of 20 scans were used in order to obtain good quality spectra by making a pellet of the sample with KBr. The procedure consisted of grinding the sample with KBr in an agate mortar and pestle and compressing the sample in an evacuable KBr die by applying a pressure

of 5 tons for 5 min in a hydraulic press, Techno search instrument M-15 KBr press (KBr pellet method). The pellet was placed in the light path and the spectrum was obtained.

4.4.4 Differential scanning calorimetry (DSC):

The phase transition of the pure drug, excipients, physical mixture, and all Lyophillized formulation batches were studied by thermogram obtained by using Differential scanning calorimeter (Dupont 2000, model SDT-2960, USA). An empty aluminum pan was used as reference. DSC measurements were performed at the heating rate of 10 ºC/min from 25 to 350 ºC using aluminum sealed pan. Sample weight was kept between 5-10 mg. During the measurement, the sample cell was purged with nitrogen gas.(29)

4.4.5 X-Ray powder diffraction study (XRD):

The crystalline nature of pure drug and all Lyophillized formulation batches were examined by studying its X-Ray diffraction patterns by using powder X-Ray diffractometer (PW- 3710 BASED). It was determined whether the obtained formulation after precipitation is a coprecipitate of individual substances or whether it becomes co-crystal. The operating parameters for instrument were Cu filtered K (α) radiations, a voltage of 40 kV, current of 25 mA and receiving slit of 0.2 In. The instrument was operated over 2θ scale. The angular range was 5 to 50º (2θ) and counts were accumulated for 0.8 second at each step.(30)

4.4.6 Powder density:

The powder density of all lyophilized formulations was determined by pouring a known mass of powder under gravity into a calibrated measuring cylinder and recording the volume occupied by the powder. The tapped density of the dried powders was determined by tapped density measurements on the same samples until no further change in the powder volume was observed. Measurements were performed in triplicate. (10)

Carr's Index values for each Lyophilized powder were derived from poured density and tapped density data, according to given formula. The Carr's Index value gives an indication of powder flow; a value less than 25 % indicates a fluid powder, whereas a value greater than 25 % indicates cohesive powder as shown in the table 11.(31)

Carr's Index (%) = 100 (Tapped density – Poured density)/ Tapped density

table 6: flow properties of powder

Sr. No.	Carr's Index (%)	Flowability
1	5-12	Excellent
2	12-18	Good
3	18-21	Fair
4	21-25	Poor, fluid
5	25-32	Poor, cohesive
6	32-38	Very poor
7	40<	Extremely poor

4.4.7 Formulation development and in vitro powder aerosolization:

The in vitro aerosolization and deposition properties of DPI blended with different carriers are determined using an Andersen cascade impactor (ACI) (Copley Scientific Limited, Nottingham, UK) with a Rotahaler® (Glaxo, UK) device. Experiments are carried out with a preseparator at air flow rates of 30 and 60 L/min. In each experiment, 8mL of the extracting solvent is poured inside the preseparator. A coating of 1% (w/v) solution of silicon oil in hexane is used on the impaction plates to prevent particle bounce and re-entrainment. Depending upon actual drug content, the representative powder from each batch was filled into hard gelatin capsule (size 3, Capsugel, Germany) manually so that each capsule contained 50 µg Rb.(32) About 25 mg of L1, 20 mg of M1 and 20 mg of K1 samples of powders were weighed and loaded into size 3 hard gelatin capsule, which were individually installed in a Rotahaler® device.(33)

Rotahaler® is used as the inhaler to aerosolize the powder inside the ACI. An actuation time of 4 and 8 s is used for flow rates of 60 and 30 L/min, respectively, for each capsule to completely disperse all the particles. Experimental runs are conducted in triplicate. Particles remaining in the capsule, inhaler and different parts of the ACI are extracted using the same solvent used for the blend homogeneity test mentioned in the earlier section. The solutions are also assayed in a similar way.

Each batch was analyzed in triplicate and the following parameters were used to characterize the deposition profiles of the drug:

- The emitted dose (ED), which was the sum of drug collected from all parts of the ACI.
- The fine particle dose (FPD) defined as the amount of drug deposited in the lower stages of the ACI.
- The fine particle fraction (FPF) or respirable fraction (RF) calculated as the amount deposited in the lower stage as a percentage of the emitted dose (amount emitted into upper and lower stages excluding the amount remaining in the device).[34]

4.4.8 In vitro drug release:

The in-vitro drug release of all the Lyophilized formulations was investigated by dissolution study. An accurately weighed amount of DPI equivalent to 50 µg of Rb was added into membrane bag which is put into 900 ml of dissolution medium; Phosphate buffer pH 7.4 and drug release was investigated using the USP rotating paddle dissolution apparatus (Lab India 2000) at 100 rpm and 37 ºC. A percent release study was continued from 5 min. to 3 hrs. The final volume in all cases was 900 ml. The samples were withdrawn from the dissolution medium at various time intervals. 5 ml of sample was withdrawn from dissolution medium and subjected to UV Spectrophotometric analysis at 207 nm (λmax of Rb). All the samples were analyzed in triplicate.[35]

5. RESULT AND DISCUSSION:

1. PREFORMULATION STUDY:

5.1.1 DRUG AND EXCIPIENT AUTHENTICATION:

5.1.1.1 Fourier transform infrared spectrometry (FTIR)[36]

The following Figure 7 shows the FTIR spectra of Ribavirin obtained by FTIR spectrometry of the procured sample, was concordant with the standard reference as shown in Table 7. Hence, it was confirmed that the procured drug sample was authentic.

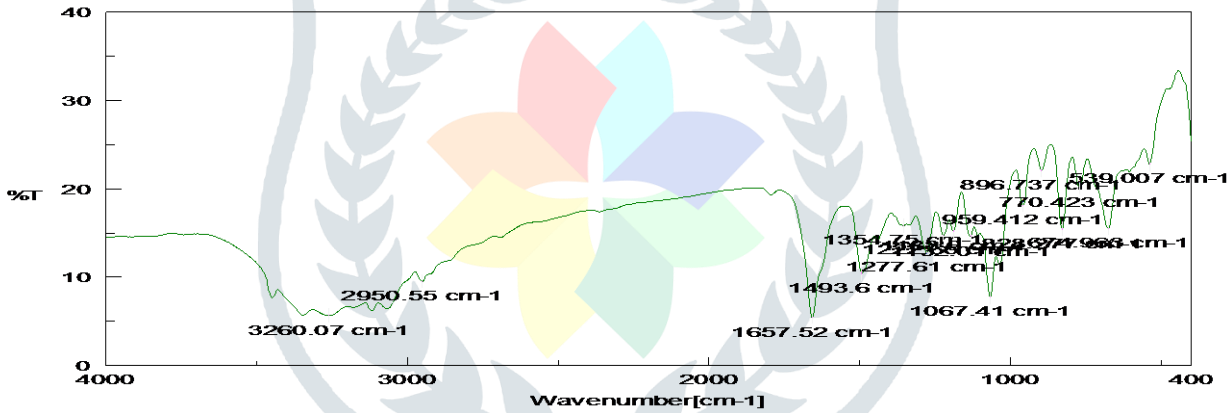

figure 7: ftir spectra of procured ribavirin.

table 7: authentication of procured ribavirin by ftir spectrometry

Type of vibration and Functional group/structure	Wavenumber (cm^{-1})	
	Reference	Observed
O-H Str	3200-3400	3260
Alkyl C-H Str	2900-3000	2950
Alkyl C-H Def	1300-1500	1493
Aromatic C-H Def	1350-1600	1354
C=O Str	1650-1750	1657
C-O Str in ether	1100-1400	1067, 1277
N-H Def	1575-1625	1608
C-O Str in primary alcohols	1000-1000, 900-1000	1021, 997
Out of Plane Def	700-850	737, 770, 896

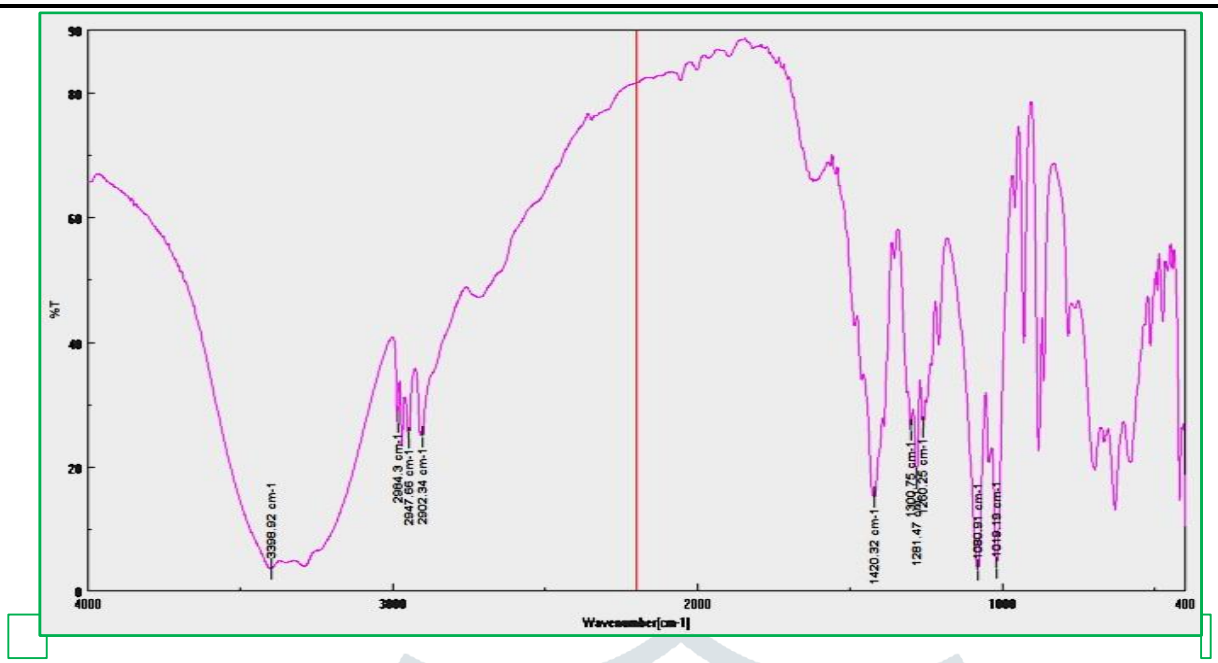

figure 8: ftir spectra of procured mannitol

table 8: authentication of procured mannitol by ftir spectrometry

Type of vibration and Functional group/structure	**Wavenumber (cm^{-1})**
O-H Str	3398
Alkyl C-H Str	2984, 2947, 2902
Alkyl C-H Def	1420
C=O Str	1578
C-O Str coupled with O-H Def in Primary alcohols	1300, 1291, 1260, 1080, 1044

FTIR spectra of Mannitol shown in Fig. 8, the strong peak at 3398 cm^{-1} could be assigned to the O-H str. The characteristic bands of Mannitol are shown in Table 8.

figure 9: ftir spectra of procured pvpk-30

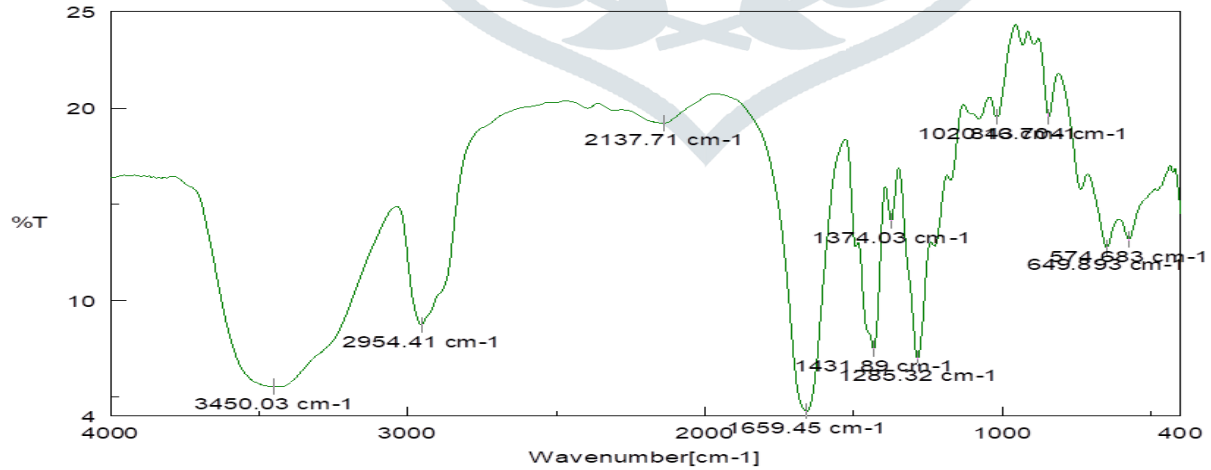

table 9: authentication of procured pvpk-30 by ftir spectrometry

Type of vibration and Functional group/structure	Wavenumber (cm^{-1})
O-H Str	3450
Alkyl C-H Str	2954
Alkyl C-H Def	1431, 1374
C-O Str in Ether	1285

Fig. 9 shows the FTIR spectra of the PVPK-30 obtained, with all characteristic bands given in Table 9. The strong peak at 3450 cm^{-1} could be assigned to the O-H str.

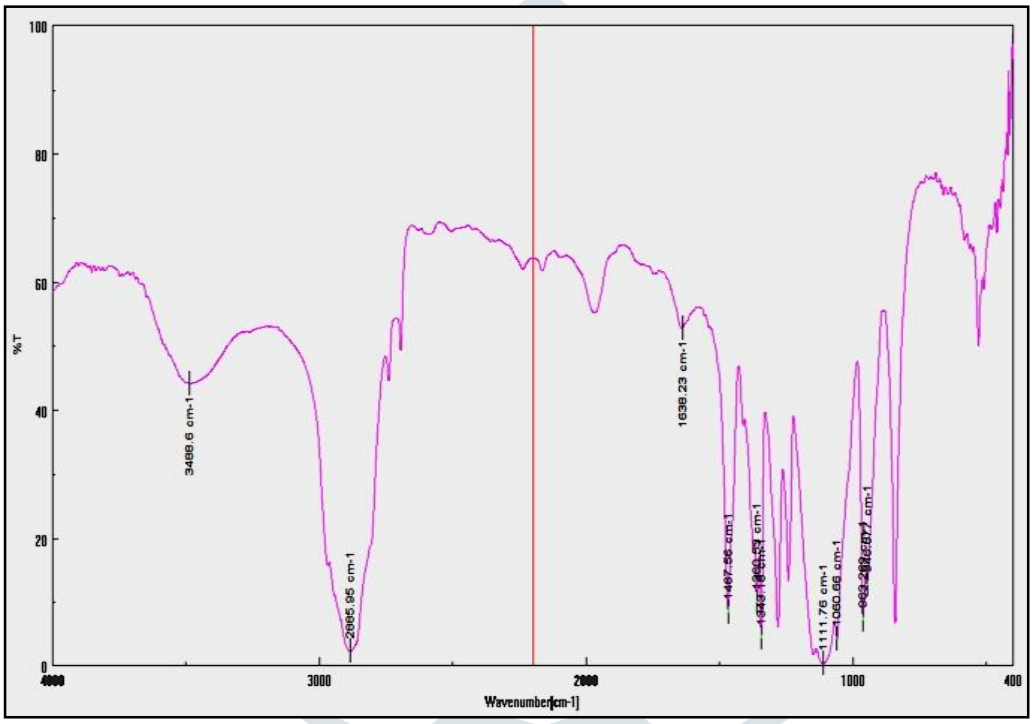

figure 10: ftir spectra of procured poloxamer 188

table 10: authentication of procured poloxamer 188 by ftir spectrometry

Type of vibration and Functional group/structure	Wavenumber (cm^{-1})
O-H Str	3488
Alkyl C-H Str	2855
Alkyl C-H Def	1467, 1360, 1343
C-O Str in ether	1111
C-O Str coupled with O-H Def in Primary alcohols	1360 (m), 1060 (s)

FTIR spectra of Poloxamer 188 shown in Fig. 10 showed all the characteristic bands. The strong peak at 3488 cm^{-1} could be assigned to the O-H str. The characteristic bands of Poloxamer 188 are shown in Table 10.

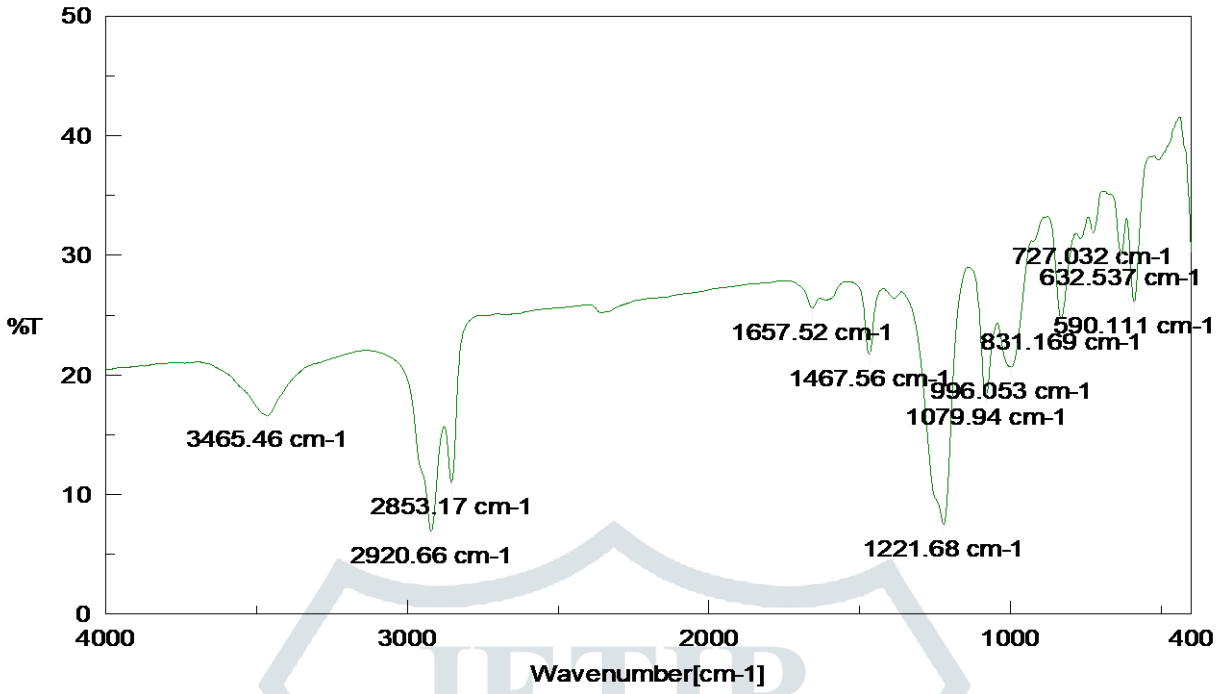

figure 11: ftir spectra of procured sls.

table 11: authentication of procured sls by ftir spectrometry

Type of vibration and Functional group/structure	**Wavenumber (cm^{-1})**
O-H Str	3465
Alkyl C-H Str	2853
Alkyl C-H Def	1467, 1221
C-O Str in ether	1079
C-O Str coupled with O-H Def in Primary alcohols	1221 (m), 996 (s)

FTIR spectra of SLS shown in Fig. 11, the strong peak at 3465 cm^{-1} could be assigned to the O-H str. The characteristic bands of SLS are shown in Table 11.

From the FTIR spectra of all the procured samples (Fig. 7-11) and characteristic bands (Table 7-11), it is found that the spectra obtained by FTIR spectrometry, was concordant with the standard reference of respective sample. Hence, it was confirmed that the procured drug and excipient samples were authenticate.

5.1.1.2 Melting point determination:

Following given is the melting range of pure drug (RIBAVIRIN) and all excipients used for formulation of DPI. From the Table 12, it is clear that pure drug and all the excipients comply with the reference melting point respectively.[37]

table 12: melting range of drug&excipient

Sr. No.	Drug/Excipient	Reference Melting Range in ^{0}C	Observed Melting Range in ^{0}C
1	Ribavirin	168-172°C	170°C
2	Pvpk-30	150-152°C	150°C
3	Mannitol	166-168°C	166°C
4	SLS	204-207°C	205°C
5	Poloxamer 188	52-57°C	55°C

As per the Table no.12,drug containing Ribavirin melting point observed at 170°C,also Pvpk at 150°C, Mannitol at 166°C, SLS at 205°C and Poloxamer melting point observed at 55°C.

5.1.1.3 Solubility Study: -

The solubility of drugs was checked by using different solvents, with the help of the reported data drug solubility obtained as follows.

table no.13 - solubility of drug in different solvents

Sr. No	Solvent	Solubility
1	Water	+++
2	Ethanol	+
3	Ethyl Acetate	_ _
4	Chloroform	_ _
5	Phosphate buffer pH=7.4	+++

+ Partially soluble, ++ sparingly soluble, +++ freely soluble**,** -- Practical Insoluble**.**

As per the Table no.13 shows Ribavirin is freely soluble in Water & Phosphate Buffer pH 7.4, while Practical insoluble in Ethyl Acetate and Chloroform. Ribavirin was Practical soluble in Ethanol.

5.1.2.1 Standard calibration curve of Ribavirin in Phosphate buffer (pH 7.4)[38,39]

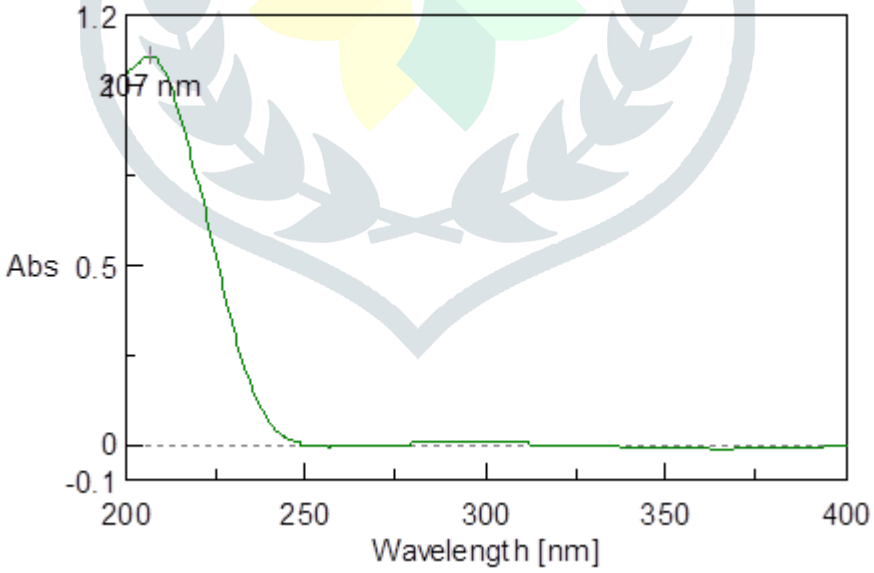

figure 12: λmax for ribavirin

Ribavirin showed an absorbance peaks at 207 nm. The linear regression data for the calibration curve showed a good linear relationship over the concentration range 2-10 µg/ml respectively with respect to absorbance of Ribavirin.

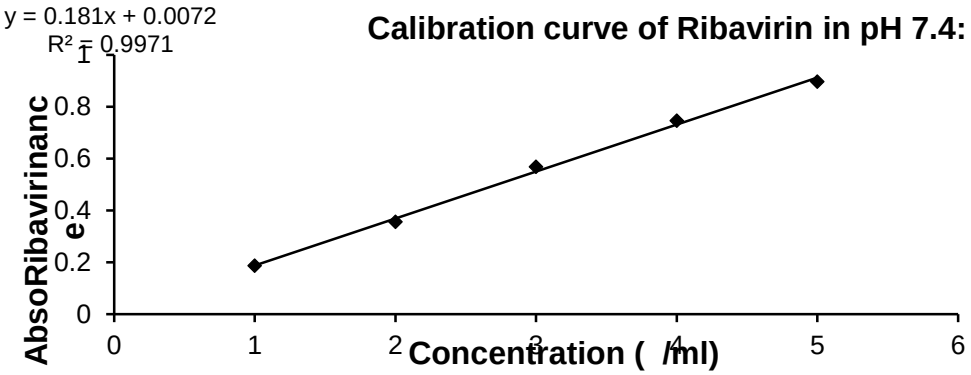

figure 13= calibration curve of ribavirin in phosphate buffer 7.4

5.1.2.2 Standard calibration curve of Ribavirin in Water:-

The standard solution of Ribavirin in Water was prepared by diluting stock solution in range of 2, 4, 6, 8 and 10µg/ml. The calibration curve of Ribavirin was plotted by using Water as blank and measured at 225nm. The standard calibration curve and value of R^2 of Ribavirin in Water are shown in figure 14.

table.14: readings for calibration curve of ribavirin in water.

Sr. no	Conc.(µg /ml)	Absorbance of Ribavirin
1	2	0.09
2	4	0.17
3	6	0.25
4	8	0.32
5	10	0.39

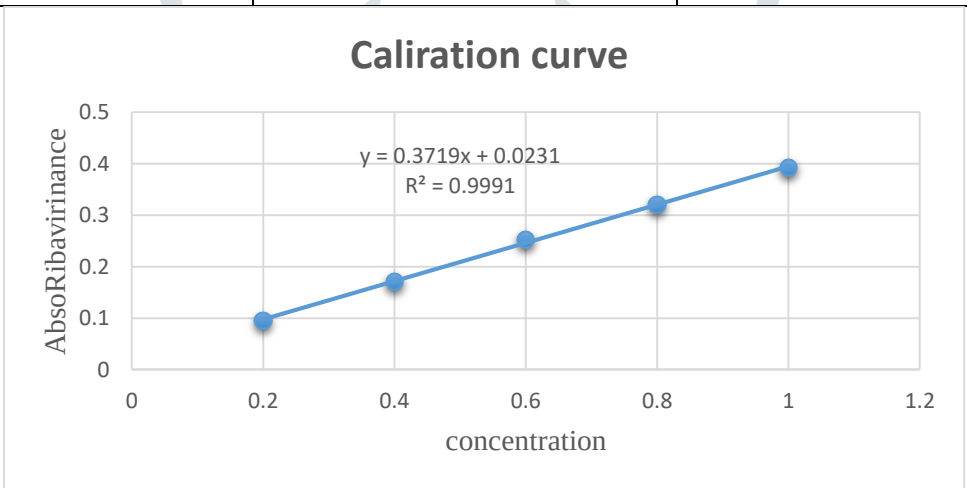

figure 14= calibration curve of ribavirin in water

5.1. COMPATIBILITY STUDY:

5.1.2 By FTIR spectrometry:

The possible interaction between the drug and excipients was studied by IR spectroscopy at first, second and third week. The infrared (IR) spectroscopy was conducted using a JASCO FTIR spectrophotometer (JASCO FTIR-410, Japan) and the spectrum was recorded in the wavelength region of 4000 to 400 $cm^{-}1$. The IR spectra of pure Ribavirin, Pvpk-30, Mannitol, SLS, Poloxamer 188 and its physical mixture are shown in Fig.15 Characteristic peaks for physical mixture was compared with the reference spectra's of pure drug. The results revealed no considerable changes in the IR peaks of Ribavirin, when mixed with excipients. Such interaction was also studied at same temperatures upto 3 weeks. Hence IR spectroscopy results showed that the drugs are compatible with given excipients.

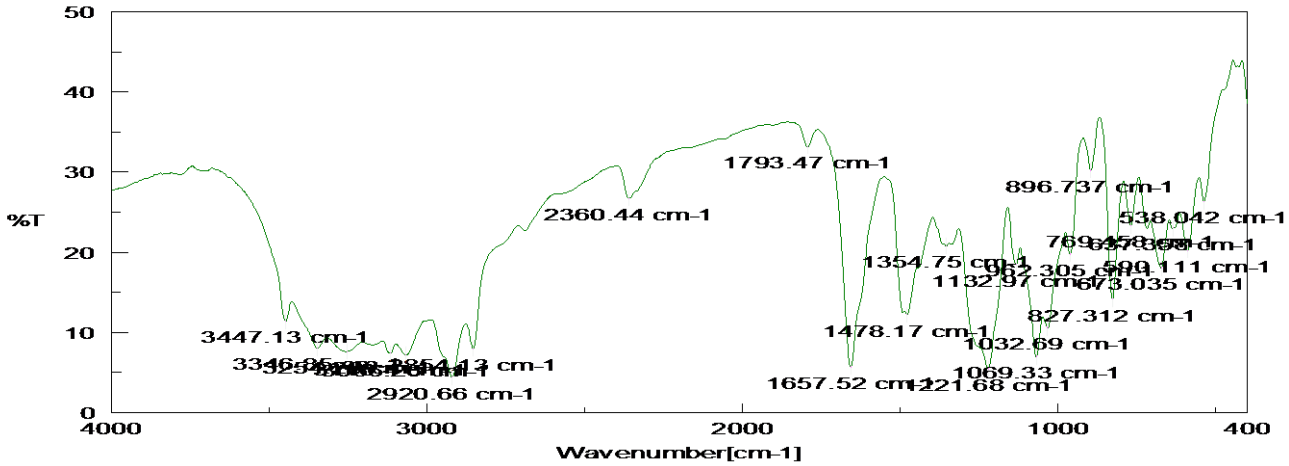

figure 15: 1st week compatibility study of ribavirin and sls by ftir spectrophotometry

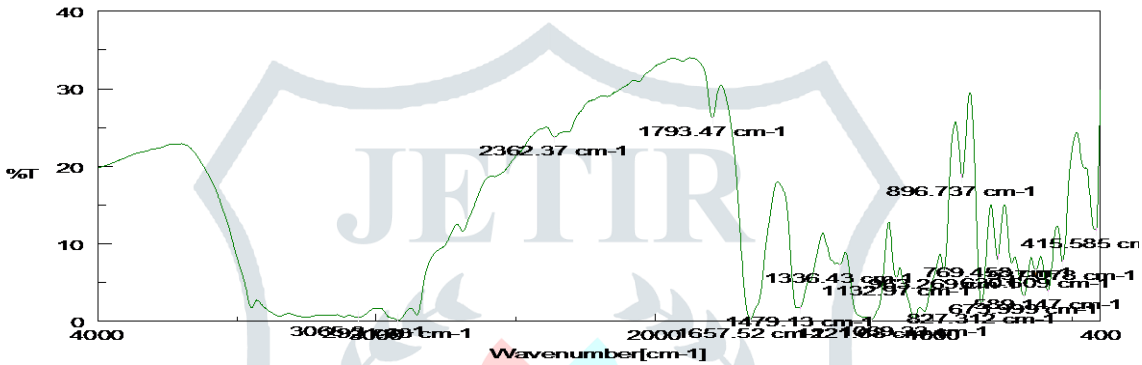

figure 16: 2nd week compatibility study of ribavirin and sls by ftir spectrophotometry

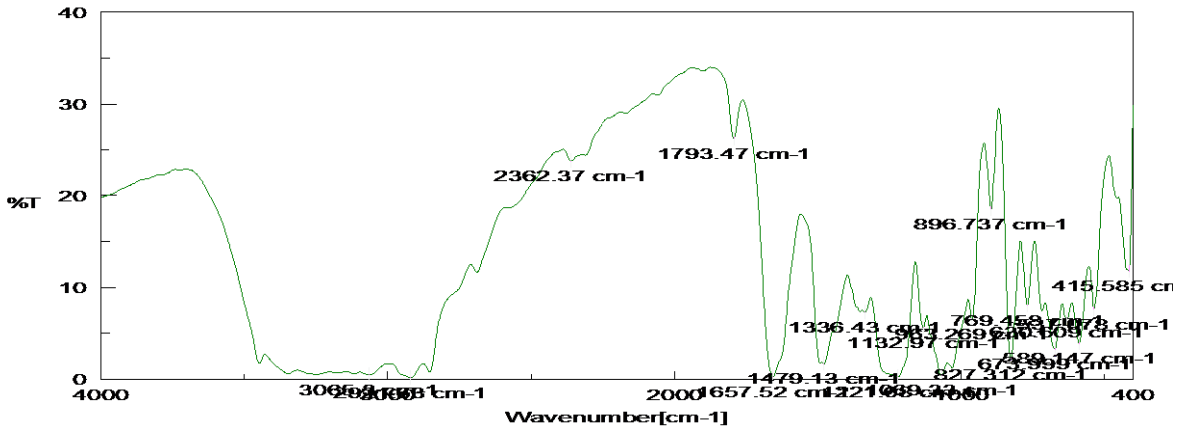

figure17: 3rdweek compatibility study of ribavirin and sls by ftir spectrophotometry

table 15: compatibility study of ribavirin & sls after 1st, 2nd, 3rd week by ftir spectrophotometry

Type of vibration and Functional group/structure	Wavenumber (cm-1)		Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
	Pure Drug	1st	2nd	3rd
O-H Str	3260	3447,3346,3254	3440	3440
Alkyl C-H Str	2950	2920	3065	3065
Alkyl C-H Def	1493	1478	1479	1479
Aromatic C-H Def	1354	1354	1336	1336
C=O Str	1657	1657,	1657	1657
C-O Str in ether	1067,1277	1132,1032	1132,1121	1132,1121
N-H Def	1608	1657	1657	1657
C-O Str in primary alcohols	1021,997	962	963	963
Out of Plane Def	737,770,896	769,827,896	779,827,896	779,827,896

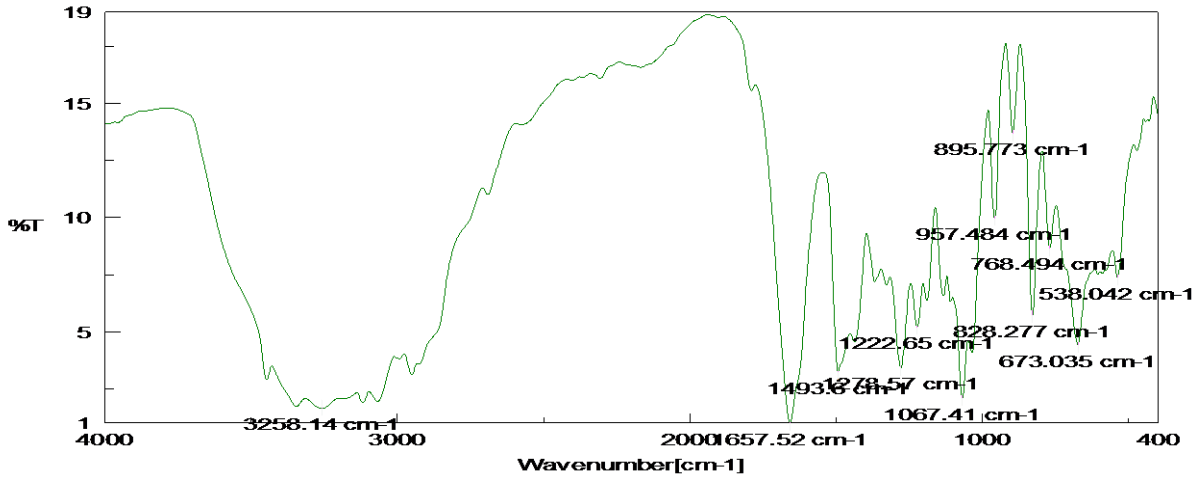

figure 18: 1st week compatibility study of ribavirin and pvpk by ftir spectrophotometry

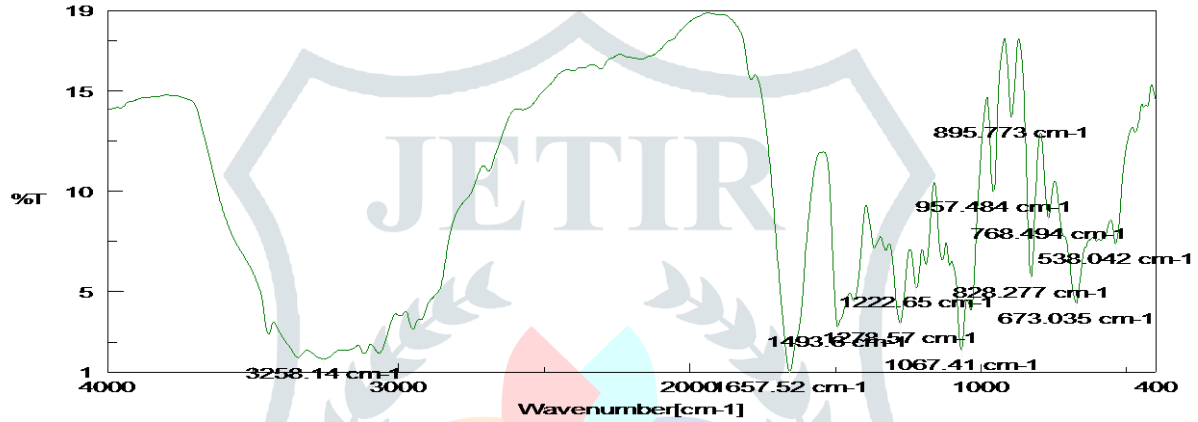

figure 19: 2nd week compatibility study of ribavirin and pvpk by ftir spectrophotometry

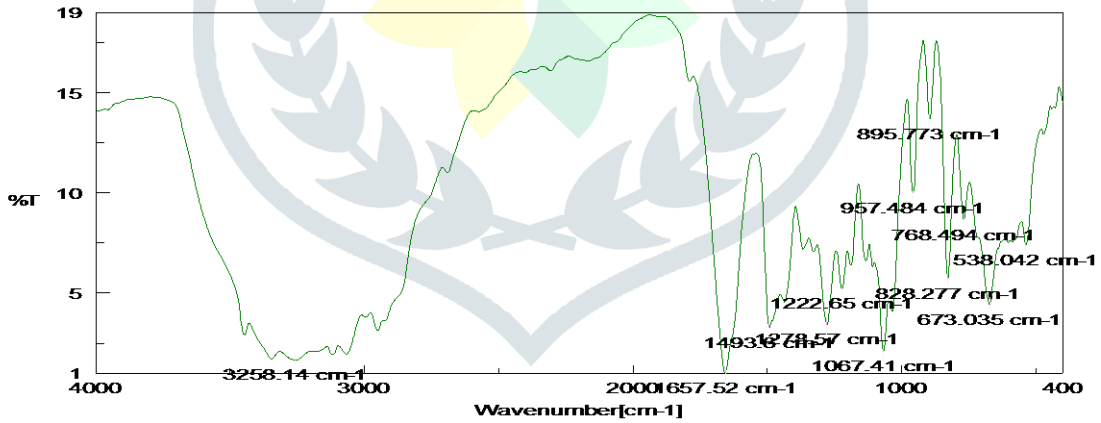

figure 20: 3rd week compatibility study of ribavirin and pvpk by ftir spectrophotometry

table 16: compatibility study of ribavirin &pvpk after second week by ftir spectrophotometry

Type of vibration and Functional group/structure	Wavenumber (cm-1)		Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
	Pure Drug	1st	2nd	3rd
O-H Str	3260	3447,3346,3254	3447,3361	3258
Alkyl C-H Str	2950	2920	2920	3000
Alkyl C-H Def	1493	1478	1478	1493
Aromatic C-H Def	1354	1354	1354	1493
C=O Str	1657	1657,	1657	1657
C-O Str in ether	1067,1277	1132,1032	1132,1032	1278,1222
N-H Def	1608	1657	1657	1657
Out of plane	737,770,896	768,783	768,783	768,828
C-O Str in primary alcohols	1021,997	895,957	895	957

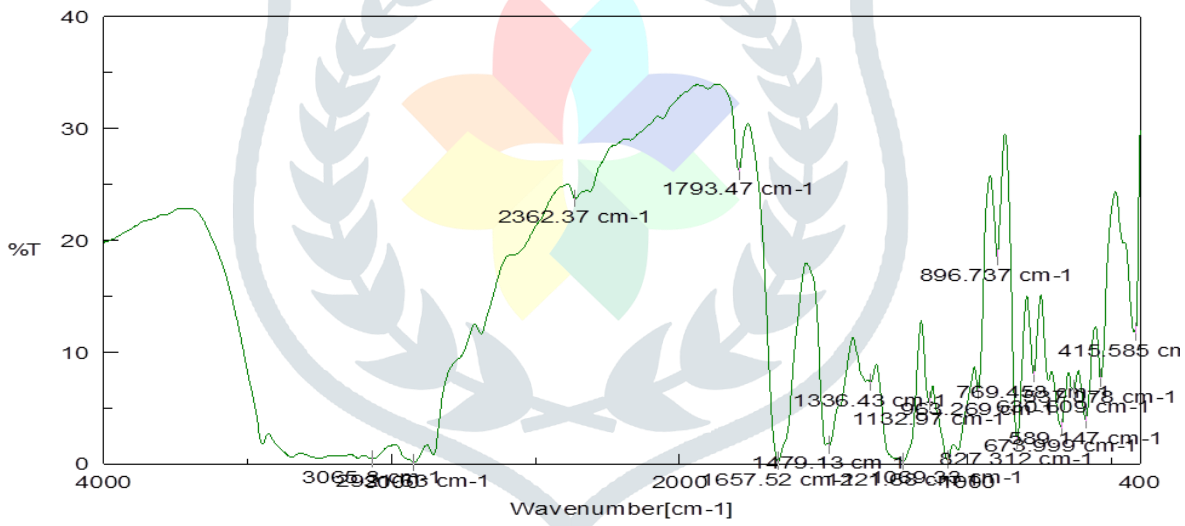

figure 21: 1st week compatibility study of ribavirin and f68 by ftir spectrophotometry

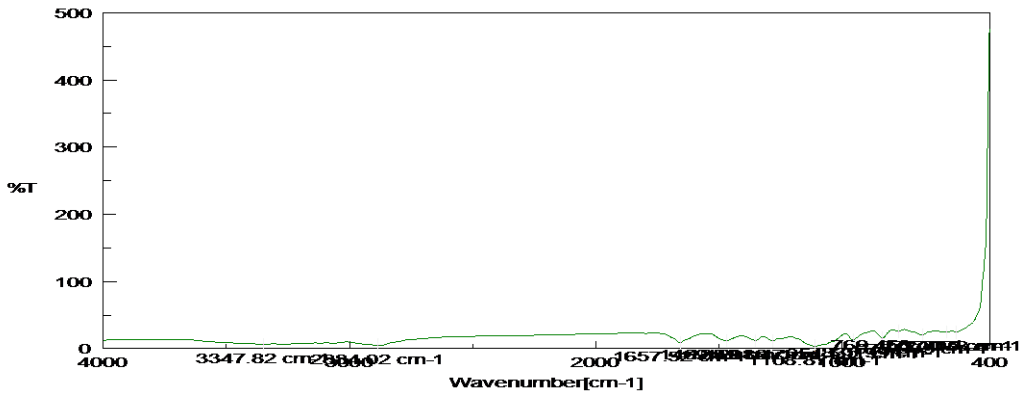

figure 22: 2nd week compatibility study of ribavirin and f-68 by ftir spectrophotometry

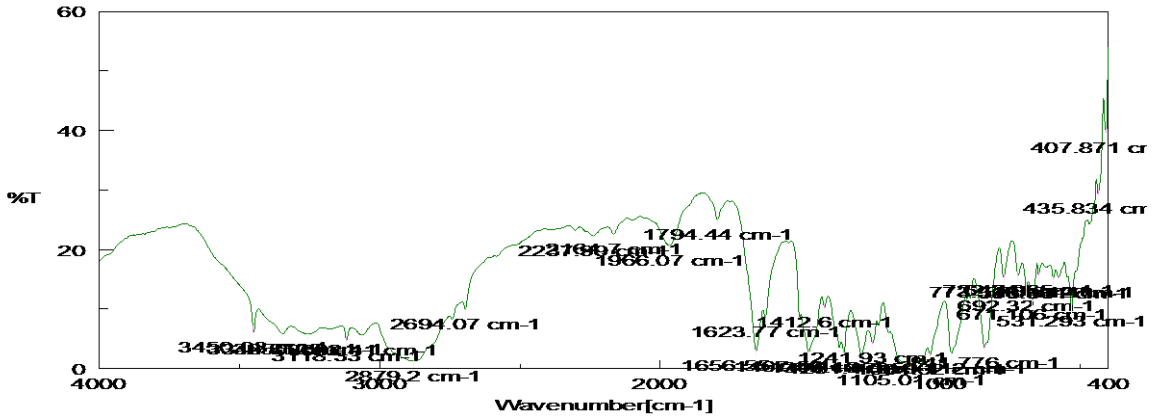

figure 23: 3rd week compatibility study of ribavirin and f-68 by ftir spectrophotometry

table 17: compatibility study of ribavirin & f-68 after third week by ftir spectrophotometry

Type of vibration and Functional group/structure	Wavenumber (cm-1)		Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
	Pure Drug	1st	2nd	3rd
O-H Str	3260	3347	3347	3450,3118
Alkyl C-H Str	2950	3000	3000	3000
Alkyl C-H Def	1493	1460	1460	1546,
Aromatic C-H Def	1354	1315	1315	1412
C=O Str	1657	1657	1657	1656
C-O Str in ether	1067,1277	1108	1108	1241
N-H Def	1608	1623	1623	1656

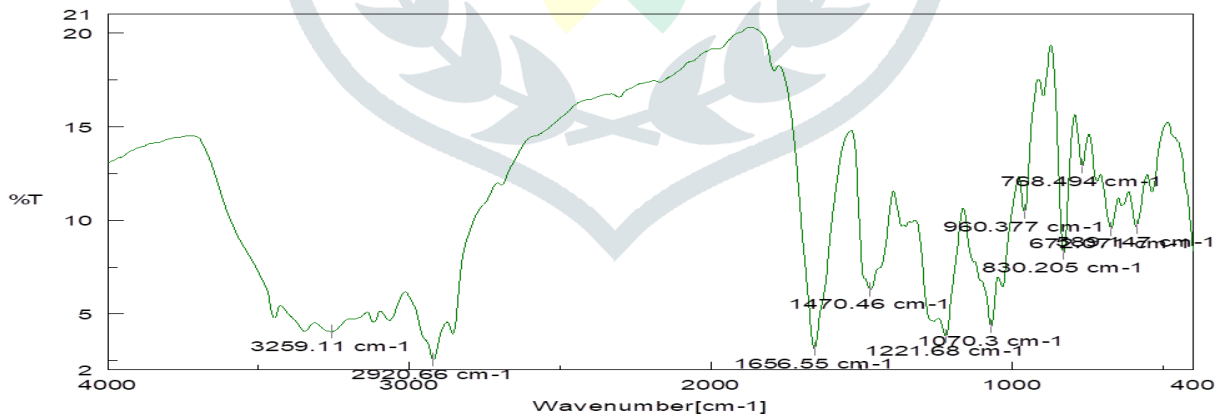

figure 24: 1st week mixture compatibility study.

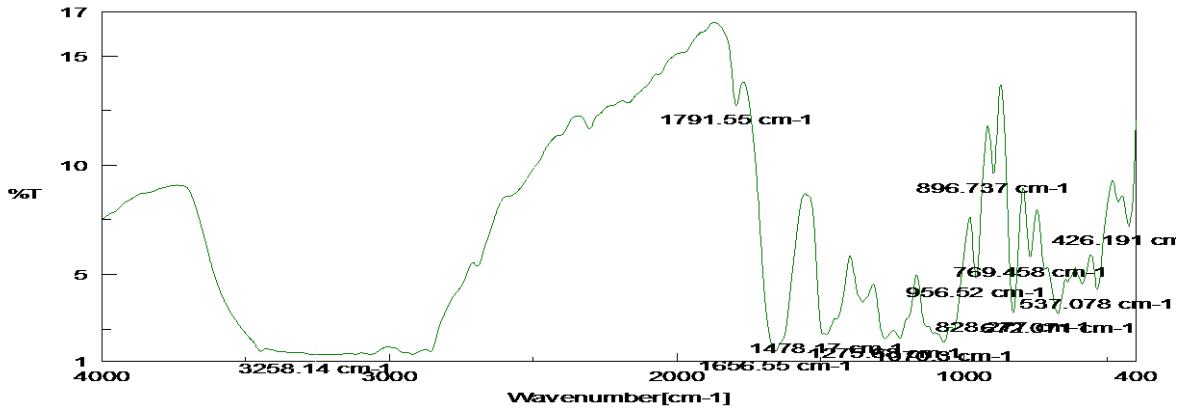

figure 25: 2nd week mixture compatibility study.

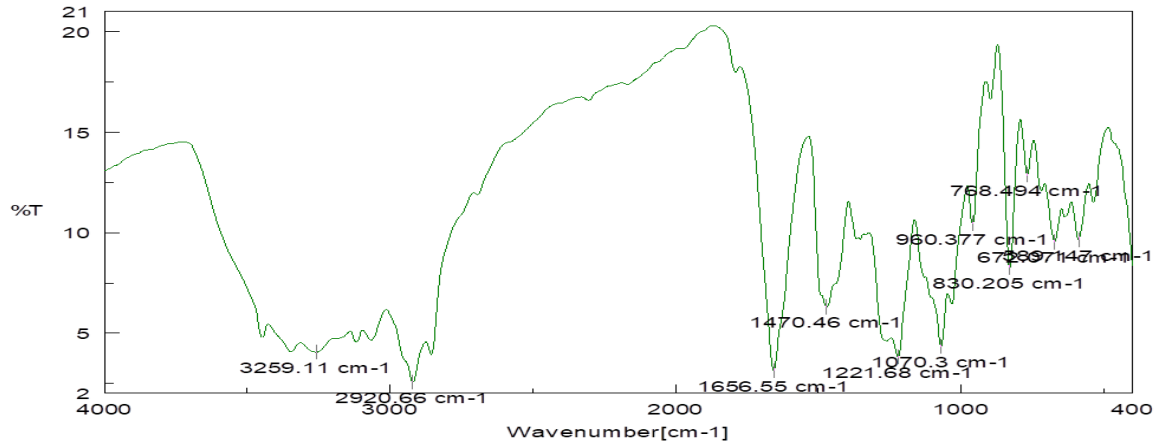

figure 26: 3rd week mixture compatibility study

table 18: compatibility study of mixture by ftir spectrophotometry

Type of vibration and Functional group/structure	Wavenumber (cm-1)		Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
	Pure Drug	1st	2nd	3rd
O-H Str	3260	3259	3258	3259
Alkyl C-H Str	2950	2920	3000	2920
Alkyl C-H Def	1493	1470	1478	1470
Aromatic C-H Def	1354	1470	1656,1478	1470
C=O Str	1657	1656	1656	1656
C-O Str in ether	1067,1277	1221	1221	1221
N-H Def	1608	-	-	-

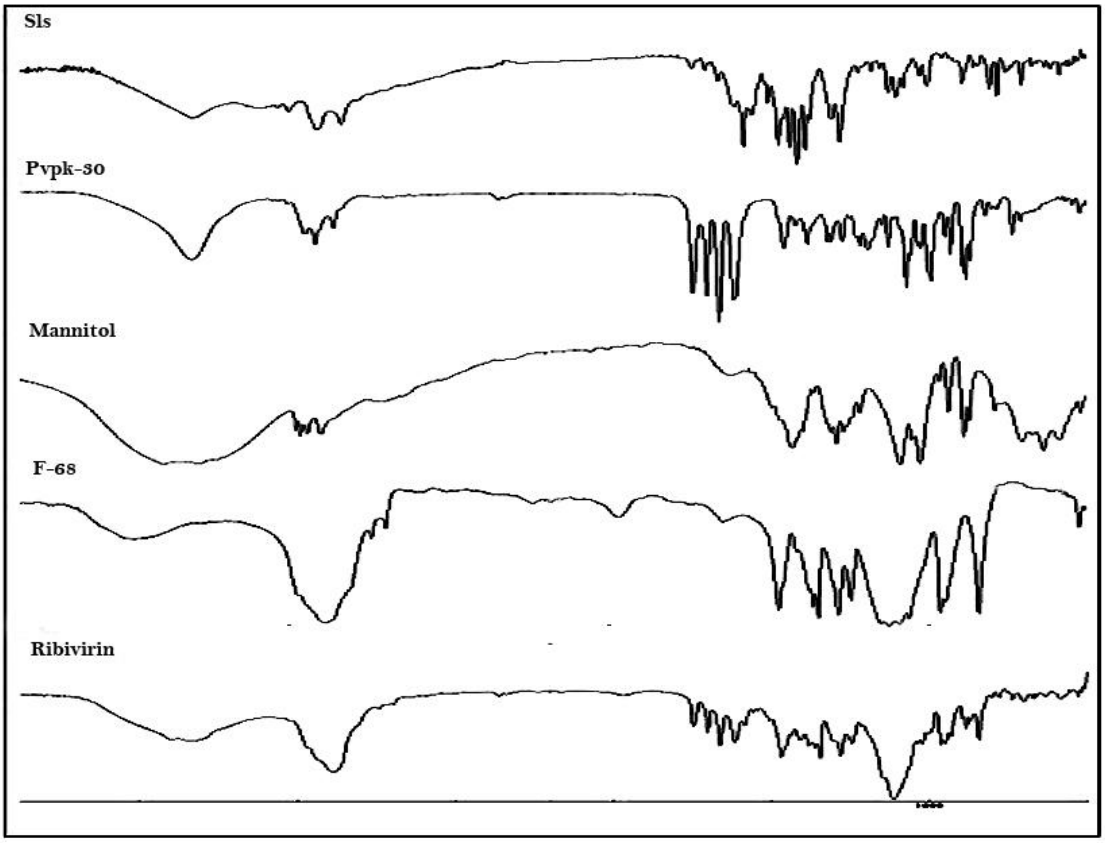

figure27: ftir spectra of ribavirin, sls, mannitol, poloxamer 188 and pvpk-30

5.2 Freeze drying:

Freeze drying was employed in this research as a means of controlled particle size production. Freeze drying was conducted using a Lab scale Freeze drying unit from DELVAC (Mumbai) equipped with thermocouples for inlet and outlet temperatures, as well as controllers for pump and aspiration rates. The Freeze dryer was equilibrated with solvent system prior to every run. An integrated nozzle cleaning device was utilized between runs to prevent clogging of the Freeze dryer. The aqueous suspension was pumped into the Freeze drying chamber using a peristaltic pump at a rate of 5%. Freeze drying was conducted at inlet temperature of 120^0C. At higher temperatures, excessive collection of the particle was observed in the Freeze chamber.(40)

It was found, within the limitations of the experimental design, that no sole parameter of Freeze drying process was responsible for controlling the particle size of the dried product. The particle size of the Freeze dried material was affected by the following factors; the aspiration speed, feed pump speed and atomizing pressure. However, the interaction between the aspirator speed and feed pump speed did affect particle size, i.e. when both the aspirator speed and feed pump speed were at higher level, a large particle size resulted. This would be expected as the concentration of the material in the atomized droplet would be greater, combined with the effect of increased airflow which would result in a reduced drying time, so reducing shrinkage and thus increasing particle size. It was also observed that when atomization pressure was kept below 20 psi the particle size did affect.

The percentage yield of the Freeze dried material was affected by the following factors; the aspirator speed and feed concentration and the interaction between the pump and the feed concentration.

5.3 CHARACTERIZATION OF DRY POWDER FOR INHALATION (DPI):

5.3.1 Particle size:

The particle size of all Freeze dried formulations was found in the range of 0.5 to0.9 µm (Table 46).while that of Freeze dried formulation derived from dispersion containing mannitol as an excipient was in the range of 0.5 to 0.9 µm.

The polydispersity index (PDI) is also an important parameter as it gives an indication about the width of particle size distribution as well as the long-term stability of dispersion. A PDI value of 0.1–0.25 indicates a narrow size distribution whereas a PDI value greater than 0.5 indicates a very broad distribution.(6) The PDI value obtained for all batches of DPI was found to have very broad distribution for A to F batches formulations (Table 46).

table 19: particle size of dpi

Batch code	Mean Diameter (µm)	PDI
A	0.259	0.564
B	0.281	0.523
C	0.395	0.593
D	0.942	0.893
E	0.573	0.707
F	0.451	0.578

From the observations it was concluded that the formulations prepared by using Mannitol as an excipient gives larger particle size, while batch A & B gives small particle size as compared to other batches .Also uniform particle size of batch A & B as observed from polydispersity index.

The observed reasons for the particle size and its distribution pattern are crystalline nature mannitol because of which degree of conversion of crystalline form of Mannitol into amorphous was more as compared to that of other. Also, based upon the observations from Table 46; poloxamer concentration affects the particle size; as in mannitol batches. The poloxamer 188 acts as a stabilizer in certain amount which will give small particle size. In case of lactose, poloxamer concentration was found to be optimum up to 1:1(Drug: Poloxamer) proportion, below and beyond this proportion; little increase was found in particle size observations.[41,42]

As discussed in the introductory part of the formulation, batch A & B formulations; Narrow particle size distribution was observed because of SLS-Poloxamer 188 combination, as Poloxamer 188 is used as a good dispersing agent.

5.3.2 Percentage yield and Drug loading:

Percentage yield and actual drug content are mentioned in the Table 47, which showed as the poloxamer concentration was increased, the yield of the product increased. The main reason of getting the low product yield is sticky nature, adherence of Freeze dried particles on to the wall of cyclone chamber which is difficult to collect because of small particle size and poor handling properties of DPI. Presence of poloxamer was found to increase the flow properties of A and B series which is beneficial during the collection of Freeze dried particle, to increase the percent yield of respirable DPI.

table 20: percentage yield (%) and drug content of freeze powders

Batch Code	% Yield	Theoretical drug Content % (w/w)	Drug content - A	1	2	3	Mean	Actual Drug conten-t% (w/w)
A	97.5	2.05	0.19					
B	59.50	2.05	0.35					
C	62.11	2.05	0.56	0.58	0.54	0.55	0.55	0.55
D	64.60	2.05	0.71					
E	87.5	2.05	0.90					
F	74.50	2.05	0.98					

The method used for preparation of suspension was Emulsion diffusion method which enables the production of small particles in the micrometer range acceptable for pulmonary drug delivery. During the conversion of suspension into dry powder by using Freeze drying some amount of the powder may has gone from the scrubber bag of the Freeze dryer to exhaust.

All the formulations showed satisfactory drug loading from 2.05 mg so as to get the appropriate dose containing 50µg of Ribavirin from the individual respirable DPI.

5.3.5 DSC analysis:

DSC analysis of the Ribavirin, Physical mixture and Freeze dried formulations were performed in order to characterize the physical state of the drug and excipient before and after Freeze drying are shown in Fig 28 (a).

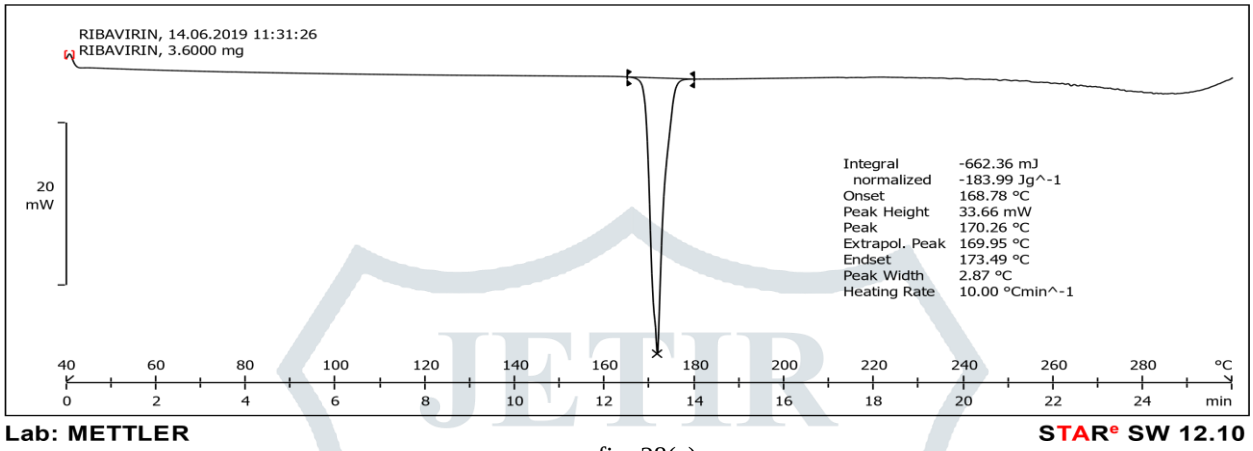

fig. 28(a)

Fig.28 (a) shows DSC scan for Ribavirin; an endothermic peak was observed at 170.26^{0}C which is quite more than its melting range, as it melts with decomposition.

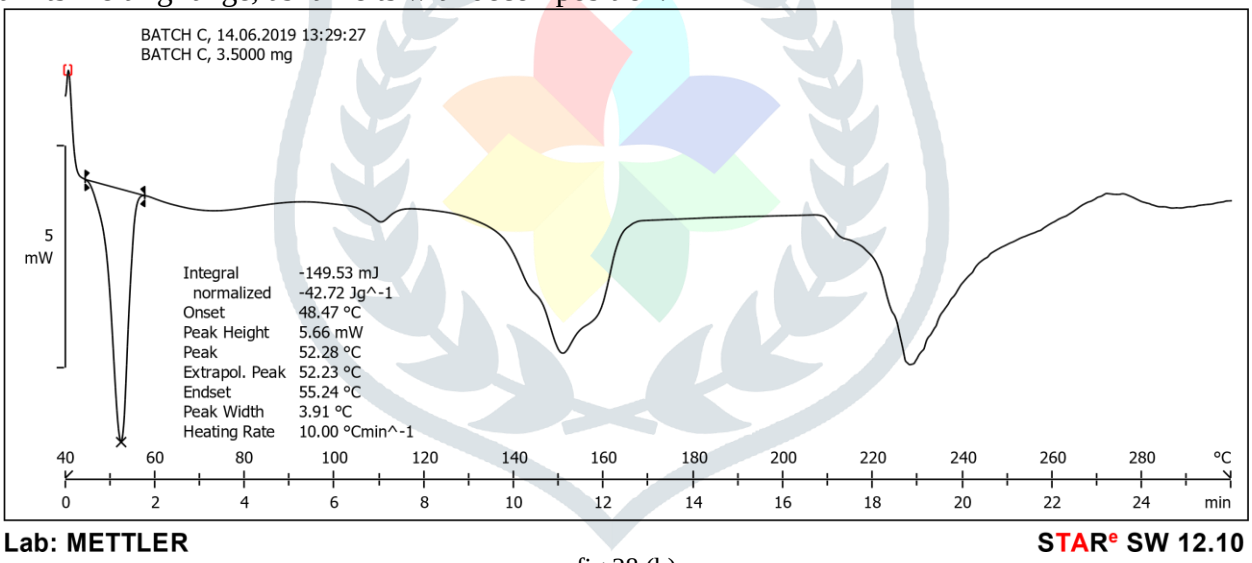

fig.28 (b)

Thermogram of Physical mixture (Figure 28-b) shows indicative sharp peak of poloxamer 188 at 52.28 ^{0}C, broad endotherm at 140-170^{0}C for Ribavirin and at 228.85^{0}C for SLS which suggest that interaction of Ribavirin & SLS. Also, absence of sharpness of peak indicates the amorphous nature of drug after Freeze drying.

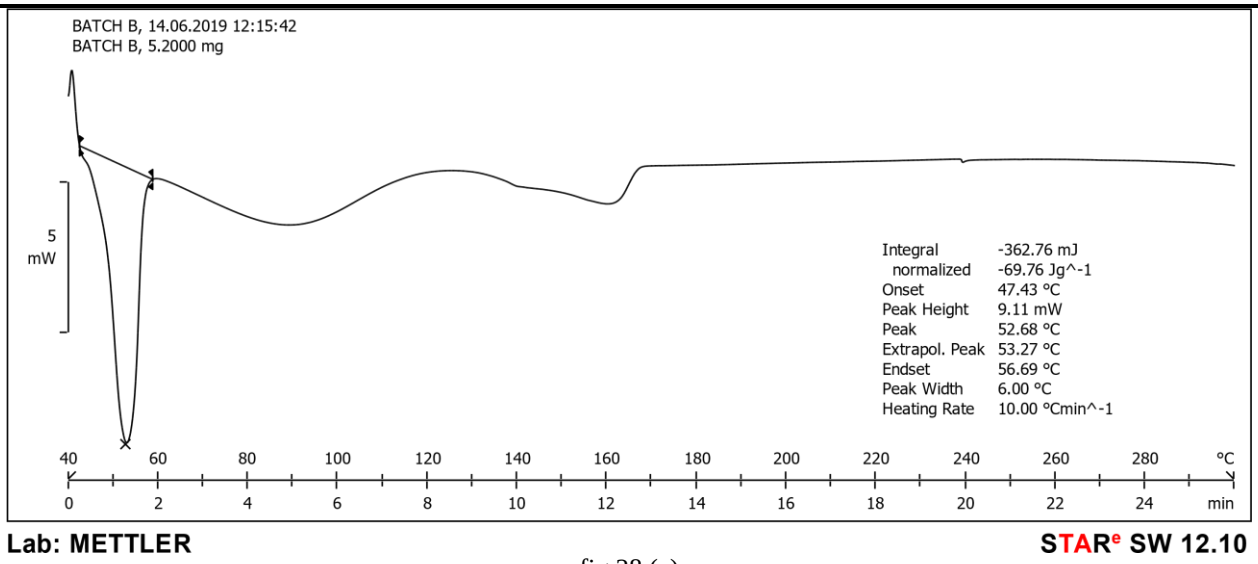

fig.28 (c)

Thermogram of Physical mixture (Figure 28-c) shows indicative sharp peak of poloxamer 188 at 52.68 ^{0}C, broad endotherm at 140-170^{0}C for Ribavirin and no peak observed for SLS, which suggest that interaction of Ribavirin & SLS.

5.3.6 XRD measurements:

The crystalline nature of pure drug and all Freeze dried formulation batches were examined by studying its X-Ray diffraction patterns. High intensity peaks of Ribavirin at 17.3^o, 22.2^o and 24.6^o which confirm that drug is crystalline in nature.

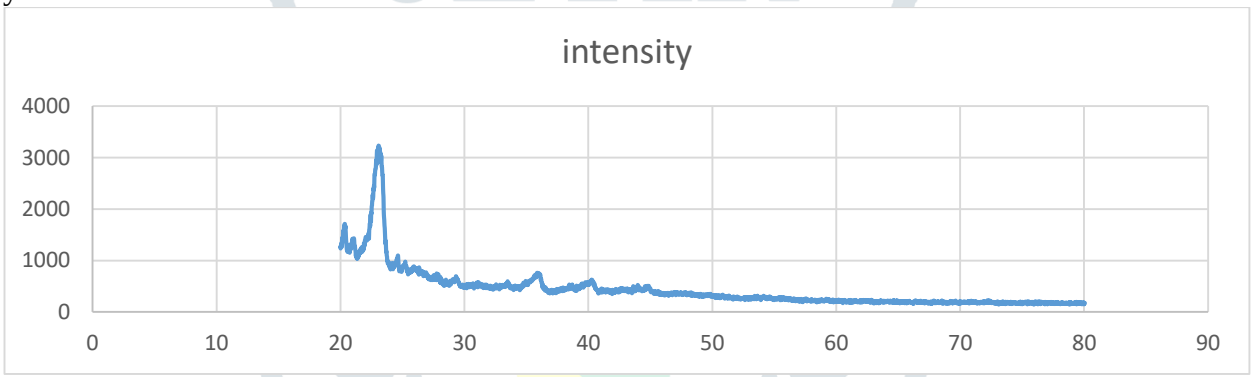

fig. 29. shows xrd patterns of batch a formulations

.

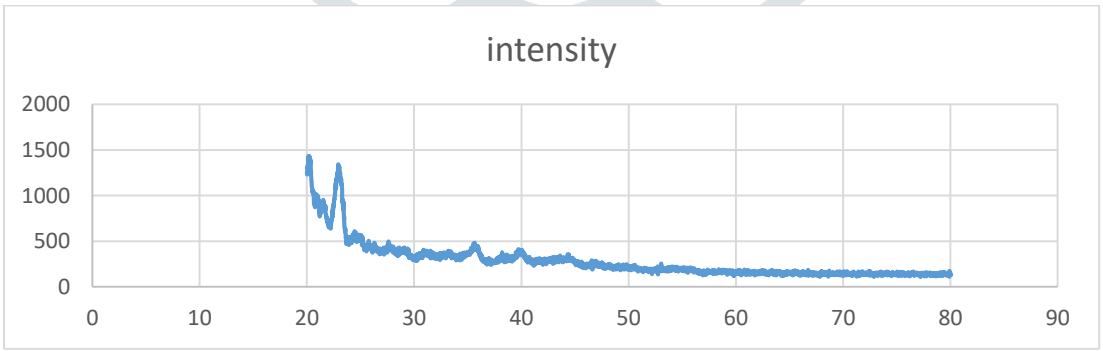

fig.30 shows xrd patterns of batch b formulations

From observations, it is clear that degree of crystallinity of Batch B is reduced as compare to Batch A and it is shifted towards amorphous nature. Batch B formulation tends to Amorphous in nature. The probable reason is the effect of stabilizer in reducing surface tension of droplet may have role in conversion of crystalline nature of pure drugs into amorphous one during Freeze drying process. In the Batch A intence peak observed at 23.17°& Batch B intense peak at 20.24°.

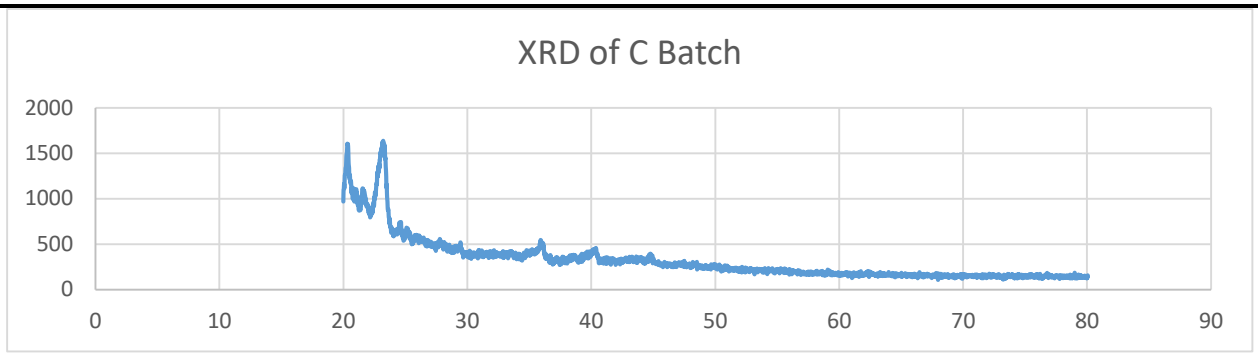

fig.31 shows xrd patterns of batch c formulations.

Suggest further decrease in crystallinity of pure drugs, as peak intensity is less than Other. The intense peak of Batch C is more than Batch B i.e, 23.29°.

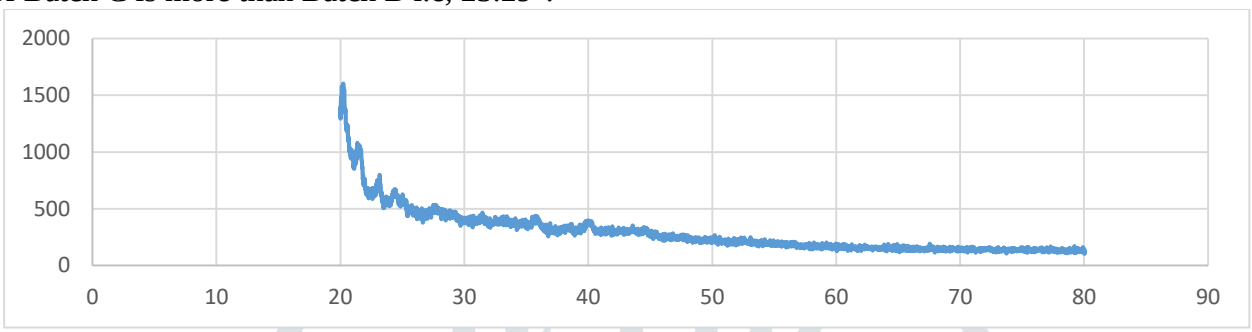

figure 32: xrd pattern of batch d formulations

Fig. 32 shows XRD patterns of Batch D formulations. From observations in figure, it is clear that degree of crystallinity of pure drugs; Ribavirin is further reduced than Batch D formulations and it is shifted towards amorphous nature. So, the Batch D formulation tends towards amorphous but still partial crystalline.

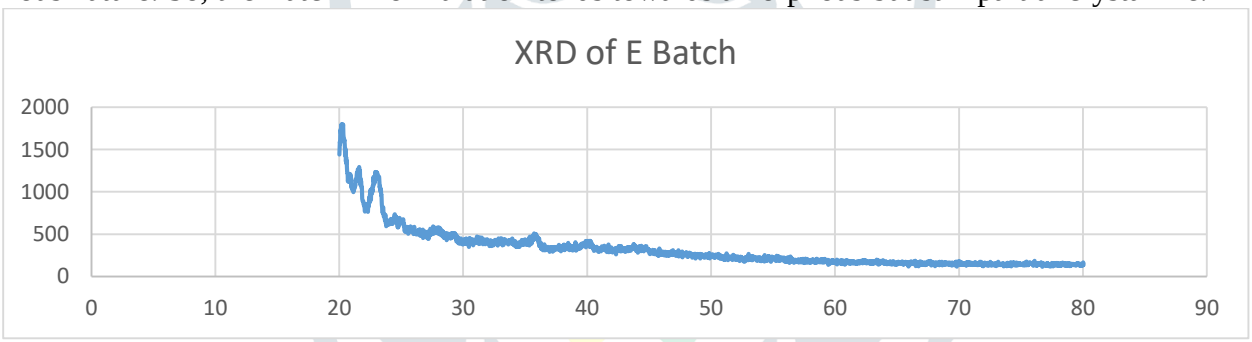

figure 33 pattern of batch e formulation

Suggests further increase in amorphous nature of pure drugs, as peak intensity in Batch E is same that of Batch D. This indicates the effect of stabilizer in the formulation, as batch E is with only Sls as an excipient while D contains without SLS, Pvpk along with poloxamer 188.

The Batch A formulation was found to be partial crystalline with peak intensity, B is less than A and more than E. From this observation it gives an idea regarding the optimum concentration of stabilizer in the formulation, as concentration of Poloxamer is less in C than in A batch which may help to get stable formulation.

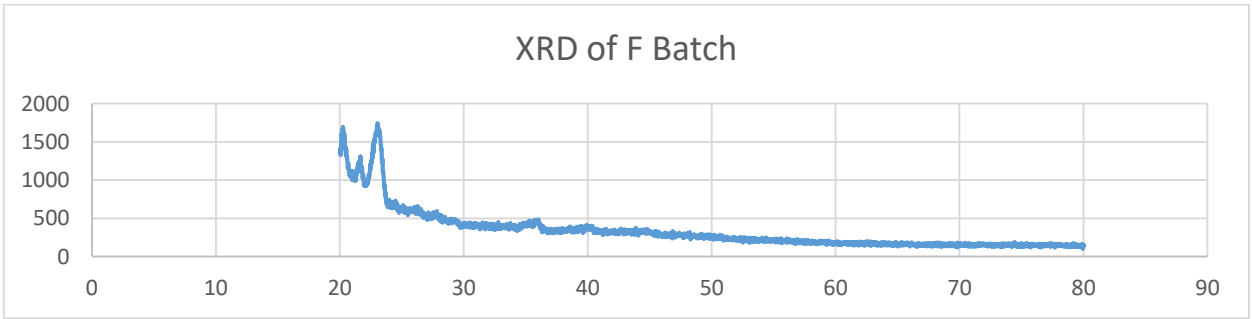

figure 34: xrd pattern of batch f formulations

The amorphous nature of formulation is exclusively because of excipient combination. As previously known, PVPK,SLS, F-68 is responsible for increase of droplet size during atomization procedure in Freeze drying and SLS helps to disintegrate the dispersion uniformly so as to get stable but amorphous formulation.

All Freeze dried formulations showed less intensive peak confirming that drugs are converted in amorphous nature. Results showed that as the drug to excipient ratio increases upto certain level, crystallinity

decreases. Also, it reveals that presence of mannitol gives more amorphous formulation than with lactose, whereas SLS proved best among all excipients as per amorphous nature of formulation is considered.(43)

Above discussed XRD pattern is due to proper dispersion of drug particle into the excipient matrix. This is in good agreement with previous DSC results. It has been known that transforming the crystalline state to the amorphous state leads to a high energy state and high disorder, resulting in enhancing solubility and dissolution rate. Analysis of relative degree of crystallinity (RDC) helps to study the change of the crystalline to amorphous nature.

$$RDC = \frac{Highest\ Peak\ intensity\ of\ formulation}{Highest\ Peak\ intensity\ of\ drug}$$

table 21: relative degree of crystallinity (rdc)

Sample	Angle 2θ	Peak intensity	RDC
A	23.17	3300	1.06
B	20.24	1400	0.45
C	23.29	1600	0.53
D	20.02	1600	0.53
E	20.02	1800	0.58
F	23.05	1700	0.54

It was determined whether the obtained formulation precipitate of individual substances or whether it becomes crystal. In the case of cocrystal formation, one would expect different physic-chemical properties of the formulation such as different thermal behavior and different XRD patterns. From all XRD patterns of pure drugs and Freeze dried formulations, it seems that; some binding between the two molecules might occur, but cocrystal formation would be rather unlikely. In all XRD measurements, the formulation shows a partly crystalline pattern masking the crystalline peaks of pure drugs. Therefore it is assumed that the formulation resulted from precipitation.(44)

5.3.7 Powder density:

Powder flow is important in dry powder aerosol formulation for both the filling of gelatin capsules or devices and for subsequent release of drug from the dry powder inhaler. Tapped density of a formulation is associated with good aerosolization; as more porous particles hold better aerodynamic property over solid particles of the same dimensions. Table 53 shows the values for Carr's Index which is used as an indication of powder flow properties; a value less than 25% indicates a fluid flowing powder, whereas a value greater than 25% indicates cohesive powder characteristics. (45)

table 22: tapped density, carr's index and flow ability of freeze dried powders

Batch code	Poured density (g/cm^{-3})	Tapped density (g/cm^{-3})	Carr's Index (%)	Flow ability
A	24	21	12.5	Good
B	14	12	14.28	Good
C	30	27	10	Excellent
D	20	18	10	Excellent
E	26	21	19.23	Fair
F	28	23	17.85	Good

From the observations given in Table 22, Batch B & A series formulations seem to have good powder flow and Batch C & D have excellent powder flow, tapped density distribution is uneven. The difference in powder flow characteristics was because of excipient nature in the formulation. Increased concentration of poloxamer indicates poor powder flow.

It would, however, be expected that the Freeze dried material would have better flow properties than that of the micronized material because of its nature, there being fewer points for physical contact. Poor flowability may have been due to differences in the surface energies of the individual components in the formulation. Also, although the surface area of the Freeze dried particles was pitted, protruded; increasing the total surface area for contact between the particles.(46)

5.3.8 In vitro powder aerosolization:

The aerodynamic behavior of the microparticle (DPI) was estimated with Anderson cascade impactor (ACI) making it possible to study the in vitro deposition profile of the representative Freeze dried formulations. The emitted dose (ED), fine particle dose (FPD), fine particle fraction (FPF) or respirable fraction (RF) of the Freeze dried powders are displayed in Table 23.

table 23: in vitro powder aerosolization properties of dpi

Batch code	ED (%)	FPD (µg)	FPF (%)
A	67.05	47.17	39.29
B	78.12	63.48	43.76
C	87.44	72.09	54.14

The amount of drug deposited in the inhaler device and throat regions were 4.2% for A, 3.29% for B and 2.19% for C Freeze dried systems.

It indicates that very less deposition in the inhaler and throat region (upper stage) supporting the highly dispersible nature of these powders; following inhalation, minimal deposition in the oropharyngeal region are obtained, thereby reducing the potential for local side effects. All the powders tested in this study would be expected to deliver a high proportion of emitted dose of the total capsule content to the pulmonary region following inhalation, with limited deposition in the device would therefore be expected to perform well during inhalation and may even be of use for the delivery of drugs for lower airways or systemic uptake.

Co-precipitates of Ribavirin showed a FPF of 22% when formulated with lubricant and 36% with mannitol carrier. For the commercial product Seretide®, which is a tertiary mixture of APIs with lactose; FPF of about 20% was found. It is due to low flowability and high adhesiveness of the powder.(47)

Further increase in FPF in case of C batch formulation is due to improved flow property with the incorporation of poloxamer 188. Surface roughness of A batch formulation particles is likely to have contributed to the cohesiveness than that of B batch formulations and high dispersibility with poloxamer 188. Another contributing factor may be the crystallinity of the mannitol and possibly of the ICSs (as confirmed by XRD).(48)

Both A and B batch formulation provides an innovative approach for combination formulations at appropriate doses without the need of physical blending. The powders with mannitol as an excipient showed high aerosol performance and uniform deposition of the two drugs as compared to C batch formulations.

The relative good performance of the C series formulations in the ACI was principally attributed to high SLS concentrations. It has previously been shown to enhance a Freeze dried formulation's aerosolization properties-the solid state glidant activity of previously used as a rational for any improvement in aerosolization performance. Here the action of SLS in reducing surface active force which is attributed to SLS particle fracture which produced rougher morphologies and reduced inter-particulate contact points for aggregation. The use of Poloxamer 188 as a potential dispersing agent and drug release enhancer has also been explored with marginal success.(49)

5.3.9 In vitro drug release:

Although, several in vitro models for the prediction of respirable fraction and site of deposition in the lung following pulmonary administration (e.g. Twin stage impinge, MSLI, Anderson cascade impactor, Next generation impactor, etc.), there is no readily available in vitro model to predict the rate and extent of drug dissolution in the lung following inhalation. However, for a sustained release DPI, it becomes essential to evaluate the release of an API molecule from the formulation matrix as a function of time.

Currently, no pharmacopoeia methodology exists for the evaluation of the in vitro release rates from respirable dry powders. To study the dissolution pattern of all Freeze dried formulation, In vitro dissolution study was carried out using USP rotating paddle dissolution apparatus (Lab India 2000). The dissolution

medium used was Phosphate buffer (pH 7.4). The dissolution method used in this study has previously been used in this research area.[50,51]

The rate of drug release from the formulation depended on the drug to excipient binding while processing, as adhesive force between drug-excipient becomes more than cohesive force between drug molecules themselves. This can be explained by a decreased amount of drug present close to the surface and also by the fact that the amount of uncoated drug decreases with higher excipient concentration. Furthermore, smaller microspheres have a larger surface area exposed to dissolution medium, giving rise to faster drug release. The initial rapid drug release can be attributed to the formation of solid dispersion of the drug where the drug would have higher solubility and hence dissolution rates.

table 24: release profile of batch a & b formulation

Time in min	% CDR of A Batch	% CDR of B Batch
0	0	0
10	16.8563	63.4972
20	17.8450	85.5046
30	20.3055	88.7622

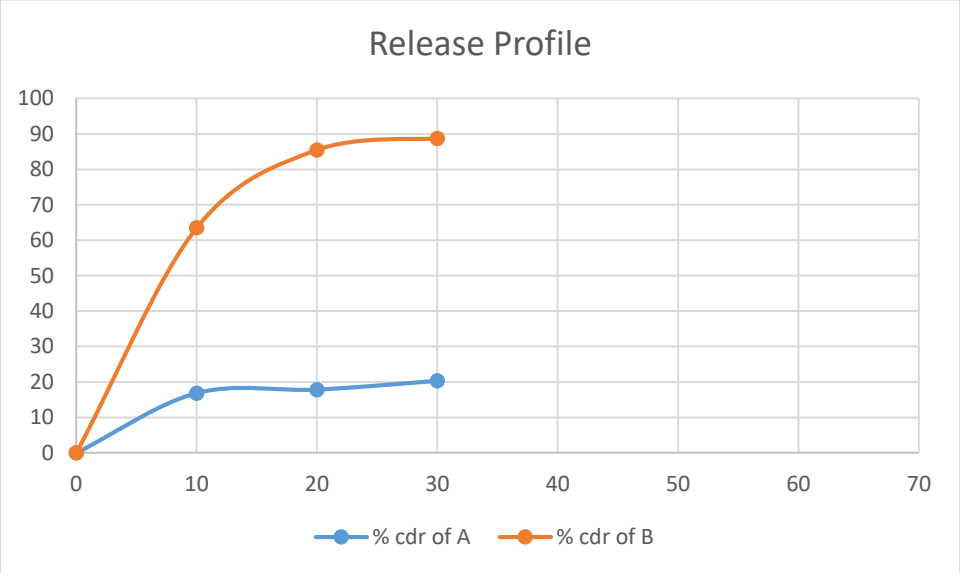

fig.35- drug release profile of a batch and b batch

In the formulation Batch B drug release in the first 30 min was in the range of 88.76% (Table 24) while that of Batch A formulations was in the range of 20.30%. An initial burst effect was observed due to the drug located on or near the surface of the microspheres. The pores formed during rapid evaporation of the solvent may also lead to the rapid release of the drug.

The Marketed formulation of Ribavirin Tablet, Capsule [54] shows drug release time within 45 min. but optimize batch B drug release time within 30 min. This is the main advantages of DPI Formulation over Tablet & capsule. Which make shows easy to administered, safe, effective formulation.

6. REFERENCES:

1) Dalby R, Suman J.2003, Inhalation therapy: technological milestones in asthma treatment, Adv. Drug Deliv. 55, 779-791.

2) Jaspart S, Bertholet P, Piel G, Dogne J, Delattre L, Evrard B,2007; Solid lipid microparticles as a sustained release system for pulmonary drug delivery, Eur. J. Pharm. Biopharm; 65, 47-56.

3) Hickey A, Thompson D., Pharmaceutical Inhalation Aerosol Technology, 2004; 2nd ed. USA: Marcel Dekker.

4) Manca M.et al,2009; Chitosan and PLGA microspheres as drug delivery system against pulmonary Mycobacteria infections (dissertation), Department of pharmacochemico technology, University of Cagliari, 16-24.

5) Elversson J.,2005; Spray-dried powders for inhalation: particle formulation and formulation concepts (dissertation), Faculty of pharmacy: Uppsala University; 11-15.

6) Ozer A,et al ,2007; Alternative applications for drug delivery: Nasal and pulmonary routes, Nanomaterials and Nanosystems for Biomedical Applications, 99-112

7) Martin G, Timsina M, Marriott C, Ganderton D, Yianneskis M.,1994; Drug delivery to the respiratory tract using dry powder inhalers, Int. J. Pharm., 101, 1-13.

8) Misra A, Chougule M, Padhi B, Jinturkar K.,2007; Development of Dry Powder Inhalers, Recent Patents on Drug Delivery & Formulation, 1, 11-21.

9) Islam N, Gladkib E.,2008; Dry powder inhalers (DPIs)—A review of device reliability and innovation, Int. J. Pharm., 238-244.

10) Scheuch G et al,2007; Clinical perspectives on pulmonary systemic and macromolecular delivery, Adv. Drug Deliv. Rev., 58, 996-1008.

11) Julia Christina Kasper, Dissertation on "Lyophilization of Nucleic Acid Nanoparticles," Ludwig-Maximilians-University, Munich, Page No 3-5.

12) John G. Day, Glyn N. Stacey,2008; Methods in molecular biology™ 368, Cryopreservation and Freeze-Drying Protocols, second edition, Human press, Totowa New Jersey, 15-22.

13) GR. Nireesha, L. Divya,2003; 'Lyophilization/Freeze Drying - An Review', International journal of novel trends in pharmaceutical sciences, (3), 87- 93.

14) Gannu Pravee Kumar, Review paper 'Fundamentals and Applications of Lyophilization', Journal of Advanced Pharmaceutical Research. 2011, 2(4), 157-169.

15) Gennaro RA, Lippincott Williams & wilkins, 2000; Remington: The science and practice of pharmacy, (21), 1.

16) Jeff SJ. 2009; Basic Cycle Development Techniques for Lyophilized Products, 35, 126-128.

17) Adams GD, Irons LI.1993; Some implications of structural collapse during freeze drying using Erwinia caratovora l-asparaginase as a model. J Chem Biotechnol, 58, 71– 76.

18) Rey S, Xialoin T & Michael J.2004; Study of optimization of the freeze dried product: practical advice pharmaceutical research. Eur. J. Pharm, 78- 94.

19) Serigo A et al; 2003; Heat and mass transfer scale-up issues during freeze drying, I: Atypical radiation and the edge vial effect. AAPS Pharm Sci Tech, 4(2), 114-127.

20) Lam T et al ; 2004; An unexpected pH effect on stability of moexipril lyophilized powder. Pharm Res, 6, 971-975.

21) Rendolph S, et al; 2005; Effect of ultrasonically induced nucleation on ice crystals size and shape during freezing in vials. Chem. Eng. Sci,, 65- 88.

22) Indian pharmacopoeia, The Indian Pharmacopoeia Commission, 1996; (2), A-8.8, 668.

23) Indian pharmacopoeia, The Indian Pharmacopoeia Commission, 2007; (2), 1141-1143.

24) A. Healy et al ;2006; Physicochemical and in vitro deposition properties of salbutamol sulphate/ipratropium bromide and salbutamol sulphate/excipients Lyophillized mixtures for use in dry powder inhalers, Int. J. Pharm; 322, 22-30.

25) Y. Michael, et al; 2000; The physicochemical properties of salmeterol and fluticasone propionate in different solvent environments, Int. J. Pharmacy, 200, 279–288.

26) S. Lakshmana et al ;2008; Compatibility studies between duloxetine hydrochloride and tablet excipients using thermal and non-thermal methods, J. Pharm. Res., 7(1), 20-23

27) Shashikant Dhole et al,2014;"formulation and evaluation of Nano suspension delivery system ",international journal of science, 2(7),1-18 .

28) K. Hadinoto, Zhu K.,2007; Drug release study of large hollow nanoparticulate aggregates carrier particles for pulmonary delivery, Int. J. Pharm., 341, 195-206.

29) M. Kumon et al;2010; Can low-dose combination products for inhalation be formulated in single crystalline particles? Eur. J. Pharm. Sci., 40, 16-24.

30) R. Westmeier,et al,2008; Combination particles containing Salmeterol xinafoate and Fluticasone propionate: formulation and Aerodynamic assessment, J. pharm. Sci., 97(6), 2299-2309.

31) Carr, Classifying flow properties of solids, Chemical Engineering, 1965; 72, 69-72.

32) T. Learoyd et al ; 2009; Sustained delivery by Leucine modified chitosan spray-dried respirable powders, Int. J. Pharm., 372, 97-104.

33) M.D. Jones et al,2010; An investigation into the relationship between carrier- based dry powder inhalation performance and formulation cohesive-adhesive force balances, Eur. J. Pharm. Biopharm., 69, 496-507.

34) R. Westmeier et al,2008; Combination particles containing Salmeterol xinafoate and Fluticasone propionate: formulation and Aerodynamic assessment, J. pharm. Sci., 97(6), 2299-2309.

35) M.S. Hassan, Lau R.et al 2010;, Inhalation performance of pollen-shape carrier in dry powder formulation with different drug mixing ratios: Comparison with lactose carrier, Int. J. Pharm., 386, 6-14.

36) P.Seville et al 2008 ; Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate, Eur. J. Pharm. Biopharm., 68, 224-234.

37)J.R Dyer et al 2002; Application of absorption spectroscopy of organic compounds, 11th edition, New Practice Hall of India, Pvt. Ltd, 22-57.

38) Indian pharmacopoeia, The Indian Pharmacopoeia Commission, 1996; 2, A-8.8, 668.

39) ICH topic Q-2B validation of analytical procedure; Methodology CPMP/ICH/281/95) the European agency for the evaluation of medicinal products, human medicines evaluation unit,1-9.

40) Beckett A. H., Stenlake J. B. et al 2004;, Principle Pharmaceutical Chemistry, CBS Publishers New Delhi, 275-314.

41) A. Souza et al 2009; Influence of Freeze drying conditions on the physical properties of dried pulp tomato., Ciencia e Tecnologia de Alimentos, Campinas, 29 (2), 291-294.

42) H.Ali, York P., Blagden N. et al 2009; Preparation of hydrocortisone nanosuspension through a bottom-up nanoprecipitation technique using microfluidic reactors, Int. J. Pharm., 375, 107-113.

43) A.Hickey et al 2004; Aerosol Dispersion of Respirable Particles in Narrow Size Distributions Produced by Jet-Milling and Freeze-Drying Techniques. Pharm. Res., 21(7), 1200-1206.

44) H. Steckel, Brandes H. et al 2004; A novel Freeze-drying technique to produce low density particles for pulmonary delivery. Int. J. Pharm., 278, 187-195.

45) G. Pilcer et al 2010; Formulation strategy and use of excipients in pulmonary drug delivery, Int. J. Pharm., 392, 1-19.

46) R. Westmeier et al 2008; Combination particles containing Salmeterol xinafoate and Fluticasone propionate: formulation and Aerodynamic assessment, J. pharm. Sci., 97(6), 2299-2309.

47) Carr. Classifying flow properties of solids, Chemical Engineering, 1965, 72, 69-72.

48) Y. Gonnissen et al 2008; "Coprocessing via Freeze drying as a formulation platform to improve the compactability of various drugs". Eur. J. Pharm. Biopharm. 69, 320–334.

49) R. Westmeier et al 2008; Combination particles containing Salmeterol xinafoate and Fluticasone propionate: formulation and Aerodynamic assessment, J. pharm. Sci., 97(6), 2299-2309.

50) M. Kumon et al 2010; Can low-dose combination products for inhalation be formulated in single crystalline particles?, Eur. J. Pharm. Sci., 40, 16-24.

51) T. Learoyd et al 2009; Sustained delivery by Leucine modified chitosan Freeze-dried respirable powders, Int. J. Pharm., 372, 97-104.

52) P. Seville et al 2008; Chitosan-based Freeze-dried respirable powders for sustained delivery of teRibavirinutaline sulfate. Eur. J. Pharm. Biopharm., 68, 224-234.

53) S. Jaspart et al 2007; Solid lipid microparticles as a sustained release system for pulmonary drug delivery, Eur. J. Pharm. Biopharm., 65, 47-56.

54) www.Drugbank/Rabitol release pattern .2016;9;1-13

10. SUMMARY AND CONCLUSION:

Dry powder for inhalation (DPI) formulations containing Ribavirin as an API was prepared by using Emulsion-Diffusion method to get the suspension having micron size particles. The solvent used for diffusion method was distilled water and Antisolvent was ethyl acetate. As ethyl acetate was having good behavior for API's among different solvents; it was expected that the particle size of freeze dried particles should be in micrometer range (2-5 µm).

It was observed, within the limitations of the experimental design, that no sole parameter of Freeze drying process was responsible for controlling the particle size of the dried product to greater extent, so all parameters while performing operations of Freeze drying were kept constant. Six different formulations containing different excipients were prepared to compare their efficacy

It was found that particle size of D batch was increased than that of A and B batch with increase in surface smoothness and reduction in tendency for aggregation while A & B batch showed smaller particle size among all formulations. This can be predicted as SLS &Poloxamer 188 combination gives more amorphous powder after Freeze drying as compared to remaining formulations. But, formulations containing particle size in micron range; that is expected for pulmonary delivery.

The FTIR studies suggested that there were no changes in the structure of Ribavirin induced by solvent displacement as well as Freeze drying technique. As per the analysis suggested that the powders formed after Freeze drying were spherical, little angular, amorphous in nature as compared to individual drugs (Ribavirin) before processing. And it was confirmed by DSC and XRD measurements.

Powder density and Carr's index value showed that B batch formulations were of average good Flow ability than other batches. Also, E batch formulation showed fair flow property. This suggested about the use of excipients to optimize the flow property of formulations.

The amount of drug deposited in the inhaler device and throat regions were 4.2%, 3.29%, 2.19% for Freeze dried systems. It indicates that very less deposition in the inhaler and throat region (upper stage) supporting the highly dispersible nature of these powders; following inhalation, minimal deposition in the oropharyngeal region are obtained, thereby reducing the potential for local side effects.

Addition of poloxamer 188 to both A, remarkably improved the FPF as compared to commercial product. If compared with lactose as an excipient; co-Freeze drying of APIs with mannitol may provide a simple alternative method for effective processing of combination formulations for inhalation containing lactose. Investigations of B series demonstrate that it was possible to generate highly respirable powders that exhibit further better aerosolization with maximum FPF than that of A batch formulations.

It was found that increased Freeze-drying thermal efficiency, achieved through the use of a surfactant, correlated with the improved aerosolization properties of a powder. *In vivo*, these powders would be predicted to deposit predominately in the central to peripheral region of the lung after inhalation, with minimal oropharyngeal deposition and reduced incidence of oropharyngeal side-effects. Once deposited in the lung, these powders would be anticipated to deliver the sustained release of both active agents, and offer the opportunity to reduce dose frequency and the number of formulations involved in a regimen.

As expected, the SLS- Poloxamer combination of excipients exhibited sustained release characteristics; increasing the concentration of SLS was associated with a more sustained release profile. While, formulations containing Pvpk and Mannitol was found to have immediate release in dissolution media. Hence, it concludes that, it is possible to develop the modified release drug delivery system for inhalable drugs based Freeze dried powders with satisfactory yields of Freeze dried formulations as well as testing the mucoadhesivity of the Freeze dried formulation. Also, all the formulations were quite stable at accelerated storage conditions.

Thus, Dry powder for inhalation (DPI) formulations containing Ribavirin as an API showed excellent performance with sustained release for SLS-Polaxomer combination than that of immediate release for Pvpk and mannitol in all evaluation aspects. Also, the comparison between the respirable dry powder containing mannitol as excipients;

The Marketed formulation of Ribavirin Tablet, Capsule etc. shows drug release time within 45 min. but optimize batch B drug release time within 30 min. This is the main advantages of DPI Formulation over Tablet & capsule. Which make shows easy to administered, safe, effective formulation.