FORMULATION AND EVALUATION OF DRY POWDER INHALER USING ANTIINFLAMATORY AND BRONCHODILATOR DRUG

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In

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I wish him all the success in life.

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This is to certify that the project thesis entitled "FORMULATION AND EVALUATION OF DRY POWDER INHALER USING ANTI INFLAMMATORY AND BRONCHODILATOR" was carried out by Mr. Sanjay Saha, at the R & D Centre of Macleod's Pharmaceuticals, Mumbai, under my guidance as M. Pharm. Final year project work. He is bearing Gauhati University Registration No. 048363 Of 2007-08, at the Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science (G.I.P.S.), Azara, Hatkhowapara, Ghy-17, during the academic year 2012-13. Neither this work, nor any part of it has been submitted for any degree/ diploma or any other academic award anywhere before.

I wish him all success in life.

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DECLARATION

I hereby declare that the matter embodied in the dissertation entitled "*FORMULATION AND EVALUATION OF DRY POWDER INHALER USING ANTI INFLAMMATORY AND BRONCHODILATOR DRUGS"* is a bonafide and genuine research work carried out by me under the joint supervision of Dr. Kapileshwar Swain, *VP Macleods Pharmaceutical, Mumbai* & Bhanu P Sahu *Asst Prof*., Department of Pharmaceutics, *Girijananda Chowdhury Institute of Pharmaceutical Scienc, Hatkhowapara, Azara, Guwahati-17*. The work embodied in this thesis is original and has not been submitted the basis for the award of degree, diploma, associate ship or fellowship of any other university or institution.

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To,
Lord Almighty
And
My very loving family

Preface

Dry Powder Inhaler (DPI) technology has a significant impact in the treatment of various respiratory disorders. DPI formulations consist of a micronized drug (<5µm) blended with an inert coarse carrier, for which lactose is widely used to date. DPIs are one of the inhalation devices which are used to target the delivery of drugs to the lungs. Drug delivery via DPI formulations is influenced by the physico-chemical characteristics of lactose particles such as size, shape, surface roughness and adhesion forces. Commercially available DPI formulations, which utilise lactose as the carrier.

The objective of the project was to prepare "*DRY POWDER INHALER USING ANTI INFLAMMATORY AND BRONCHODILATOR DRUG*" This study was carried out to investigate the effect of weight fraction of fine lactose as a carriers on aeroionisation behaviour of Formoterol fumarate and Mometasone furoate dry powder inhaler.Deffierent grade of commercial lactose monohydrate were belened in different ratios with fixed amount Formoterol fumarate and Mometasone furoate and evaluated for Pharmaceutical Performance and invitro deposition of Formoterol fumarate and Mometasone furoate Dry powder inhaler and stability studies..Better invitro depositon was observed formulation having lactose ratio (70:30),that is 70% coarse lactose (Lactohale 200) and 30% lactose fine (Lactohale) .The pharmaceutical performance were found to be satisfactory and stable. The study showed henceforth that suitable blend of lactose can be used for formulation of Formoterol fumarate and Mometasone furoate dry powder inhaler with enhanced effectiveness.

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ABBREVIATION

Abbreviation	Remarks
API	Active Pharmaceutical Ingredients
LABA	Long acting beta agonist
ICS	Inhaled cortico steroids
COPD	Chronic Obstructive Pulmonary Disease
MDI	Metered Dose Inhalers
FPF	Fine Particulate Fraction
MMAD	Mass Median Aerodynamic Diameter
HFA	Hydro Fluoro Alken
CFC	Chloro Fluoro Carbon
RH	Relative Humidity
DUSA	Dosage unit sampling apparatus
ICH	International Community for Harmonization
DPI	Dry Powder Inhalers
µm	Micro meter
FPF	Fine Particulate Fraction
SFC	Supercritical fluid crystallization
FPD	Fine Particulate Dose
RH	relative humidity
nN	nanoNewtons
SBS	salbutamol sul-phate
GCD	gamma cyclodextrin
DMCD	dimethyl-beta-cyclodextrin
BDP	Baclomethason

SX	salmeterol xinafoate
FP	fluticasone propionate
ACI	Andersen cascade impactor
MSLI	multi-stage liquid impinger
NGI	Next generation impactor
FEV	forced expiratory volume

CHAPTER –1

INTRODUCTION

CHAPTER 1 INTRODUCTION

1.1.BACKGROUND

Pulmonary drug delivery is an important research area in the field of drug delivery technology. It is the preferred mode of drug delivery in the treatment of various disorders such as asthma and chronic obstructive pulmonary disease. The alveoli region in the lungs has a large surface area and a highly permeable membrane for the absorption of drugs into the blood. An advantage of pulmonary drug delivery includes direct access of the drug to the lungs, hence it is one of the routes of choice of drug administration of large molecules which degrade in the gastrointestinal fluid and are subjected to first-pass metabolism in the liver. Pulmonary delivery also utilizes minimal drug dose to produce the desired effect and provides a rapid pharmacological response with minimal side effects. [1] It is a needle-free delivery system capable of administering a variety of therapeutic substances. [2]

There are large numbers of devices available to target the delivery of drugs to the lungs. Currently three major types of inhalers that are widely used for pulmonary drug delivery are Nebulizers, Metered Dose Inhalers (MDIs) and Dry Powder Inhalers (DPIs) and these devices use different mechanisms of delivering the drug into the lungs. [3]

The majority of dry powder inhalers are breath-actuated devices. [4] They are easy to use and do not require co-ordination of actuation and inhalation. Unlike other inhalers, DPIs do not use liquid propellants. They are portable, patient friendly and do not require spacers. [5-6] DPIs today are an expanding area of interest of pharmaceutical companies and are seen as the most promising mechanism for pulmonary drug delivery. Many diseases and disorders of the lungs, such as chronic obstructive pulmonary disease (COPD) and asthma, have been successfully treated

through the use of inhalation-based formulations due to the localized delivery/action of active pharmaceutical ingredients (APIs) to the lungs. In recent years, there has been a growing interest in the development of inhalation therapy to address other disease states given the avoidance of first-pass metabolism via delivery to the lungs.

1.2.ORGANIZATION OF THE RESPIRATORY SYSTEM

The respiratory system can be divided into two components: Upper respiratory system and Lower respiratory system (Figure.1). The upper respiratory system consists of nose, nasal cavity, paranasal sinuses and pharynx. These passageways filter, warm and humidify incoming air thereby protecting the more delicate surfaces of the lower respiratory system and it cools and dehumidifies outgoing air. The lower respiratory system includes the larynx, trachea, bronchi, bronchioles and alveoli of the lungs [8]

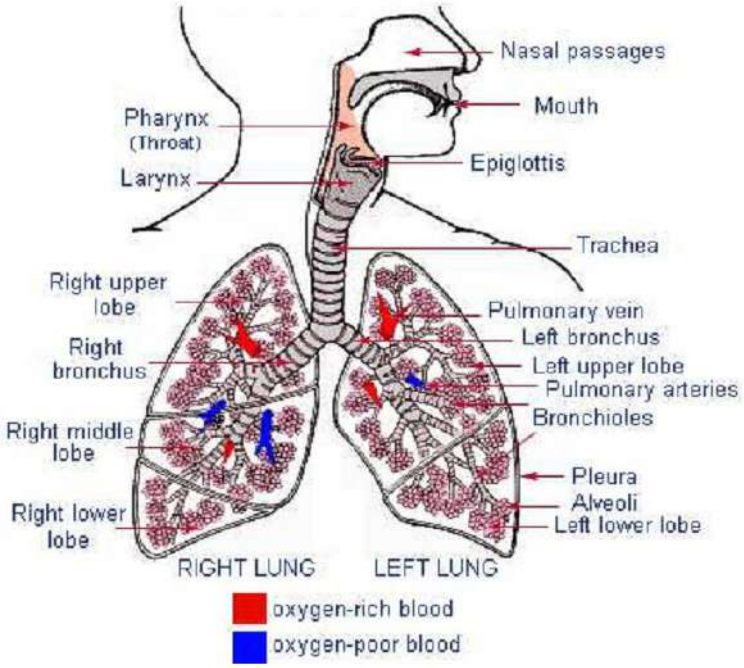
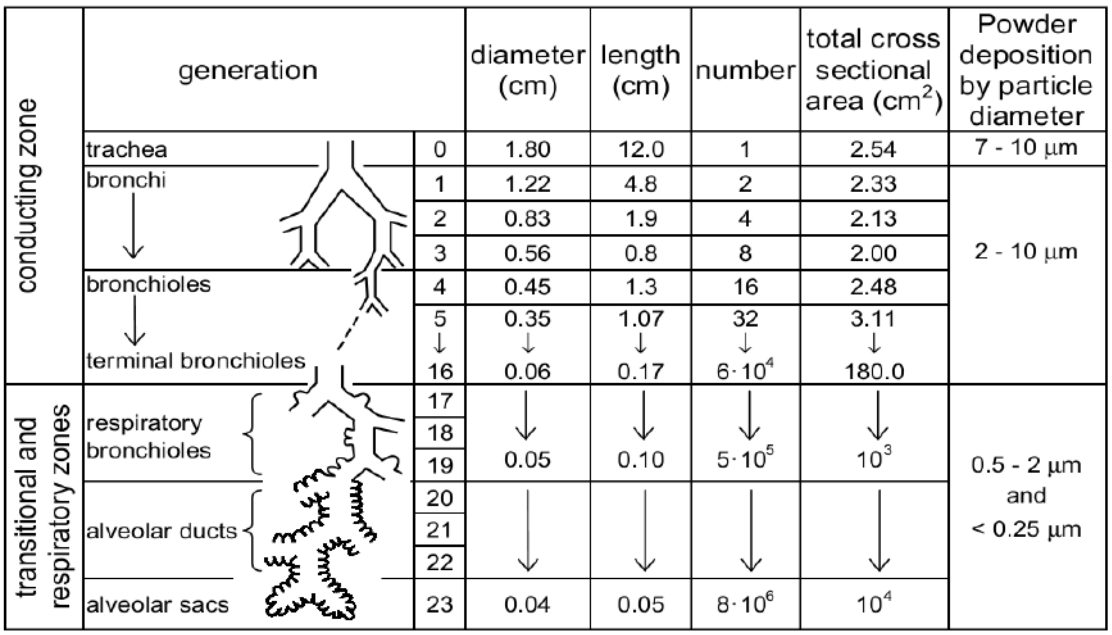


Figure 1. The Components of the Respiratory System [8]

CHAPTER 1 INTRODUCTION

The respiratory tract consists of a conducting portion and respiratory portion. The conducting portion begins at the entrance to the nasal cavity and extends through passageways of pharynx, larynx, trachea, bronchi and bronchioles to the terminal bronchioles. The respiratory portion of the tract includes the delicate respiratory bronchioles and the alveoli, air-filled pockets within the lungs where all gas exchange between air and blood occurs. Gas exchange can occur quickly and efficiently because the distance between the blood in an alveolar capillary and the air inside an alveolus is generally less than 1 µm and in some cases as small as 0.1 µm.The surface e area of the lungs involved in gas exchange ranges from 70 m^2 to 140 m^2 which is roughly 35 times the surface area of the body. Filtering, warming and humidification of the inhaled air begin at the entrance to the upper respiratory system and continue throughout the rest of the conducting system. By the time the air reaches the alveoli, most foreign particles and pathogens have been removed and the humidity and temperature are within acceptable limits. [8]A schematic representation of the human airways and the relationship with particle deposition is illustrated in figure 2. From the trachea, the airways bifurcate repeatedly for more than 17 generations before terminating in the alveolar sacs. [9]



	generation		diameter (cm)	length (cm)	number	total cross sectional area (cm^2)	Powder deposition by particle diameter
conducting zone	trachea	0	1.80	12.0	1	2.54	7 - 10 μm
	bronchi	1	1.22	4.8	2	2.33	2 - 10 μm
		2	0.83	1.9	4	2.13	
		3	0.56	0.8	8	2.00	
	bronchioles	4	0.45	1.3	16	2.48	
	terminal bronchioles	5 ↓ 16	0.35 ↓ 0.06	1.07 ↓ 0.17	32 ↓ $6 \cdot 10^4$	3.11 ↓ 180.0	
transitional and respiratory zones	respiratory bronchioles	17	↓	↓	↓	↓	0.5 - 2 μm and < 0.25 μm
		18					
		19	0.05	0.10	$5 \cdot 10^5$	10^3	
	alveolar ducts	20	↓	↓	↓	↓	
		21					
		22					
	alveolar sacs	23	0.04	0.05	$8 \cdot 10^6$	10^4	

Figure .2: A schematic representation of airway branching in the human lung [9]

1.3 DEPOSITION OF PARTICLES IN THE LUNGS

To achieve a desired therapeutic effect from aerosols, an adequate amount of drug must reach the alveolar sacs of the respiratory airways. The dynamic behaviour of aerosol particles is governed by the laws of aerosol kinetics. The dominant mechanisms of depositing aerosol particles into the respiratory tract include inertial impaction, sedimentation (gravitational deposition), and Brownian diffusion,. Inertial impaction and sedimentation are the most important for large particle deposition (1 μm < MMAD < 10 μm). A brief description of each mechanism of deposition is given below. [10]

1.3.1 Inertial impaction

A particle carried in the air stream has its own momentum and when the aerosol stream meets an obstacle or bends in the respiratory tract, the direction of the gas flow changes. Hence the particles with high momentum may impact with the object in front

of them and leads to the deposition of the particles in the respiratory tract. This is the main mechanism of the deposition of particles larger than 5 µm in diameter. It usually occurs in the upper respiratory tract (entrance of trachea) and at the conducting airway bifurcations. [11]

1.3.2 Gravitational sedimentation

This mechanism means the settling of the particles under the action of gravity. It occurs primarily for particles with a diameter ranging between 0.5 and 5 µ m. It is a predominant mechanism of deposition in the smaller airways, bronchi, bronchioles and alveoli but it can also occur in the upper respiratory tract. [12]

1.3.3. Brownian diffusion

Random motion of particles caused by collision with the gas molecules results in deposition of the inhaled particle by Brownian diffusion. As the particle size decreases, deposition by Brownian diffusion increases and is the dominant mechanism of deposition for particles less than 0.5µ m. It mainly occurs in the acinar region of lung but can also be found in the nose, mouth and pharyngeal airways for very small particles (< 0.01 µm). [13]

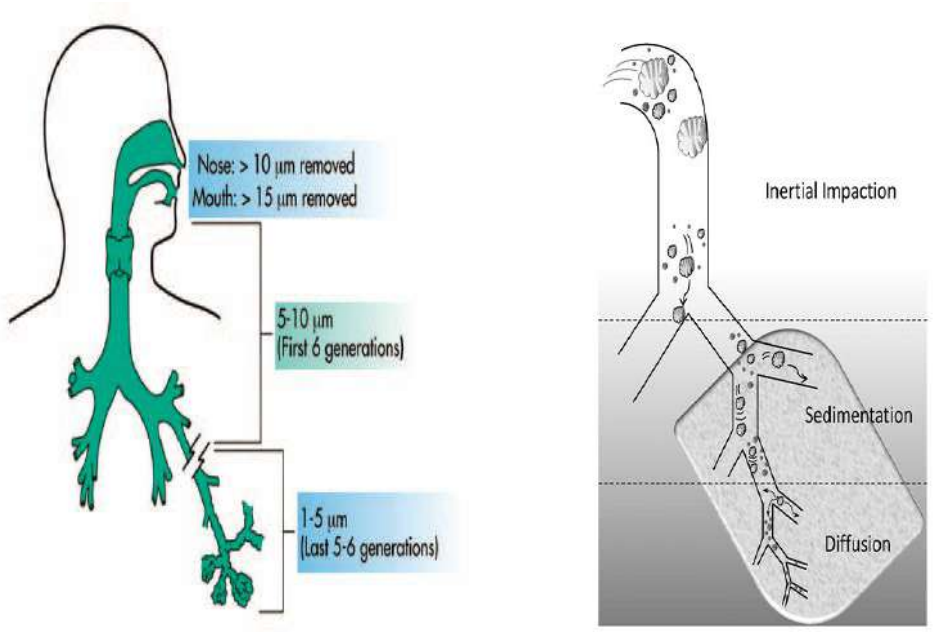


Figure. 3.Schematic diagram representing particle deposition in the lungs according to different mechanisms related to particle size: inertial impaction, sedimentation and diffusion. [14]

1.4. EXPRESSIONS USED TO DEFINE DRUG LUNG DEPOSITION

1.4.1. Fine particle dose (FPD) and Fine particle fraction (FPF)

The aerodynamic evaluation methods of fine particles permit the determination of the fine-particle dose (FPD), which corresponds to the mass of drug particles that have an aerodynamic diameter less than 5 µm. Such particles can theoretically be deposited in the deep lung after inhalation. The fine-particle fraction (FPF), which is the percentage of the FPD usually related to either the nominal dose (total drug mass contained in the device) or the recovered drug (sum of the drug collected in the device and in the different parts of the impingers or impactors after inhalation). [15]

1.4.2 Emitted dose (ED)

The emitted dose expresses the drug mass exiting the device after inhalation. In some cases, FPF can be calculated from emitted dose instead of total or recovered dose of drug from impingers or impactors. The ability of the powder to be fluidised by the airflow through an inhaler is usually indicated by the emission dose, whilst the FPD and FPF measure the capability of the formulation to be fluidised and deagglomerated in time to release the drug from the carrier to be deposited in the appropriate level of the impactors and impingers. [15]

1.4.3. Mass median aerodynamic diameter (MMAD)

Mass median diameter of an aerosol means the particle diameter that has 50% of the aerosol mass residing above and 50% of its mass below it. The concept of aerodynamic diameter is central to any aerosol measurements and respiratory drug delivery. The aerodynamic diameter relates the particle to the diameter of a sphere of unit density that has the same settling velocity as the particle of interest regardless of its shape or density. The mass–mean aerodynamic diameter (MMAD) is read from the cumulative distribution curve at the 50% point. [16]

1.4.4. Dispersiblity

The dispersibility is calculated as the ratio of FPD to emitted dose. [17]

1.4.5. Geometric standard deviation (GSD)

The degree of dispersion in a lognormally distributed aerosol is characterized by the geometric standard deviation (GSD). A larger GSD implies a longer large particle size tail in the distribution. [18] GSD for a well-functioning stage should ideally be less than 1.2 (the GSD for ideal size fractionators would be 1.0 and indicates a monodisperse aerosol). [19] GSD is a measure of the variability of the particle diameters within the

aerosol and is calculated from the ratio of the particle diameter at the 84.1% point on the cumulative distribution curve to the MMAD. For a log-normal distribution, the GSD is the same for the number, surface area or mass distributions. [20]The GSD was determined as

$$GSD = \frac{\sqrt{sizeX}}{\sqrt{size\ Y}}$$

Where sizes X and Y are particle sizes for which the line crosses the 84% and 16% mark, respectively. [21]

1.5. RESPIRATORY DRUG DELIVERY DEVICES

A good delivery device has to generate an aerosol of suitable size, ideally in the range 0.5-5 µm and a reproducible drug dosing. It must also protect the physical and chemical stability of the drug formulation. Moreover, the ideal inhalation system must be a simple, convenient, inexpensive and portable device. [22] Inhaled drug delivery devices can be divided into three principal categories: nebulizers, pressurized metered-dose inhalers (MDIs) and dry powder inhalers (DPIs), each class with its unique strengths and weaknesses.

1.5.1. Nebulizers

Nebulizers typically consist of a reservoir containing a solution or suspension of drug connected to a facemask or mouthpiece through which the patient breathes normally. The drug-containing liquid is aerosolized by ultrasound or application of compressed gas. Nebulizers are both effective at delivering drug, and capable of delivering very large doses. However, they are typically bulky and the administration of the drug takes a relatively long time. Consequently, they are used in the home or in a hospital setting. Nebulizers do not compete directly with dry powder inhalers (DPIs) and

pressurized metered dose inhalers (pMDIs) as portable delivery devices though recent developments have reduced their size and improved their metering and drug delivery rate. [23-24]

1.5.2 Pressurized metered-dose inhalers (pMDIs)

Pressurized metered-dose inhalers (pMDIs) are the most widely used devices for pulmonary drug delivery. While chlorofluorocarbons (CFCs) were employed as the propellants in pMDI formulation for decades, but in accordance with the Montreal Protocol of 1987,[25] chlorofluorocarbon propellants are being replaced by hydrofluoroalkane propellants that do not have ozone depleting properties concerns about their ozone depletion potential have prompted the search for more environment-tally friendly alternatives. The biocompatible, on-ozone depleting hydrofluoroalkanes (HFAs) have been selected as the replacements to CFCs Whereas HFAs and CFCs have similar densities and vapour pressures, several of their physicochemical properties are significantly different. There are two basic types of pMDI formulations: (i) solution-based, in which the active ingredients are dissolved in the propellant; (ii) dispersion-based, where the active ingredients are suspended in the propellant. pMDIs consist of several components, the active substance formulated with propellant and excipients, a container, a metering valve crimped onto the container, an actuator that connects the metering valve to an atomization nozzle, and a mouth piece. Additionally, holding chambers or spacers may also form part of the delivery system by connection to the actuator mouthpiece. [26] The change from CFCs to HFAs in metered-dose inhalers was not a straight forward exchange. Indeed, substantial new technology had to be developed to make the HFAs suitable for use in metered-dose inhalers.[27] Transition to HFA propellants led to the development of MDI actuators

with improved delivery efficiency, Valves have been improved to function in HFA propellants, meet more stringent regulatory requirements on dosing uniformity and extractables/leachables, Canister technologies (e.g. novel coatings) have been developed to reduce drug degradation and deposition.[28-29] However, more significant advances, related to the direct operation of metered-dose inhalers, were required not only to improve patients' comfort, but also to increase the effectiveness of the devices. Based on this objective, breath-actuated pressurized inhalers, breath-coordinated metered-dose inhalers, and velocity-modifying inhalers were developed. [30] In recent years increasing attention has been paid to the issues of electrostatic charge and facemasks related to valve holding chambers. Recently developed, HFA-soluble excipients expand the range of drug molecules, which can be formulated into highly respirable solution MDIs. Some of these excipients, such as oligolactic acid, interact with the drug to form drug/excipient complexes that are highly soluble in HFA propellants. This approach leads to significant improvement in the solubility of the drug. This allows poorly soluble drugs to be formulated with little or no co-solvent, thus increasing the drug delivery efficiency. [31]

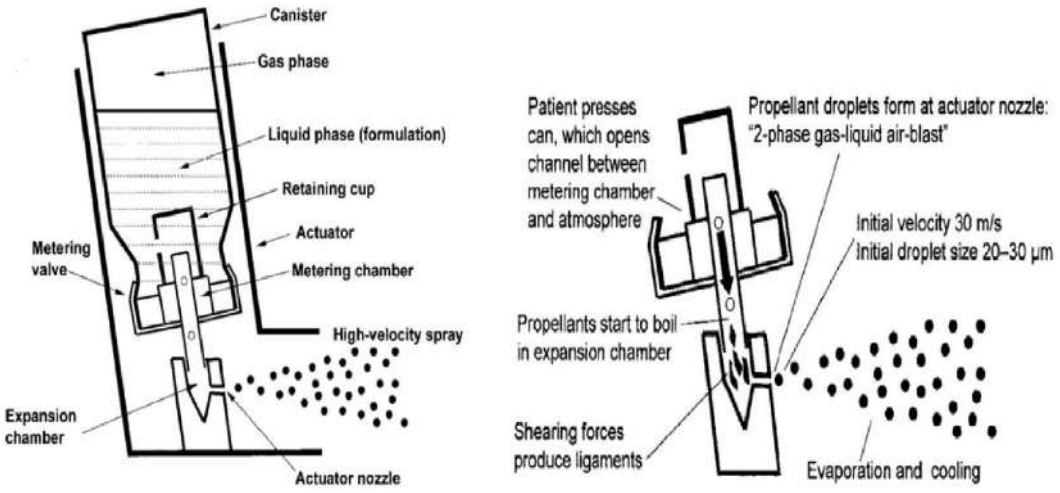


Figure 4: Schematic representation of a typical pressurized metered-dose inhaler. [32]

1.5.3. Dry powder inhaler

The problems encountered with MDIs, the design and uses of alternative inhalers that do not use propellants were developed. These devices combine powder technology with device design in order to disperse dry particles as an aerosol in the patient's inspiratory airflow. [33] Dry powder inhalers (DPIs) are devices through which a dry powder formulation of an active drug is delivered for local or systemic effect via the pulmonary route. Dry powders for inhalation are formulated either as loose agglomerates of micronized drug particles with aerodynamic particle sizes of less than 5µm or a carrier based interactive mixtures with micronized drug particles adhered onto the surface of large lactose carriers. For topical respiratory drug delivery, a particle size of 2-5 µm yields optimal benefits, where as for systemic effect particle size of less than 2 µm is needed for drug deposition in the small peripheral airways. Particle size greater than 5 µm may also result systemic effects due to impaction in the throat (i.e., oropharyngeal delivery) and oral absorption). The powder formulation is aerosolized through a DPI device, where the drug particles are separated from the carrier (from drug–carrier mixtures) or deagglomerates drug particles, and the dose is delivered into the patient's deep lungs. In these systems, particle size and flow property, formulation, drug–carrier adhesion, respiratory flow rate and design of DPI devices extensively influence the performance. [34]

For DPIs, the dose received by the patient is dependent on four interrelated factors. [35]

(i). The properties of the drug formulation, particularly powder flow, particle size and drug carrier interaction.

(ii). The performance of the inhaler device, including aerosol generation and delivery.

(iii). Correct inhalation technique for deposition in the lungs.

(iv). The inspiratory flow rate.

1.5.3.1. Historical background of dry powder inhaler

To learn about the history of DPIs actually have to travel back to *before* the first MDIs hit the market in the 1950s. “the first patent for a DPI was made in 1864 by Newton. The medicine he used was potassium chlorate. His use of potassium chlorate as a therapy was probably unfortunate because this is now considered to be a lung irritant. However, this device is probably the earliest recorded dry powder inhaler. However, while Newton's device wasn't a commercial success, "He observed that the powder needed to be finely pulverized and that it had to be kept dry -- principles that still apply to dry powder inhalers today. By the 1940s pharmaceutical companies learned that systemic injection of asthma medicines like epinephrine and atropine caused significant side effects. . They were in an all out race to develop a device that allowed asthmatics to inhale medicine and, thus, generate an immediate effect with fewer side effects. A patent from 1949, by M.R. Fields, described the first dry powder inhaler to be used for the administration of a pharmaceutical agent. The so-called Aerohaler was the first commercially available dry powder inhaler for the delivery of isoprenaline sulphate. The first single dose dry powder inhaler with a hard gelatin capsule technology was initially developed for the inhalation of relatively large amounts of drug, being 50 mg of disodium cromoglycate (Spinhaler, 1971). Later, DPI's found their application in inhalation therapy as a CFC-free alternative for the older p-MDI's. However, nowadays DPI's seem to have a much larger potential, because of the high lung deposition that can be attained and their suitability for

pulmonary delivery of therapeutic peptides and proteins, which can subsequently become systemically available. [36-37]

1.5.3. 2. Advantages of dry powder inhaler

As DPIs has been motivated by the desire for alternatives to pMDIs, so advantages of DPI over pMDI is given as follows; [38]

(i).Require little or no coordination of actuation and inhalation:

Incorrect use of pMDIs found that poor coordination of actuation and inhalation caused decreased asthma control in a substantial proportion of patients treated with corticosteroid pMDIs. Whereas DPIs are activated by the patient's inspiratory airflow, they require little or no coordination of actuation and inhalation.

(ii).Formulation Stability:

Since DPIs are typically formulated as one-phase, solid particle blends, so they are preferred as stable formulation. Dry powders are at a lower energy state, which reduces the rate of chemical degradation and the likelihood of reaction with contact surfaces. By contrast, pMDI formulations, which include propellant and co solvents, may extract organic compounds from the device components.

(iii).Propellant-free design:

pMDI contains propellants such as chlorofluorocarbons and hydrofluoroalkanes which are ozone-depleting and greenhouse gases respectively. Production of CFC propellants was banned from 1st January 1996 in order to stop the depletion of ozone layer. So pMDI were replaced by DPI which do not contains propellant. So DPI's are environmental friendly formulation.

1.5.3. 3. General requirements of DPI

DPIs have to meet the following requirements;

(i) Particle Size of API: Active Compound must be size about 1 to 5 µm. Such micro fine particles can be obtained by micronization, controlled precipitation from suitable solvent or by spray drying, if the process conditions are suitable.

(ii) Drug content uniformity: It is important that each capsule or blister in a single dose system contain the same amount of powder and medication while in a multi-dose system; the reservoir must release the same amount of powder and drug every time.

(iii) Content uniformity at different airflows: The dose has to be released in exactly the same way at low breathing and at a high breathing rate.

(iv) Stability of powder against humidity and temperature: Major ingredient of DPI is lactose which must be protected against particle size growth. The main property responsible for particle size growth is an undesired combination of temperature and relative humidity which is controlled by storage in the correct packaging is important for stability.

(v)Flow ability: Almost all active ingredients have poor flow ability; the good flow has to be supplied by the carrier.

1.5.3. 4. Principles of operation

Most DPIs contain micronized drug blended with larger carrier particles, which prevents aggregation and helps flow. To generate the aero-sol, the particles have to be moved. Movement can be brought about by several mechanisms. Passive inhalers employ the patient's inspiratory flow. When the patient activates the DPI and inhales, airflow through the device creates shear and turbulence; air is introduced into the

powder bed and the static powder blend is fluidized and enters the patient's airways. There, the drug particles separate from the carrier particles and are carried deep into the lungs, while the larger carrier particles impact in the oropharynx and are cleared. Thus, deposition into the lungs is determined by the patient's variable inspiratory air-flow. Inadequate drug/carrier separation is one of the main explanations for the low deposition efficiency en-countered with DPIs.Dose uniformity is a challenge in the performance of DPIs. [37] Figure 4 shows the principles of DPI design.

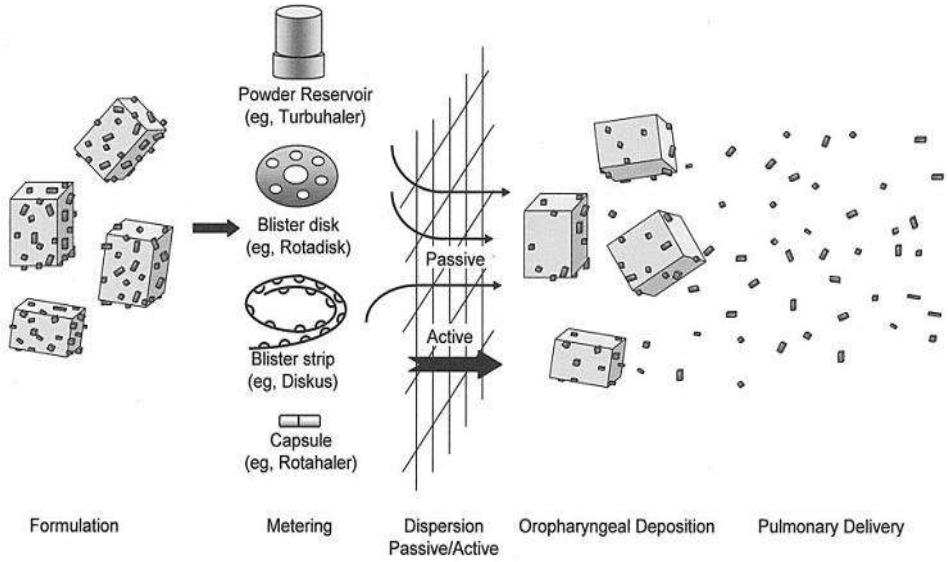


Figure. 5. Principle operation of dry powder inhaler design. [37]

1.5.3.5. Formulation aspects

Dry powder formulations either contain the active drug alone or have a carrier powder mixed with drug. Particle size of drug should be less than 5 µm. It should be in the range of 2-5 µm. Generally the drug particle size is not well controlled during bulk drug production. The drug particle size must be reduced in a separate unit operation. There are various size reduction techniques such as milling, spray drying, and supercritical fluid extraction. There are various types of mills used for size reduction of drugs but few of them are suitable for DPI to reduce the size in the range of 2-5 µm

such as fluid-energy mills, such as the jet mill; high-peripheral-speed mills, such as the pin-mill; and the ball mil.[38]The requirement to use micronized drug with small(ideally less than 5 μm) particle achieved good aerodynamic properties of the dispersed powder is confounded by the need to develop formulation that fill easily and accuratlly.It is also important that changes in the physical nature of the formulation on transportation and storage not adversely affect during formulation development. The inclusion of carrier can aid in the handling of the formulation and may impart some aerodynamic benefits also. The goal of delivering micronized powders is a challenging one. Because, these type of powder are highly cohesive. Their high interparticulate forces make them difficult to deaggregate, hence need for high inspiratory flow rate and turbulent airflow within DPI.Incorporation of carrier may aid the deaggregation process, but it can also lead to problem absorption of atmospheric moisture. Controlled temperature and humidity studies of salts form and lactose (or other suitable carrier) combination are essential during formulation development.[39] Various techniques' were develop to improve formulation performance by development of tertiary expcepient like magnesium stearate and leucine. The inclusion of magnesium stearate in the DPI formulation as a ternary additive helped in improving the performance of the formulations. Magnesium stearate can form film layers which can adhere to drug-excipient particles and can interfere with inter-particle bonding as a result o f hydrophobic coating. [40]The use of leucine also in the DPI formulation as a ternary additive has helped in improving the performance of the DPI formulations. This is possibly due to antiadherent action of the material. [41]

1.5.3. 6. Carriers used in DPI

Carrier particles are used to improve drug particle flow ability, thus improving dosing accuracy and minimizing the dose variability observed with drug formulations alone while making them easier to handle during manufacturing operations. With the use of carrier particles, drug particles are emitted from capsules and devices more readily, hence, the inhalation efficiency increases. Design of the carrier particle is important for the development of DPIs.Carrier particles should have several characteristics such as physicochemical stability, biocompatibility and biodegradability, compatible with the drug substance and must be inert, available and economical. [42] Lactose is the most common and frequently used carrier in DPI formulations accordingly nowadays various inhalation grades of lactose with different physico-chemical properties are available on the market. The advantages of lactose are its well-investigated toxicity profile, physical and chemical stability, compatibility with the drug substance, its broad availability and relatively low. Lactose, in particular alpha-lactose monohydrate, is typically used as 'the' carrier in dry powder inhalers. [43]

Due to several drawbacks of lactose and modified lactose as a carrier for dry powder inhalers, there is an urgent need to find suitable alternative carriers for better drug dispersibility in DPIs.Alternative carriers like mannitol, glucose, sorbitol, maltitol and xylitol as potential carriers in DPI formulations. Of all the sugars evaluated, mannitol seemed to be a promising carrier for DPIs whereas sorbitol, maltitol and xylitol sugars were not able to generate desirable FPF due to their hygroscopic nature. [44]Other carriers‟ viz. crystallized mannitol (Pearlitol 110 C), spray-dried mannitol (Pearlitol 100 SD), crystallized maltitol (Maltisorb P90) and spray-dried lactose (Lactopress SD 250) has been used for two drugs: micronized terbutaline sulfate and micronized formoterol fumarate. It was found that crystallized forms of the carrier offered lower adhesion and better release of the active ingredient

than spray-dried forms. The crystallized mannitol produced maximal fine particle dose. [45]

1.5.3. 7. Production methods of various grades of lactose

Lactose is a natural disaccharide consisting of galactose and glucose and is present in the milk of most mammals. Commercially, lactose is produced from the whey (residual liquid from the milk following cheese and casein production) of cows' milk. Lactose can be obtained in either of two basic isomeric forms, namely α-and β-lactose, or in an amorphous form. α-Lactose exists both in monohydrate and in anhydrous forms, the former being the most thermodynamically stable form.α-Lactose monohydrate is prepared by crystallisation from supersaturated solutions below 93.5 °C. Its crystalline shape can be a prism, a pyramidal or a tomahawk and is dependent on the precipitation and crystallisation methods. Anhydrous lactose (typically containing 70–80% anhydrous β-lactose and 20–30% anhydrous-lactose) is most often produced by roller drying a lactose solution above 93.5 °C. Next, both resulting products are milled to decrease particle size and sieved to select an appropriate PSD. Spray-dried lactose is obtained by spray-drying a suspension of α-lactose monohydrate crystals in water in a lactose solution. In recent years, supercritical fluid crystallization (SFC) technologies have gained increasing attention in the pharmaceutical industry due to their capability and versatility of producing micro-fine particles to predetermined specifications. [46]As can be seen, in, Table 1, there is a wide variety of lactose with different physico-chemical properties that could be used in DPI formulations.

Table 1: Characteristics of different grades of inhalation lactose obtained by different methods [47]

Processing		Lactose monohydrate Sieved		Lactose monohydrate Milled		Lactose monohydrate Micronised	
Roughness		+		++		++	
Surface Amorphous		+		++		+++	
Water uptake (at high RH)		+		+		+	
Fine content below 10µm		+		++		++++	
Shape		Tomahawk		Tomahawk		Tomahawk	
Size µm d(0.5)	~60	~130	200–220	50–100	50–60	17	>5
Carr's Index%	16-25	11–15	1–15		32–37	>38	>38
Examples of commercially available lactoses	Respitose SV003, Inhalac 250, Lacto-Sphere MM50	Inhalac 120, Lactohale LH 100	Lacto-Sphere MM250, Inhalac 70	Lactohale LH 200, Lactose MH Inhalation 120M	Respitose ML001, Lactose MH Inhalation 80M	Respitose ML006	Lactohale LH 300, Lacto-Sphere MM3, Respitose MC.

+ For the lowest and ++++ for the highest. NR means “non reported”.

1.5.3. 8. Lactose engineering for application in DPIs

(i) Surface modification

Iida et al. prepared lactose carrier particles for dry powder inhalations by its surface modification with aqueous ethanol solution and evaluated the inhalation efficiency of

salbutamol sulfate from its mixture with modified lactose. The degree of adhesion between drug particles and carrier particles and the separation characteristics of drug particles from carrier particles in air flow were assessed by the ultracentrifuge separation and the air jet sieve methods, respectively. It was shown that the average adhesion force between the surface-treated lactose carrier and drug particles was considerably lower than that of powder mixed with the un-treated lactose carrier, indicating better drug separation from carrier and consequently an improvement of in vitro inhalation properties. The authors claimed that surface-smoothing of lactose by aqueous ethanol solution resulted a well balanced drug-carrier adhesion force so that the drug particles could be emitted together with the carrier particles and efficiently separated in airflow after emission. [48]

(ii) Shape modification

Lactose crystallization from Carbopol gel in different conditions (named Carbo lactose) produced more regular shape lactose with smoother surface as compared with the control lactose. The Carbo lactose caused a higher and reproducible salbutamol sulphate emission and fine particle fraction after aerosolisation via a Rotahaler® tested in multi-stage impinge. It was concluded that engineered crystal growth under controlled conditions can enhance the potential inhalable fraction of drug from dry powder inhalers. More recently, the use of more elongated crystals of lactose was also found to produce a higher inhalation efficiency of albuterol sulfate.However, the improvement in drug dispersion that can be achieved by increasing the elongation ratio of the carrier particles is limited. [49]

(iii) Composite lactose

Lactose carrier particles were prepared by fusing sub units of lactose (2, 6 or 10 μm prepared by spray drying) in saturated lactose slurry, sieve fractioned to obtain a 63–

90 µm carriers and used for inhalation of salbutamol sulphate. The surface morphology and physicochemical properties of the composite carriers were considerably different from regular α-lactose monohydrate. In all cases, the composite carriers resulted in improved drug aerosol performance. It was suggested that composite based carriers are a potential route to control drug-carrier adhesion forces and variability thus allowing more precise control of formulation performance. [50]

(iv) Engineered lactose-mannitol mixture

Mannitol and lactose were co-crystallised to prepare crystals with more desirable characteristics than either lactose or mannitol alone appropriate for application as carriers in the formulations of salbutamol sulphate DPIs. In vitro deposition evaluation showed that crystallized carriers resulted more efficient delivery of salbutamol sulphate compared to formulations containing commercial grade carriers. It was concluded that simultaneous crystallization of lactose-mannitol can be a new approach to enhance inhalation performance of DPI formulations. [51]

1.5.3. 9. Adhesive–cohesive forces of interactions between the drug and lactose

The performance of dry powder inhaler (DPI) formulations can be a strong function of the balance of cohesive and adhesive forces experienced by the drug particles under stresses induced in the flow environment during aerosolization. [52]

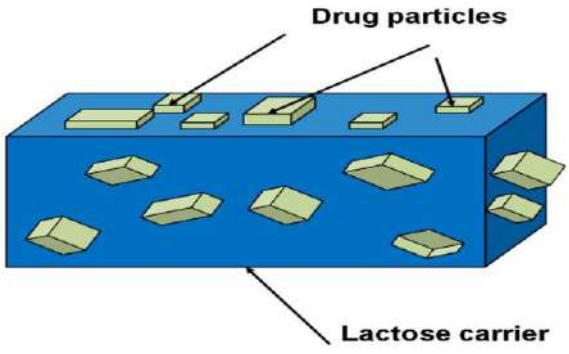


Figure. 6 .Diagram of an idealized view of drug adhered to lactose carrier. [52]

(i). Interparticulate interactions

Interparticulate interactions (both cohesion, i.e., drug–drug, and adhesion, i.e., drug–carrier) are dominated by physical forces of interaction: i) vanderwaals forces, which are considered to be the most substantial forces, ii) electrostatic charges, iii) capillary forces and, iv) mechanical interlocking .Other interactions are determined by chemical forces, such as acid–base interaction forces and hydrogen bonding . The different interparticulate interactions between the drug and lactose carrier are illustrated in figure. 5.

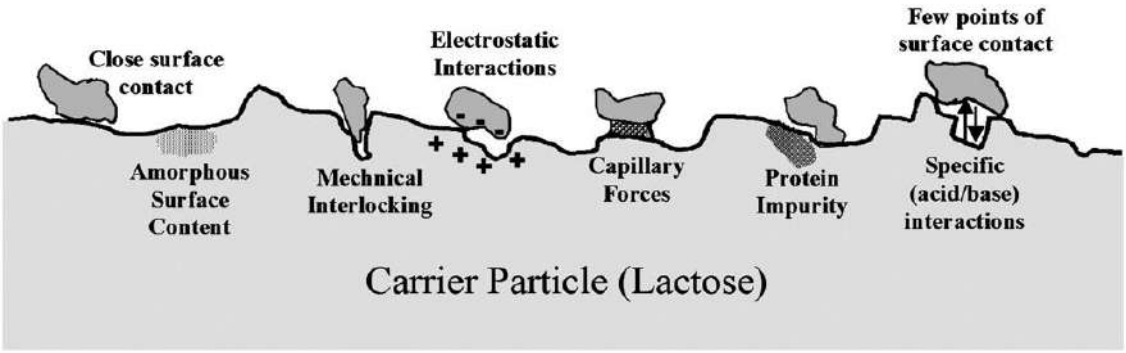


Figure.7. Illustration of the different causes of interactions between micronised particles (i.e., drug or fine excipients) and lactose carrier particles [47]

The order of magnitude of the physical forces varies with particle size, shape, surface properties, the hardness of the adhering particle, surface roughness, contamination of the carrier particle, the intensity (and duration) of the press-on forces during mixing, and the relative humidity (RH) . In the case of formulations consisting of micronized particles (e.g., drug and fine lactose) and a larger carrier, the size difference between the fine particle and the carrier allows a type of adhesion to be considered that is basically the same as that between a sphere and a flat surface. The magnitude of the adhesive force for this situation is proportional to the diameter of micronized particle and varies following the distance between micronised particle and the carrier (for

vander Waals forces and electrical charges) or following the surface tension of the liquid between particles (for capillary forces) . [47]

(ii). Vander Waals forces and mechanical interlocking.

The dominant interaction forces between micron-sized particles in a powder are vander Waals forces of attraction. Vander Waals forces dominate gravitational forces when the separation distance between particles is sufficiently small (b100 nm) and the particle size is sufficiently small (b10 μm), as is the case in formulations with drug and carriers. On the other hand, the intimate contact area could substantially increase the van der Waals forces of attraction and may cause mechanical interlocking when protuberances fit into cavities.

(iii). Capillary forces.

Capillary forces or "meniscus forces" arise due to the formation of a liquid concave-shaped meniscus (liquid bridge) around the contact area of two neighbouring particles .When two particles enter into contact with each other, a narrow slit is created around the contact surface. The attractive force caused by the concave-shaped meniscus is due to the surface tension of the liquid around the periphery of the meniscus, which pulls the particles together. Capillary forces have been reported to vary from a few nanoNewtons (nN) to a few hundred nN for organic drug crystals over a range of humidities. The magnitude of these forces, involved in adhesion and cohesion, varies according to drug or carrier physico-chemical properties, such as shape and size, as well as the roughness and chemical properties of the surface, but especially according to the environmental RH. [47]

(iv). Electrostatic charges.

Electrostatic charges occur when two dissimilar surfaces are brought into contact and then separated, resulting in oppositely charged surfaces due to a charge transfer between a donor and an acceptor. When the contact is made by a short collision or by intense friction, the resulting charging phenomenon is called “triboelectrification”. Contact charging is classified into three categories according to the contacting materials: metal–metal, metal–insulator, and insulator–insulator contacts. In the pharmaceutical field, most drugs and excipients are organic crystals, which present high resistivity and poor conductivity and therefore behave as insulators under ambient conditions. The contact surface may be metal (the mixer vessel) or insulating materials (the device's plastic components as well as the excipient or drug particles during mixing and fluidisation). Triboelectrification, arising from manufacturing processes (mixing, handling, and filling), is considered to be a nuisance because it limits powder flow ability during the industrial process by increasing the adhesive–cohesive forces between powder particles, which decrease aerosol performance. The materials of the mixer vessel (stainless steel) and the device constituents (polypropylene and acetal) influence adhesion as well as the magnitude and polarity of the charges acquired by the powders as a function of the nature of the powder. Furthermore, the addition of a fine-lactose excipient (b10 μm) to coarse lactose decreases the magnitude of triboelectrification during mixing. The resulting electrostatic charges are involved in particle deposition in the lungs and can be used to enhance lung deposition. The lactose grade (sieved or milled), inhaler device and capsule material have a strong effect on both the magnitude and the polarity of electrostatic charges generated by the aerosolisation of dry powders. The design and deaggregation mechanisms of the inhaler have an impact on the triboelectrification of

drug and lactose carrier particles. In addition, external factors such as storage RH affect the magnitude of electrostatic charges of drug particles during inhalation and therefore have an impact on the in vitro aerodynamic performance. Static (i.e., within the powder bed) and dynamic (i.e., during inhalation) electrifications of dry powder for inhalation are important factors that must be taken into consideration and must be better understood to improve the use of DPIs. It is important to evaluate the triboelectrification of the dry-powder formulation arising from manufacturing processes, which could influence the adhesion forces affecting flow properties and in vitro aerodynamic performance, and that from powder aerosolisation, which could increase particle deposition in the lung. [47]

1.5.3. 11. Basic design of dry powder inhaler devices

The inhalation device is important in achieving adequate delivery of inhaled drug to lungs. The device should be easy to use, in expensive and portable. The device must provide an environment where the drug can maintain its physicochemical stability and produce reproducible drug dosing. The device should be designed to deliver high fine particle fraction (FPF) of drugs from the formulations. However, devices with higher resistance need a higher inspiratory force by the patients to achieve the desired air flow. This could be difficult for patients with severe asthma and for children and infants.[34]Dry powder inhaler devices are classified by dose type into single-unit dose, multi-dose reservoirs, and multi-unit dose, as illustrated schematically in Fig. 6. -In a single-unit dose device,

The drug is formulated as a micronized drug powder and carrier system and supplied in individual gelatine capsules, which are then inserted into the inhaler for a single

dose and removed and discarded after use. There are two types of multi-dose devices, reservoir type devices and multi-unit dose devices. [53]

-The multi-dose reservoir device

The formulation in bulk, and has a built in mechanism to meter individual doses from the bulk upon actuation. Newer devices of this type attempt to address issues such as reducing the flow rate dependent dose emission and of moisture ingress into the reservoir from patient exhalation or environmental humidity during the life of the product as these are common issues with the reservoir type device.

-The multi-unit dose device

Sealed doses packaged in a manner that the device can hold multiple doses without having to reload. Typically, the packaging consists of replaceable disks or cartridges, or strips of foil-polymer blister packaging that may or may not be reloadable. This pre-packaged does have the advantage of being protected from the environment until use and ensuring adequate control of dose uniformity. [54]

(a). Ideal Characteristics for DPI Device [34]

(i). A device which is simple to use, convenient to carry, contains multiple doses, protects the drug from moisture and has a indicator (audiovisual) of doses remaining.

(ii) Dose delivery which is accurate and uniform over a wide range of inspiratory flow rates

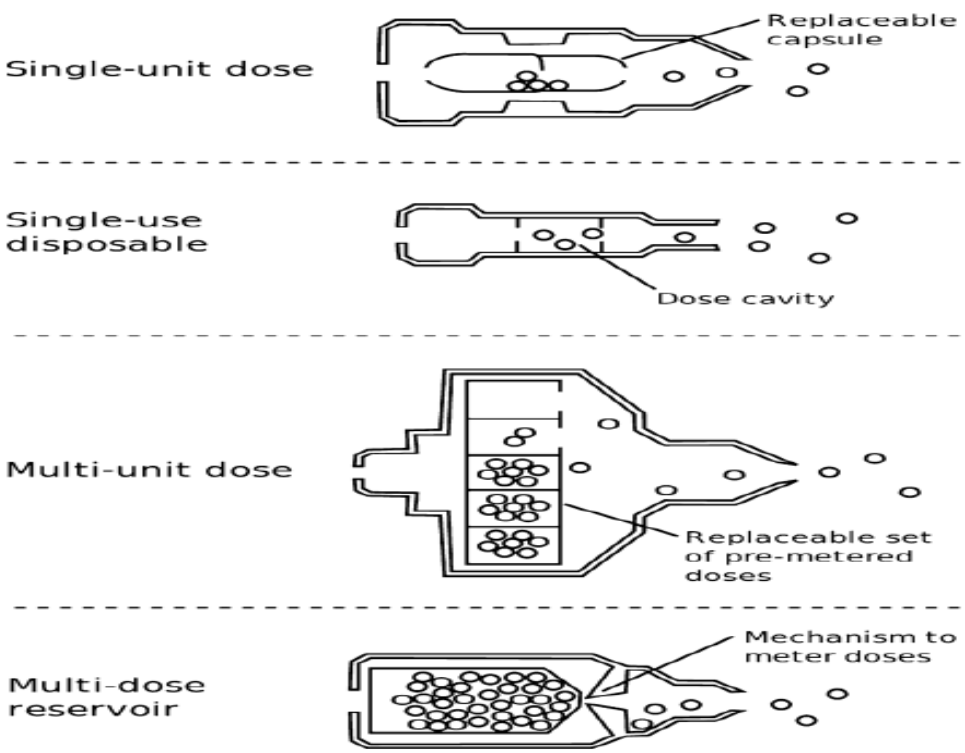


Figure 8. Illustration of four dose design options available for dry powder inhalers. [53]

(iii). Consistent dose delivery throughout the life of the inhaler and consistency of dose when compared to other similar inhalers.

(iv). Optimal particle size of drug for deep lung delivery.

(v). Suitability for a wide range of drugs and doses.

(vi) Minimum adhesion between drug formulation and devices.

(vii) Product stability in the device.

(viii) Cost-effectiveness.

- Unit-dose devices

The Spinhaler® (Aventis) was the first dry powder device, described in 1971. It has a mechanism for piercing the capsule. The cap of the capsule fits into an impeller, which rotates as the patient breathes through the device, projecting particles into an

airstream (Fig. 7). Shear force and relative motion are the predominant mechanisms of Powder deggregation. [55]

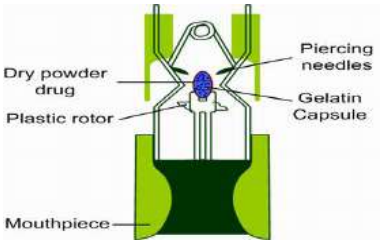


Figure 9: Schematic presentation of the Spinhaler® [56]

- Multi-dose devices

The Diskhaler® (GlaxoSmithKline) employs individual doses packaged in blister packs on a disk cassette. Following piercing, inspiratory flow through the packaging depression containing the drug induces dispersion of the powder. The aerosol stream is mixed with a bypass flow entering through two holes in the mouthpiece that, together with a grid, gives rise to turbulence that promotes deagglomeration. [55]

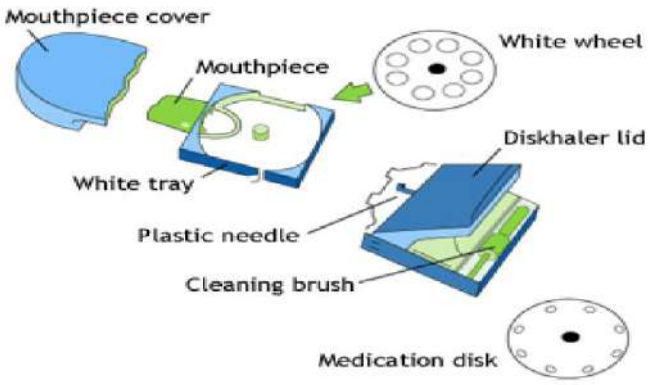


Figure 10: Schematic presentation of the Diskhaler® [57]

(b) Resistance flow of device

A wide range of dry powder inhaler (DPI) devices are currently available on the market to deliver drugs into lungs with a view to maximize drug delivery with low

variability. The performance of the DPI system depends not only on the powder formulation but also the inhaler device. However, devices are much less explored than the powder formulations. There is a wide range of passive (breathe driven) and active (power driven) single or multiple dose DPI devices in the market. The market is currently driven by passive devices which rely on the inspiratory airflow of the patient for powder dispersion into individual particles.DPI device has a different air flow resistance that governs the required inspiratory effort by the patient. The higher the resistance of the device, the more difficult it is to generate an inspiratory flow great enough to achieve the maximum dose from the inhaler. However, deposition in the lung tends to increase while using high-resistance inhalers. [58]

The intrinsic resistance to airflow through a DPI is a fixed property that determines the airflow rate through the inhaler in response to the inspiratory effort of the patient. Each inhaler has a unique resistance and current inhalers have a wide range of resistance values. The peak inspiratory flow rate (PIFR, measured in l/min) influences the efficiency of the inhaler in lifting particles of the drug formulation from the drug chamber or capsule, and it will also affect the efficiency of deagglomeration of the particles and the amount of drug reaching into the lungs.

A patient's inhalation effort will generate a higher PIFR through an inhaler with a low airflow resistance than through an inhaler with a high airflow resistance. In high-resistance DPIs, interpatient variability in PIFR may be reduced compared to that through low resistance devices, leading to more reproducible drug delivery. However, fine particle production is a function of specific inhaler design, not simply airflow rate through the inhaler, and neither inhaler airflow resistance nor PIFR alone can predict how efficiently a DPI will deliver a drug to the lungs. [59]

Table 2: Different type of DPI devices with their resistance [60]

Inhaler	Manufacturer	Resistance(H_2O/L/s)
Rotahaler	GlaxoSmithKline	0.015
Spinhaler	Sanofi-Aventis	0.016
Diskhaler/Diskus	GlaxoSmithKline	0.032/0.034
HandiHaler	Boehringer Ingelheim	0.042
Turbuhaler	AstraZeneca	0.044
Novolizer	Viatris	0.028
Inhalator	Boehringer Ingelheim	0.051-.062

1.5.3. 12. physicochemical characterization or evaluation of dry powder formulation.

The DPIs must be characterised to find out its physicochemical properties with the suitable analytical method.

(i) Physical parameter like Crystallinity of the drug particles can be examined by X-ray diffractrometer and by thermograms of differential scanning colorimetry. Bulk density, Tap density, and Carr's index have to be determined to evaluate powder flow ability. Particle morphology can be measured by scanning electron microscopy and dynamic and static image analysers. Recently, Raman imaging systems are used for the measurement of particle size, crystalline and shape. Particle size and its distribution can be measured with laser diffraction techniques and photo correlation spectroscopy.

(II) Water content in the blend can be measured by using Automatic Karl-Fischer Titrator.

(iii) Dosage unit sampling apparatus (DUSA) is used for sampling and testing of dry powder inhaler.

(iv) Drug content and solubility can be analysed with LC-MS, HPLC, UV or other suitable system. Total delivered dose and dose uniformity, particle size and shape are determined by scanning electron microscope.

(v) DPI design enable the dose to be dispensed independent of inspiratory flow rate between 30 L/min and 90 L/min. DPIs with medium resistance to airflow are designed to operate at an optimum rate of 60 L/min. The next generation impactor (Copley Scientific, UK), Anderson cascade impactor (Copley Scientific, twin impinger or aerosizer are used to determine particle size distribution, to estimate respirable fraction and for the aerosolization and deposition properties in vitro. The multistage cascade impactors were used to determine the mass-weighted aerodynamic particle-size distribution and the data was used to calculate the mass median aerodynamic diameter of the samples.

(vi) Magnetic resonance imaging was utilised to explains the inter and intra subject variation in oropharyngeal deposition.

(vii) A stability study has to be carried out for the final formulations as per ICH guidelines. Drug contents and thermal behaviour of the formulation should be monitored during the stability studies. Magnetic resonance imaging was utilised to explains the inter and intra subject variation in oropharyngeal deposition.

1.5.3.13. Novel dry powder inhalers

The large dependence on high inspiratory flow rates for the operation of the first dry powder inhalers led to the development of new technologies based on passive and active powder dispersion mechanisms. In both cases, the objective is to facilitate de-agglomeration of drug particles, resulting in greater lung deposition. Devices using passive mechanisms include Novolizer® (Meda, Sweden) and Airmax® (Yamanouchi, Netherlands) the air classifier technology has been described as the most efficient passive powder dispersion mechanism currently used in dry powder inhalers. In this case, multiple supply channels generate a tangential airflow that results in a cyclone within the device during inhalation. Novolizer® uses this technology and, when compared to Turbuhaler®, showed a greater degree of budesonide deposition in the lung and lower drug deposition rates in the oropharynx.A similar mechanism is used in the Airmax®. This inhaler has a separator within which the airflow generates a cyclone similar to that observed in the Novolizer®, and this device also has greater efficacy than Turbuhaler®with respect to total drug that is delivered to the lungs, according to studies with salbutamol and budesonide.The technology of dry powder inhalers has developed to use energy as a key element in the process of particle de-agglomeration. Storage of mechanical energy in systems based on springs or compressed-air chambers was one of the alternatives found in some devices. Exubera® (Nektar Therapeutics, USA), for example, uses an air chamber that is actuated by the patient through a kind of manual pump. The effectiveness of this device, which was designed for aerosolizing insulin. Battery-powered, electrically driven systems have also become attractive options. Spiros® is a dry powder inhaler that operates appropriately even at very low inspiratory flow rates, exactly because it uses this principle to operate a twin-blade impeller that aerosolizes the drug.

Advancements in these inhalers also include new types of powdered formulations of drugs through the production of micro particles by spray-drying techniques, resulting in porous particles, with low geometric diameter and high potential for lung deposition. Similar porous particles may be coupled to long-sized carrier molecules to reach the lungs with similar efficacy. Drug encapsulated liposome's are also a prospect of further improvement of drugs used in these devices. [31

Table 3: Currently available DPI device in market [40]

Device	DPI type	Company	Delivery method	Drug(s)
Breath-actuated single unit dose				
Spinhaler®	Single dose	Aventis	Capsule	Sodium cromoglycate
Rotahaler®	Single dose	GlaxoSmithKline	Capsule	Salbutamol sulphate and Beclomethasone dipropionate
Inhalator®	Single dose	Boeringher-Ingelheim	Capsule	Fenoterol
Aerolizer®	Single dose	Novartis	Capsule	Formoterol
TwinCaps®	Single dose	Hovione	Capsule	Zanamivir
Breath-actuated multiple dose				
Turbuhaler	Multi-dose	Astra Zeneca	Reservoir	Salbutamol sulphate Terbutaline sulphate Budesonide
Diskhaler®	Multi-dose	GlaxoSmithKline	Blister	Salmeterol xinafoate Beclomethasone dipropionate Fluticasone propionate Zanamivir
Diskus®	Multi-dose	GlaxoSmithKline	Strip pack	Salbutamol sulphate

				Salmeterol xinafoate Fluticasone propionate
Aerohaler®	Multi-dose	Boeringher-Ingelheim	Reservoir	Ipratopium bromide
Easyhaler®	Multi-dose	Orion Pharma	Reservoir	Salbutamol sulphate Beclomethasone dipropionate
Eclipse®	Multi-dose	Aventis	Capsule	Sodium cromoglycate
Novolizer®	Multi-dose	ASTA	Reservoir	Budesonide
Twisthaler®	Multi-dose	Schering-Plough	Reservoir	Mometasone furoate

Table 4: Future/next generation DPIs (approved or in development stage)

Device	DPI type	Company	Delivery method	Drug(s)
Aspirair	Multi-dose	Vectura	Powder/Active	Apomorphine hydrochloride
MicroDose	Multi-unit	MicroDose/ 3M	Electronic activated	Insulin
Conix One	Single dose	Cambridge Consultant	Foil seal	Vaccines
Cyclovent	Multi-dose	Pharmachemie	Reservoir	Morphine
Spiros	Multi-unit	Dura	Blister/Active	Albuterol sulphate

CHAPTER – 2

LITERATURE REVIEW

CHAPTER 2 LITERATURE REVIEW

The Dry powder inhaler (DPI) was introduced to treat respiratory diseases many years ago. From the last few decades so many researches are going on for the development of zero defect DPIs. Some of the works are mentioned below.

Aquino RP et al (2012) developed gentamicin dry powder inhaler by using leucine and evaluated formulation parameters aerosol performance and invitro toxicity on Cufi1 cells. Aerodynamic properties were analyzed both by Single Stage Glass Impinger and Andersen Cascade Impactor. Moreover, the potential cytotoxicity on bronchial epithelial cells bearing a CFTR F508/F508 mutant genotype (CuFi1) was tested. Results indicated that leucine may improve the aerosol performance of G-dried powders. The maximum fine particle fraction (FPF) of about 58.3% was obtained when water/isopropyl alcohol 7:3 system and 15-20% (w/w) of leucine were used, compared to a FPF value of 13.4% for neat G-dried powders. [61]

Somchai sawatdee et al (2006) developed isoniazid as dry powder aerosol for delivery to the lower airways and studied the susceptibility of M.bovis and M. tuberculosis to the formulations. Isoniazid was formulated with trehalose, mannose and lactose by physical mixing and spray drying techniques. All formulations were evaluated for delivery efficiency and stability. Susceptibility tests of Mycobacterium species to the drug formulations were carried out. Isoniazid mixed with fine trehalose, micronised mannose or fine lactose produced the formulations which gave fine particle fraction (< 5 µm) of over 60%.The different size of carrier particles strongly affects the deposition of isoniazid to the lower airways in vitro. The content of all isoniazid dry powder formulations was almost 100%of the theoretical content and found to be stable over 3 months storage. [62]

Flore Depreter et al (2010) developed highly dispersible and dry formulations of insulin for use in dry powder inhalers (DPIs) using high-pressure homogenisation (HPH) and spray-drying. Several formulations were evaluated, including formulations spray-dried without excipients and formulations coated with lip-ids. A physiological lipid composition based on a mixture of cholesterol and phospholipids was used to form the coating film around micronized drug particles. The resulting powders exhibited a size and shape suitable for the deep lung deposition of drugs, and good aerodynamic features were obtained for the different formulations tested, with fine particle frac-tions between 46% and 63% vs. 11% for raw insulin powder. The presence of a lipid coating of up to 30% (w/w) did not significantly affect the aerodynamic behaviour, and the coated formulations also exhibited decreased residual moisture content of between 2.3% and 3.7% vs. 4.8% for raw insulin, which should improve the long-term stability of the protein formulations. No degradation of the insulin mole-cule occurred during the HPH/spray-drying process, as it was shown using an HPLC method (insulin con-tent between 98.4% and 100.5%). [63]

Bhavna et al (2009) developed nano sulbutamol dry powder inhaler. Nanoparticle DPI is known to have deeper lung penetration compared to micronized DPI. The objective of this study was to make nano-salbutamol sul-phate (SBS) DPI, radiolabel it with Tc-99m using a novel surface labeling methodology, characterize the formulation and assess its in vitro and in vivo deposition in healthy human volunteers to estimate its bio-availability in the target area. Nano-SBS with a mean particle of 60.71 ± 35.99 nm was produced using liquid anti-solvent precipitation method. They have found drug particles were spherical, pure and crystalline. Ander-son cascade impaction showed that blend formulations of Nano-SBS exhibited significantly higher respi-rable fraction of 45.2% compared to the known behaviour of micronized

salbutamol sulphate blends.Nano-SBS formulations showed nearly 2.3-fold increase in total lung deposition compared to micronized SBS. The in vivo deposition data and the ratio of peripheral to central lung deposition (P/C) of 1.12 ± 0.4 indicate that Nano-SBS is evenly distributed within different lung regions. As demonstrated for SBS, nano-sizing may enhance regional deposition and thus provide an attractive particle engineering option for the development of blend formulations for inhalation delivery. [64]

Srichana T et al (2001) investigated cyclodextrin as a potential potential drug carrier for dry powder inhaler formulation. Two types of cyclodextrin were chosen; gamma cyclodextrin (GCD) and dimethyl-beta-cyclodextrin (DMCD) as carriers in dry powder formulations. Salbutamol was used as a model drug and a control formulation containing lactose and the drug was included. A twin-stage impinger (TSI) was used to evaluate in delivery efficiency of those dry powder formulations. The toxicity of cyclodextrin complexes was investigated in the rat by monitoring blood urea nitrogen (BUN) and urinary creatinine, as well as determining haemolysis of human red blood cells. They have found that GCD and DMCD are able to promote salbutamol delivery in dry powder inhaler compared to a formulation containing lactose. In addition, GCD is relatively safe in the rat if the amount of GCD in the formulation is similar to this experiment. [65]

Yang Y et al (2012) developed dry powder inhaler (DPI) formulation of hybrid nanoparticles composed of poly(lactic-co-glycolic acid) and soybean lecithin as the polymer and lipid constituents, respectively. The hybrid nanoparticles are transformed into inhalable microscale nanocomposite structures by a novel technique based on electrostatically-driven adsorption of nanoparticles onto polysaccharide carrier

particles, which eliminates the drawbacks of conventional techniques based on controlled drying (e.g. nanoparticle-specific formulation, low yield). First, they engineer polysaccharide carrier particles made up of chitosan cross-linked with tripolyphosphate and dextran sulphate to exhibit the desired aerosolization characteristics and physical robustness. Second, investigated the effects of nanoparticle to carrier mass ratio and salt inclusion on the adsorption efficiency, in terms of the nanoparticle loading and yield, from which the optimal formulation is determined. Desorption of the nanoparticles from the carrier particles in phosphate buffer saline is also examined. Lastly,they characterized aerosolization efficiency of the nanocomposite product in vitro, where the emitted dose and respirable fraction are found to be comparable to the values of conventional DPI formulations.[66]

Selvam P et al (2012) developed budesonide nanocluster dry powder inhaler.The resulting budesonide NanoCluster powders exhibited a large emitted fraction and a high fine particle fraction (FPF) from a Monodose® dry powder inhaler. In this work,excipients were added premilling or post milling and the performance of budesonide NanoCluster dry powders was investigated. Sodium chloride, Pluronic®, or ethanol was added prior to milling due to their ability to modify surface tension or ionic strength and thereby affect the attrition/agglomeration process. Lactose or l-leucine was added after milling because these are known to modify powder flow and dispersion. The chemical stability of budesonide was maintained in all cases, but the physical aerosol properties changed substantially with the addition of excipients. In all cases, they have found that the addition of excipients led to an increase in the size of the budesonide NanoClusters and tended to reduce the emitted fraction and FPF. [67]

Sultana S et al (2012) developed alendronate nanoparticles as a dry powder inhaler for the treatment of osteoporosis.The precipitation technique was used to prepare non-polymeric alendronate nanoparticles. The influence of various formulation parameters on the average particle size was investigated and the effect of various stabilizers (PVA, tween, chitosan, alginate, PEG, HPMC, poloxomers) was evaluated. The selection of surfactant was a key factor to produce particles with desired properties. Poloxomer F68 was found best in achieving the minimum particle size and providing physical stability to the drug. On basis of preliminary trials, a central composite design was employed to study the effect of independent variables, drug concentration (X_1), antisolvent volume (X_2), stirring speed (X_3), and stabilizer concentration (X_4) on the average particle size. The drug and stabilizer concentrations exhibited a more significant effect on a dependent variable. The particle size varied from 62 to 803.3 nm depending upon the significant terms. The validation of optimization study, performed using six confirmatory runs, indicated very high degree of prognostic ability of response surface methodology, with mean percentage error (±SD) as -2.32 ± 2.47. The minimum particle size (44.11 nm) was predicted at 10 mg/ml drug concentration, 20 ml antisolvent volume, 925 rpm stirring speed, and 8.5% stabilizer concentration with 98.16% experimental validity. Respirable fraction for optimized nanosized alendronate (43.85% ± 0.52%) was significantly higher when compared with commercial alendronate (17.6 ± 0.32). [68]

Nutan shah et al (2011) developed sustained release dry powder inhaler , the spray-dried combination powders showing the sustained release of two chemically distinct therapeutic agents. Spray dried formulations were produced from 70% v/v aqueous ethanol formulations containing salmeterol xinafoate, fluticasone propionate, leucine

as a surfactant and HPMC as a drug release modifier. The aim of this work is to use leucine-HPMC combination as an excipient to impart sustained release profiles to drugs. The influence of leucine-HPMC combination as an excipient on spray drying thermal efficiency and drug release profile was investigated. Also, resultant powders were subjected to physical characterization. Sustained drug release profiles were observed in dissolution tests for both agents; increased HPMC concentration associated with increased duration of drug release. The controlled co-delivery of long acting β-2 agonist and glucocorticoid molecules underlines the capability of spray drying to produce respirable combination particles with sustained release for pulmonary delivery. [69]

S.P.Shah et al (2004) developed liposomal Amphotericin B dry powder inhaler and optimized for treatment of invasive lung fungal infection.Liposomes were prepared by reverse phase evaporation technique using ethyl acetate and ethanol(1:1) as organic solvents. Drug lipid ratio was 1:10 with membrane composition of hydrogenated soyaphosphatidylcholine; cholesterol and either saturated soyaphosphatidylglycerol (7:3:0.5) or stearylamine (1:1:0.1) was used to prepare negatively (AMB1) and positively (AMB2) charged liposomes, respectively. Liposomes were extruded through 2 µm polycarbonate membrane, separated from unentrapped drug and subjected to lyophilization using Tris buffer containing cryoprotectants in various mass ratios. Sucrose was found to be the best cryoprotectant for liposomal AMB in a mass ratio of lipid: sucrose at 1:5 for AMB1 and AMB2, respectively. Sorbolac 400 and sieved Pharmatose 325 M (500#) in (liposome: carrier ratio to be 1:6) resulted in 22.6 ± 2.2% and 16.8 ± 2.2% FPF for AMB1 and AMB2, respectively. [70]

***Traini D. et al (2012*)** prepared dry powder inhaler of two micronized drugs like salbutamol sulphate and baclomethasone Five different blends of SS:BDP ratios of 0:100, 25:75, 50:50, 75:25, and 100:0 (w/w) were prepared. Aerosolization performance was evaluated using a multistage impinger and a Rotahaler® device. Results: The median SS particle diameter was larger than BDP (4.33 ± 0.37 µm compared to 2.99 ± 0.15 µm, respectively). The SS appeared to have a ribbon-like morphology, while BDP particles had plate-like shape with higher cohesion than SS. This was reflected in the aerosolization performance of the two drugs alone, where SS had a significantly higher fine particle fraction (FPF) than BDP (12.3%, 3.1% and 2.9%, 0.2%, respectively). The study of cohesion versus adhesion for a series of SS and BDP probes on SS and BDP substrates suggested both to be moderately adhesive.The FPF of the two drugs from the binary blends, at all three ratios, were similar.Such observations indicate that when these two drugs are formulated as a binary system, the resulting powder structure is altered and the aerosolization performance of each drug is not reflective of the individual drug performance. Such factors could have important implications and should be considered when developing combination dry powder inhalation systems. [71]

Martin GP et al (2012) evaluated aerodynamic deposition of combination dry powder inhaler by using three deferent imapactor. The aerodynamic deposition of the combination dry powder inhaler (DPI) Seretide Accuhaler, which contains salmeterol xinafoate (SX) and fluticasone propionate (FP), was assessed using the Andersen cascade impactor (ACI), multi-stage liquid impinger (MSLI) and next generation impactor (NGI) and the results were compared. Significant differences in the particle size distributions were observed when the same formulation was tested using various

impactors. The ACI was found to be less suitable for DPI testing at flow rates considerably higher than 28.3Lmin (-1) due to the significant overlap in the cut-off curves of the pre-separator and stage 0. This was not the case with the MSLI but the data derived were limited by the relatively small number of stages. Deposition data determined by the three impactors were significantly different. The NGI produced good resolution and minimal inter-stage overlap and was regarded as the impactor of choice for DPI testing. [72]

Price R et al (2009) investigated the engineering of inhalation particles incorporating, in each individual particle, a combination of a long-acting beta-agonist and a glucocorticosteroid in a pre-determined and constant ratio for delivery via a dry powder inhaler (DPI).Individual crystalline particles containing both the glucocorticosteroid fluticasone propionate (FP) and long-acting beta-agonist salmeterol (SX) were prepared, in a ratio of 10:1, using the solution atomization and crystallization by sonication (SAX) process. Combination drug particles were characterized by particle size, morphology, crystallinity and aerosolisation efficiency using inertial impaction.Combination drug particles were spherical and crystalline, with a median diameter of 4.68 +/- 0.01 micron. Aerosolisation of formulations containing combination drug particles resulted in greater uniformity in delivery ratios of both actives across all stages of the impactor before and after storage. [73]

Misra A et al (2009) developed insulin dry powder inhaler with deferent grade of lactose. The powder was incorporated with lactose of different grades and their combinations as carriers to deliver using an inhaler device. Solid-state characteristics of the carrier as well as the drug powder were assessed by particle size and distribution measurement. The flow properties such as moisture content, powder

density, angle of repose, and carr's compressibility index of the powder mixture were determined. The aerosol behaviour of the powder was studied by dispersion using rotahaler(c) connected to a twin-stage impinger (TSI) and an eight-stage Andersen cascade impactor (ACI) operating at different flow rates of 30-90 l/min. The in vivo performance was studied by deliverance to the respiratory tract of guinea pigs. The coarser particles of lactose in fractions of carrier containing a wide particle size distribution impacted in the preseperator of cascade impactor, and only the particle less than 10 microm size entered stage 0-stage 7. Formulation containing 1:1 mixture of Respitose ML006 (62 %< 50 microm) and Respitose ML003 (37.8 %< 50 microm) as carrier imparts well deaggregation of insulin, and higher deposition leads to 52.3% of fine particle fraction at 60 Lit/min and in vivo bioavailability of 82%. Hence, it was concluded that the availability of insulin for systemic absorption depends on the particle size of the drug as well as the carrier lactose. [74]

CHAPTER – 3

RATIONALE

CHAPTER 3 RATIONALE

3.1.ASTHAMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Asthma is a chronic inflammatory disease of the airways characterised by reversible airway obstruction associated with exacerbations of coughing, wheezing, chest tightness, difficult breathing, and airway hyper responsiveness. Asthma is predominantly a disease of young individuals. Asthma has a high incidence. It is estimated, that over 100 million people worldwide have asthma. Epidemiological studies in The Netherlands have revealed that 10 -20% of the general population report respiratory symptoms like wheezing and shortness of breath that could indicate the disease asthma and Chronic Obstructive Pulmonary Disease (COPD), which refers primarily to chronic bronchitis and emphysema, is characterised by chronic airway obstruction causing progressive loss of lung function. COPD is associated with symptoms of chronic cough and purulent sputum COPD is strongly related to a history of smoking and is predominantly a disease of older patients. Asthma differs from COPD in that there is a greater reversibility, spontaneously and after treatment with bronchodilators or corticosteroids. Some patients with asthma have progressive irreversible airway obstruction and therefore have a form of COPD, and some patients may have coexistent asthma and COPD.The term Chronic Obstructive Lung Disease (COLD) is used as definition for combined asthma and COPD.The goal of respiratory therapy is to improve the patient's quality of live by achieving and maintaining control of symptoms, preventing exacerbations, attaining normal lung function, maintaining normal activity levels, including exercise, and avoiding adverse effects from respiratory medication. [75-76]

3.2 DRUGS COMMONLY USED TO TREAT ASTHMA AND COPD

Drugs used to treat asthma are bronchodilators and are classified as β2 adrenoreceptor agonists, Xanthines, Muscarinic receptor antagonists and Corticosteroids.

(I) β2 adrenoreceptor agonists dilate the bronchi by a direct action on β2 adrenoreceptors on the smooth muscle. Two categories of β2 adrenoreceptor agonists are used in asthma, Short acting agents which have onset of action within 30 minutes and duration of action lasts for 4-6 hours. e.g. Salbutamol and Terbutaline. Long acting agents have the duration of action for 12 hours. E.g. Salmeterol.

(II) The three naturally occurring pharmacologically active Xanthine drugs used in the treatment of asthma are Theophylline, Theobromine and Caffeine.

(III) The muscarinic receptor antagonists widely used as an anti-asthmatic drug is Ipratropium,tiotropium bromide.

(IV) Other classes of drugs which can be used for the treatment of asthma are anti-inflammatory drugs and corticosteroids such as Fluticasone, Budesonide and Beclomethasone. These inhaled corticosteroids reduce swelling and tightening in the airways. However β2 adrenoreceptor agonists form the first line drugs of choice for the treatment of asthma. [77-78]

3.3 NEED FOR STUDY

The confirmation of destruction, an international agreement was made to ban the use of CFCs by 2005.After the ban, development of alternate replacement propellants became necessary, and subsequently hydrofluoroalkanes (HFAs) came into existence. Large safety studies conducted with animals and humans showed that the new HFA propellants (specifically HFA-134a and HFA-227) were as safe as or safer than the

CFCs. However, HFAs presented new technical challenges (e.g. the elastomeric materials in the metering devices and some surfactants are not compatible with HFAs.) which needed to be resolved before receiving marketing authorization. These are the reasons which are responsible for limiting the use and development of pMDI, and eventually leading towards development of alternate inhalation systems, such as Dry Powder Inhalers. [79] Combination drugs are chosen for development of dry powder inhaler for this project. Combination medications contain both an inhaled long-acting bronchodilator (LABA) and an inhaled corticosteroid. This means that two areas of asthma can be effectively treated at the same time: (1) the bronchodilator works by widening your airways, making it easier for you to breathe, and (2) the inhaled steroid reduces and prevents inflammation of your airways. [80]

The combination of LABA and ICS should be considered when: [81]

- ✓ Symptoms or sub-optimal lung function persist on ICS alone.
- ✓ It is desirable to reduce the current dose of ICS while maintaining optimal asthma control.
- ✓ Initiating asthma treatment in a patient with moderate to-severe asthma in whom rapid symptom improvement is needed.

CHAPTER – 4

AIM AND OBJECTIVE

CHAPTER 4 AIM AND OBJECTIVE

4.1. AIM

- Formulation of dry powder inhaler containing corticosteroids and beta 2 agonist.
- Evaluation of prepared dry powder inhaler.

4.2. OBJECTIVE

Objective of this present study are

- To develop a dry powder inhaler with combination of two APIs, Formoterol fumarate and Mometasone furoate , using different grade of coarse and fine lactose monohydrate and to evaluate them for better in vitro deposition.
- Use of different grades of lactose monohydrates like Lactohale 200, 210,300, Sorbolac 400, Respitose SV010, Respitose ML001, Respitose ML006,Pharmatose 100M, and of evaluation their compatibility study .
- Formulations and characterization of the DPIs.
- Stability study of optimized formulation.

CHAPTER – 5

PLAN OF WORK

CHAPTER 5 PLAN OF WORK

PLAN OF WORK

Stage 1

- ✓ Literature review
  - ➢ Product
  - ➢ API
  - ➢ Excipient
- ✓ Selection of drug and excipient.
- ✓ Characterization APIs and excipient.

Stage 2

- ✓ Designing of prototype type formulation with different grades of lactose.
- ✓ Preparation of dry power inhaler formulation containing selected combination drug with different grade of lactose.
- ✓ Felling of dry powder blend in hard gelatin capsule (size 3) by hand filling capsule machine.

Stage 3

- ❖ Evaluation of prepared dry powder inhaler containing combination drugs

✓ Evaluation of physical parameter of prepared Dry powder inhaler(DPI)

- ➢ Locking length of the capsule
- ➢ Average net content
- ➢ Uniformity of weight
- ➢ Device performance

- ❖ Evaluation of chemical parameter of prepared dry powder inhaler
- ➢ Uniformity of content
- ➢ Uniformity of the delivered dose by Dose uniformity sampling apparatus.
- ➢ *In vitro* deposition by Twin stage liquid impinger.
- ➢ Assay
- ➢ Water content by Karlfisher titration method

Stage 4

- ➢ Stability studies of optimized formulation
    - ➢ Physical appearance
    - ➢ Device performance
    - ➢ Water content by Karlfisher method
    - ➢ Assay
    - ➢ *In vitro* deposition by Twin stage liquid impinger.

CHAPTER – 6

DRUG & EXCEIPIENTPROFILE

6.1 DRUG PROFILE [77, 82, 83, 84, 96]

6.1.1 Formoterol fumarate (LABA)

Description: white powder, slightly yellow powder

BCS classification: Class III

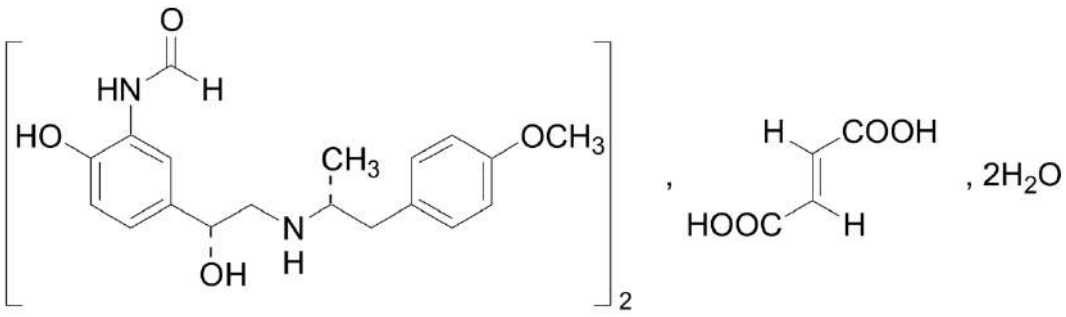
Category: Adrigenic beta agonist, Bronchodilator.

Chemical formula: $(C_{19}H_{24}N_2O_4)_2$, $C_4H_4O_4$, $2H_2O$

Molecular weight: 840.91

Melting point: 138-140°C

Chemical structure:



Chemical name: N-[2-hydroxy-5-(1-hydroxy-2-{[1-(4-methoxyphenyl)propan-2 yl]amino}ethyl)phenyl]formamide.

Protein binding: 61% to 64%

LogP : 2.2

Half life : 12 hours

6.1.1. 1. Pharmacodynamics:

LABA is a long-acting selective beta2-adrenergic receptor agonist (beta2-agonist). Inhaled LABA acts locally in the lung as a bronchodilator. In vitro studies have shown that formoterol has more than 200-fold greater agonist activity at beta2-receptors than at beta1- receptors. Although beta2-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta1-receptors are the predominant receptors in the heart, there are also beta2-receptors in the human heart comprising 10%-50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta2- agonists may have cardiac effects.

6.1.1.2. Mechanism of action

The pharmacologic effects of beta2-adrenoceptor agonist drugs, including LABA, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibit the release of pro-inflammatory mast-cell mediators such as histamine and leukotrienes. LABA also inhibits histamine-induced plasma albumin extravasation in anesthetized guinea pigs and inhibits allergen-induced eosinophil influx in dogs with airway hyper-responsiveness.

6.1.1.3. Absorption

Rapidly absorbed into plasma following administration by oral inhalation. It is likely that the majority of the inhaled LABA delivered is swallowed and then absorbed from the gastrointestinal tract.

6.1.1.4. Metabolism

Metabolized primarily by direct glucuronidation at both the phenolic or aliphatic hydroxyl group and O-demethylation followed by glucuronide conjugation at either phenolic hydroxyl groups. Minor pathways involve sulfate conjugation of Formoterol fumarate and deformylation followed by sulfate conjugation. The most prominent pathway involves direct conjugation at the phenolic hydroxyl group. The second major pathway involves O-demethylation followed by conjugation at the phenolic 2'-hydroxyl group. Four cytochrome P450 isozymes (CYP2D6, CYP2C19, CYP2C9 and CYP2A6) are involved in the O-demethylation of Formoterol fumarate

6.1.1.4. Route of elimination

Eliminated in the urine (59% to 62%) and feces (32% to 34%). About 10% is excreted unchanged in the urine, and about 15% to 18% is excreted in the urine as conjugates

6.1.1.5. Indications and Usage

For use as long-term maintenance treatment of asthma in patients 6 years of age and older with reversible obstructive airways disease, including patients with symptoms of nocturnal asthma, who are using optimal corticosteroid treatment and experiencing regular or frequent breakthrough symptoms requiring use of a short-acting bronchodilator. Not indicated for asthma that can be successfully managed with occasional use of an inhaled, short-acting beta2-adrenergic agonist. Also used for the prevention of exercise-induced bronchospasm, as well as long-term treatment of bronchospasm associated with COPD.

6.1.1.6.Adverse –effects

Tremor, dizziness, insomnia, dysphonia, headache, nervousness, Hypertension, tachycardia, chest pain, Nausea, dyspepsia, abdominal pain, irritation of the throat and mouth, Bronchitis, respiratory infection, dyspnea, tonsillitis, Viral infection (most likely in children)

6.1.2 Mometasne Furoate (ICS)

Description: white or almost white powder.

BCS classification: Class II

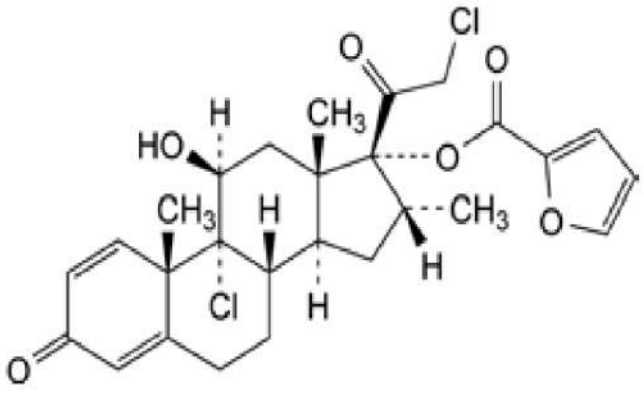
Category: Anti-inflammatory, Anti-allergic agent

Molecular weight: 521.4

Melting point: 218-220

Chemical formula: $C_{27}H_{30}Cl_2O_6$

Chemical structure:



Chemical name: 9, 21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate

Protein binding: 98-99 %

LogP : 2.1

Half life : 5.8 hours

6.1.2.1. Pharmacodynamics:

ICS is a medium-potency synthetic corticosteroid with antiinflammatory, antipruritic, and vasoconstrictive properties. Studies in asthmatic patients have demonstrated that ICS provides a favorable ratio of topical to systemic activity due to its primary local effect along with the extensive hepatic metabolism and the lack of active metabolites. Though effective for the treatment of asthma, glucocorticoids do not affect asthma symptoms immediately. Maximum improvement in symptoms following inhaled administration of ICS may not be achieved for 1 to 2 weeks or longer after starting treatment. When glucocorticoids are discontinued, asthma stability may persist for several days or longer. ICS has been shown in vitro to exhibit a binding affinity for the human glucocorticoid receptor which is approximately 12 times that of dexamethasone, 7 times that of triamcinolone acetonide, 5 times that of budesonide, and 1.5 times that of fluticasone. The clinical significance of these findings is unknown.

6.1.2 .2.Mechanism of action

Unbound corticosteroids cross cell membranes and bind with high affinity to specific cytoplasmic receptors. Inflammation is decreased by diminishing the release of leukocytic acid hydrolases, prevention of macrophage accumulation at inflamed sites,

interference with leukocyte adhesion to the capillary wall, reduction of capillary membrane permeability, reduction of complement components, inhibition of histamine and kinin release, and interference with the formation of scar tissue. The anti-inflammatory actions of corticosteroids are thought to involve phospholipase A_2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes. ICS has been shown in vitro to exhibit a binding affinity for the human glucocorticoid receptor which is approximately 12 times that of dexamethasone, 7 times that of triamcinolone acetonide, 5 times that of budesonide, and 1.5 times that of fluticasone.

6.1.2 .3.Absorption

Rapidly absorbed into plasma following administration by oral inhalation. It is likely that the majority of the inhaled LABA delivered is swallowed and then absorbed from the gastrointestinal tract.

6.1.2 .4.Metabolism

Hepatic. Extensive metabolism to multiple metabolites. There are no major metabolites detectable in plasma. Upon in vitro incubation, one of the minor metabolites formed is 6ß-hydroxy-ICS. In human liver microsomes, the formation of the metabolite is regulated by cytochrome P-450 3A4.

6.1.2.5.Route of elimination

Eliminated in the urine (59% to 62%) and feces (32% to 34%). About 10% is excreted unchanged in the urine, and about 15% to 18% is excreted in the urine as conjugates

6.1.2 .6. Indications and Usage

The inhaler is indicated for the maintenance treatment of asthma as prophylactic therapy. The nasal spray is indicated for the treatment of the nasal symptoms of seasonal allergic and perennial allergic rhinitis

6.1.2.7. Adverse –effects

Adults and children ≥12 years of age previously receiving bronchodilators and/or inhaled corticosteroids: Headache,allergic rhinitis, pharyngitis,upper respiratory tract infection,sinusitis, oral candidiasis, dysmenorrhea, musculoskeletal pain, back pain,dyspepsia. Adults and children ≥12 years of age previously receiving oral corticosteroids: Musculoskeletal pain, oral candidiasis, sinusitis, allergic rhinitis, upper respiratory infection, arthralgia, fatigue, depression, sinus congestion. Children 4–11 years of age previously receiving bronchodilators and/or inhaled corticosteroids: Fever, headache, allergic rhinitis, pharyngitis, upper respiratory tract infection, abdominal pain.

6.2 EXCEPIENT PROFILE [85, 86]

6.2.1 Lactose monohydrate

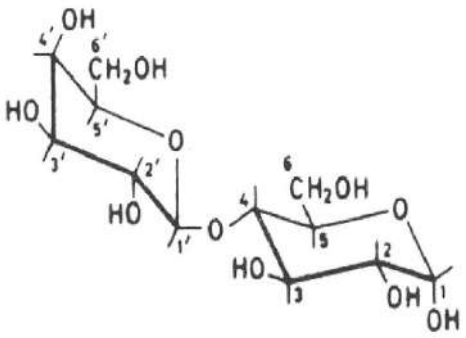
Description: In the solid state, lactose appears as various isomeric forms, depending on the crystallization and drying conditions, i.e. a lactose monohydrate, b-lactose anhydrous, and a-lactose anhydrous. The stable crystalline forms of lactose are a-lactose monohydrate, b-lactose anhydrous and stable a-lactose anhydrous.

Lactose occurs as white to off-white crystalline particles or powder. Lactose is odourless and slightly sweet-tasting; a-lactose is approximately 20% as sweet as sucrose, while b-lactose is 40% as sweet.

Category: Dry powder inhaler carrier; lyophilization aid; tablet binder; tablet and capsule diluent; tablet and capsule filler.

Chemical formula : $C_{12}H_{22}O_{11}.H_2O$

Chemical structure:



Chemical name: O-b-D-Galactopyranosyl-(14)-a-D-glucopyranose monohydrate

Molecular weight: 360.31

6.2.1.1.Typical properties

Density (bulk): 0.36-0.81 g/cm^3

Density (tapped): 0.54-1.02 g/cm^3

Loss on drying: Typically 0.2% for Monohydrate 80M, Monohydrate Impalpable; and 0.1–0.2% for Meggle products.

Melting point: 201–202^0 C

Solubility: Practically insoluble in Chloroform, Ethanol, Ether and freely soluble in water

6.2.1 .2.Stability and Storage Conditions

Mould growth may occur under humid conditions (80% relative humidity and above). Lactose may develop a brown coloration on storage, the reaction being accelerated by warm, damp conditions. Lactose should be stored in a well-closed container in a cool, dry place

6.2.1 .3.Incompatibilities

A Maillard-type condensation reaction is likely to occur between lactose and compounds with a primary amine group to form brown, or yellow-brown-coloured products. Lactose is also incompatible with amino acids, amphetamines and lisinopril

6.2.2.4. Different grade of commercial grade lactose

(i) Lactohale 200

Lactohale® 200 is milled lactose which enables the mean particle size to be tightly controlled. Milled Lactose that has a degree of cohesion, Irregular shaped particles with various amount of fine particles. The fine content can also be controlled by removing the fines by air classifying and then blending fines back in with the coarse to meet a particular target.

Table 5: Particle size distribution of lactohale 200

Particle size by Sympatec laser diffraction	
D10	5-15 µm
D50	50-100 µm
D90	120-160 µm

(ii).Lactohale® 300

Micronized lactose for use as a fine fraction or for producing agglomerates with drug substance, highly cohesive material. Micronized lactose with a D50 below 5 microns.

Lactohale® 300 is used to develop customer specific lactose blends and for agglomerates of drug and microfine lactose particles.

Table 6: Particle size distribution of lactohale 300

Particle size by Malvern laser diffraction	
D10	-----
D50	<5µm
D90	≤ 10µm

(iii). Lactohale 210

Lactohale 210 is a milled lactose, having particle size D 90 of 50µm.

(iv). Respitose® ML001

Respitose® ML001 is milled inhalation grade lactose with a relatively broad particle size distribution.Particle size by Sympatec laser diffraction

Table 7: Particle size distribution of Respitose ML001

Particle size by Sympatec laser diffraction	
D10	4µm
D50	55 µm
D90	170 µm

(v). Respitose® ML006

Respitose® ML006 is fine milled inhalation grade lactose with a narrow particle size distribution.Particle size by Sympatec laser diffraction

Table 8: Particle size distribution of Respitose ML006

Particle size by Sympatec laser diffraction	
D10	2 µm
D50	17 µm
D90	45 µm

(vi). Respitose® SV010

Respitose® SV010 is coarse sieved inhalation grade lactose with a relatively broad particle size distribution.

Table 9: Particle size distribution of Respitose SV010

Particle size by Sympatec laser diffraction	
D10	50 µm
D50	105 µm
D90	175 µm

(vii) Sorbolac 400

Sorbolac 400 is milled lactose having narrow distribution of the particle sizes, high storage stability; variation from batch to batch is minimal, good miscibility.

Table 10: Particle size distribution of Sorbolac 400

Particle size by Sympatec laser diffraction.	
D95	<45µm
D99.5	<63µm

(vii).Pharmatose 100M

Pharmatose 100M is sieved lactose having relatively narrow size distribution

Table 11: Particle size distribution of Pharmatose 100M

Particle size by Sympatec laser diffraction.	
D10	>63µm
D50	>150µm
D90	> 250µm

CHAPTER – 7

INSTRUMENTATION

CHAPTER 7 INSTRUMENTATION

7.1 MALVERN AND SYMPATEC

Knowledge of the particle size distribution (PSD) of raw ingredients can be very important in pharmaceutical formulation, particularly for quality control to ensure batch-to-batch reproducibility. For this study, its primary purpose was to determine the PSDs of the micronized drugs in ensuring their suitability for inhalation formulations. Several techniques are available for particle sizing: sieving, examination by various forms of microscopy, time of flight analysis, impaction, and low angle laser light scattering (LALLS). During the laser diffraction measurement, particles are passed through a focused laser beam. These particles scatter light at an angle that is inversely proportional to their size. The angular intensity of the scattered light is then measured by a series of photosensitive detectors. Laser diffraction particle size analyzers calculate particle size from the angle of light scattered by a stream of particles passing through a laser beam. The size limits and sensitivity of a laser diffraction particle analyzer depend on the number and placement of detectors in the instrument.The volume particle size distribution was measured with a Malvern Mastersizer 2000 and Sympa-TEC particle size analyser. (Malvern, Worcestershire, U.K.) equipped with dry and wet sampling systems (Scirocco 2000 and Hydro 2000 S, respectively), the latter being used for measurements. A 2 mW 632.8 nm Helium Neon laser, along with a shorter wavelength 466 nm blue light source for improved fine particle resolution, was used. Detection limits for the equipment range from 0.02-2000 µm and 0.25 to 3500 µm for Sympa-TEC particle size analyser (RODOS). [88, 89, 90]

Figure 11: Malvern Mastersizer 2000

Figure 12: Sympatec (Rodos)

7.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC (or just LC), is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture. The basic operating principle of HPLC is to force the analyte through column of the stationary phase (usually a tube packed with small spherical particles with a certain surface chemistry by pumping a liquid (mobile phase) at high pressure through the column .The use of pressure increases the linear velocity (speed) giving the components less time to diffuse within the column, leading to improve resolution in the resulting chromatogram. [91]

---Isocratic and gradient elution.

- ❖ Isocratic elution

--- Eluent composition remains constant

--- Single solvent or single solvent mixture

- ❖ Gradient elution:

--- Eluent composition (and strength) changed

--- Increases separation efficiency

--- Decreases retention time

--- Peak shape is improved (Less tailing)

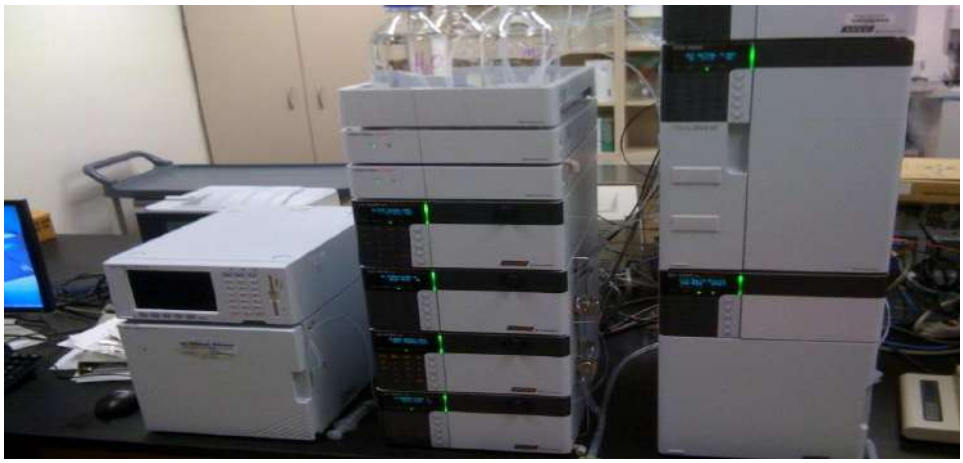


Figure 13: Simadzu HPLC system

7.2 DEVICE

The dry powder inhaler devices used were medium resistance, single dose capsule device Myhaler device is a mouthpiece inhaler instrument. It is a two-piece device made up of plastic. It is a breath-activated device that can be used by patients suffering from asthma. This device is very simple to use and also very cost effective. Machaler also single dose system with piercing type device. That uses two metal pins to puncture the ends of a size 3 capsule. [92] The device is shown in figure 14 & 15

Figure 14: MyHaler

Figure 15: MacHaler

7.4 KARL FISCHER TITRATOR

The Water Determination Test (Karl Fischer Method) is designed to determine water content in substances, utilizing the quantitative reaction of water with iodine and sulfur dioxide in the presence of a lower alcohol such as methanol and an organic base such as pyridine. There are two determination methods different in iodine-providing principle: the volumetric titration method and the coulometric titration method. In the volumetric titration method, iodine required for reaction with water is previously dissolved in water determination TS, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water in a sample.

Figure 16: Karlfisher titrator.

In the coulometric titration method, first, iodine is produced by electrolysis of the reagent containing iodide ion, and then, the water content in a sample is determined by measuring the quantity of electricity which is required for the electrolysis (i.e., for the production of iodine), based on the quantitative reaction of the generated iodine with water. Generally, the apparatus consists of an automatic burette, a back- titration flask, a stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current. [93]

7.5 DOSE SAMPLING UNIT APPARATUS:

The delivered dose is the dose delivered from the inhaler to the patient. Dose uniformity sampling apparatus (DUSA) was used to determine the Uniformity of delivered dose from the inhalation device.

A dose collection apparatus consists of a filter-support base with an open mesh filter-support, such as a stainless steel screen. A sample collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the sample collection tube and the mouthpiece. One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler. The dose collection apparatus must be capable of quantitatively capturing the delivered dose. The filter-support base is designed to accommodate 25 mm diameter filter disks. The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter

sample tube was connected to a flow system according to the scheme specified in figure 17. [94, 96]

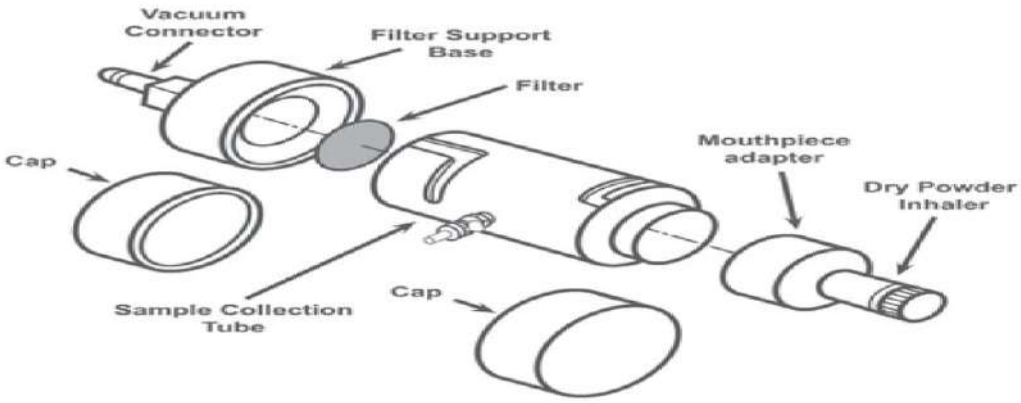


Figure 17: Dose unit sampling apparatus (DUSA)

7.6 TWIN STAGE LIQUID IMPINGER

The TSLI is the first device which was used to assess pulmonary drug delivery through inertial impaction. It has two stages and a representative throat. It first appeared in the British Pharmacopoeia and is used for the initial screening of inhalation devices and formulations. These wet stages of the impinger make it less prone to errors resulting from particle re-entrainment and they also create a humid environment similar to that in the lungs. The development is described of the twin impinger, a two-stage separation device for assessing the drug delivery from metered dose inhalers and other oral inhalation delivery devices. The discharged aerosol is fractionated by firing through a simulated oropharynx and then through an impinger stage of defined aerodynamic particle size cut-off characteristics. The fine (pulmonary) fraction which penetrates is collected by a lower impinger. It is demonstrated that this device is able to assess individually the fine particle delivery of both components of two-drug aerosols. Formulations showing undue agglomeration or serious crystal growth of drug are readily detected.

The twin impinger is shown to be a valuable device for routine quality assessment of aerosols during product development, stability testing and for quality assurance and comparison of commercial products. [94, 96, 97]

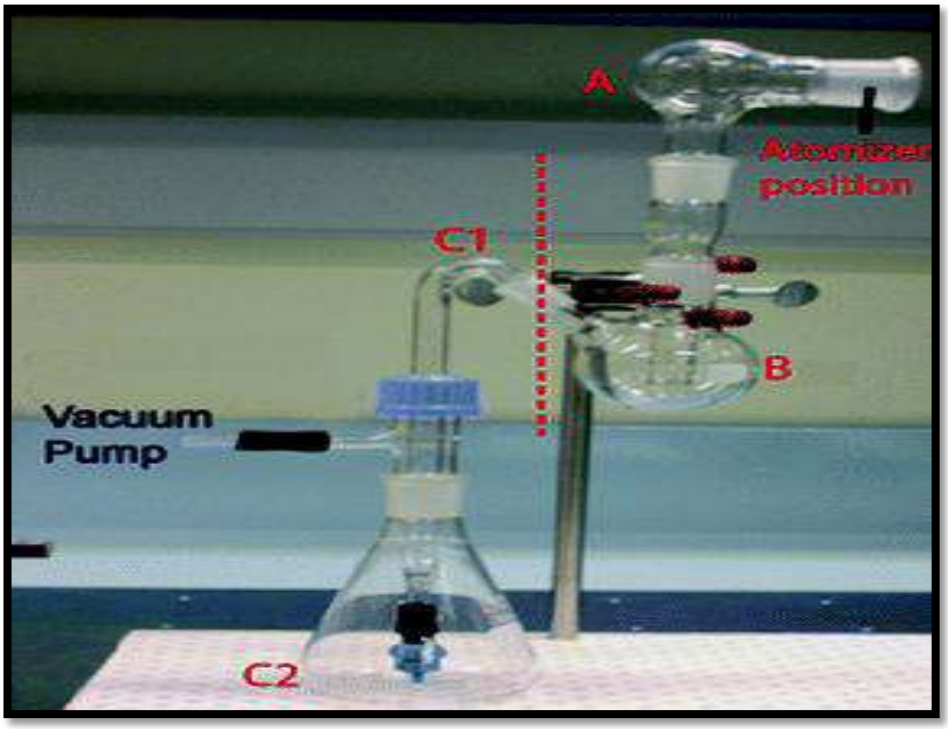


Figure 18: Twin stage liquid impinger

CHAPTER – 8

MATERIAL &METHOD

CHAPTER 8 MARERIALS AND METHOD

7.1 MATERIALS

7.1.1. Drugs, Excipients, Chemicals, Packaging materials and instrument

The materials used are shown in table 5, 6, and 7

Table 12: List of Drugs, Excipients and Chemicals

Sl.no	*Drugs*	*Source*
1	Formoterol Fumarate (LABA)(Micronized)	VAMSI LAB, Mumbai
	Mometasone Furoate (ICS) (Micronized)	SYMBIOTIC,Pune
2	*Excipients*	*Source*
	Lactose monohydrate	
	a)Lactohale 200	DFE pharma, Netherlands
	b) Lactohale 210	DFE pharma, Netherlands
	c) Lactohale 300	DFE pharma, Netherlands
	d) Sorbolac 400	Meggle Pharma,
	e) Pharmatose 100M	DFE pharma, Netherlands
	f) Respitose ML006	DFE pharma
	g) Respitose ML001	DFE pharma
	h) Respitose SV010	DFE pharma
3	*Chemicals*	*Source*
	Sodium dihydrogen orthophosphate	Merck, Mumbai
	Acetonitrile	Merck, Mumbai
	Orthophosphoric acid	Merck, Mumbai
	Water	Millipore

Table 13: Packaging material details

Sl.no	MATERIAL	SOURCE
1	Capsule size"3"	Natural capsules ltd, Bangalore
2	30 ml HDPE container	Shriji containers and clousers, Mumbai
3	7 layer wad	Shriji containers and clousers, Mumbai
4	Desiccant -silica gel	Ap silica gel,Pune

Table 14: List of Equipments /Instruments

Sl. No.	Instruments	Name & Place
1	Digital balance	Shinko Sansui, Japan
2	Electronic Digital balance	AW 120 – SHIMADZU, Japan
3	Tap density tester	Electro lab tap density tester.
6	Capsule filling machine	Crompton Greaves,11G6502
7	Myhaler and Machaler	Macleods pharmaceuticals ltd
8	HPLC	LC2010AHT,Shimadzu,Japan
9	Sympa-TEC particle size analyser	RODOSM SN 214, Germany
10	Malvern	Master sizer 200
11	FT-IR	Bruker Alpha (Model no. 10059736), Germany
12	XRD	X-part, japan
13	DSC	Perkin Almer, USA
13	Vernier calipers	Micutoyo absolute
14	DUSA	Copley scientific, UK
15	TSLI	Copley scientific, UK
16	Stability chamber	Thermolab
17	Karl Fisher apparatus	Labindia 1-077

7.2. METHODS

7.2.1. Preformulation studies:

7.2.1.1. Characterization of drugs [96]

Formoterol fumarate (LABA) and Mometasone furoate (ICS)

(i). Physical Characteristics:

The Physical appearances of the drugs were characterized and recorded using descriptive terminology.

(ii) Solubility studies:

Solubility studies of Formoterol fumarate and Mometasone furaote were performed by dissolving weight quantity of APIs in different solvent as per IP. And result of solubility study of Formoterol fumarate and Mometasone furoate are shown in table 22 and table 23 respectively..

(iii). Identification:

Identification of drugs was carried out by TLC and IR spectrophotometry. Identification of Mometasone furaote (ICS) was done by TLC and Formoterol fumarate (LABA) was done by IR spectroscopy method.

(iv). Loss on drying.

Approximately 1 gm of each APIs were weighed and kept for checking the loss on drying on a moisture sensitive balance at 105°C for 3 mins. Losses of percentage moisture content were determine for individual APIs and recorded and result are shown in table 24.

(v). Melting point determination.

Melting point of Formotrol fumarate and Mometasone furoate were determined by Capillary tube method by taking small amount of each drug in a capillary tube closed at one end. The capillary tube was placed in a melting point apparatus and the

temperature at which drug melts was recorded. This was performed thrice and an average value were noted.

(vi). Assay:

Assay of Formoterol fumarate and Mometasone furoate were determined by potentiomatricaly and U.V. method. For assay of Mometasone furoate, weighed amount (40 mg) of Mometasone furaote dissolved in 100 ml of ethanol (95%), and from that withdraw 2 ml sample and further diluted to 100 ml with ethanol (95%) and measured the absorbance at wavelength 249nm and calculated the content of Mometasone furoate taking absorbtivity 481nm and assay of Formoterol fumarate (LABA) was carried out by potentiomatricaly.For end point determination Solution of Formoterol fumarate (LABA) titrated with 0.1M percholric acid and results are show in table 25.

(vii). Particle size analysis of APIs

Particle size of Formoterol fumarate and Mometasone were determined by lasser diffraction method. A small amount (about 10 mg) of Formoterol fumarate and Mometasone furoate were dispersed separately in 10 ml of solvent and sonicated for 3 min to avoid agglomeration. Particle sizes of both drug were measured by laser diffraction (Mastersizer 2000).Sample measurement time was 10 second. The particle size distribution was calculated and represented as a volume distribution, and was also characterized by the 10th, 50th and 90th percentile of the cumulative particle undersize frequency distribution and result are shown in table 26

7.2.1.2. Charecterization of excipient

(i).Bulk density: [98]

Weighed the empty 100ml measuring cylinder and the powder sample was filled in the measuring cylinder 50 to 70 ml and calculate the weight of the sample. Then density was calculated by the following formula.

Bulk density (g/ml) = weight of sample in gms/volume occupied by the sample.

(ii) Tapped density [98]

The measuring cylinder which was used for the bulk density calculation already filled with sample was taken. Cylinder was then subjected to a fixed number of taps 500, 750 and 1250 until the powder bed had reached the constant volume. The final volume was recorded and the tap density was calculated by the following formula

Tapped density (g/ml) = weight of sample in gms / volume occupied by sample

(iii) Hausner's ratio [98]

Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula:

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

Lower Hausner ratio (<1.25) indicates better flow properties than higher ones (>1.25).

(iv) Carr's index [98]

Carr's index is a dimensionless quantity, which is also useful for predicting the flow behavior. Apparent bulk density was determined by pouring the samples into

a graduated cylinder. Tapped density was determined by placing a graduated cylinder containing a known mass of powder on a mechanical tapper apparatus (Electro lab tap density tester). Samples were tapped until no further reduction in volume of the sample was observed. Relationship between powder flow ability & % compressibility is shown in Table 15. Carr's index was calculated using the given formula**.**

Compressibility index = tapped density - bulk density / tapped density X 100

Table 15: Relationship between powder flowability and %compressibility

% Compressibility range (Carr's index)	Flow description
5-15	Excellent (free flowing granules)
16-18	Good (free flowing granules)
19-21	Fair (powdered granules)
22-35	Poor (very fluid powders)
36-40	Very Poor
>40	Extremely poor

Result of this particular test are shown in table 27

(v) Particle size analysis of lactose monohydrate.

Particle size of lactose monohydrate was done by sympatec dry method. Carefully assembled the flow cell and the lens into the main unit in the instrument. Volume dispersion cell was connected to the main unit. The instrument was switched on after feeding the required parameters with pressure of 2 bars and feed rate of 50% for proper cleaning of lenses. The back ground was measured and 1 gm of sample was added to the dry accessor and measured the sample by applying air pressure of 2 bar, feed rate of 50%, during the measurement laser obscurtion should be between 2 to

6%. The particle size distribution was calculated and represented as a volume distribution, and was also characterized by the 10th, 50th and 90th percentile of the cumulative particle undersize frequency distribution and results are shown in table 28.

7.2.1.3. Compatibility study of Drugs and Excipient.

(i) Differential scanning calorimetry (DSC) study

DSC thermograms of pure drugs, and lactose monohydrate were recorded using Perkin Elmer instrument (Perkin Elmer, Jade, USA). About 5 mg of sample was placed on the 50 µL of aluminium pan in a hermetically sealed condition. The measurement was performed in a nitrogen atmosphere (20 mL/minute) between DSC temperatures at 50 to 300 ^{o}C at a heating rate of 10 ^{o}C/minute. An empty sealed pan was used as reference.

(ii). FTIR study

The excipients were mixed individually with the pure drugs in a 1:0.1 ratio and the spectra were recorded using FTIR Bruker Alpha. The scanning range was 400-4000 cm^{-1} and the resolution was 2 cm^{-1}.

7.3. Analytical method

Standard chromatogram of pure Formoetrol fumarate and Mometasone furoate were obtained using HPLC analysis

(i). Buffer Preparation (pH 3)

Accurately 1.45 gm of sodium dihydrogen orthophosphate anhydrous was dissolved in 1000 ml of water and mixed, the pH of the solution was adjusted to 3.0±0.05 with

orthophosphoric acid and mixed, and the solution was filtered through 0.45 micrometer nylon filter.

(ii). Mobile Phase Preparation

Mobile phase A

Buffer pH 3.0

Mobile Phase B

Acetonitrile

(iii). Diluent preparation

Buffer pH 3 and acetonitrile was mixed in the ratio 65:35 v/v and degassed by sonication.

(iv). Preparation of Standard stock solution:

-Stock Solution of Formoterol fumarate: (Stock solution A)

Weighed and transferred 12 mg of Formoterol fumarate standard in 200ml volumetric flask, 150 ml of the diluent was added and sonicated to dissolve, allowed to equilibrate to room temperature and diluted to volume with diluent and mixed. Final concentration was 6 µg/ml

-Stock Solution of Mometasone Furotae: (Stock solution B)

Weighed and transferred 40 mg of Mometasone Furoate standard in 100ml volumetric flask, 70 ml of the diluent was added and sonicated to dissolve, allowed to equilibrate

to room temperature and diluted to volume with diluent and mixed. Final concentration was 400µg/ml

(v) Standard preparation of Formoterol fumarate and Mometasone furoate

Transferred 1 mL of solution A and 5 mL of solution B to 100 mL volumetric and make up the volume with diluents.

7.2.2.1. Chromatographic Conditions

Column	: Hypersil BDS C18
Column dimensions	: 250mm× 4.6mm × 5µ
Flow Rate	: 1.2 ml/min
Injection Volume	: 100µl
Mobile Phase	: Buffer pH 3.0: Acetonitrile
Column Oven Temperature	: Ambient 25°C
Sample Cooler Temperature	: Ambient 25°C
Detector	: UV
Wavelength	: 230 nm
Run Time	: 20 minutes
Pump mode	: Gradient

Table 16: Gradient Programme for HPLC

Time (min)	Mobile phase A	Mobile phase B	Comments
0 → 5	70	30	Isocratic
5 → 7	70 → 30	30-70	Linear gradient
7 → 14	30	70	Isocartic
14 → 16	30 → 70	70 → 30	Linear gradient
16 → 20	70	30	Re- equilibration

Initially, column was equilibrated with mobile phase for sufficient time until stable baseline is obtained. Injected blank in single, standard preparation in 5 replicates and peak area responses for five replicates were recorded and result are shown in table 29

7.2.3. FORMULATION DESIGN

The formulation were prepared as per formula shown in table 17, 18 and 19

Table 17: Formulation chart of dry powder for inhalation of Formoterol fumarate and Mometasone furoate using variable percentage of Lactohale 200 and 210

Formulation	Active ingredient	Dose + 10% oa mcg	Lactohale 210 (%)	Lactohale 210mg/capsule	Lactohale 200 mg/capsule
F1	LABA+ ICS	6.6 +440	0	0	24.56
F2	LABA+ ICS	6.6 +440	5	1.25	23.31
F3	LABA+ ICS	6.6 +440	7	1.75	22.81
F4	LABA+ ICS	6.6 +440	10	2.5	22.06
F5	LABA+ ICS	6.6 +440	20	5	19.56
F6	LABA+ ICS	6.6 +440	30	7.5	17.06

Table 18: Formulation chart of dry powder for inhalation of Formoterol fumarate (LABA) and Mometasone furoate (ICS) using different grades of fine lactose

formulation	Active ingredient	Dose +10 % oa mcg	Fine grade lactose	Fine grade lactose mg/capsule	Lactohale 200 mg/capsule
F6	LABA+ ICS	6.6 +440	Lactohale 210	7.5	22.81
F7	LABA+ ICS	6.6 +440	Lactohale 300	7.5	22.81
F8	LABA+ ICS	6.6 +440	Sorbolac 400	7.5	22.81
F9	LABA+ ICS	6.6 +440	Respitose ML006	7.5	22.81

Table 19: Formulation chart of dry powder for inhalation of Formoterol fumarate (LABA) and Mometasone furoate (ICS) using different grades of coarse lactose

Formulation	Active ingredient	Dose + 10% oa mcg	Lactohale 210 mg/capsule	Coarse grade lactose	Coarse grade lactose mg/capsule
F6	LABA+ ICS	6.6 +440	7.5	Lactohale 200	22.81
F10	ICS+LABA	6.6 +440	7.5	Pharmatose 100M	22.81
F11	ICS+LABA	6.6 +440	7.5	Respitose ML001	22.81
F12	ICS+LABA	6.6 +440	7.5	Respitose SV010	22.81

O.a: Over age, LABA: Formoterol fumarate ICS: Mometasone furote

7.2.3.1. Preparation of Drug Loaded Dry Powder Inhalations

An accurately weighed amount of ICS and LABA was mixed separately in each case with lactose monohydrate in geometric progress. and passed through 60# mesh and blended in polybag and filled in to size “3” hard gelatin capsules with partial filling manual capsule filling machine with fill weight of 25 mg per capsules. Step involve in preparation of dry powder inhaler as follows is given in figure no 9.

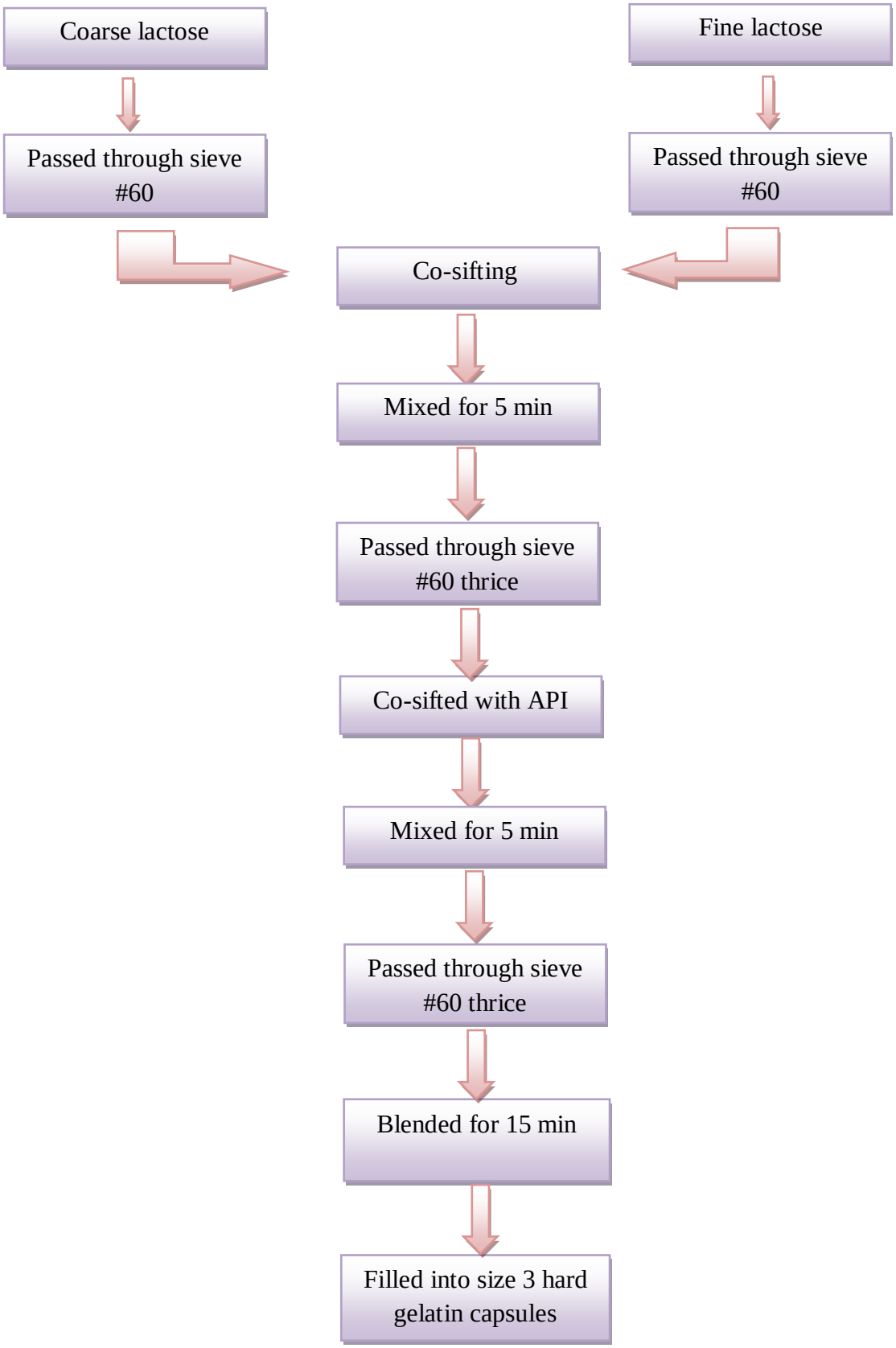


Figure 19: Formulation flow chart

7.2.3. 2. Characterization of formulation by XRD

The X-ray powder diffraction study was performed to know the crystallinity of the APIs and various grades of lactose carriers. The spectra were recorded using shimadzu superior TD 3000 X-ray diffractometer. The samples were run at regular scan with following parameter.

Table 20: Optimum parameters for XRD

Regular scan	Range	0-50 degrees 2 theta
	Scan mode	Continuous scan
	Step size(2 theta)	0.01 deg
	Scan step time(S)	50
	Measurement temp	25^0c
	Detector	X accelerator active length 2.122^0
	X ray source	C_u 15406A^0

7.2.3. 3. Evaluation of Prepared Dry Powder Inhaler.

(i). Physical appearance. [99]

Accurately 20 capsules of the formulation were placed in a clear and dry Petri dish against white background and inspected for particulate matter, color change of the blend, sticking of blend to the walls of the inner capsule shell and also observed for the softening of the capsules shell.

(ii) Flow property. [98]

Flow property of individual formulation was carried out as per above mention procedure in section 7.2.1.2 and results are shown in table no.31

(iii). Locking length of the capsule [99]

The locking length of the capsule was measured by vernier calipers and recorded the reading for each formualation.For determination locking length 10 capsules were randomly selected and recorded the reading from that average locking length was measured and the average locking length of each formulation are show in table no 32

(iv) Average net content: [99]

Randomly 20 capsules were weighed, removed the content from each capsule as completely as possible .Weighed together accurately the emptied shell and calculated the average net content.Using following formula for each formulation and the result of this test given in table 33

Average net content (mg) = $(W_t - W_e/20)$

Where, W_t = Weight of 20 filled capsules in mg

W_e = Weight of 20 empty capsules in mg

(vi) Uniformity of Weight. [98]

Weighed accurately 20 capsules individually taking care to preserve the identify of each capsules. Removed the contents of each capsule as completely as possible. Weighed accurately the emptied shells individually and calculate for each capsule the net weight of shell from the respective gross weight. Calculate the % deviation of weight of each unit from the average weight for each formulation and result are given in table 34

Net content of the individual unit $(W_I) = (W_T - W_E)$

% deviation of each unit $=100-(W_I/W_A*100)$

Where,

W_T= weight of the individual filled capsules (mg)

W_E=weight of the empty capsules in mg

W_I=net content of the individual unit (mg)

W_A=average net content of the capsule in mg

(vii) Device performance.

For observation of device performance, following two devices were selected and experiment was performed as mention below.

- Using Myhaler

10 capsules was operated in Myhaler and observed for brittleness of capsules .Took clean and dried Myhaler device and each time one capsule was taken and placed into Myhaler with cap of the capsule in upward position and turn the body of Myhaler in Clock wise direction until the cap and body of the capsule got separated and detached the parts of and observed the capsule for any brittleness.

- Using Machaler

10 capsules was operated in machaler and observed for brittleness of capsules .Took clean and dried machaler device and each time one capsule was taken and placed into machaler by sliding the cap of machaler and later closed and operate the machaler and observed for the brittleness of the capsule. Results of device performance are showing in table. 35

(vii) Uniformity of drug content: [98]

Drug content uniformity was performed to ensure that the Formoterol fumarate and Mometasone furoate were blended uniformly with the lactose upon powder mixing. Content uniformity measurements were undertaking by randomly sampling ten

samples of approximately 25 ± 2 mg from the powder blend into the capsules. This test was carried out in 10 individual capsules and results are show in table 36, 37 and 38.

Sample preparation:

The content of one capsule was transferred to 10 ml volumetric flask. The inner walls of the emptied capsules were washed to remove any adherent with mobile phase and transferred the washing to the same volumetric flask. 6ml of the diluents(65:35::Buffer pH 3.0 : Acetonitril) was added and sonicated for about 10 min, equilibrated to room temperature and diluted to volume with diluent and mixed. The sample was analyzed by HPLC and was compared with the standard. The percentages of Formoterol fumarate (LABA) and Mometasone furoate (ICS) per capsule were found out by using the formula given below.

$$\textbf{\% of LABA /Capsule} = A_T/A_S \times W_S/100 \times 5/100 \times 10/1 \times P/LC \times 1000$$

Where,

$A_{T=}$ Area of the sum of the peaks due to Formoterol fumarate (LABA) in the chromatogram of the sample preparation.

A_s= Mean area of sum of peaks due to Formoterol fumarate (LABA) standard chromatogram of standard preparation

W_s= Weight of Formoterol fumarate (LABA) reference/working standard taken for standard preparation in mg

P= % assay of Formoterol fumarate (LABA) reference/ standard on as is basis

LC = Label claim of Formoterol fumarate (LABA) in a capsule (mcg)

And

$$\% \text{ of ICH/Capsule} = A_T/A_S \times W_S/200 \times 1/100 \times 10/1 \times P/LC \times 1000$$

Where,

$A_{T=}$ Area of the sum of the peaks due to Mometasone furoate (ICS) in the chromatogram of the sample preparation.

A_s= Mean area of sum of peaks due to Mometasone furoate (ICS) standard chromatogram of standard preparation

W_s= Weight of Mometasone furoate (ICS) reference/working standard taken for standard preparation in mg

P= % assay of Mometasone furoate (ICS) reference/ standard on as is basis

LC = Label claim of Mometasone furoate (ICS) in a capsule (mcg)

(vii) Uniformity of the Delivered Dose [96]

The delivered dose is the dose delivered from the inhaler to the patient. Dose uniformity sampling apparatus (DUSA) was used to determine the Uniformity of delivered dose from the inhalation device. Inhaler for use was connected it to the inlet of the apparatus using a mouthpiece adapter to ensure an airtight seal. One port of a differential pressure meter was connected to the pressure reading point P1, and the other was open to the atmosphere. Pump was switched on, 2-way solenoid valve was opened and flow control valve was adjusted until the pressure drop across the inhaler is 4.0 kPa (40.8 cm H_2O) as indicated by the differential pressure meter and the apparatus was operated at an air flow rate of 100 l/min for 2.4 s.This test was carried out in the test on 10 individual capsules. Air was drawn through the inhaler using the

predetermined conditions. Quantitatively contents of the apparatus were collected and determine the amount of active substance. The results of percentage drug delivered from each capsule are given in table 39, 40, and 41 and the results of acceptance criteria are shown in the table 42, 43, and 44.

- *Sample preparation:*

After aspiration of the sample using sample adapter of the apparatus, body part of apparatus was detached, washed with diluent and collected into 25ml volumetric flask, volume was made up with diluent, mixed. Sample preparation was injected and chromatogram was recorded by following the system suitability parameters and compared with the standard.

$$\% \text{ LABA} = A_T/A_S * W_S/200 * 1/250 * P/LC * 1000$$

$$\% \text{ ICS} = A_T/A_S * W_S/100 * 20/250 * P/LC * 1000$$

Where,

$A_{T=}$ Area of the sum of the peaks due to Formoterol fumarate (LABA) and Mometasone Furoate (ICS) the chromatogram of the standard preparation

A_s=Mean area of sum of peaks due to Formoterol fumarate (LABA) and Mometasone Furoate (ICS) the chromatogram of standard preparation

W_s= Weight of Formoterol fumarate (LABA) and Mometasone furoate (ICS) reference / working standard taken for standard preparation in mg.

P= % assay of ICS and LABA reference/ standard on as is basis.

LC = Label claim of Formoterol fumarate (LABA) and Mometasone furoate (ICS) in a capsule (mcg).

(ix). In vitro deposition by TSLI. [96]

In the TSLI test, 7ml of diluent was introduced in stage 1 and 30 of the same solvent was placed in stage 2 of the apparatus .The capsule to be tested was placed in device attached to the throat piece of the impinger. The assemble was checked and when found to be airtight and vertical; the vacuum pump was switched on .After the pump had run for 5seconds at 60ml/min, the dose was released. The pump was allowed to run for another 5 seconds at 60ml/min following the release of the dose. The deposition test was repeated until ten capsules had been actuated in the same manner. The inhaler body, capsule shell and mouthpiece were washed with the same solvent. The sample thus obtained was used to measure the amount of drug retained in the each inhaler device, the upper stage (Stage 1) that is the non-respirable fraction and lower stage (Stage 2) that is the respirable fraction of the impinger. The test was carried out in 10 capsules **.**After completion of the test on 10 capsules, apparatus was dispatched and the washing was collected into three different portions and result are shown in table 45,46 and 47.

Table 21: Stages indicating the deposition of the emitted dose in TSLI

Sample preparation	Stage	Parts of apparatus
Sample preparation 1	D1	Washing of inhaler , mouth piece adapter
Sample preparation 2	S1	Wash the inner surface of the inlet tube to the upper impingement chamber and its outer surface that projects into the chamber with diluent, collecting the washing in the upper impingement chamber
Sample preparation 3	S2	Wash the inner surface of the inlet tube to the lower impingement chamber and its outer surface that projects into the chamber with diluent, the washing in the lower impingement chamber

The rinsing of stage was transferred to a separate 50 ml volumetric flask and diluted with diluent to the volume. Sample preparation was injected and chromatogram was recorded by following the system suitability parameters and compared with the standard. The entire samples were analyzed by HPLC validated method and content of Fromoterol fumarate (LABA) and Mometasone furoate (ICS) using following formula

Calculations:

$$\% \text{ LABA} = A_T/A_S*W_S/100*1/50*50/10*P/LC*1000$$

$$\% \text{ ICS} = A_T/A_S*W_S/100*5/50*50/10*P/LC*1000$$

Where,

$A_{T=}$ Area of the sum of the peaks due to Formoterol fumarate (LABA) and Mometasone Furoate (ICS) the chromatogram of the standard preparation

A_s=Mean area of sum of peaks due to Formoterol fumarate (LABA) and Mometasone Furoate (ICS) the chromatogram of standard preparation

W_s= Weight of Formoterol fumarate (LABA) and Mometasone furoate (ICS) reference / working standard taken for standard preparation in mg.

P= % assay of ICS and LABA standard on as is basis.

LC = Label claim of Formoterol fumarate (LABA) and Mometasone furoate (ICS) in a capsule (mcg)

(x) Assay

Sample preparation:

Mixed and weighed the content of 20 capsules. Weighed accurately and transfer the mixed content equivalent about 60µg of formoterol fumarate to 100 ml volumetric flask. About 70 ml of diluent was added and sonicated for 10 min, equilibrated to room temperature; volume was made up with diluent and mixed. Equilibrate the column with mobile phase for stable base line. Blank and prepared sample were injected and chromatogram was recorded.

Calculations:

LABA (% label claim) =A_T/As*Ws/200*1/100*100/W_T*A_W/LC*P*1000

ICS (% label claim) =A_T/As*W/100*20/100*100/W*A_W/LC*P*1000

$A_{T=}$ Area of the sum of the peaks due to Formoterol fumarate (LABA) and Mometasone Furoate (ICS) the chromatogram of the standard preparation

A_s=Mean area of sum of peaks due to Formoterol fumarate (LABA) and Mometasone Furoate (ICS) the chromatogram of standard preparation.

W_s= Weight of Formoterol fumarate (LABA) and Mometasone furoate (ICS) reference / working standard taken for standard preparation in mg.

P= % assay of ICS and LABA reference/ standard on as is basis.

LC = Label claim of Formoterol fumarate (LABA) and Mometasone furoate (ICS) in a capsule (mcg)

W_T= Weight of sample taken for sample preparation (mg) for Formoterol fumarate (LABA) and Mometasone furoate (ICS)

A_W= Average net content of a capsule (mg).

(xi) Water content by Karlfisher titration.

Transferred about 50 ml of a mixture of methanol to the titration vessel and titrate with Karl Fischer reagent to detect any moisture that may present in the formulation. Quickly add about 100 mg of powder, mix and again titrate with the Karl Fischer reagent (sulphur dioxide, imidazole base and iodine). [99] Calculate the water content of the specimen, in %, taken by the formula and result are shown in table 51

% Moisture content = BF x 100/W

Where

W = Weight of the Sample, in mg.

B = (Burette reading) Volume of the KF reagent, in ml.

F = the water equivalence factor of KF reagent, in mg.

(xii). Stability studies of the optimized formulation (F6)

Drug decomposition or degradation occurs during storage, because of chemical alteration of the active ingredients or due to product instability, leading to lower concentration of the drug in the dosage form, hence the stability of pharmaceutical preparation need to be evaluated. The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and relative humidity (RH) conditions.

A drug formulation is said to be stable if it fulfills the following requirements:

- It contains at least 90% of the stated active ingredient.
- It does not exhibit softening of the capsules.
- It does not exhibit discoloration of the blend.
- It does not exhibit any brittleness of the capsules when operated in the device.

The formulation were packed in HDPE 30cc container and sealed and studies were carried out for 90 days by keeping at 40 $\pm$ 2^{o}C and 75 $\pm$ 5% RH

Samples were withdrawn on 30th, 60th & 90th day and checked for changes in physical appearance, Device performance and evaluated for drug, water content, and deposition of the emitted dose and result are shown in table 49, 50 51,52 and 53.