FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION

A Dissertation Submitted to
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
BRANCH-1-PHARMACEUTICS

Submitted by
ABINAYA M
REGISTRATION No. 261711401

Under the guidance of
Dr. S. MOHAN, M.Pharm, Ph.D.,
PRINCIPAL & HEAD
Department of Pharmaceutics

KARPAGAM COLLEGE OF PHARMACY
OTHAKKALMANDAPAM, COIMBATORE-641032.

NOVEMBER 2019

KARPAGAM COLLEGE OF PHARMACY

Othakkalmandapam, Coimbatore- 641 032, Tamil Nadu, India

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** is a bonafied research work done by **Ms. ABINAYA M (Reg. No: 261711401)** in partial fulfillment for the award of degree of **Master of Pharmacy in Pharmaceutics.** The Research work was carried out in **Department of Pharmaceutics, Karpagam College of Pharmacy** and submitted to ***The Tamilnadu Dr.M.G.R. Medical University, Chennai*** under the supervision and guidance of **Dr. S. Mohan, M. Pharm, Ph.D.,** during the academic year 2018-2019. The results embodied in this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

PRINCIPAL

KARPAGAM COLLEGE OF PHARMACY

Othakkalmandapam, Coimbatore- 641 032, Tamil Nadu, India

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** is a bonafied research work done by **Ms. ABINAYA M (Reg. No: 261711401)** in partial fulfillment for the award of degree of **Master of Pharmacy in Pharmaceutics.** The research work was carried out in **Department of Pharmaceutics, Karpagam College of Pharmacy** and submitted to ***The Tamilnadu Dr.M.G.R. Medical University, Chennai*** under the supervision and guidance of **Dr. S. Mohan**, Professor and Principal during the academic year 2018-2019. The results embodied in this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

Dr. S. RAMKANTH, M. Pharm, Ph.D.,
Professor& Head,
Department of Pharmaceutics

KARPAGAM COLLEGE OF PHARMACY

Othakkalmandapam, Coimbatore- 641 032, Tamil Nadu, India

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** is a bonafied research work done by **Ms. ABINAYA M (Reg. No: 261711401)** in partial fulfillment for the award of degree of **Master of Pharmacy in Pharmaceutics.** The research work was carried out in **Department of Pharmaceutics, Karpagam College of Pharmacy** and submitted to ***The Tamilnadu Dr. M.G.R. Medical University, Chennai*** under my supervision and guidance during the academic year 2018-2019. The results embodied in this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

RESEARCH GUIDE
Dr. S. MOHAN, M. Pharm, Ph. D.,

KARPAGAM COLLEGE OF PHARMACY

Othakkalmandapam, Coimbatore- 641 032, Tamil Nadu, India

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** is a bonafied research work done by **Ms. ABINAYA M (Reg. No: 261711401)** in partial fulfillment for the award of degree of **Master of Pharmacy in Pharmaceutics.** The research work was carried out in **Department of Pharmaceutics, Karpagam College of Pharmacy** and submitted to ***The Tamilnadu Dr.M.G.R. Medical University, Chennai*** under our **Co-Guidance** during the academic year 2018-2019. The results embodied in this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

Mr. C. Benedict Jose M.Pharm.,
Associate Professor,
Department of Pharmaceutics

Mrs. R Gayathri M.Pharm.,
Associate Professor,
Department of Pharmaceutics

KARPAGAM COLLEGE OF PHARMACY

Othakkalmandapam, Coimbatore- 641 032, Tamil Nadu, India

I hereby declare that this dissertation work entitled **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** submitted by me, in partial fulfillment for the Degree of **Master of Pharmacy in Pharmaceutics** to ***The Tamilnadu Dr.M.G.R. Medical University, Chennai*** is the result of my original and independent research work carried out under the guidance of **Dr. S. MOHAN, M.Pharm, Ph.D., Department of Pharmaceutics, Karpagam College of pharmacy, Coimbatore** during the academic year 2018-2019.

The work is original and the dissertation either in part or full has not been submitted by me or any other person to any University/Institute in any part of thesis/ dissertation/ monograph.

I hereby further declare that the **Department of Pharmaceutics, Karpagam College of pharmacy, Coimbatore** shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic or research purpose.

Signature of the Candidate

Ms. ABINAYA M
Reg. No. 261711401

KARPAGAM COLLEGE OF PHARMACY

Othakkalmandapam, Coimbatore- 641 032, Tamil Nadu, India

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** submitted by **Ms. ABINAYA M (Reg No:261711401)** to ***The Tamilnadu Dr.M.G.R. Medical University, Chennai*** in partial fulfillment for the Degree of **Master of Pharmacy in Pharmaceutics** is a bonafied work carried out during the academic year 2018-2019 by the candidate at the **Department of Pharmaceutics, Karpagam College of Pharmacy, Coimbatore** and was evaluated by us.

Examination Centre:-

Date:-

Internal Examiner **External Examiner**

ACKNOWLEDGEMENT

"Research is to see what everybody else has seen, and to think what nobody else has thought"

-Albert Szent-Gyorgyi

Apart from my effort, the success of this dissertation mainly depends on the encouragement and guidance of many others. I take this opportunity to express my gratitude to the people who have been instrumental behind the successful completion of this task.

First and foremost, I wish to express my thanks to holiness for his love and unseen guidance throughout my work.

Any successful completion of task would incomplete without mentioning people who made it possible with their guidance and encouragement.

I would like to express my profound thanks to my beloved Parents father **Mr. K. Mani** and mother **Mrs. M. Renuga Devi** to bring me this world and encouragements in all my life carriers and a special gratitude to my brother **Mr. M. Vignesh Kumar** for his support and encouragements in all these two years.

I extend my thanks to **Dr. Vasanthakumar, Chairman, Karpagam Academy of Higher Education, Coimbatore** for the facilities provided by him to carry out the study in efficient manner.

I proudly take this opportunity to express my deep sense of gratitude to my guide **Dr. S. Mohan, M.Pharm, Ph.D., Principal, Karpagam college of Pharmacy, Coimbatore,** for his valuable encouragement and guidance to complete this project.

I am particularly thankful to **Dr. S. Ramkanth, M.Pharm, Ph.D., Professor and Head, Department of Pharmaceutics Department, Karpagam College of pharmacy** for his heartly and valuable guidance during the course of my work.

I express my sincere thanks to my esteemed co-guide **Mrs. R. Gayathri, M.Pharm., Associate Professor, Department of Pharmaceutics, Karpagam college of Pharmacy** for her valuable suggestions, encouragements, motivation, inspiring, guidance and co-operation throughout my dissertation work.

I want to express my gratitude to my beloved staff **Mr. C. Benedict Jose, M.Pharm., Associate Professor, Department of Pharmaceutics, Karpagam college of Pharmacy** for his support, guidance and encouragements throughout my dissertation work.

I want to express my heartfelt thanks to **Mr. G. Prakash, M.Pharm., Associate Professor, Department of Pharmaceutical Chemistry, Karpagam College of Pharmacy** for his encouragement throughout the research work.

I express my sincere thanks to **Dr. G. Nagaraja Perumal**, M.Pharm, Ph D., Professor and Head, Department of Pharmacology, **Dr. C Senthil Kumar** M.Pharm, Ph D., Professor, Department of Pharmacology and **Dr. Asha Jose** M.Pharm, Ph D., Associate Professor, **Department of Pharmacology**, Karpagam College of College of Pharmacy for their support and motivation during my *in vivo* studies.

I want to express my gratitude to **Mr. Antony Doss, Mrs. Sivasankari and Mr. Joseph** Laboratory Technician, Department of Pharmaceutics for their sincere concern and help.

I want to express my thanks to **all Faculties of Karpagam College of Pharmacy,** who directly or indirectly gave a helping hand to me while carrying out the dissertation.

I want to express my gratitude to **Library staff** and **Lab Technician of Karpagam college of Pharmacy** and all those helped me in the completion of my entire work.

I would like to express heartfelt thanks to my friends **Mr. R Prince, Ms. A Ramya Devi, Mr. M. Aravind** and **First year M.Pharmacy Students from Department of Pharmaceutics and Pharmacology (2018-2020) batch.**

I express my sincere and heartfelt indebtedness to the ***University Research Council,* The Tamilnadu Dr.M.G.R. Medical University, Chennai for funding this Research work.**

Finally I would like to express heartfelt thanks to **Nature of god** to give invisible guidance throughout my life carriers, studies and dissertation work.

-ABINAYA M

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

1) DPI - Dry Powder Inhaler
2) DPs – Dry powders
3) COPD - Chronic Obstructive Pulmonary Disease
4) API - Active Pharmaceutical Ingredient
5) pMDIs - Pressurized Metered Dose Inhalers
6) CFC – Chlorofluorocarbons
7) GI – Gastro intestinal
8) µm – Micrometre
9) nm – Nanometre
10) % - Percentage
11) ml – Milli litre
12) s – Second
13) NRT - Nicotine Replacement Therapy
14) min – Minute
15) hr – Hour
16) g – Gram
17) 5 HTP – 5 Hydroxy Tryptamine
18) Kg – Kilogram
19) ℃ - Celsius
20) L – Litre
21) λ max – Maximum absorbance of wavelength
22) cm – Centimetre
23) cm^2 – Square centimetre
24) cm^3 – Cubic centimetre
25) RH – Relative humidity
26) HPLC – High Performance Liquid Chromatography
27) FE-SEM - Field Emission Scanning Electron Microscope
28) EDS -Energy dispersive Spectroscopy
29) XRD – X Ray Diffraction
30) FT-IR – Fourier Transform-Infrared Spectroscopy
31) DSC – Differential Scanning Colorimetry

32) UV – Ultraviolet/ Visible
33) Mcg – Micro gram
34) ACI - Anderson cascade impactor
35) RF - Respirable fractions
36) ED - Emission dose
37) D_{ae} – Aerodynamic diameter
38) < - Lesser than
39) > - Greater than
40) kDa – Kilo Dalton
41) IAEC - Institutional Animal Ethical Committee
42) IV – Intravenous
43) µl – Micro litre
44) NaOH – Sodium hydroxide
45) w/v – Weight per volume
46) MMAD- Mass Median Aerodynamic Diameter

1. INTRODUCTION

Pulmonary drug delivery by Dry Powder Inhalers (DPIs) has recently become subject of active research, because of its propellent free nature, better patient compliance, high dose carrying capacity, drug stability and patent protection. It is involved to realize full potential of lungs for local and systemic treatment of pulmonary diseases like asthma, Bronchitis, Pulmonary embolism, Pulmonary hypertension, Chronic Obstructive Pulmonary Disease(COPD), Embolism, Respiratory diseases, etc. DPIs are devices through which a dry powder formulation of an active drug is delivered for local or systemic effect via the pulmonary route. Although DPIs are complex in nature and their performance relies on many aspects including the design of inhaler, the powder formulation and the airflow generated by the patient. DPI is mainly classified into Active and Passive. Majority of DPI's are passive breath- actuated devices. They depend on the patient inspiration to operate. There is no need to co-ordinate breathing with the activation. The patient simply inhales deeply to access the drug. Passive DPI can be sub divided in to two categories; Pre metered (Single or multidose) where the dose is pre-measured during manufacture. Ex.: Blister, Capsule. Device metered in which the drug is contained in reservoir within the device which pre-measures every dose on actuation. In last decade, performance of DPIs has improved significantly through the use of engineered drug particles and modified excipients systems.[1-6]

The dry powder platform comprises of devices that generate an direct inhalation of dry powder from particle size range of 1 to 5µm drug powder, or mixtures with excipients. Excipients used in DPI are used as carrier for Active Pharmaceutical Ingredient (API). Most commonly used carrier is Lactose Monohydrate18. The development of DPIs has been motivated by the desire for alternatives to pressurized Metered Dose Inhalers(pMDIs) , to reduce emission of ozone-depleting and greenhouse gases chlorofluorocarbons (CFC) and hydrofluoroalkanes respectively that are used as propellants.[7,8]

1.1. Advantages of DPI over pMDIs:

1.1.1 Require little or no coordination of actuation and inhalation

Inaccurate utilization of pMDIs is still a predominant issue. It was 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. This has frequently resulted in better lung delivery than was achieved with comparable pMDIs [9].

1.1.2. 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. [7]

1.1.3. 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. [10]

Other advantages of DPI are as follows

1. High drug dose carrying capacities. DPIs can deliver a range of doses from less than 10 mg to more than 20 mg via one short inhalation.

2. Minimal extra pulmonary loss of drug due to low oropharyngeal deposition, low device retention and low exhaled loss.

3. Reduces extracellular enzyme levels compared to GI tract due to the large alveolar surface area.

4. Provides local action within the respiratory tract and are non-invasive.

5. Avoids hepatic first-pass metabolism.

6. Better patient compliance, simple to use and convenient to carry and do not require spacers.[7-10]

1.2. Disadvantages:

1.Respirable dose dependent on inspiratory flow rate.

2. Humidity may cause powders to aggregate and capsules to soften.

3.Dose lost if patient inadvertently exhales into the DPI.[11]

1.3.Ideal characteristics of DPI:

Characteristics of ideal DPI systems will include most or all of the following attributes

1. Simple, comfortable and easy to use.
2. It should be compact and economical.
3. Highly reproducible fine particle dosing.
4. Reproducible emitted doses.
5. Accurate and uniform delivery of doses over extensive range of inspiratory flow rate.
6. Optimal particle size of drug for deep drug lung delivery
7. Powder should be physically and chemically stable.
8. Suitability for a extensive range of drugs and doses.
9. Minimum adhesion between drug formulation and devices.
10. Minimal extra-pulmonary loss of drying.
    - Low oropharyngeal deposition.
    - Low device retention.
    - Low exhaled loss.
11. Multidose system Powder protected from external environment and usable in all environments.
12. Overdose protection.
13. Indicating the number of doses delivered or remaining.

No DPIs achieved all of these characteristics; however considerable research is being conducted to improve their performance characteristics wherever necessary.[13,14]

1.4. Principle of DPI design:

The formulation typically consist of micronized drug blended with large carrier particles dispensed by metering system. An active or passive dispersion system entrains the

particles into the patient's airways, where drug particles separate from the carrier particles and are carried into the lung. 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 (Fig. 1). Deposition of drug into the lungs is determined by the patient's variable inspiratory airflow. Inadequate drug carrier separation leads to low deposition of drug into the lung which affects the efficiency of DPI. [15]

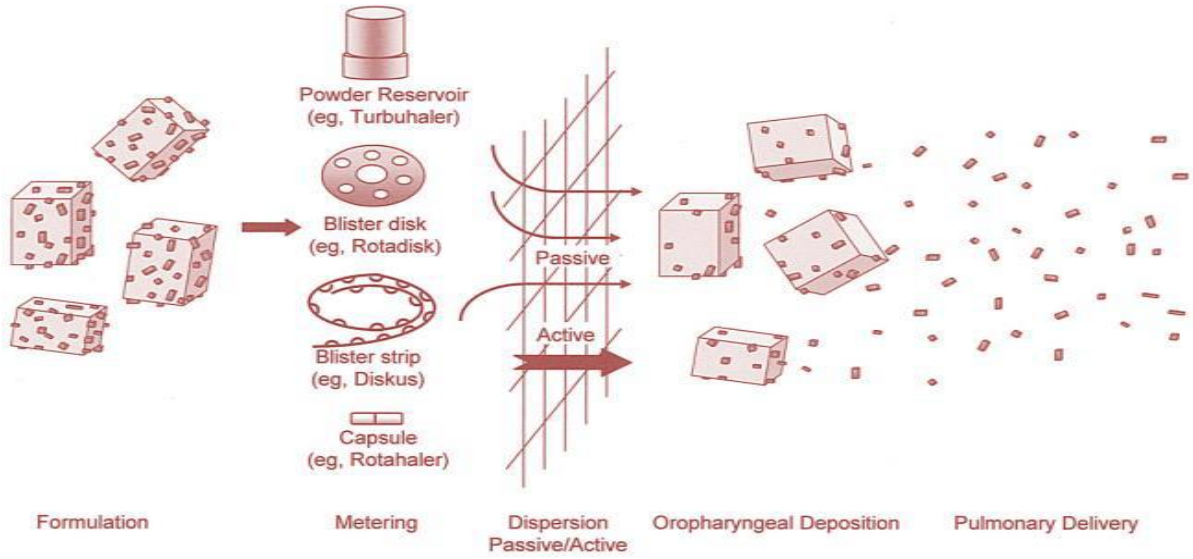


Figure No. 1: Principle of DPI design

1.5.General requirements of DPI:

DPIs have to meet the following requirements;

a. Particle Size of API

Active Compound must be inhalable for systemic action. In order to reach deep into the lungs the particles must possess the particle size in the range of 1 to 5µm. Such micro fine particles can be obtained by Micronization, controlled precipitation from suitable solvent or by spray drying if the procedure conditions are suitable.

b. Drug content uniformity

In order to assure that the patient gets the same dose of drug every time of inhalation, it is necessary 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.

c. Content uniformity at different airflows

As the drug delivery from a DPI depends on the patient's breathing pattern the dose has to be released in exactly the same way at low breathing and at a high breathing rate. Content uniformity at different airflows is therefore extremely important for a DPI.

d. Stability of powder against humidity and temperature

Because the particle size distribution of lactose is extremely important for the action of a DPI, the lactose must be protected against particle size growth. The main property responsible for particle size growth is an undesired combination of temperature and relative humidity. Controlling the temperature and relative humidity followed by storage in the correct packaging are important for stability. [16-18]

1.6.Formulation processes in production of DPI:

Three major processes are involved in the delivery of drugs from DPIs, namely, **i)** the detachment of drug particles from the carrier, **ii)** their dispersion in the air flow and **iii)** deposition in the respiratory tract. Thus, any factor that affects any of these processes could ultimately influence the bioavailability of the inhaled drug. These include the design of inhaler devices, airway physiology, inhalation manoeuvres and the powder formulation (table 1)

Table No. 1: Formulation process in DPI production

Design of inhaler devices	Rotahaler®, Spinhaler®, Inhalator®, Turbohaler®, Diskhaler®, Diskus®, Accuhaler®, etc.
Powder formulation	Drug-drug interaction, drug-carrier interaction, particle size and shape, surface texture, crystallinity, hygroscopicity, density, etc.
Inhalation manoeuvre	Flow rate, tidal volume, breath-holding, breathing frequency, air acceleration, etc.
Physiology of respiratory tract	Geometry, lung function, pulmonary ventilation, mucus secretion, etc.

1.6.1.Powder production methods (Micronization):

Dry powders for inhalation are mostly produced by mechanical Micronization although spray-drying has been investigated as an alternative means of generating particles suitable for deep lung penetration. Powders produced using different methods would be expected to have different physical, if not chemical, properties and these, as has been stated, include particle size, size distribution, shape, crystalline properties, surface texture and energy, etc. These physical properties are critical in the behaviour of particles, both before and after inhalation.[19-25]

1.6.1.1.Attrition milling:

Attrition milling has long been used as the main method to prepare inspirable particles. Opposing jets of compressed air at different pressures force the particles to impact with one another and this violent attrition causes the reduction in particle size. A cyclone separation mechanism is used to collect the resultant micronized powder. To obtain particles that can reach the lower airways, large quantities of energy are needed during micronization and this energy can affect the material being processed. Thus, micronized particles often have both a high surface energy and electrical charge. Attrition milling produces amorphous regions onto the particle surfaces and crystalline disorder to chemical stability of macromolecule such as proteins and peptides. Apart from that it may induce physico-chemical instability of certain drugs that are sensitive to compaction.[26-31]

1.6.1.2.Fluid energy mill:

Fluidized Energy mill, also known as micronizers, or jet mills operates by particles impaction and attrition. A fluid or milling gas, usually air or inert gas is injected as a high pressure jet through nozzles at the bottom of the loop. The powder particles in the mill are accelerated to high velocity. The kinetic energy of the air plus the turbulence created causes inter particle (particle-particle collision) and particle-wall contact resulting in particle size between 2 and 10µm. The fluidized effect transports the particles to a classification zone where the size classifier retains the particles until sufficiently fine to be removed. Fluidized

energy can also be used in milling of thermolabile materials and it is the choice of mill when higher degree of drug purity is required.[32-37]

1.6.1.3.Spray drying:

Spray-drying has been employed as a routine technique for the production of pharmaceutical particles for decades. Spray-drying constitutes a single step process that transforms a solution or suspension into a fine powder. The feed material in spray-drying is a liquid, and drying is accomplished by atomising this into a hot drying medium. Spray drying involves droplet formation and rapid evaporation of the solvent. Generally, spray-drying produces hollow spherical particles, resulting in a powder with low bulk density compared to the initial material. However, particles manufactured in this manner demonstrate poor flow characteristics. Furthermore, the need to provide heat during particle formation by spray-drying makes it less suitable for thermosensitive compounds. Most importantly, spray-drying is able to produce particles of a size suitable for inhalation and thus, spray-dried particles may be directly formulated into dry powder aerosols. The denaturation of proteins during spray-drying may be minimised by carefully controlling the operational parameters.[38]

1.6.1.4.Nano Spray drying:

Nano spray drying creates particles in the nanometric size range. The particle sizes have been reduced to 300 nm, yields up to 90% with the sample volume of 1 ml. These expanded limits are possible due to new technological developments to the spray head, the heating system, and the electrostatic particle collector. However, the smallest particles produced are in the sub-micrometre range common to fine particles rather than the nanometer scale of ultrafine particles. The functional principle is basically the same as with normal spray dryers. There are just different technologies that are used to do similar things. The drying gas enters the system via the heater. A new kind of heater system allows for laminar air flow. The spray head sprays the fine droplets with a narrow size distribution into the drying chamber. The droplets dry and become solid particles. The solid particles are separated in the electrostatic particle collector. The exhaust gas is filtered and sent to a fume hood or the environment. The inlet temperature is controlled by a temperature sensor. [38]

1.6.1.5.Crystallization:

Crystallization separates a solid substance from solution by means of changing the physical or chemical properties of the material concerned. A compound may crystallize from a solution when its solubility in that solvent is exceeded, i.e. the solution becomes supersaturated. Supersaturation can be achieved in many ways, some of which involve the evaporation of the solvent, cooling the solution or by producing additional solute as a result of chemical reaction or a change in the solvent system (by the addition of a poor solvent for the crystallising material). Crystallisation provides an important means of preparing inspirable particles and has advantages over both micronization and spray-drying in that it may produce particles of high crystallinity and of predetermined size, size distribution and shape. Crystallization may be carried out in either conventional solvents such as ethanol or in a supercritical fluid. [39,40]

1.6.2.Formulation:

Formulation of DPI mainly includes three steps;

1.6.2.1.API Production

Particle size is an important parameter in case of API DPI . Particle size of API should be in the range of 1-5µm. Generally the API particle size is not well controlled during bulk drug production. It must be reduced in a separate unit operation to achieve aerodynamic particle size of drug. There are various size reduction techniques namely milling, spray drying, and supercritical fluid extraction.

There are various sort of mills used for size reduction of drugs but few of them are appropriate for DPI to reduce the size in the range of 2-5µm such as fluid-energy mills (eg., jet mill), high-peripheral-speed mills (eg., pin-mill and ball mill).[16,41]

1.6.2.2.Formulation of API with or without carriers:

DPI is generally formulated as a powder mixture of coarse carrier particles and micronized drug particles with aerodynamic particle diameters of 1–5µm. Carrier particles are used to improve drug particle flowability, 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. Moreover, usually no more than a few milligrams of a drug needs to be delivered (e.g., between 20μg and 500μg of corticosteroids for asthma therapy), and thus carrier provides bulk, which improves the handling, dispensing, and metering of the drug. During insufflation, the drug particles are detached from the surface of the carrier particles by the energy of the inspired air flow that overcomes the adhesion forces between drug and carrier. The larger carrier particles impact in the upper airways, while the small drug particles go through the lower parts of lungs (Pilcer et al., 2012). Consequently, it has been stated that the efficiency of a DPI formulation is extremely dependent on the carrier characteristics and the selection of carrier is a crucial determinant of the overall DPI performance.[42]

Although carrier–drug mixtures represent the most common formulations, the use of aggregates composed solely of drug particles could avoid some of the problems encountered with the formulations where coarse carrier particles are involved. The Turbohaler® designed by Astra is a typical example of a carrier-free dry powder inhaler device where loosely agglomerated drug particles are employed as the powder formulation. Carrier free formulations are especially advantageous over the carrier–drug mixtures for patients who are intolerant to carriers such as lactose. However, it is more difficult to process carrier-free formulations than the drug–carrier mixtures since the inhaled drug normally requires a unit dose of the order of micrograms. [43-46]

1.6.2.3.Integration of the formulation into device

After the formulation has been blended, it is filled into capsules, multi-dose blisters, or reservoirs for use with the inhaler device. The filling process is automated and depends on the nature of the metering system. [16]

1.7.Cigarette smoking:

Cigarette Smoking is the most significant cause of avoidable health harm. The great majority of regular smokers are dependent on cigarette smoking, and not simply addicted to nicotine [48] . Smoking is highly contextual and associated with certain rituals. These start with the opening of a packet, followed by the lighting process and then the sight and smell of smoke. After inhaling smoke from a modern cigarette, arterial nicotine levels increase

markedly within 15 seconds [49] .This bolus of nicotine activates the brain-reward system by increasing dopamine release [50] .This brain reward system is a common pathway for pleasurable activities like sexual activity, eating and most drugs of addiction [51] .This peak in plasma nicotine level, and the transient activation of the reward system, is followed by a gradual fall in nicotine levels into a state of withdrawal [52] that is, in turn, relieved by the next cigarette. Dependence arises from the temporal association of the rituals and sensory inputs with the repeated stimulation and relief of withdrawal [47] . This required association explains why Nicotine Replacement Therapy (NRT) products, that deliver nicotine slowly and do not produce high plasma nicotine levels, have minimal addictive potential. [53]

The NRT formulations available in India include gum, patches and oral inhaler. Nicotine nasal spray and a sublingual tablet or lozenges are not presently available in India.

Gums contain nicotine (2 mg or 4 mg per piece) in a resin base. The gum should be chewed slowly, and then left between the cheek and gum. Over the next 20–30 minutes, the gum should be chewed intermittently and repositioned. Because nicotine is poorly absorbed in an acid environment, acid drinks such as fruit juices should be avoided. While extra doses may not rapidly increase nicotine levels, the process of their use is a ritual that is in some ways analogous to smoking, and this may be an advantage.

Nicotine transdermal patches are designed to release nicotine slowly. Immediately after application, there may be relatively rapid transfer of nicotine from the adhesive layer. Patches are applied each morning. The 16-hour preparations are useful for smokers who experience insomnia or other nocturnal symptoms. Local skin reactions are the commonest adverse effect. This can be minimized by rotation among a number of sites of application, but can be severe enough to require discontinuation.

The inhaler is a plastic cartridge that is inserted into a mouthpiece. Gaseous nicotine is released by deep inhalation through the mouthpiece. Twenty minutes after the first deep inhalation, the device has released about 4 mg of nicotine. This process, as with patches and gums, does not release nicotine rapidly [54] ,but it does replicate some of the smoking rituals. After use, the device is spent and cannot be reused or recycled.

1.7.1.Side effects, precautions and contraindications

Nausea, insomnia and dry mouth are common early symptoms. The time to peak plasma level is three hours. Therefore, if insomnia is prominent, the evening dose may be

taken early, but at least eight hours after the morning dose. Seizures are the major side effect of concern. Bupropion is absolutely contraindicated in patients with a history of epilepsy, and there is a relative contraindication in conditions that might increase the risk of seizures, such as type 1 or 2 diabetes. If it is to be used in patients with such conditions, it should only be after careful consideration of the risks and alternative treatment options, balanced against the benefits of cessation in the individual. Hypersensitivity reactions are the other adverse effects of concern. Facial edema has been reported, as has a serum-sickness-like reaction. [55]

1.7.2.L Tryptophan and L Tyrosine in smoking cessation:

From literarures it is cleared that in spite of many formulations available for smoking cessation, withdrawal symptoms is majorly causing difficult in quitting of the smoking behaviour.

Hence treatments that reduce the immediate effects of smoking withdrawal have potential for helping smokers quit. Serotonin-enhancing substances, such as tryptophan and high-carbohydrate diets, have been used in clinical disorders to relieve negative effect, a classic symptom of cigarette withdrawal says Deborah J. Bowen et al.[60] They used oral/-tryptophan plus a high-carbohydrate diet, in conjunction with more traditional smoking cessation treatment techniques, to ameliorate the smoking withdrawal syndrome. [60]

Munafo et al., investigated the impact of the administration of a tyrosine-depleting amino acid mixture compared to a balanced mixture on measures of mood, craving and selective processing of smoking-related cues in healthy cigarette smokers instructed to abstain from smoking for 12 hr prior to, and during, the experiment. These results tentatively support for the role of dopaminergic neurotransmission in mediating the response of cigarette smokers to smoking-related cues.[70]

Tyrosine is an amino acid precursor to norepinephrine and dopamine, and tryptophan is a precursor amino acid for serotonin. [56-59] Manipulations of tryptophan and tyrosine levels in plasma may affect release of serotonin, dopamine and, to a lesser extent norepinephrine in rodent brain and may affect depressed mood in humans says Yekta Dowlati et al 2019.[61]

2. LITERATURE REVIEW

2.1 Bowen DJ *et al.*, (1991) studied with Serotonin enhancing substance-Tryptophan along with high-carbohydrate diets to relieve negative effect which is the classic symptom of cigarette withdrawal. In this research work they used tryptophan (50mg/kg/day) and carbohydrate diets to ameliorate the smoking withdrawal syndrome and to improve abstinence rates. Subjects were randomly assigned to receive either tryptophan (n=16) or placebo (n=15). Standard smoking cessation treatment was identical for the experimental and control groups and consisted of four 2-hr weekly sessions of multicomponent group therapy. Smoking behaviour, symptoms of nicotine withdrawal and negative effect were assessed during a 2-week withdrawal period. Tryptophan treated subjects who could not fully abstain were able to smoke fewer daily cigarettes. Reported anxiety and other withdrawal symptoms were lower in the tryptophan group compared with controlled subject. From these data they suggested that serotonin- enhancing substances showed promising use as an adjunct to existing smoking cessation programs.[60]

2.2 Yekta Dowlati *et al.*, (2019) have reported that twenty-one healthy cigarette smokers received either active dietary supplement followed by washout and placebo or the same in reverse order. The dietary supplement in their protocol composed of monoamine precursors (2 g tryptophan, 10 g tyrosine) and blueberry antioxidants (blueberry juice with blueberry extract). Vulnerability to depressed mood was measured by the change in scores of depressed mood on the visual analog scale (VAS) following the sad mood induction paradigm (MIP). There was a significant increase in VAS depressed mood scores after the sad MIP during supplement and placebo, but no difference between active and placebo conditions. There was also a significant increase in urge-to-smoke scores after sad MIP during supplement and placebo but no difference between active and placebo conditions. Reliability of the increase in VAS after MIP was very good. The dietary supplement had negligible effect on depressed mood, but sad MIP is a very reliable method that can be applied in future studies to assess other interventions for preventing dysphoric mood during early cigarette withdrawal.[61]

2.3 Viswanath V Venugopalan *et al.*, (2011) investigated that depletion of acute Phenylalanine / Tyrosine reduces motivation to smoking cigarette at the stages of addiction. In this method Dopamine (DA) synthesis was transiently decreased in 3 groups of abstinent smokers. Compared to nutritionally balanced control mixture, Acute Phenylalanine/Tyrosine Depletion decreased the self-administration of nicotine containing cigarettes and this was found to be occurred in all the three groups of smokers. From the result they suggested that DA influenced the willingness to sustain effort for nicotine reward. In the transition from sporadic to addicted use, the role of DA in the motivation to seek drug may change less than previously proposed.[62]

2.4 John R Hughes *et al.*, (2004) assessed the effect and safety of antidepressant medications to aid long-term smoking cessation. The medications used in their research was bupropion; doxepin; fluoxetine; imipramine; lazabemide; moclobemide; nortriptyline; paroxetine; S-Adenosyl-L-Methionine (SAMe); selegiline; sertraline; St. John's wort; tryptophan; venlafaxine; and zimeledine. From the study they concluded that the antidepressants bupropion and nortriptyline aided long-term smoking cessation. Adverse events with either medication appear to rarely be serious or lead to stopping medication. Evidence suggested them that the mode of action of bupropion and nortriptyline was independent of their antidepressant effect and that they were of similar efficacy to nicotine replacement.[63]

2.5 Brian Hitsman *et al.*, (2005) studied the effect of acute tryptophan depletion (ATD), which transiently reduced brain serotonin, on negative symptoms and cigarette smoking topography in schizophrenic smokers. Nicotine-dependent schizophrenics (n=11) and nonpsychiatric controls (n=8) were examined after ingesting comparable mixtures that do and do not deplete plasma tryptophan. They found that ATD influenced smoking topography in both schizophrenics and nonpsychiatric controls in a manner suggestive of increased desire to smoke. Schizophrenics exhibited increased puff duration and decreased cigarette duration. Controls displayed increased puff duration. ATD did not produce changes in negative symptoms of depression. They compromised brain serotonin via ATD appears to intensify smoking behaviour in nicotine-

dependent individuals directly, rather than indirectly through changes in either mood or psychopathologic symptoms.[64]

2.6 Michele Pergadia *et al.*, (2004) conducted Double-blind trial on the effects of tryptophan depletion on depression and cerebral blood flow in smokers. They found that Tryptophan depletion (TD) showed increased negative mood in smokers, particularly those with recurrent depression. Decreased bilateral cerebral blood flow to the inferior frontal (IF) lobe following TD relative to placebo was associated with increased depressed mood (r=−.653, P<.05). Among smokers, a decrease in brain serotonin is associated with increased depressed mood and with focal bilateral decreases in IF activity. Chronic nicotine exposure appears to be associated with cortical responses suggestive of depressive vulnerability.[65]

2.7 Mary Perugini *et al.*, (2003) examined the affective and neuroelectric correlates of smoking abstinence and cigarette smoking following depletion of the serotonin precursor, tryptophan. In a randomized, double-blind two session (tryptophan depletion [TD] vs. non-depletion), placebo-controlled design, spectrally analysed electroencephalogram (EEG), self-ratings of withdrawal symptoms and mood states were assessed in 18 male cigarette smokers before smoking abstinence, 5 h post smoking abstinence and again following sham smoking and the smoking of one cigarette. Compared to a nutritionally balanced amino acid (AA) mixture containing tryptophan (i.e., placebo mixture), oral ingestion of a similar mixture devoid of tryptophan resulted in a 70% reduction of plasma tryptophan but failed to alter the appearance or reversal (by acute cigarette smoking) of withdrawal symptoms, negative mood states and increased slow wave EEG in male smokers deprived of cigarettes. Their results, although not supported a role for the serotonergic system in acute smoking and early smoking abstinence symptoms, are discussed in relation to the neuropharmacology of smoking behaviour and they suggested this for future work.[66]

2.8 Verner Knott *et al.*, (2012) studied that in depression prone individuals, tryptophan depletion induces mood lowering and an asymmetric frontal alpha similar to that seen in depression. Nicotine treatment counteracts depressed mood

and frontal EEG changes accompanying transient reductions in serotonin. Posterior EEG alpha augmentation induced with nicotine may play a separate role in the emotional modulating effects of tobacco smoking. Their findings indicated that in depression prone individuals, nicotine acts to stabilize the mood lowering and associated frontal functional asymmetry elicited by an acute decrease in brain serotonin.[67]

2.9 Brian Hitsman *et al.*, (2007) examined the effects of a serotonergic challenge on the attentional salience of various cues associated with cigarette and hypothesized that cigarette-related word cues would be more distracting after acute tryptophan depletion than after a placebo challenge. They also hypothesized that smokers vulnerable to recurrent depression would show greater attentional bias towards these cues than smokers without a history of depression. In their study thirty-four smokers diagnosed as having (***n*** = 15) or lacking (***n*** = 19) a history of DSM-IV major depressive disorder (MDD) underwent acute tryptophan depletion (ATD) and placebo challenges in double-blind and counterbalanced order 1 week apart. Five hours after consumption of each mixture, subjects completed a modified Stroop task to measure attentional bias to smoking-related, positive affect, and negative affect word cues. Stroop interference was calculated as a difference score between latencies for the motivationally salient and the neutral (furniture) word lists. Finally they concluded that acutely compromising central serotonergic neurotransmission via ATD heightens the attentional salience of cigarette-related cues, perhaps by triggering reward and motivational deficits underlying nicotine dependence.[68]

2.10 Ivan Berlin *et al.*, (2005) assessed the effect of high doses of oral glucose on tobacco craving, withdrawal symptoms, plasma TRP and blood serotonin concentrations in temporarily abstinent smokers. This is done because oral glucose showed decrease in tobacco craving in many but not all previous studies. Glucose ingestion may facilitates entry of tryptophan (TRP), the unique source of brain serotonin, into the brain, glucose's action seems to be opposite of rapid TRP depletion. Hence in their study aspartame 0.6 g/200 ml (A, placebo), glucose 32.5 g/200 ml (G32.5) and 75 g/200 ml water (G75) were administered

to 12 healthy smokers after an overnight abstinence in a crossover, double blind study and questionnaires were assessed. The result was analysed and they concluded that Glucose attenuates tobacco craving and withdrawal symptoms in temporarily abstinent smokers. This was accompanied by a decrease in plasma TRP and a sex dependent increase in blood serotonin.[69]

2.11 **Marcus R Munafo *et al.*, (2007)** investigated the impact of the administration of a tyrosine-depleting amino acid mixture compared to a balanced mixture on measures of mood, craving and selective processing of smoking-related cues in healthy cigarette smokers instructed to abstain from smoking for 12 hr prior to, and during, the experiment. A modified stroop task was used by them to index selective processing of smoking-related cues. They observed evidence for an increase in subjective craving among males, and an attenuation of the selective processing of smoking-related cues compared to control cues among females, in the tyrosine-depleting condition compared to the balanced condition. No effects of mixture were observed on measures of subjective mood. Their results tentatively supported for the role of dopaminergic neurotransmission in mediating the response of cigarette smokers to smoking-related cues. In addition, their results also provided further evidence for sex differences in the factors that maintain cigarette smoking, in particular with respect to conditioned reinforcement of smoking behaviour, and suggested the relationship between subjective craving and selective processing of smoking-related cues may differ in males and females.[70]

2.12 **Patrick F Sullivan *et al.*, (2001)** investigated the association between two markers in the seventh intron of the tryptophan hydroxylase gene (TPH C218A and C779A) in a population-based case control study of 780 genotyped subjects. As in prior studies, the two markers were in strong linkage disequilibrium. The phenotypes they studied were smoking initiation and progression to nicotine dependence. Allele, genotype, and estimated haplotype frequencies for each marker were highly significantly different for smoking initiation ($P < 0.0004$ for each comparison) and were non- significant for progression to nicotine dependence. An empirical test suggested them that the positive results were

unlikely to have resulted from population stratification. Their results were similar to those of Lerman et al. [2001: Am J Med Genet (Neuropsychiatr Genet) 105:000–000] in associating these TPH markers with a construct related to smoking initiation but dissimilar in the variable implicated. If their results replicate in other samples, the serotonergic system may be involved in the etiology of smoking initiation given the rate-limiting role of TPH in the biosynthesis of serotonin.[71]

2.13Durk Fekkesa *et al.*, (2001) investigated whether in healthy subjects L-tryptophan may serve as a precursor for the endogenous synthesis of the β-carboline norharman. For this purpose subjects, smokers as well as non-smokers, received 0 or 1.2 g of an oral dose of tryptophan. In their study smokers started the experiment 2 h after cessation of smoking. Plasma levels of tryptophan and norharman were measured 100 and 125 min after the start of the experiment. The levels of both compounds were significantly higher in the group receiving tryptophan. Norharman concentrations in the plasma of smokers were significantly higher than in the non-smoking subjects under both experimental conditions. Their results added some proof to the hypothesis that in humans tryptophan may serve as a precursor for the synthesis of norharman.[72]

2.14Ju Wang *et al.*, (2010) revealed a significant genetic contribution to the risk of smoking initiation and progression (SI/P), nicotine dependence (ND), and smoking cessation (SC). Further, numerous genes have been implicated in these smoking-related behaviors, especially for ND. However, no study has presented a comprehensive and systematic view of the genetic factors associated with these important smoking-related phenotypes. By reviewing the literature on these behaviors, they identified 16, 99, and 75 genes that have been associated with SI/P, ND, and SC, respectively. They then determined whether these genes were enriched in pathways important in the neuronal and brain functions underlying addiction. They identified 9, 21, and 13 pathways enriched in the genes associated with SI/P, ND, and SC, respectively. Together, their findings showed significant genetic overlap among these three related phenotypes. Although identification of susceptibility genes for smoking-related behaviors is still in an

early stage, the approach used in this study has the potential to overcome the hurdles caused by factors such as genetic heterogeneity and small sample size, and thus should yield greater insights into the genetic mechanisms underlying their complex phenotypes.[73]

2.15 Douglas M. Ziedonis *et al.*, (1997) performed smoking cessation program for 24 smokers with schizophrenia. Fifty percent completed the program, 40% decreased use by 50%, and 13% remained abstinent (carbon monoxide verified) for 6 months. Nicotine replacement, motivational enhancement therapy, and relapse prevention behavioral therapy were important components of their treatment. They conducted study in the CMHC outpatient programs in which about 750 patients were diagnosed with schizophrenia or schizoaffective disorder, and about 70% of these individuals smoke. Hall found low motivation to quit smoking among their sample of 300 chronic psychiatric patients. About 85% were in the precontemplation or contemplation stage of the Prochaska and DiClemente (1983) readiness for change model. Their clients were not interested in stopping smoking in the next 6 months. Interestingly, about three-fourths of the patients in their study presented in the contemplation stage (admit smoking is a problem, but probably will not want to quit in the next 6 months).They summarised that smoking cessation treatment can work, but more study is required in this area. MET seemed to improve outcomes, and nicotine replacement was an essential ingredient of treatment.[74]

2.16 Mark F.McCarty *et al.*, (1982) studied that nicotine increases the release and turnover of catecholamines in the brain, and many features of the tobacco withdrawal syndrome — such as drug craving, poor concentration, impaired motor performance, drowsiness, fatigue, increased appetite with hyperphagia — may reflect diminished central catecholaminergic tone. Support of central catecholamine synthesis with the nutrients tyrosine and glucose tolerance factor (which enhance brain tyrosine levels) may lessen tobacco withdrawal symptoms and may increase the chance of success in smoking cessation programs. Nutritional measures of their type would probably be safer and more appropriate for long-term use than central-stimulant drugs.[75]

2.17 **Alice Simon *et al.*, (2016)** prepared engineered particles of rivastigmine hydrogen tartrate (RHT) to characterize the physicochemical and aerodynamic properties, in comparison to a lactose carrier formulation (LCF). They prepared microparticles from ethanol/water solutions containing RHT with and without the incorporation of L-leucine (Leu), using a spray dryer. Dry powder inhaler formulations prepared by them were characterized by scanning electron microscopy, powder X-ray diffraction, laser diffraction particle sizing, ATR-FTIR, differential scanning colorimetry, bulk and tapped density, dynamic vapour sorption and *in vitro* aerosol deposition behaviour using a next generation impactor. They altered smooth-surfaced spherical morphology of the spray dried microparticles by adding Leu, resulting in particles becoming increasingly wrinkled with increasing Leu. Powders presented low densities. Their glass transition temperature for formulation was sufficiently high (>90 °C) to suggest good stability at room temperature. As Leu content increased, spray dried powders presented lower residual solvent content, lower particle size, higher fine particle fraction (FPF < 5 μm), and lower mass median aerodynamic diameter (MMAD). The LCF showed a lower FPF and higher MMAD, relative to the spray dried formulations containing more than 10% Leu. Spray dried RHT powders presented better aerodynamic properties, constituting a potential drug delivery system for oral inhalation.[76]

2.18 **Zeng XM *et al.*, (2001)** investigated the interdependence of carrier particle size, surface treatment of the carrier, and inclusion of fines on the drug delivery from dry power inhaler formulations. In their study two size fractions (< 63 and 63-90 micron) of alpha-lactose monohydrate were subjected to treatment with 95% (v/v) ethanol to introduce small asperities or cavities onto the otherwise smooth surface without substantially changing the particle shape. After blending with albuterol sulfate [ALB; volume median diameter (VMD), 1.9 micron; geometric standard deviation (GSD), 1.5], the solvent-treated lactose produced a fine particle fraction (FPF; < 6.18 microm) and dispersibility of the drug that was significantly (ANOVA $p < 0.01$) lower than that which resulted from formulations containing untreated lactose of a similar size fraction, after aerosolization at 60 L min(-1) via a Rotahaler. The findings of their study

indicated the presence and characteristics of the finer fraction of lactose carrier particles dominate over the particle size and surface smoothness of the carrier particles in determining dispersion and deaggregation of drugs from dry powder formulations for inhalation.[77]

***2.19*Xiao-FeiYang *et al.*, (2015)** used antibiotics in dry powder inhalation for the treatment of lung infections as it has attracted drastically increasing attention offering rapid local therapy at lower doses and minimal side effects. In their study, aztreonam (AZT) was used as the model antibiotic and spray-dried to prepare powders for inhalation. Amino acids of glycine (GLY), histidine (HIS) and leucine (LEU) were used by them as excipients to modify the spray-dried particles. In comparison with the AZT spray-dried powders, HIS-modified spray-dried powders showed increased compressibility, indicating larger distance and less cohesion between particles; while the LEU-modified spray-dried particles showed a hollow structure with significantly decreased densities. The fine particle fraction for HIS- and LEU-modified powders was 51.4% w/w and 61.7% w/w, respectively, and both were significantly increased (one-way ANOVA, Duncan's test, P <0.05) compared to that of AZT spray-dried powders (45.4% w/w), showing a great potential to be applied in clinic.[78]

***2.20*Yingtong Cui *et al.*, (2018)** studied with Netilmicin (NTM) which is one of the first-line drugs for lower respiratory tract infections (LRTI) therapy, but its nephrotoxicity and ototoxicity caused by intravenous injection restrict its clinical application. Due to the nature of NTM hygroscopicity that hinders its direct use through DPI, they used L-leucine (LL) into NTM dry powder to reduce its moisture absorption rate and improve its aerosolization performance. They prepared NTM DPIs using spray-drying with different LL proportions. The particle size, density, morphology, crystallinity, water content, hygroscopicity, antibacterial activity, in vitro aerosolization performance, and stability of each formulation were characterized by them. NTM DPIs were suitable for inhalation and amorphous with a corrugated surface.They summarized that leucine can be used to enhance the dispersibility and aerosolisation properties of spray-dried powders for pulmonary drug delivery.[79]

***2.21*P.C. Seville *et al.*, (2007)** investigated the use of the amino acids arginine, aspartic acid, leucine, phenylalanine and threonine as 'dispersibility enhancers' in spray-dried powders for inhalation. Parameters such as spray-dried yield, tapped density, and Carr's Index were not predictive of their aerosolization performance. In addition, whilst the majority of amino acid-modified powders displayed suitable particle size distribution for pulmonary administration and potentially favourable low moisture content, in vitro particle deposition was only enhanced for the leucine-modified powder. They summarized that leucine can be used to enhance the dispersibility and aerosolization properties of spray-dried powders for pulmonary drug delivery.[80]

***2.22*T E G K Murthy *et al.*, (2010)** developed Dry powder inhalers (DPIs) for Triotropium Bromide with a view to treat bronchospasm associated with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema effectively. They prepared the formulations with different grades of Lactose monohydrate like Lactohale 300, Sorbolac400, Inhalac 230, Respitose SV003, DCL11 and Flowlac100 and evaluated for Physical appearance, Average fill weight per capsule, content uniformity, Uniformity of delivered dose, Emitted dose, Moisture content, Assay ,and Locking length. The influence of composition of DPI and overages on performance of DPI were studied them. The better fine particle fraction was obtained from the DPIs formulated with 10:90 ratio of fine lactose (Lactohale 300): coarsey lactose (Respitose SV003) and having 20% w/w overages.[81]

***2.23*Marcelde Matas *et al.*, (2008)** investigated the use of artificial neural networks (ANNs) for generating models to predict the relative lung bioavailability and clinical effect of salbutamol when delivered to healthy volunteers and asthmatic patients from dry powder inhalers (DPIs). They used ANN software to model in vitro, demographic and in vivo data from human subjects for four different DPI formulations containing salbutamol sulfate. In 12 volunteers, a model linking the in vitro aerodynamic characteristics of the emitted dose and volunteer body surface area with the urinary excretion of drug and its metabolite in the 24 h period after inhalation was established. In 11 mild asthmatics, a predictive model

correlating in vitro data, baseline lung function, body surface area and age with post-treatment improvements in forced expiratory volume in 1 s (FEV1) was also generated. Models validated using unseen data from individual subjects receiving the different DPI formulations were shown to give predictions of in vivo performance. The squared correlation coefficients (R^2) for plots comparing predicted and observed in vivo outcomes were 0.83 and 0.84 for urinary excretion and lung function data, respectively. They finally concluded that ANN models have the potential to predict the in vivo performance of DPIs in individual subjects.[82]

***2.24*Joanna Muddle *et al.*, (2017)** investigated the feasibility of using artificial neural networks (ANNs) to predict fine particle fraction (FPF) based on formulation device variables. Thirty-one ANN architectures were evaluated for their ability to predict experimentally determined FPF for a self-consistent dataset containing salmeterol xinafoate and salbutamol sulfate dry powder inhalers (237 experimental observations). Principal component analysis was used to identify inputs that significantly affected FPF. Orthogonal arrays (OAs) were used to design ANN architectures, optimized using the Taguchi method. The primary OA ANN r2 values ranged between 0.46 and 0.90 and the secondary OA increased the r2 values (0.53-0.93). The optimum ANN (9-4-1 architecture, average r2 0.92 ± 0.02) included active pharmaceutical ingredient, formulation, and device inputs identified by principal component analysis, which reflected the recognized importance and interdependency of these factors for orally inhaled product performance. The Taguchi method was effective for them at identifying successful architecture with the potential for development as a useful generic inhaler ANN model, although this would require much larger datasets and more variable inputs.[83]

***2.25*Deepak J Singh *et al.*, (2015)** prepared surface modified lactose (SML) particles using force control agents, and studied in vitro performance on dry powder inhaler (DPI) formulation of Fluticasone propionate. With a view to reduce surface passivation of high surface free energy sites on the most commonly used DPI carrier, α- lactose monohydrate, effects of various force control agents such

as Pluronic F-68, Cremophor RH 40, glyceryl monostearate, polyethylene glycol 6000, magnesium stearate, and soya lecithin were studied by them. They prepared DPI formulations with SML showed improved flow properties, and atomic force microscopy (AFM) studies revealed decrease in surface roughness. The DSC and X-ray diffraction patterns of SML showed no change in the crystal structure and thermal behavior under the experimental conditions. The fine particle fraction (FPF) values of lactose modified with Pluronic F-68, Cremophor RH 40, glyceryl monostearate were improved, with increase in concentration up to 0.5%. Soya lecithin and PEG 6000 modified lactose showed decrease in FPF value with increase in concentration. Increase in FPF value was observed with increasing concentration of magnesium stearate. Two different DPI devices, Rotahaler® and Diskhaler®, were compared to evaluate the performance of SML formulations. FPF value of all SML formulations were higher using both devices as compared to the same formulations prepared using untreated lactose. One month stability of SML formulations at 40°C/75% RH, in permeable polystyrene tubes did not reveal any significant changes in FPF values.[84]

***2.26*P.S. Uttekar *et al.*, (2013)** prepared microparticles for Dry Powder Inhalation, produced by environmentally driven Amphiphilic crystallization technique process by using different nonionic surfactants at different concentration and at different processing parameters, for inhalation therapy. Budesonide, as one of the inhaled glucocorticosteroids, is widely used in the treatment of asthma by pulmonary delivery. The objective of their work was to developed microcrystals by using Amphiphilic crystallization process with different nonionic surfactants at different processing parameters. Salvation of Budesonide in aqueous Cremophor EL and Tween 20(1:1) was investigated by them using HPLC. A response surface type central composite design were employed using Design-Expert 5.0 software (StatEase, QD Consulting, Penzance, UK) with the factors investigated were stirrer speed, antisolvent addition rate, Cremophor EL and Tween 20. The crystals were filtered and freeze dried. They optimized the process variables for narrow particle size distribution (PSD). Cremophor EL and Tween 20 were added as the stabilizers. Their freeze dried crystals were subjected to XRD, DSC and SEM analysis for stability. The PSD also depended on the

balance of meso and micromixing determined by the crystallization conditions. Their optimized formulation was identified and characterized to determine their suitability for pulmonary delivery by using MSLI. Optimized formulation showed the highest FPF loaded and FPF emitted of 42 (1%) and 69 (3%) respectively, depositing mainly on stages 3 and 4, with much lower amounts collected on the higher stages of the MSLI.[85]

2.27 **Marina Andrade-Lima *et al.*, (2012)** evaluated the pharmaceutical equivalence of a test formulation (fixed-dose combination of budesonide and formoterol fumarate in a single capsule dispensed in an Aerocaps® inhaler) in relation to a reference formulation (budesonide and formoterol fumarate in two separate capsules dispensed in an Aerolizer® inhaler). They performed an in vitro study in which identification/quantification of the active ingredients by HPLC was performed and determined dose uniformity and aerodynamic particle size distribution in the test and reference formulations. In their test formulation, the content of budesonide and formoterol was 111.0% and 103.8%, respectively, compared with 110.5% and 104.5%, respectively, in the reference formulation. In the test formulation, dose uniformity regarding budesonide and formoterol was 293.2 µg and 10.2 µg, respectively, whereas it was 353.0 µg and 11.1 µg in the reference formulation. Their values were within the recommended range for this type of formulation (75-125% of the labeled dose). The fine particle fraction (< 5 µm) for budesonide and formoterol was 45% and 56%, respectively, in the test formulation and 54% and 52%, respectively, in the reference formulation. Hence they concluded that for both of the formulations tested, the levels of active ingredients, dose uniformity, and aerodynamic diameters were suitable for use with the respective dry powder inhalers.[86]

2.28 **Abolghasem Safdar *et al.*, (2016)** prepared the inhalable micro particle from propranolol by spray drying method. They chose this because prescription of propranolol by the oral route of administration suffers from the hepatic first pass metabolism effect. This phenomenon caused to decrease the oral bioavailability of the propranolol. It is possible to eliminate the hepatic first pass metabolism effect by prescription of the propranolol inhalable micro particle via the

pulmonary system. The different solvent as the spray drying vehicle such as water and the mixture of the water and ethanol were applied by them for preparation of the propranolol inhalable micro particle during the spray drying process. The physical characteristics of the micro particles such as true and bulk density, size, shape and aerodynamic behavior of the particles were evaluated by the in vitro test. In the in vivo tests they insufflated the inhalable microparticles to the lung of the rats. The plasma samples 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8 hours after insufflation of the drug to the lung of the animal, intravenous and oral administration of drug were collected by them. The concentration of propranolol in the plasma samples were measured by the HPLC method. The pharmacokinetic parameters of the drug such as AUC0-60, Tmax, Cmax, T1/2, Ke, Ka, Vd and absolute bioavailability of drug were calculated. The results of the in vitro tests showed that the type of the spray drying vehicle has the significant effect on the physical characteristics and the aerodynamic behavior of the propranolol inhalable micro particles. The presence of 75% ethanol in the spray drying vehicle caused to increase in fine particle fraction. The value of the fine particle fraction for these micro particles was ±35.771. Their results of the in vivo tests showed that the rate and extent of the pulmonary absorption of propranolol is more than the oral rout of administration. The presences of the different excipients in the spray drying vehicle and the type of the spray drying vehicle have the significant effect on the pulmonary absorption of the drug. They concluded that it is possible to reach 0.69+0.27 as the value of the absolute bioavailability by prescribing the propranolol via the pulmonary system.[87]

***2.29*Rayehe Teymouri Rad *et al.*, (2019)** prepared inhalable tadalafil nanocomposites as a dry powder formulation by spray drying technique for increasing bioavailability and treatment efficacy, as well as decreasing systemic side effects. They used D-optimal design for optimization of formulation parameters. Microparticle size, morphology, crystallinity, density, solubility, redispersion (%), and in-vitro inhalation performance of tadalafil nanocomposites were investigated by them as physicochemical characteristics. Pharmacokinetic parameters were also evaluated by them in plasma and lung tissue of Wistar rats after intratracheal insufflation and compared with a control

group receiving an oral tadalafil marketed product (dose=10 mg/kg). Their suggested optimum formulation contained stable amorphous particles with almost rounded shape and corrugated surface that were completely redispersed in the lung simulated medium with the mass median geometric diameter of 3.2µm, density of 1.4g/cm3, fine particle fraction based on emitted dose (%) of 57.2 ± 6.5 %, and 13.7-fold enhancement in dissolution rate. Their in-vivo studies showed that the ratio of AUC0-24h lung /AUC0-24h plasma, achieved in the treated group after intratracheal insufflation, was significantly higher than the control group that means high local drug concentration and more efficacy. Besides, plasma data analysis indicated high value of MRT (2.3-fold) and t_{max} (3.7-fold) after intratracheal insufflation of tadalafil nanocomposites in comparison with the conventional oral route, indicating longer retention of tadalafil molecules in the lungs and their slower entry to the systemic blood circulation. They concluded that inhalable tadalafil nanocomposites can be introduced as an alternative to oral tadalafil in the treatment of PAH.[88]

***2.30*Birendra Chaurasiya *et al.*, (2018)** considered delivery of inhalational dry powders (DPs to the lung of mice is pivotal for pre-clinical pharmacokinetic and pharmacodynamic investigations. Although several devices have been reported, their application is always limited by many factors, including complicated design, high price, commercially discontinued status, as well as requirement of special skills. So they have introduced a simple device for non-invasive and precise delivery of DPs in mice. They set up the self-made device using a 20 G cannula tube and a 1 mL syringe. Subsequently, they validated in terms for proper installation, delivery of dry powder and safety. Taken together, they believed that this device will be helpful in pre-clinical studies, especially in laboratory experiments, for respiratory drug delivery in small animal models.[89]

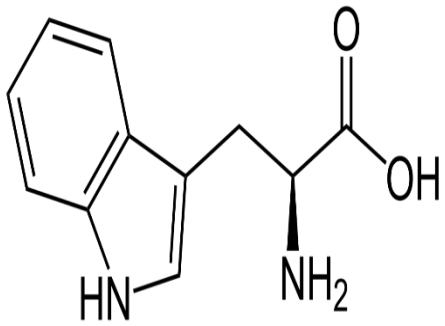
***2.31*Duret C *et al.*, (2012)** evaluated the ability of the Penn-Century Dry Powder Insufflator for mice (DP-4M) to reproducibly, uniformly, and deeply deliver dry powders for inhalation in the mouse lung. Itraconazole-based dry powder formulations produced by spray-drying were different in terms of composition (different ratios of drug and mannitol, with or without phospholipids), but

relatively similar in terms of particle size and mass median aerodynamic diameter. The ability of the dry powder insufflator to disaggregate each formulation was the same, indicated by the absence of a statistically significant difference between the particle size distribution parameters, as measured by laser scattering. The emitted fraction varied in vivo compared to the in vitro condition. Their Fluorescent particle distribution in the lungs was uniform and reached the alveolar spaces, as visualized by fluorescent microscopy. In terms of drug recovery in lung tissue, a minimum administered powder mass (in this case ~1 mg) was necessary for them to recover at least 30% of the emitted dose in the lung and to obtain reproducible pulmonary concentrations. To reduce the dose administered in the lung, they preferred to dilute the active ingredient within the carrier instead of reducing the dry powder mass inserted in the sampling chamber.[90]

3. DRUG & EXCIPIENT PROFILE

3.1.L-TRYPTOPHAN

3.1.1.Structure:



3.1.2.Molecular Formula:

$C_{11}H_{12}N_2O_2$

3.1.3.Molecular mass:

204.229 g/mol

3.1.4.IUPAC Name:

(2S)-2-amino-3-(1H-indol-3-yl)propanoic acid

3.1.5.Description:

White to slightly yellowish-white crystals or crystalline powder odorless with a flat taste. An essential amino acid that is necessary for normal growth in infants and for nitrogen balance in adults. it is a precursor of indole alkaloids in plants. it is a precursor of serotonin (hence its use as an antidepressant and sleep aid). it can be a precursor to niacin, albeit inefficiently, in mammals.

Tryptophan is the least plentiful of all 22 amino acids and an essential amino acid in humans (provided by food), Tryptophan is found in most proteins and a precursor of serotonin. Tryptophan is converted to 5-hydroxy-tryptophan (5-HTP), converted in turn to serotonin, a neurotransmitter essential in regulating appetite, sleep, mood, and pain. Tryptophan is a natural sedative and present in dairy products, meats, brown rice, fish, and soybeans.

3.1.6.Physical state:

Solid

3.1.7.Melting point:

290.5°C

3.1.8.Water Solubility:

13400 mg/L (at 25 °C)

Slightly soluble in acetic acid, ethanol; insoluble in ethyl ether.

3.1.9.pH:

5.5 to 7.

3.1.10.Decomposition temperature:

282°C .When heated to decomposition it emits toxic fumes of nitric oxide.

3.1.11.pKa:

7.38 (at 25 °C)

3.1.12.Maximum Absorption (λ max):

280nm

3.1.13.Mechanism in smoking cessation:

L-tryptophan is considered an essential amino acid because our bodies can't make it. It is important for the development and functioning of many organs in the body. After absorbing L-tryptophan, our bodies convert it to 5-HTP (5-hyrdoxytryptophan), and then to serotonin. Serotonin is a hormone that transmits signals between nerve cells. It also causes blood vessels to narrow. Changes in the level of serotonin in the brain can alter mood.

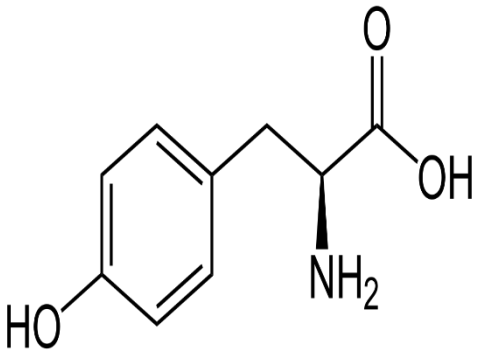
Tryptophan (50 mg/kg/day) has been used as an adjunct therapy for smoking cessation. During a two-week study, tryptophan-treated subjects experienced fewer nicotine withdrawal symptoms and were able to abstain or smoke fewer cigarettes than controls.

3.1.14.Side effects:

L-tryptophan can cause some side effects such as heartburn, stomach pain, belching and gas, nausea, vomiting, diarrhoea, and loss of appetite. It can also cause headache, light headedness, drowsiness, dry mouth, visual blurring, muscle weakness, and sexual problems.[91]

3.2.L TYROSINE

3.2.1.Structure:



3.2.2.Molecular Formula:

$C_9H_{11}NO_3$

3.2.3.Molecular mass:

181.191 g/mol

3.2.4.IUPAC Name:

(2S)-2-amino-3-(4-hydroxyphenyl)propanoic acid

3.2.5.Description:

L Tyrosine is a non-essential amino acid white solid fine silky needle like crystals. L-Tyrosine is the levorotatory isomer of the aromatic amino acid tyrosine. L-tyrosine is a naturally occurring tyrosine and is synthesized in vivo from L-phenylalanine. It is considered a non-essential amino acid; however, in patients with phenylketonuria who lack phenylalanine hydroxylase and cannot convert phenylalanine into tyrosine, it is considered an essential nutrient. In vivo, tyrosine plays a role in protein synthesis and serves as a precursor for the synthesis of catecholamines, thyroxine, and melanin.

3.2.6.Melting Point:

343°C

3.2.7.Decomposition temperature:

342-344°C

3.2.8.Solubility:

Water Solubility

479 mg/L (at 25 °C)

Slightly soluble in Acetic acid

0.479 mg/mL

3.2.9.pH:

5.66 (at isoelectric point)

3.2.10.pKa:

2.2 (at 25 °C)

3.2.11.Max absorption:

(0.5 N Sulfuric Acid): 275 nm (A= 450, 1 %, 1 CM); 294 nm (A= 800, 1 %, 1 CM)

3.2.12.Mechanism in smoking cessation:

Although tyrosine has numerous mechanisms of action, perhaps the most clinically significant is its role as a precursor for norepinephrine and dopamine synthesis. By improving the rate of neurotransmitter synthesis,5,6 tyrosine stimulates the central nervous system and acts as an antidepressant.

3.2.13.Side effects:

Tyrosine is generally safe with infrequent reports of side effects. Occasional nausea, diarrhoea, headaches, vomiting, or insomnia are reported by those taking higher doses of tyrosine (>150 mg/kg daily). Insomnia can be prevented by avoiding supplementation in the evening. Tyrosine has FDA GRAS status (generally recognized as safe), although safety studies in pregnancy have not been conducted. Therefore, patients who are pregnant or wish to become pregnant should consult a health care practitioner regarding tyrosine supplementation.[92]

EXCIPIENT PROFILE

3.3.LACTOSE

3.3.1.Nonproprietary Names

BP: Anhydrous Lactose

JP: Anhydrous Lactose

PhEur: Lactose, Anhydrous

USP-NF: Anhydrous Lactose

3.3.2.Synonyms

Anhydrous 60M; Anhydrous Direct Tableting (DT); Anhydrous DT High Velocity; Anhydrous Impalpable; Lactopress Anhydrous; Lactopress Anhydrous 250; lactosum anhydricum; lattosio; milk sugar; SuperTab 21AN; SuperTab 22AN; saccharum lactis.

Chemical Name and CAS Registry Number

O-b-D-Galactopyranosyl-(1!4)-b-D-glucopyranose [63-42-3]

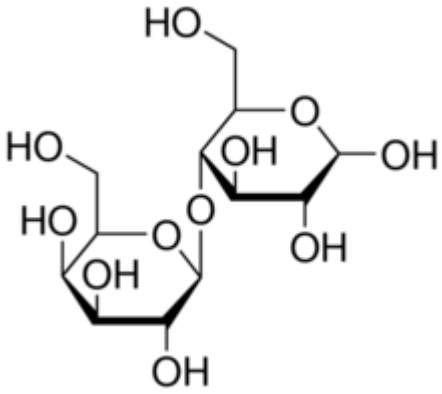
3.3.3.Empirical Formula

$C_{12}H_{22}O_{11}$

3.3.4.Molecular Weight

342.30

3.3.5.Structural Formula



3.3.6.Description

Anhydrous lactose occurs as white to off-white crystalline particles or powder. Several different brands of anhydrous lactose are commercially available which contain anhydrous b-lactose and anhydrous a-lactose. Anhydrous lactose typically contains 70–80% anhydrous b-lactose and 20–30% anhydrous a-lactose.

3.3.7.Density

0.88 g/cm^3

3.3.8.Melting point

232.08ºC

3.3.9.Solubility

Soluble in water; sparingly soluble in ethanol (95%) and ether; 40 g/100mL at 258ºC

3.3.10.Specific rotation

54.48 to 55.98

3.3.11.Stability and Storage Conditions

Mold growth may occur under humid conditions (80% RH and above). Lactose may develop a brown coloration on storage, the reaction being accelerated by warm, damp conditions;

Lactose anhydrous should be stored in a well-closed container in a cool, dry place.

3.3.12.Incompatibilities

Lactose anhydrous is incompatible with strong oxidizers. When mixtures containing a hydrophobic leukotriene antagonist and anhydrous lactose or lactose monohydrate were stored for six weeks at 408C and 75% RH, the mixture containing anhydrous lactose showed greater moisture uptake and drug degradation.(3) Studies have also shown that in blends of roxifiban acetate (DMP-754) and lactose anhydrous, the presence of lactose anhydrous accelerated the hydrolysis of the ester and amidine groups.(4) Lactose anhydrous is a reducing sugar with the potential to interact with primary(5) and secondary amines(6) (Maillard reaction) when stored under conditions of high humidity for extended periods.

3.3.13.Safety

Lactose is widely used in pharmaceutical formulations as a diluent in oral capsule and tablet formulations, and has a history of being used in dry powder inhaler formulations. Adverse reactions to lactose are largely due to lactose intolerance, which occurs in individuals with a deficiency of the enzyme lactase. Recently, the presence of milk proteins in lactose-containing dry powder inhalers, which can cause anaphylaxis in cases of severe allergy to cow's milk, has been reported.(3,4) In view of the route of administration, inhalation lactose should be tested to additional micobiological specifications, for example, endotoxins, as requested by the regulatory authorities. Inhalation lactose is typically supplied with an increased range of microbiological tests.[93]

4. AIM & OBJECTIVES

4.1.AIM:

To develop an efficient and convenient dosage form in order to eradicate the withdrawal symptoms after smoking cessation.

4.2.OBJECTIVES:

- To formulate and develop dry powder inhalation using L-Tryptophan and L-Tyrosine amino acids by Nano spray drying technique for smoking cessation therapy.
- To carry out *in vitro* study using Anderson cascade impactor for characterization of aerosol performance and Bag diffusion study to analyze percentage drug release.
- To perform *in vivo* study for the assessment of drug concentration in lung tissue and plasma pharmacokinetic study to evaluate the pharmacokinetic parameters.

5.PLAN OF WORK

5.1.PREFORMULATION STUDIES

- Selection of Raw Materials
- Construction of standard curve for L Tryptophan and L Tyrosine
- Compatibility studies
    - ✓ Fourier-Transform Infrared spectroscopic analysis(FT-IR)
    - ✓ Differential Scanning Colorimetric analysis (DSC)
- Determination of solubility of drugs
- Determination of pH and Partition coefficient

5.2.OPTIMIZATION & FORMULATION DEVELOPMENT

- Micronization of amino acids by Nano spray drying technique
- Particle size reduction of lactose by milling
- Blending and Filling

5.3.FORMULATION EVALUATION

- Physical Characterization
    - ✓ Determination of Moisture Content
    - ✓ Solid State stability studies
- Micromeritic characterization
    - ✓ Scanning Electron Microscopic analysis(SEM)
    - ✓ Elemental analysis by EDAX- Energy Dispersive Spectroscopy
    - ✓ Powder X Ray Diffraction

- ✓ Flow Characteristics
    - Bulk & Tapped density
    - Carr's index
    - Hausner ratio

5.4.CAPSULE EVALUATION

- Physical appearance
- Locking Length
- Average fill Weight per capsule
- Assay (Drug Content determination)

5.5.*IN VITRO* STUDY

- Anderson Cascade impactor
- Bag diffusion study

5.6.*IN VIVO* STUDY

- Determination of Percentage Delivered dose
- Assessment of drug concentration in lung tissue
- Plasma pharmacokinetic study

5.7.STATISTICAL ANALYISIS

6. METHODOLOGY

6.1.Materials

Table No. 2: List of Chemicals used

S.No	CHEMICALS	MAKE
1	L-Tryptophan	Nice chemicals Pvt Ltd.
2	L-Tyrosine	Nice chemicals Pvt Ltd.
3	Lactose Anhydrous (Sterile)	HiMedia Laboratories Pvt Ltd.
4	HPLC Water	HiMedia Laboratories Pvt Ltd.
5	Disodium hydrogen phosphate	HiMedia Laboratories Pvt Ltd.
6	Potassium dihydrogen phosphate	HiMedia Laboratories Pvt Ltd .
7	Sodium chloride	HiMedia Laboratories Pvt Ltd.
8	N-Octanol	HiMedia Laboratories Pvt Ltd.
9	Diethyl ether	HiMedia Laboratories Pvt Ltd.
10	Diazepam	Diastat, Valium

Table No. 3: List of instruments used

S.No	INSTRUMENTS/ EQUIPMENT	MAKE
1	Digital balance	AY 120,Shimadzu corporation.
2	Nano spray drying	B90/Buchi India Pvt.Ltd.
3	Ball mill	RETSCH
4	Double cone Blender	Shakthi Pharmatech Pvt Ltd.
5	Field Emission Scanning Electron Microscope with EDS	Tescan – Mira3 XMU
6	X-Ray Diffractometer (XRD)	X-Pert Pro, PANalytical
7	FT-IR	8400S Shimadzu
8	Differential Scanning Colorimetry	Mettler Toledo
9	Vernier caliper	Zoom Yamayo Classic
10	UV Spectrophotometer	UV-1800 Shimadzu Corporation
11	Cascade impactor	SITRA-ASTMF2101
12	Centrifuge	R8C, REMI Elektrotechnik Ltd.
13	Digital pH meter	Deluxe pH meter-101, Deep vision
14	Bath Sonicator	Labman Scientific instruments
15	Gelatin capsule size 3	ACG Pam Pharma Technologies Pvt Ltd.

6.2.METHODS:

6.2.1.PREFORMULATION STUDIES

6.2.1.1.Construction of Standard curve of L Tryptophan

Standard stock solution of L Tryptophan was prepared by dissolving 100mg of L Tryptophan in 100ml of phosphate buffer 7.4 pH as solvent.10ml of this stock solution was taken and diluted up to 100ml by using same solvent to produce a concentration of 100mcg/ml solution. Further dilutions were made from stock and the volume was made up to 10ml using the same solvent to produce 10mcg/ml, 20mcg/ml, 30mcg/ml, 40mcg/ml and 50mcg/ml up to 100mcg/ml solution respectively. Then the calibration curve was constructed by measuring absorbance at 220nm using UV visible spectrometer.

6.2.1.2.Construction of Standard curve of L Tyrosine

Standard stock solution of L Tyrosine was prepared by dissolving 100mg of L Tyrosine in 100ml of phosphate buffer 7.4 pH as solvent.10ml of this stock solution was taken and diluted up to 100ml by using same solvent to produce a concentration of 100mcg/ml solution. Further dilutions were made and the volume was made up to 10ml using the same solvent to produce 10mcg/ml, 20mcg/ml, 30mcg/ml, 40mcg/ml and 50mcg/ml up to 100mcg/ml respectively. Then the calibration curve was constructed by measuring absorbance at 283nm using UV visible spectrometer.

6.2.1.3.Compatibility studies:

Physical mixture of drug and carrier were filled in prewashed, dried plastic container and sealed. The sealed container was stored at 37ºC±0.5ºC for 28 days in stability chamber. At the end of 28 days plastic container was removed from stability chamber and subjected to drug-excipient compatibility studies by FTIR analysis and DSC.

a. Fourier-Transform Infrared Spectroscopic analysis(FT-IR):

Pure amino acids such as L Tryptophan, L Tyrosine, Anhydrous Lactose and physical mixture of Amino acids with carrier in the ratio of 1:1:1 were subjected to IR spectral studies using FTIR Spectrophotometer. A physical mixture of amino acids and carrier was mixed with desirable quantity of potassium bromide. 100mg of this mixture was

compressed to form a transparent pellet using hydraulic press at 15 tons pressure. It was scanned from 4000-400cm^{-1} in a FTIR-8400 Shimadzu, Japan. The individual spectra of drug and carrier were performed.

b. Differential scanning colorimetric analysis (DSC):

Differential scanning calorimetry (DSC) is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. It is used to determine the purity and stability of the sample. The compatibility of lactose with drug used in the formulation was determined by using DSC. Weighed samples (5±10mg, Mettler M3 Microbalance) of the individual components or drug-excipients combinations (75±150 mm sieve fraction) were scanned in Al pans pierced with a perforated lid at 10 K min in the 30 ± 2008ºC temperature range under static air, using a Mettler TA4000 apparatus equipped with a DSC 25 cell.

6.2.1.4.Determination of Solubility of drugs:

Saturation solubility of L Tryptophan and L Tyrosine were determined by shake flask method in water, pH 4.0, pH 6.8, and pH 7.4. Excess quantities of drug were added in measured quantity of distilled water and buffer, which is then incubated in orbital shaker at 37°C and at 100 rpm for 24 hrs. Solutions were filtered through Whatman filter paper. Absorbance of resulting solutions was measured on UV spectrophotometer at 220nm and 283nm respectively for L Tryptophan and L Tyrosine. Saturation solubility was then calculated by plotting measured absorbance value in calibration curve.

6.2.1.5.Determination of pH and Partition coefficient:

Drug solutions were prepared and pH was determined by using digital pH meter.

Lipophilic/hydrophilic balance is one of the most important contributing factors for optimum drug absorption and delivery. Oil-water partition coefficient gives the idea about drug's ability to cross the lipidic membrane. The partition coefficient study was performed by using n-octanol as oil phase and water (1:1) as aqueous phase. The two phases were mixed in equal quantities in a separating funnel and weighed amount of drug was added to it. Two phases were allowed to saturate for 2hr at room temperature. The two phases were separated and drug content was determined. The partition coefficient was measured by using the formula

$$\text{Log p} = \frac{\text{Conc of drug in oil phase}}{\text{Conc of drug in aqueous phase}}$$

6.2.2.OPTIMIZATION & FORMULATION DEVELOPMENT

6.2.2.1.Micronization of Amino acids by Nano Spray drying technique

20% concentration of amino acid solution was prepared individually and spray dried using Nano spray dryer maintaining inlet temperature 100 °C, measured outlet temperature 48 °C, solution feed rate 40 ml/min, aspirator rate 100% and atomising airflow 700 l/h.

The optimization of the process is done by the following parameters in the instrument.

Table No. 4 : Optimization parameters of the DPI formulation

S.No	PARAMETERS	VALUES
1	Volume	1%
2	Nozzle	4µm
3	Pump	3
4	Gas flow	119-136 L/min
5	Inlet temperature	120ºC
6	Outlet temperature	48-51 ºC
7	Spray	100%
8	Head temperature	127 ºC
9	Pressure	37-43mbar
10	Aspirator	37.95

6.2.2.2.Particle size reduction of Lactose by Milling:

The anhydrous Lactose was fed into the ball mill from the left through a 60° cone and the product is discharged through a 30° cone to the right. As the shell rotates, the balls are lifted up on the rising side of the shell and then they cascade down (or drop down on to the feed), from near the top of the shell. The solid particles in between the balls and ground are reduced in size by impact.

6.2.2.3.Blending and Filling:

The optimized formulations were blended in a shaker mixer for 1hour at 42 revolutions per minute to obtain a homogeneous mixture. The obtained mixture was weighed accurately and filled in capsule size 3.

6.2.3.FORMULATION EVALUATION

6.2.3.1.Physical characterization:

6.2.3.1.1.Moisture content determination:

Moisture content of dry powder was determined by keeping it inside the desiccator under reduced pressure by applying vacuum for 24 hours. Initial weight and the final weight after 24 hrs was noted and the moisture content was determined by using formula.

$$\text{Moisture content [\%W/W]} = \frac{\text{Initial weight- Final weight}}{\text{Initial weight}} \times 100$$

6.2.3.1.2.Solid state stability studies:

Optimized formulation was selected for stability studies. The capsules filled with dry powder inhalation was packed in High density polyethylene 30cc container and sealed and studies were carried out for 90 days by keeping at 25 ºC/60% RH and 40 ºC/75% RH. Samples were withdrawn on 30th, 60th & 90th day and checked for changes in drug content.[94]

6.2.3.2.Micromeritic characterization

6.2.3.2.1.Scanning electron microscopic analysis(SEM):

The morphology of the spray dried amino acids (L Tryptophan and L Tyrosine) and milled Lactose were studied using a scanning electron microscope at 5 kV. The particle size was optimized to 1-4µm and 50-150 µm for amino acids and Lactose respectively.

6.2.3.2.2.Elemental analysis by EDAX- Energy Dispersive Spectroscopy:

The number of elements in a drug molecule and its weightage was analysed using EDAX.

6.2.3.2.3.Powder X-ray diffraction:

The solid state nature of powders was evaluated by X-ray powder diffraction measurements using X-Ray diffractometer. Measurements were taken from 5ºC to 40ºC on the two theta scale at a step size of 0.017 per s. A minimum of two analyses was performed for each sample.

6.2.3.2.4.Flow characteristics

a) Bulk and tapped density:

Bulk density was measured by weighing the amount of powder required to occupy 1 mL volume in a graduated glass syringe.

Tapped density was then evaluated by tapping the syringe onto a level surface at a height of 5 cm, 100 times. The result was obtained by calculating the ratio of the mass to the tapped volume of the sample. The results given were the average of three determinations.[76]

b) Carr's index:

Carr's index was calculated using the formula

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

The nature of flow was inferred by comparing the data with the index given below:

Table No. 5: Flow property determination based on Consolidation index

CONSOLIDATION INDEX (%)	FLOW
5-15	Excellent
12-16	Good
*18-21	Fair to passable
*23-35	Poor
33-38	Very poor
>40	Very very poor

c) Hausner ratio:

Hausner ratio was determined from the formula below

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

The nature of flow is inferred by comparing the data given below[95]

Table No. 6: Flow property determination based on Hausner ratio

Hausner ratio	Type of Flow
<1.25	Good flow
1.25-1.5	Moderate
>1.5	Poor flow

6.2.4.CAPSULE EVALUATION

6.2.4.1.Physical appearance

The capsules were visually observed for its size, shape and integrity.

6.2.4.2.Locking length

Locking length of the capsules were checked using Vernier callipers and readings were recorded .

6.2.4.3.Averages fill weight per capsule

Open the 20 capsule without losing any part of the shell and remove the contents as completely as possible.

20 capsules were weighed and the average of fill weight was determined using the following formulae[80]

$$\text{Average fill weight (mg)} = \frac{\text{20 Capsules content in mg}}{20}$$

6.2.4.4.Assay (drug content determination):

10 capsules were weighed and transferred into a 100 ml volumetric flask to which 10 ml of water was added and kept in a sonicator to dissolve the capsule. Suitable volume of diluent(Phosphate buffer; pH 7.4) was added to the above mixture and allowed to sonicate for 10 minutes. Then the mixture was filtered through 0.45µm membrane and the drug content was determined by measuring the absorbance using UV Spectrometer at 220nm and 283nm for L Tryptophan and L Tyrosine respectively. Drug content was calculated using the formula

$$\text{Amount of drug} = A_{Sam} \times C_{Std} / A_{Std}$$

Where.,

A_{Sam} = absorbance of Sample solution

C_{Std} = Concentration of drug in standard solution

A_{Std} = Absorbance of standard drug solution

C_{Std} and A_{Std} were obtained from the standard graph.[96]

6.2.5.*IN VITRO* STUDY

6.2.5.1.Anderson cascade impactor:

The aerodynamic particle deposition was measured using Anderson cascade impactor (ACI). The ACI consisted of initiation port (IP), pre-separator (PS), seven stages and a final collection filter. The stages were coated with polypropylene glycol dissolved in hexane (2% w/w). The stages were left to dry under ambient conditions for one hour. Experiments were conducted at an air flow rate of 60L/min. The operating conditions and theoretical cut-off diameters were shown in table 7. After actuation the wash solutions from different parts were collected and quantitated for drug content by UV Spectrophotometer. The respirable fractions (RF) and emission dose (ED) were calculated to describe the inhalation properties of DPIs. Six replicates of the measurements were performed.[96]

Table 7: Operating conditions and theoretical cut-off diameters of ACI

ACI Parameters	**ACI Results**
Flow rate (L/min)	60
Time per actuation (s)	4
Volume per actuation (L)	4
Cut off diameter (µm)	
Stage 0	5.7
Stage 1	4.6
Stage 2	3.2
Stage 3	2.1
Stage 4	1.3
Stage 5	0.7
Stage 6	0.39
Stage 7	0.15
Stage 8	Filter

6.2.5.2.Bag diffusion study:

The *in vitro* release of the amino acids was determined by a dialysis bag diffusion technique. The *in vitro* release was carried out with Phosphate buffer of pH 7.4 as the diffusion medium and a dialysis membrane of 14kDa molecular weight cut off (HiMedia Mumbai, India). An aqueous dispersion equivalent to 1mg of Amino acids were placed in a dialysis bag and sealed at both ends. The dialysis bag was immersed in 250ml of diffusion medium and stirred at 100 rpm. 1ml sample was withdrawn at predetermined time intervals, and the receptor phase was replenished with an equal volume of blank after each sample was withdrawn. Drug content in aliquots were evaluated using UV method and the graph was plotted with the percent drug release versus time. Absorbance of the samples at 220nm and 283nm was determined by UV/Vis spectrophotometer with pH 7.4 Phosphate Buffer as blank. The cumulative percent drug at various time intervals was calculated and plotted against time. Experiments were done in triplicate.[97,98]

6.2.6.*IN VIVO* STUDY

This study was approved by Institutional Animal Ethical Committee Reg. No: 1762/PO/R/S/14/CPCSEA and the IAEC no is KFMSR/M.Pharm/01/2019-20.

Animals were purchased from the Kerala Veterinary and Animal Sciences University, Thiruvazhamkunnu, Kerala and housed for a period of one week at Animal house, Karpagam Faculty of Medical Sciences, Coimbatore to check the animal activities before starting the study.

6.2.6.1.Animals

Wistar Rats of both sexes weighing between 150-200 g were used for this study.

6.2.6.2.Experimental Procedure:

6.2.6.2.1.Determination of % Delivered dose:

The delivered dose of Dry powders(DPs) from device was calculated on the weight basis. Cannula tube was weighted before loading of DPs and after delivery of DPs.[98,99] The process was repeated for three times and the delivered dose of DPs from device was calculated by using the following equation,

% Delivered dose = loaded dose - remained dose in cannula tube X 100

----

loaded dose

6.2.6.2.2Assessment of drug concentration in lung tissue:

Table No. 8: Grouping for assessment of drug concentration in lung tissue

Category	**Drug**	**Dose**	**Route of administration**	**Time interval (hrs)**	**No of Animals**
Group I	DPI formulation	10mg/kg	Intratracheal	½	6
Group II				1	6
Group III				2	6
Group IV				3	6
Group V				4	6
Total number of animals					30

The animals were sacrificed at various time intervals 1/2, 1, 2, 3 and 4. At each sampling time point, lung tissues were removed, rinsed twice with normal saline (0.9% w/v), and homogenized with 20 µl of methylparaben solution (100 µg/ml) as internal standard and 20 µl of NaOH (1 N) was added to 120 µl of lung or plasma sample and vortex-mixed for 10 min. The resultant mixture was centrifuged. After centrifugation, the supernatant layer was separated and analysed by UV Spectrophotometer.[99,100]

6.2.6.2.3.Plasma Pharmacokinetic Study

Table No. 9: Grouping for Plasma Pharmacokinetic study

Group	Sample	Dose	Route of administration	No. of animals
Group 1	Control	-	-	6
Group 2	Test 1	10mg/kg	Oral	6
Group 3	Test 2	10mg/kg	Intravenous	6
Group 4	Test 3	10mg/kg	Intra-tracheal	6
Total number of animals				24

Weigh the animals 150-200g, number them and divide into different groups consisting of 6 animals in control group, 6 in test group I, 6 animals in test group II and 6 animals in test group III. The control group will be kept under observation without drug. The drug will be administered oral for test group II, intravenous for test group III and intra tracheal for test group IV. Before intratracheal administration each animal was anesthetized by intraperitoneal injection of 0.1 ml Diazepam. Then, animals were quickly fixed on the self-prepared 45° angled platform. The tongue of each animal was gently pulled out with the help of blunt plastic tipped forceps and otoscope was used to visualize the tracheal opening to insert the 20 G cannula tube. Plastic syringe was filled with different volumes of air and DPs loaded was blown into the lung of animals (Fig. 2). Air was blown with same volume for three consecutive times at an interval of 5 min. Animals were then unfixed from the platform and placed into the cage. The physical behaviours, such as nasal (or oral) bleeding, mobility and change in relative body weights, were monitored for a week and were rehabilitated. For oral and IV drug administration the drug is made as solution using sterile water for injection and administered. At various time intervals such as 30min, 1hr, 2, and 4hrs, 0.5 ml of blood will be taken from the retro orbital vein and plasma will be separated using centrifugation. The separated plasma will be determined for drug concentration using suitable analytical technique.[99,100]

7.RESULTS & DISCUSSION

7.1.PREFORMULATION STUDIES:

7.1.1.Construction of Standard curve of L Tryptophan:

Standard curve was constructed using a series of concentrations of drug solutions ranging from 10-100 mcg/ml using phosphate buffer of 7.4pH.[101] The absorbance values corresponding to the concentration were shown in table 10 and the regression value (r^2) was found to be 0.997 from the fig.3.

Table No. 10: Standard curve data of L Tryptophan by UV Spectroscopy

CONCENTRATION (mcg/ml)	ABSORBANCE (nm)
1	0.165
2	0.298
3	0.425
4	0.545
5	0.698
6	0.824
7	0.979

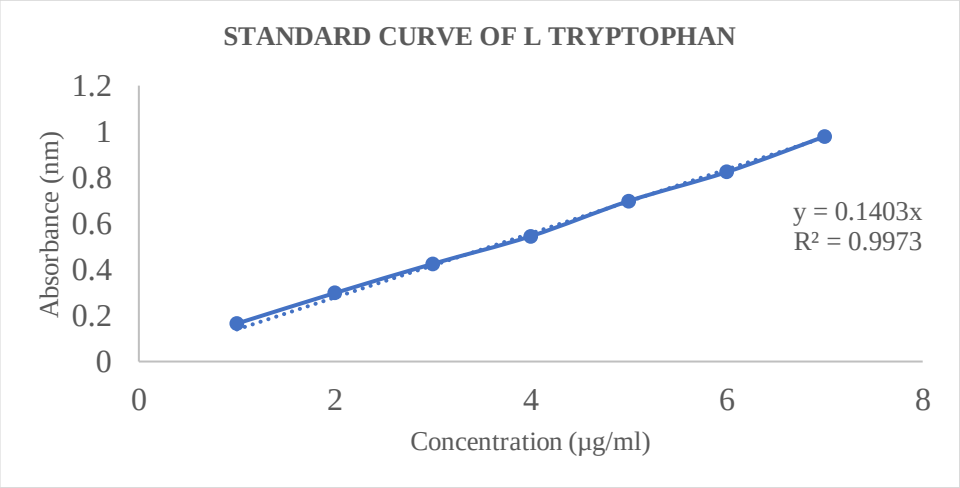


Figure No. 3: Standard Curve of L Tryptophan

7.1.2. Construction of Standard curve of L Tyrosine:

Standard curve was constructed using a series of concentrations of drug solutions ranging from 10-100 mcg/ml using phosphate buffer of pH 7.4.[102] The absorbance values corresponding to the concentration were shown in table 11 and the regression value (r^2) was found to be 0.998 from the fig.4.

Table No. 11: Standard curve data of L Tyrosine by UV spectroscopy

CONCENTRATION (mcg/ml)	ABSORBANCE (nm)
10	0.103
20	0.202
30	0.286
40	0.368
50	0.483
60	0.575
70	0.652
80	0.747
90	0.827
100	0.952

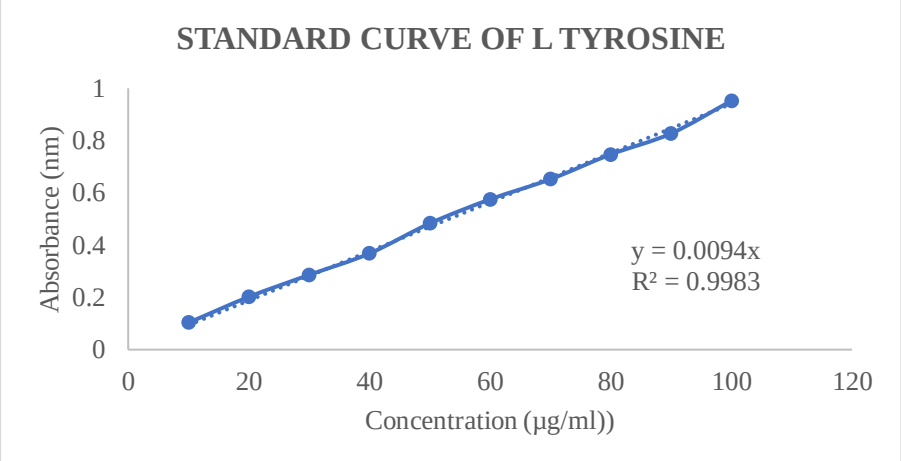


Figure No. 4: Standard Curve of L Tyrosine

7.1.3.Compatibility studies:

a. Fourier-Transform Infrared Spectroscopic analysis (FT-IR):

FT-IR analysis of L Tryptophan:

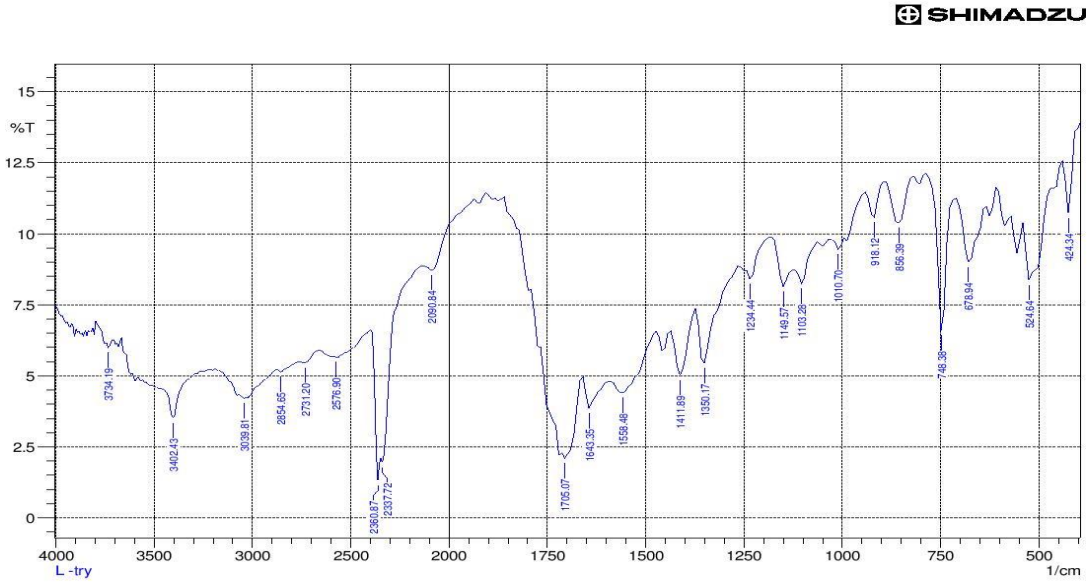


Figure No. 5: FT-IR spectrum of L Tryptophan

FT-IR analysis of L Tyrosine:

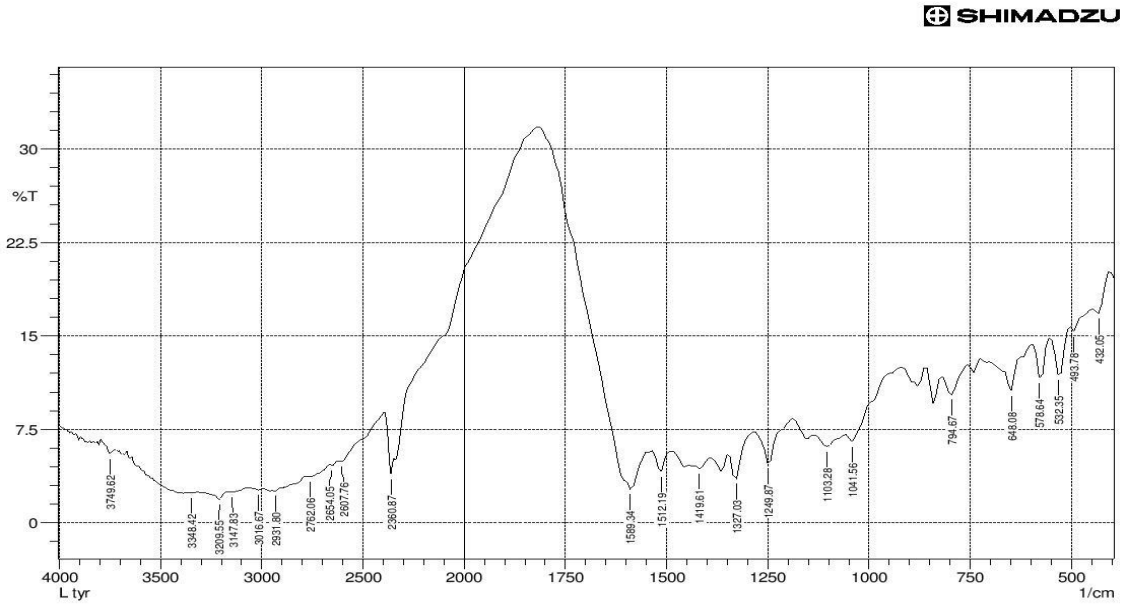


Figure No. 6 : FT-IR spectrum of L Tyrosine

FT-IR analysis of Lactose:

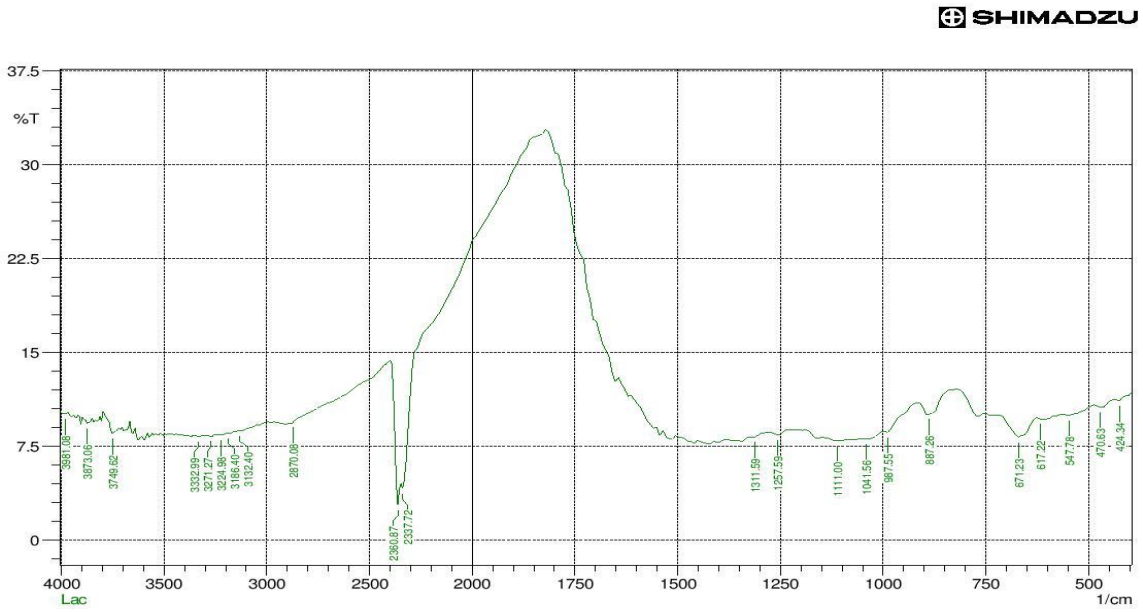


Figure No. 7: FT-IR spectrum of Lactose

FT-IR analysis of DPI formulation:

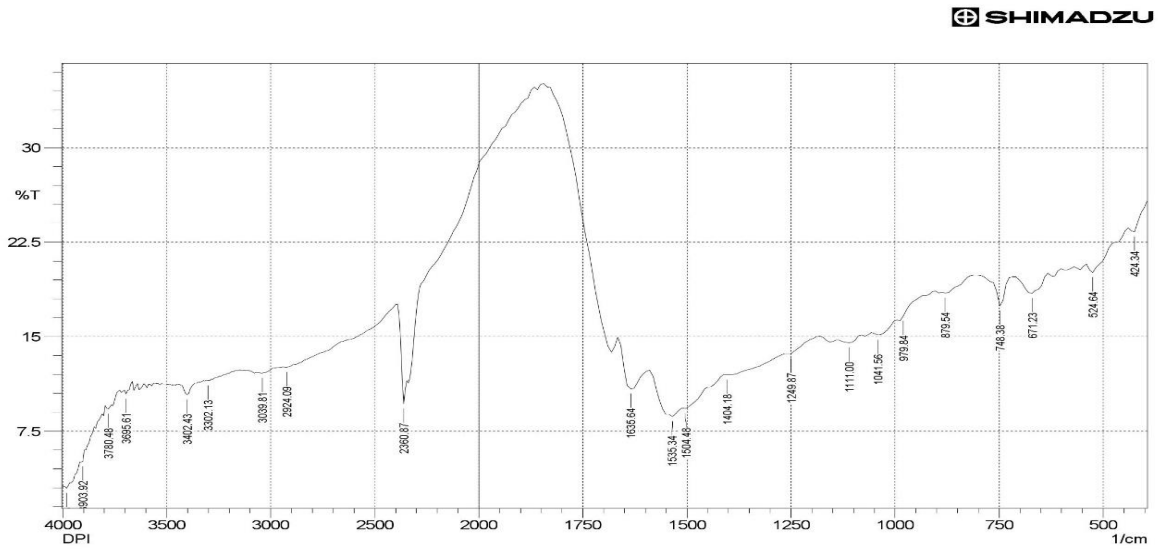
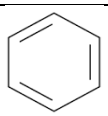


Figure No. 8: FT-IR spectrum of DPI formulation

The FT-IR spectrum of the drug and carrier mixture shown in fig. 8 indicates no change in characteristic peaks of the drug. Hence no interaction occurred between the drug and carrier.

Table No. 12: FT-IR spectrum interpretation

S.No	Functional Group	Wave number	Observed frequency			
			L Tryptophan	L Tyrosine	Lactose	Formulation
1		1600 & 1500-1430	1613.35	1512.19	-	1504.48
2	C=0	1780-1650	1643.35	-	-	1635.64
3	C-0	1250-1050	1234.44	1249.87	1111.00	1111.00
4	C-O-C	1300-1000	-	-	1257.59	1249.87
5	Ph-O-C	1250& 1040	-	-	1041.58	1041.56
6	O-H (alcohol)	3650-3200	3030.81	3209.55	3224.96	3402.43
7	O-H (COOH)	3300-2500	2576.90	3209	-	3302.13
8	N-H	3500-3300	3402.43	3348.42	-	3695.61
9	C-H	3300-2700	2731.20	2762.06	3271.27	2924.09

b. Differential Scanning Colorimetric Analysis(DSC)

DSC analysis of L Tryptophan:

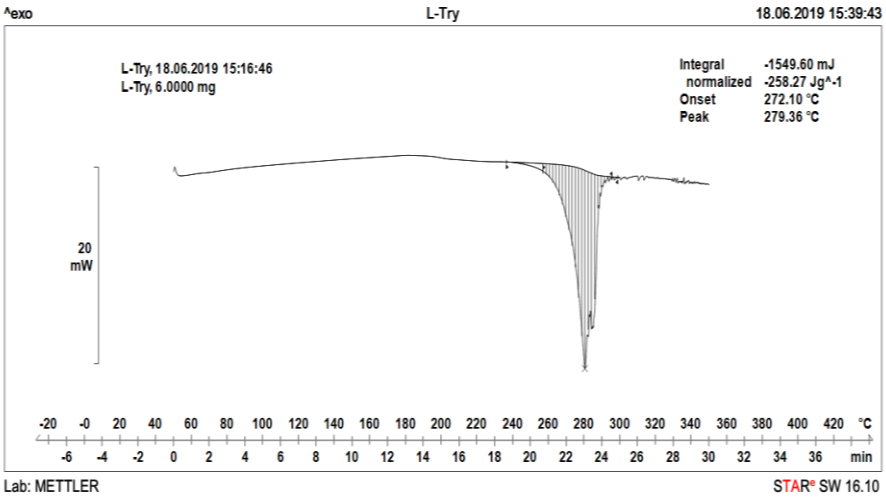


Figure No. 9: DSC spectrum of L Tryptophan

DSC analysis of L Tyrosine:

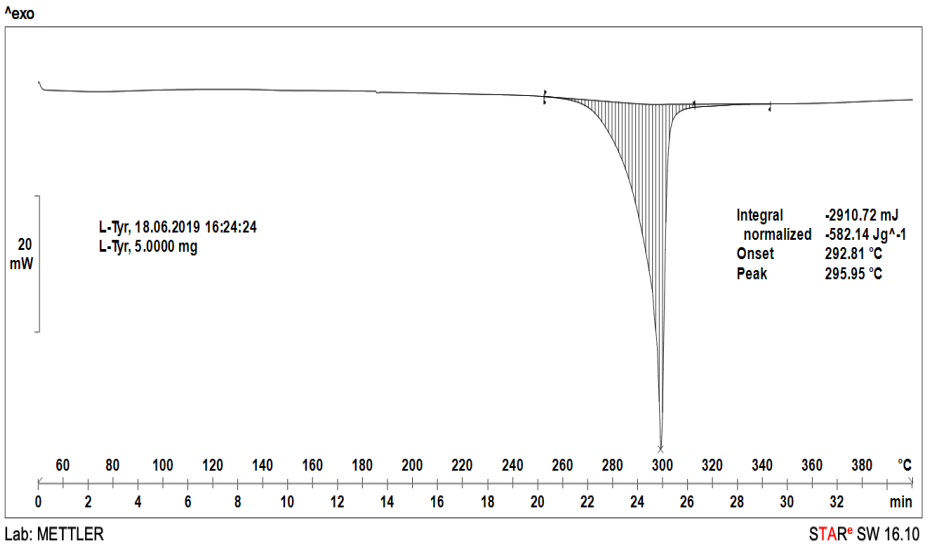


Figure No. 10: DSC spectrum of L Tyrosine

DSC analysis of Lactose:

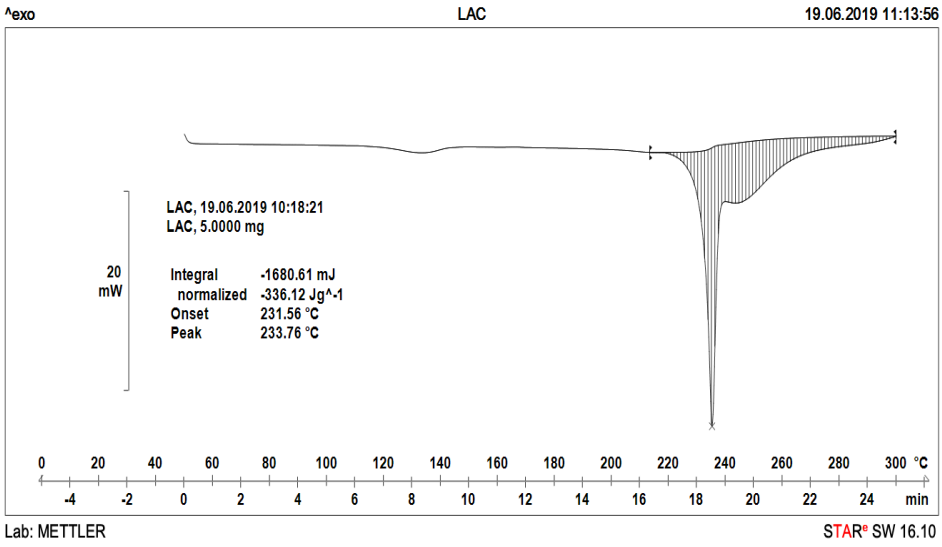


Figure No.11: DSC spectrum of Lactose

DSC analysis of DPI formulation:

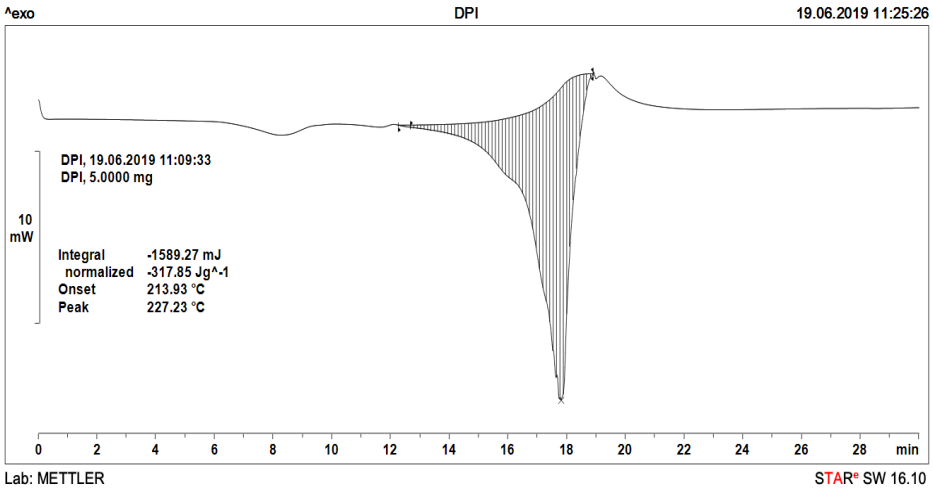


Figure No.12: DSC spectrum of DPI formulation

From the results of Fig. 9-12 it was noticed that L Tryptophan showed, a characteristic peak representing its melting point at 279.36°C, whereas for L Tyrosine and Lactose at 295.95°C & 233.76°C respectively. In the spray dried formulation the peak was observed at 227.23°C which indicated that lactose has undergone phase transition from solid to liquid at its melting point. The melting point of lactose was low compared to L Tryptophan and L Tyrosine so there was no significant peak observed above or below 227.23°C which indicated that the formulation remains stable without any interaction until 227.23°C.

7.1.4.Determination of Solubility of drugs:

Table No. 13: Solubility of drugs in solutions of different pH

S.No	pH	SOLUBILITY (mg/ml)	
		L-TRYPTOPHAN	L-TYROSINE
1	4.0	4.79	0.091
2	6.8	8.83	0.272
3	7.4	11.56	0.397
4	Water	13.75	0.432

The data for the solubility study is provided in Table 13. The solubility studies indicates that the drug solubility was observed to be dependent on pH. Solubility of drug

increases with increase in pH. Hence the drug is found to be more soluble in the distilled water.

7.1.5.Determination of pH and Partition coefficient:

Table No. 14: pH and Log P value for L Tryptophan and L Tyrosine

S.No	Drug	pH (1%)	Log P Value
1	L-Tryptophan	5.7	-0.99
2	L-Tyrosine	5.2	-1.71

The partition coefficient (P) describes the propensity of a neutral (uncharged) compound to dissolve in an immiscible biphasic system of lipid (fats, oils, organic solvents) and water. A negative value for logP means the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when logP = 0 the compound is equally partitioned between the lipid and aqueous phases; a positive value for logP denotes a higher concentration in the lipid phase (i.e., the compound is more lipophilic).

With this regard the value of log P lies -0.99 and -1.71 for L Tryptophan and L tyrosine respectively showed in table 14 indicated that the drugs were hydrophilic in nature.

7.2.OPTIMIZATION & FORMULATION DEVELOPMENT:

7.2.1.Optimized formulation:

The drugs were micronized by Nano Spray drying technique and the carrier was size reduced by ball milling.

The ratio of drug and carrier in DPI formulation are as follows:

Table No. 15: Drug to carrier ratio for DPI formulation

S.No	INGREDIENTS	RATIO (mg)
1	L Tyrosine	5
2	L Tryptophan	1
3	Lactose	4

7.3.FORMULATION EVALUATION

7.3.1.Physical Characterization:

7.3.1.1.Determination of Moisture content:

Table No. 16:Determination of moisture content

S.No	Initial weight (g)	Final weight (g)	% Moisture content
1	1	0.98	2.0
2	1	0.98	2.0
3	1	0.98	2.0
4	1	0.98	2.0
5	1	0.99	1.0

The average percentage moisture content was found to be 1.8% showed in table 16 which lied within the limit according to IP.

7.3.1.2.Solid state Stability studies:

The optimized formulation was subjected to stability studies according to ICH guidelines by storing at 25ºC/60% RH and 40ºC/75% RH for 90 days. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals.

Table No. 17: Stability studies of drug according to ICH guidelines

Stability conditions	Sampling days	Drug content L Tryptophan Mean ± SD	Drug content L Tyrosine Mean ± SD
25ºC/ 60% RH	0	99.53 ± 0.25	100.00 ± 0.10
	30	99.30 ± 0.25	99.98 ± 0.11
	60	98.98 ± 0.05	99.81 ± 0.24
	90	98.96 ± 0.11	99.00 ± 0.10
40ºC/ 75% RH	0	99.29 ± 0.10	99.98 ± 0.22
	30	99.25 ± 0.22	99.90 ± 0.18
	60	98.95 ± 0.17	98.79 ± 0.17
	90	98.89 ± 0.14	97.56 ± 0.22

***Standard deviation n = 3**

From the table 17, it is clear that drug content lies in range of 99.5-98.9 and 100-99% at 25ºC/ 60% RH for L Tryptophan and L tyrosine respectively for 0-90 days. 99.29-98.9 % and 99.98-97.56% at 40ºC/ 75% RH for L Tryptophan and L tyrosine respectively for 0-90 days. This indicates that the formulation did not undergo any chemical changes/interaction during the study period.

7.3.2.Micromeritic characterization:

7.3.2.1.Scanning Electron Microscopic analysis:

7.3.2.1.1. Scanning Electron Microscope photograph of L Tryptophan

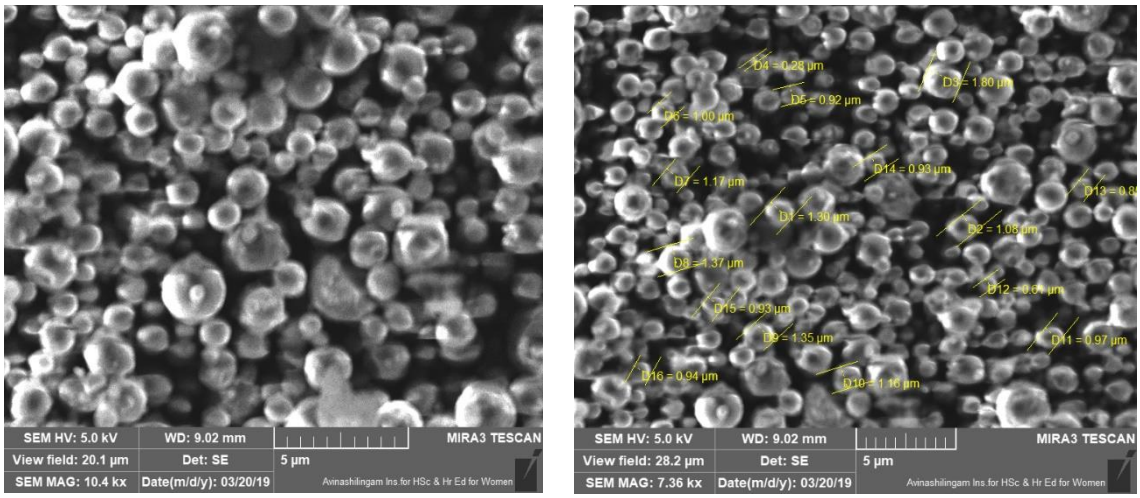


Figure No.13: SEM photographs of L Tryptophan

7.3.2.1.2. Scanning Electron Microscope photograph of L Tyrosine

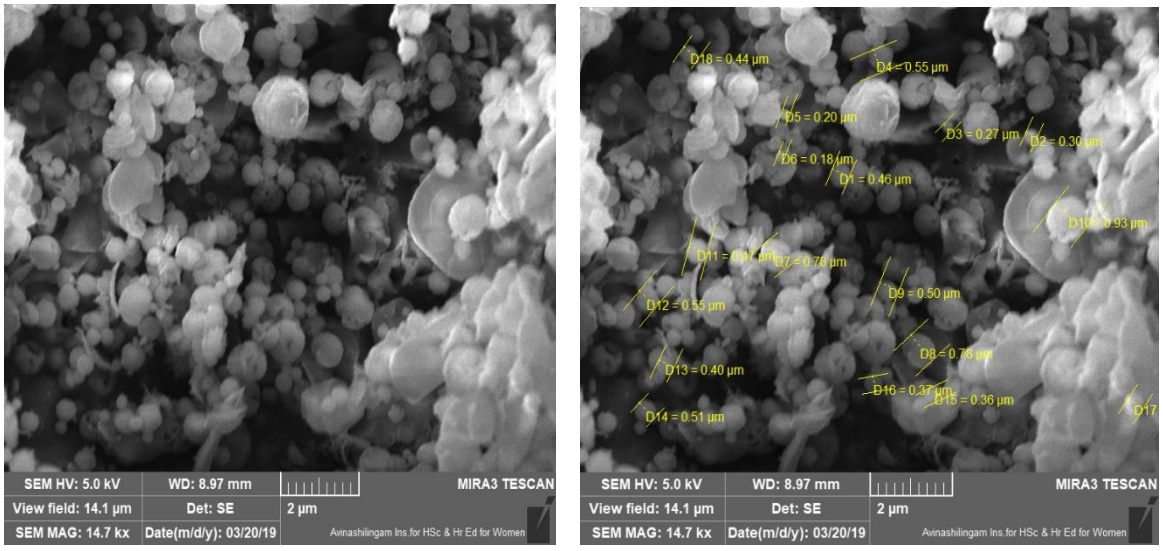


Figure No.14: SEM photographs of L Tyrosine

7.3.2.1.3.Scanning Electron Microscope photograph of Lactose:

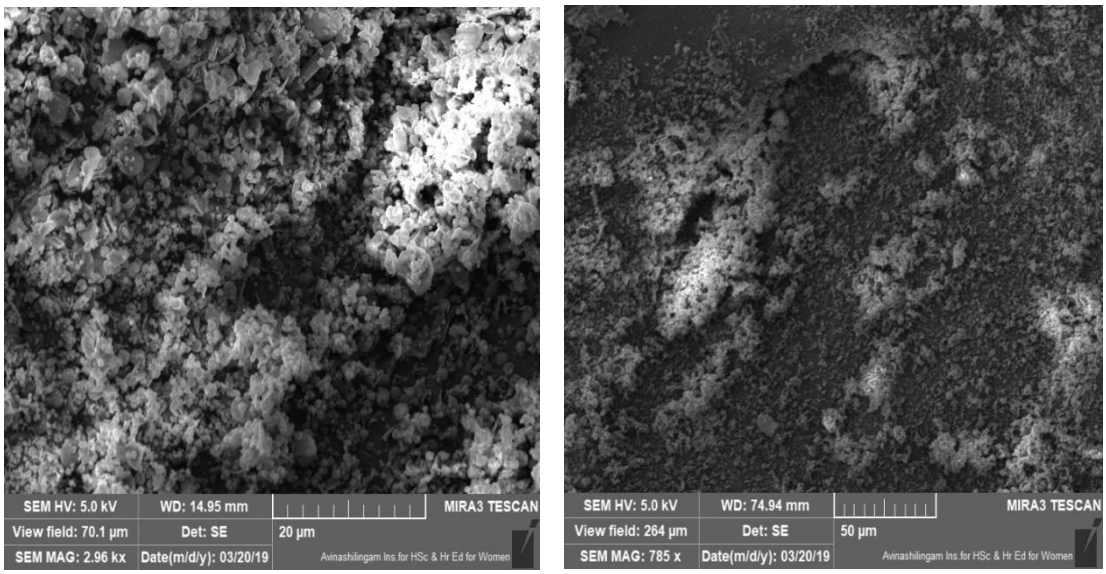


Figure No.15: SEM photographs of Lactose

SEM photographs showed that Dry powder produced by spray-drying yielded loose agglomerates of microparticles with comparable size and surfaces. The particles were of good morphological characteristics, having rough surface with particle size range of 1-3µm. The particles present in form of loose agglomerates supports the administration of free dispersion of powder in the form of inhalation.

7.3.2.2.Elemental analysis by EDAX- Energy Dispersive Spectroscopy

7.3.2.2.1.EDAX-graph L Tryptophan:

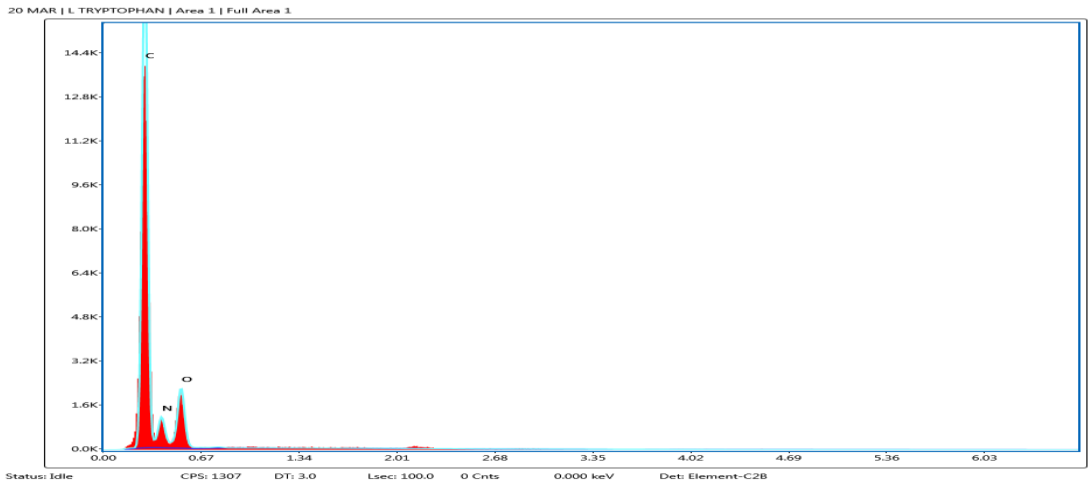


Figure No. 16: Energy Dispersive Spectroscopy graph of L Tryptophan

7.3.2.2.2.Elemental analysis L Tryptophan:

Table No. 18: Elemental analysis of L Tryptophan

Element	Weight %	Atomic %	Error %
C K	64.11	69.09	3.47
N K	16.24	15.01	15.27
O K	19.65	15.90	11.77

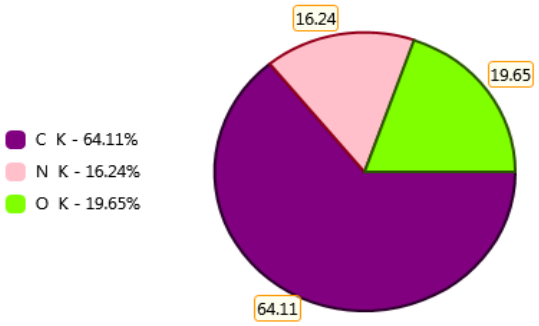


Figure No. 17: Elemental analysis graph of L Tryptophan

7.3.2.2.3.EDAX graph- L Tyrosine:

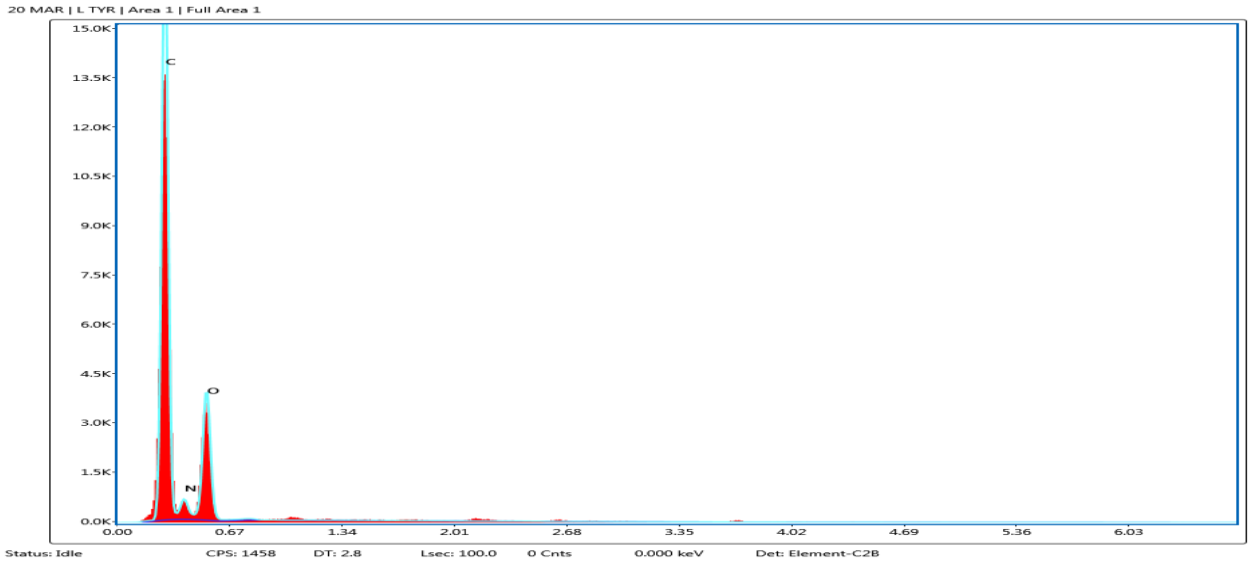


Figure No. 18: Energy Dispersive Spectroscopy graph of L Tyrosine

7.3.2.2.4.Elemental analysis L Tyrosine:

Table No. 19: Elemental analysis of L Tyrosine:

Element	Weight %	Atomic %	Error %
C K	61.58	67.49	3.80
N K	7.63	7.17	18.44
O K	30.80	25.34	9.55

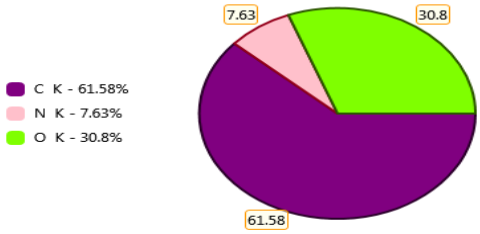


Figure No. 19:Elemental analysis graph of L Tyrosine

EDAX graph Fig16,18. indicated that the elements such as carbon, Nitrogen and oxygen are only present in the drug which confirmed the absence of impurities.

Table 18,19 and fig.17,19 indicated the weightage of the elements present in the drug L Tryptophan and L tyrosine respectively.

7.3.2.3.Powder X Ray Diffraction:

7.3.2.3.1.X Ray Diffraction analysis of L Tryptophan:

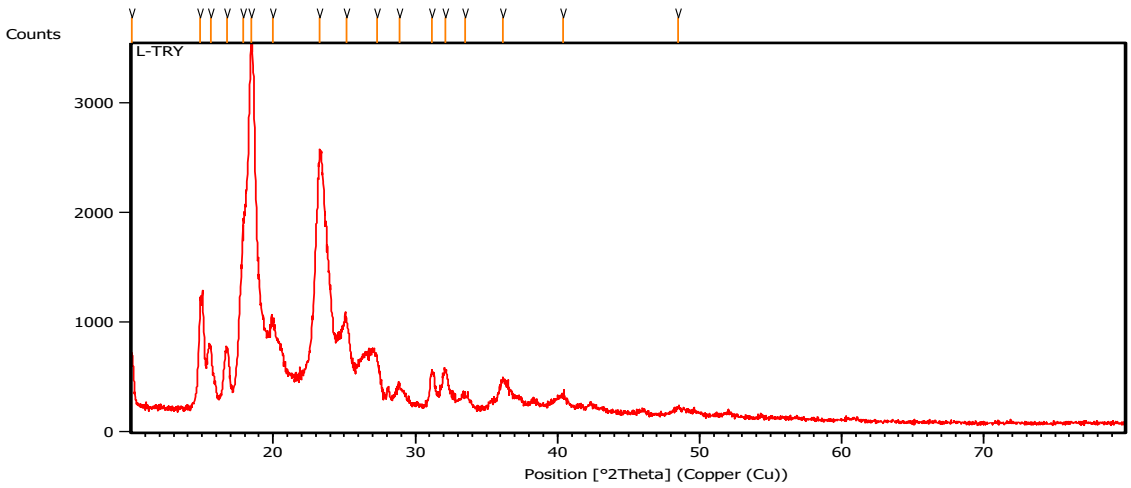


Figure No. 20: X Ray Diffraction image of L Tryptophan

Table No. 20: XRD interpretation of L Tryptophan

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
10.0801	473.02	0.2007	8.77538	15.19
14.8664	882.59	0.3346	5.95914	28.34
15.6097	498.01	0.2676	5.67703	15.99
16.7850	428.18	0.3346	5.28207	13.75
17.8791	1423.86	0.2676	4.96122	45.72
18.4817	3114.65	0.4015	4.80081	100.00
19.9708	545.21	0.3346	4.44607	17.50
23.2447	2018.90	0.3011	3.82676	64.82
25.1591	626.37	0.3346	3.53974	20.11
27.3215	329.15	0.6022	3.26429	10.57
28.9053	127.44	0.6691	3.08894	4.09
31.1967	310.06	0.3346	2.86709	9.95
32.1091	338.96	0.3346	2.78767	10.88
33.5199	105.74	0.5353	2.67350	3.39
36.1712	237.48	0.5353	2.48339	7.62
40.3948	90.50	0.7360	2.23295	2.91
48.5026	47.50	0.8029	1.87695	1.52

7.3.2.3.2.X Ray Diffraction analysis of L Tyrosine:

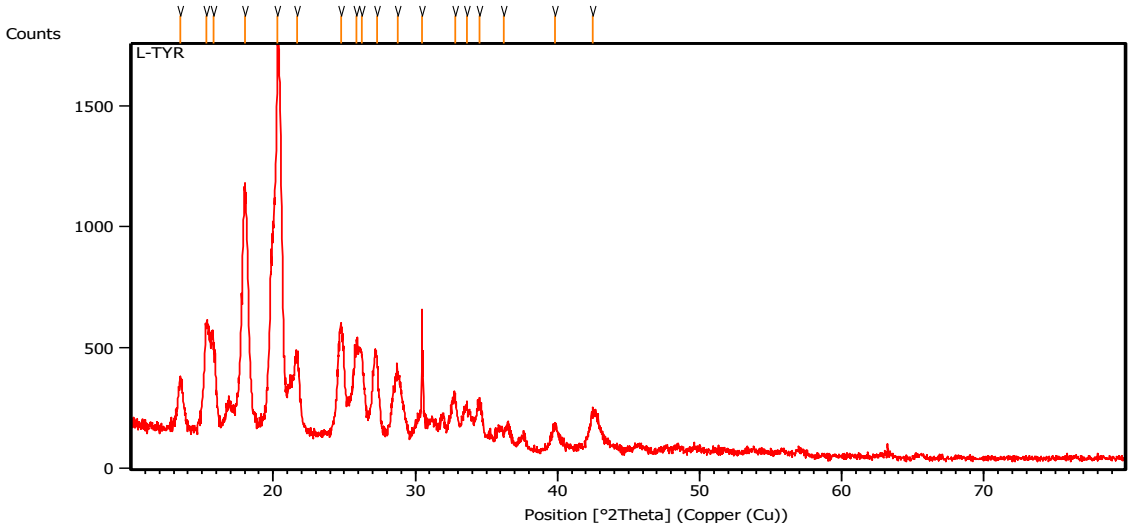


Figure No. 21: X Ray Diffraction image of L Tyrosine

Table No. 21: XRD interpretation of L Tyrosine

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
13.4908	201.79	0.3346	6.56354	12.69
15.3154	444.72	0.2676	5.78545	27.97
15.8096	352.71	0.3346	5.60570	22.19
18.0203	995.06	0.4349	4.92268	62.59
20.3212	1589.73	0.1840	4.37019	100.00
21.7070	312.23	0.3346	4.09423	19.64
24.7584	453.49	0.3011	3.59612	28.53
25.8296	354.91	0.2676	3.44935	22.32
26.2446	326.76	0.3346	3.39575	20.55
27.3042	319.67	0.3680	3.26632	20.11
28.7639	265.48	0.6691	3.10380	16.70
30.4692	548.05	0.0502	2.93387	34.47
32.7858	172.62	0.3680	2.73166	10.86
33.6070	133.61	0.4015	2.66677	8.40
34.5158	157.67	0.3346	2.59860	9.92
36.2504	53.16	0.8029	2.47814	3.34
39.8191	94.69	0.5353	2.26389	5.96
42.4917	137.62	0.6022	2.12748	8.66

The º2Th values from table 20,21 and the Diffraction pattern with the presence of strong peaks fig.20,21 shows the characteristic of crystallinity indicating that the drug retained the crystalline nature even after the process of micronization.

7.3.2.4.Flow characteristics:

7.3.2.4.1.Bulk density:

Table No. 22: Bulk density of DPI formulation

S.No	**Mass (g)**	**Bulk volume (cm^3)**	**Bulk density (g/cm^3)**
1	0.5	1	0.5
2	0.5	0.9	0.55
3	0.5	0.9	0.55
Bulk density			$0.53 g/cm^3$

7.3.2.4.2.Tapped density:

Table No. 23: Tapped density of DPI formulation

S.No	Mass (g)	Tapped volume (cm^3)	Tapped density (g/cm^3)
1	0.5	0.75	0.66
2	0.5	0.76	0.65
3	0.5	0.74	0.67
		Tapped density	0.66 g/cm^3

7.3.2.4.3.Carr's index and Hausner ratio:

Table No. 24: Carr's index and Hausner ratio for DPI formulation

Flow properties	Values	Flow
Carr's index	19.6%	Fair
Hausner ratio	1.24	Good

Powder flow is the displacement of powder particles in relation to each other, under the effect of some directional force. Particle size, shape, porosity, density and moisture content influences the flow properties of powder. Hence it is necessary to determine the flow characteristics to ensure whether the powder is freely flowing. The bulk density and tapped density of the formulation was found to be 0.53g/cm^3 and 0.66 g/cm^3 respectively in table 22,23 . Carr's index and Hausner ratio was found to be 19.6% and 1.24 respectively table 24. These results clearly shows that the prepared powders have good flow potential.

7.4.CAPSULE EVALUATION

7.4.1.Table No. 25: Evaluation parameters of capsule

S.No	EVALUATION PARAMETERS	VALUES
1	Physical appearance	Smooth and elegant
2	Locking length	15 ± 0.08 mm [n=20]
3	Weight uniformity	19.95 ± 0.17 mg [n=20]

The selected capsules were found to be stable and had a good integrity.

7.4.2.Assay (Drug content determination):

Table No. 26: Percentage drug content of L Tryptophan and L Tyrosine

S.No	Drug	Wavelength (nm)	Absorbance	Drug content (%)
1	L Tryptophan	287.40	1.367	99.53
2	L Tyrosine	274.60	0.959	100.00

From the table 26 it was found that the % Drug content was found to be 99.53 and 100% for L tryptophan and L tyrosine respectively indicating that the drug content lies within the limit.

7.5.*IN VITRO* STUDY:

7.5.1.Anderson Cascade impactor:

Table No. 27: ACI results for DPI formulation n=6

Parameters	Results
Emitted dose (%)	88.56±2.96
Respiratory fraction (%)	19.6±3.53
Total recovery	95.5±0.92
Mass median aerodynamic diameter (µm)	1.92±0.01

*Respiratory fraction calculated as ratio of total drug deposited in the lower stages of the ACI (stages 2-8) to total theoretical dose

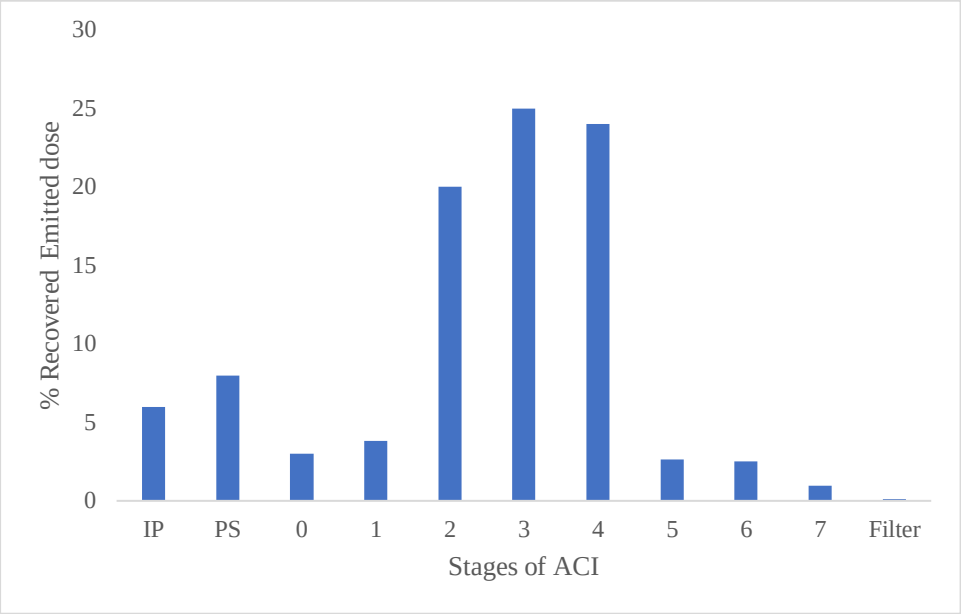


Figure No. 22: ACI Histogram for % deposition profiles in each stage for DPI formulation

Aerosolization of dry powder formulation depends on bulk flow with consideration of the desired particle size and its compatibility with the drug and carrier. In the DPI formulation tested here, the particle size tended to increase due to solid-phase interaction after blending with lactose. There is also relation between mass-median aerodynamic diameter(MMAD) and lung deposition. Anderson cascade impactor was used to determine mass weighed aerodynamic particle size distribution and the data was used to calculate the MMAD. The efficiency of drug deposition mainly depends upon the particle size. % emitted

dose, % respiratory fraction and MMAD were shown in table 28 and the ACI histogram for % deposition profiles in each stage for DPI formulation was showed in fig.22.

7.5.2.Bag diffusion study for L Tryptophan in DPI formulation:

Dose: 10mg

Wavelength : 220nm

Table No. 28: *In vitro* drug release for L Tryptophan in DPI formulation

Time (min)	Absorbance	Drug Concentration (mg/L)	Amount Released (mg)	% Released (%)
2	0.0096	10.2128	2.5532	25.5
4	0.0109	11.5957	2.8989	29.0
6	0.0126	13.4043	3.3511	33.5
8	0.0145	15.4255	3.8564	38.6
10	0.0198	21.0638	5.2660	52.7
12	0.0211	22.4468	5.6117	56.1
14	0.0231	24.5745	6.1436	61.4
16	0.0245	26.0638	6.5160	65.2
20	0.0300	31.9149	7.9787	79.8
25	0.0354	37.6596	9.4149	94.1
30	0.0370	39.3617	9.8404	98.4
45	0.0384	40.8511	10.2128	102.1
60	0.0386	41.0638	10.2660	102.7

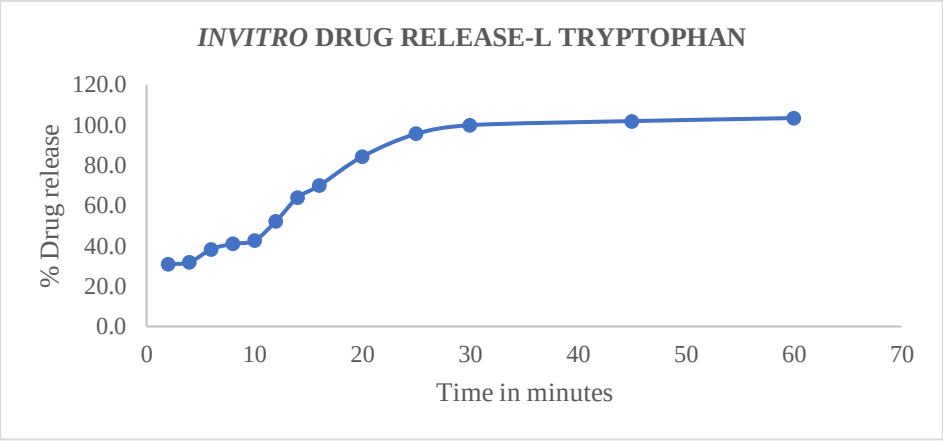


Figure No. 23: *In vitro* drug release of L Tryptophan in DPI formulation

7.5.2.Bag diffusion study for L Tyrosine in DPI formulation:

Dose: 50mg

Wavelength : 283nm

Table No. 29: *In vitro* drug release for L Tyrosine in DPI formulation

Time (min)	Absorbance	Drug Concentration (mg/L)	Amount Released (mg)	% Released (%)
2	0.2890	61.7962	15.4490	30.9
4	0.2990	63.9344	15.9836	32.0
6	0.3590	76.7641	19.1910	38.4
8	0.3840	82.1098	20.5274	41.1
10	0.3990	85.3172	21.3293	42.7
12	0.4890	104.5617	26.1404	52.3
14	0.5990	128.0827	32.0207	64.0
16	0.6540	139.8432	34.9608	69.9
20	0.7890	168.7099	42.1775	84.4
25	0.8950	191.3756	47.8439	95.7
30	0.9350	199.9287	49.9822	100.0
45	0.9540	203.9914	50.9979	102.0
60	0.9680	206.9850	51.7463	103.5

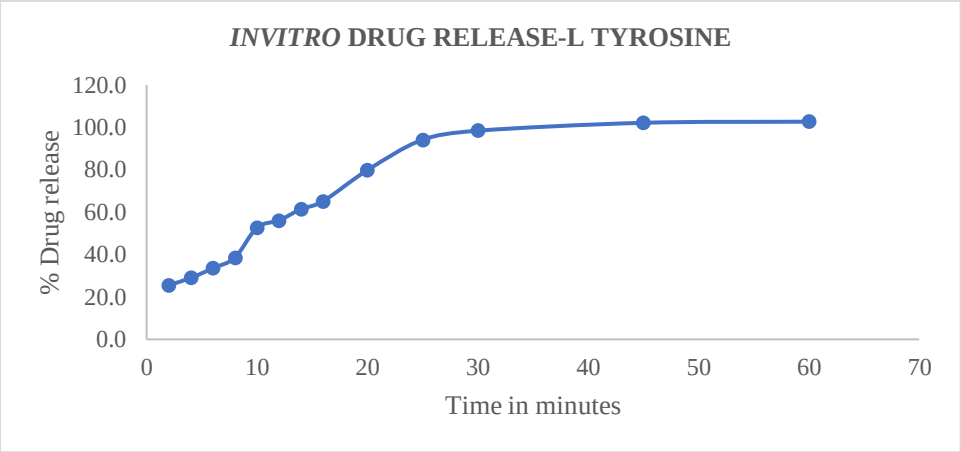


Figure No. 24: *In vitro* drug release for L Tyrosine in DPI formulation

Invitro diffusion study was carried out in Phosphate buffer at pH 7.4.and the data were recorded in tables 29,30 and figures 23,24. The amino acids were released completely within 30 minutes. The release of the amino acid was gradual and attained a steady state concentration after 30 minutes up to 1hour.

7.6.*In vivo* study:

7.6.1.Determination of Percentage delivered dose:

$$\text{Percentage Delivered dose} = \frac{\text{Loaded dose} - \text{Remained dose in canula tube}}{\text{Loaded dose}} \times 100$$

Table No. 30: Percentage delivered dose

S.No	LOADED DOSE (mg)	REMAINED DOSE (mg)	DELIVERED DOSE (%)
1	10	1.6	84.0
2	10	1.1	89.0
3	10	0.5	95.0
4	10	1.2	88.0
5	10	1.6	84.0
6	10	1.3	87.0
Average		1.216 (12.166%)	87.83

The influence of the retained drug in the device and the drug delivered from the subsequent applications of the same device for the drug delivery was also studied. The results were showed in table 32. The average % delivered dose and remained dose was 87.83% and 12.166% respectively which indicated that 87.83% dose had been transferred from the device.

7.6.2.Assessment of Drug Concentration in Lung tissue:

Table No. 31: Drug concentration in lung tissue

Time (in minutes)	Drug Concentration in Lung Tissue (n=6) (µg/ml)
30	4.971 ± 0.04
60	3.524 ± 0.07
120	2.074 ± 0.04
180	0.726 ± 0.07
240	0.310 ± 0.05

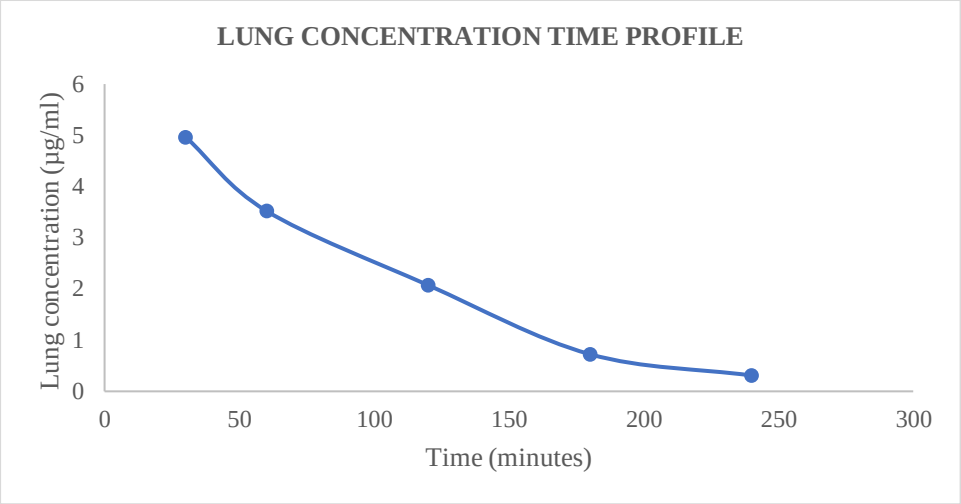


Fig. 25: Lung concentration time profile of DPI formulation

Assessment of drug concentration in the lung region is an important criteria for DPI formulation as the drug is intended to be deposited in the lungs. Lung concentration time profile is shown in fig.27. The concentration of drug was 4.971, 3.524, 2.074, 0.726, 0.310µg/ml at 30,60,120,180 and 240 minutes respectively showed in table 32. Decrease in concentration of drug in the lung tissue at each time interval indicated that the drug diffuses to systemic circulation after deposition.

7.6.3.Plasma Pharmacokinetic study:

Table No. 32: Plasma drug concentration at different time intervals with respect to route of drug administration

ROUTE OF DRUG ADMINISTRATION	TIME (in minutes)	PLASMA DRUG CONCENTRATION (in)
Intravenous	30 60 120 240	10.78 ± 0.71 6.069 ± 0.10 4.34 ± 0.04 2.90 ± 0.04
Oral	30 60 120 240	5.498 ± 0.29 6.042 ± 0.10 3.823 ± 0.33 2.225 ± 0.06
Intratracheal	30 60 120 240	6.179 ± 0.09 7.024 ± 0.05 8.29 ± 1.56 4.399 ± 0.05

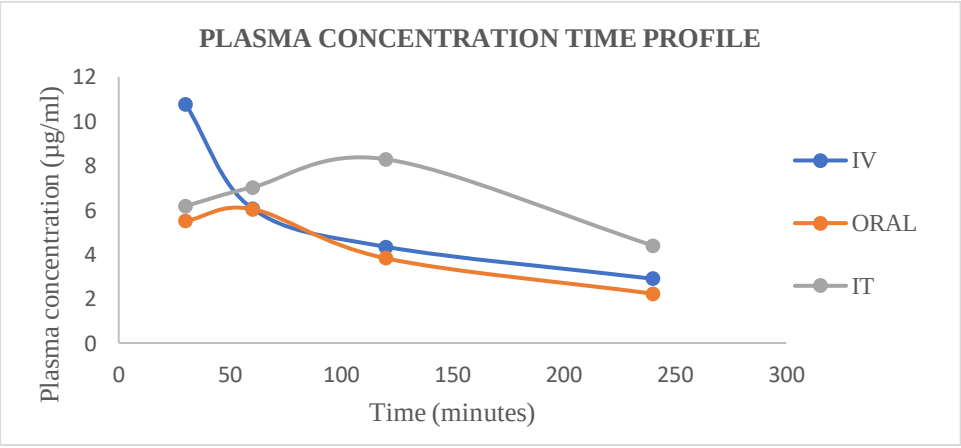
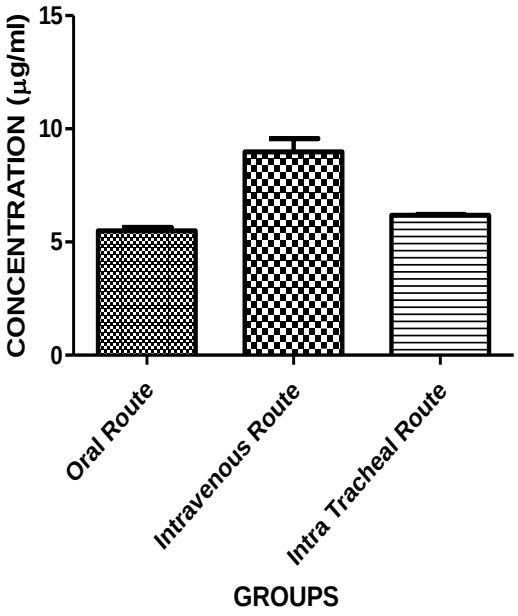
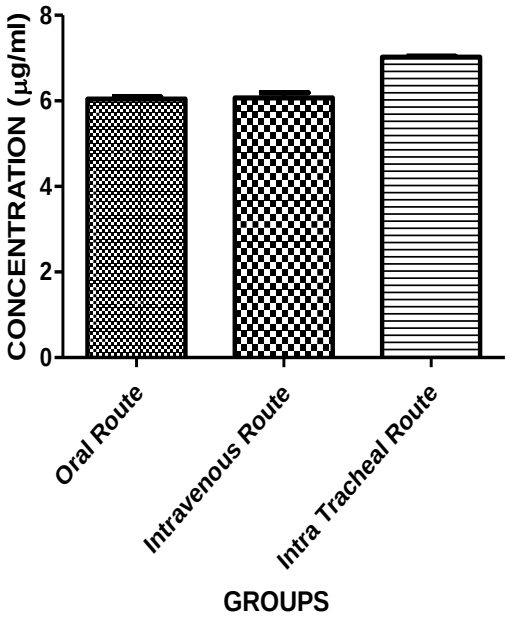


Figure No. 26: Plasma concentration time profile for different routes of drug administration

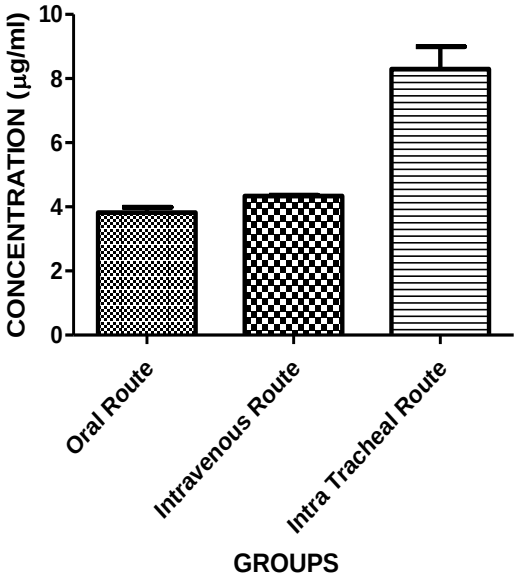
30 minutes



60 minutes



120 minutes



240 minutes

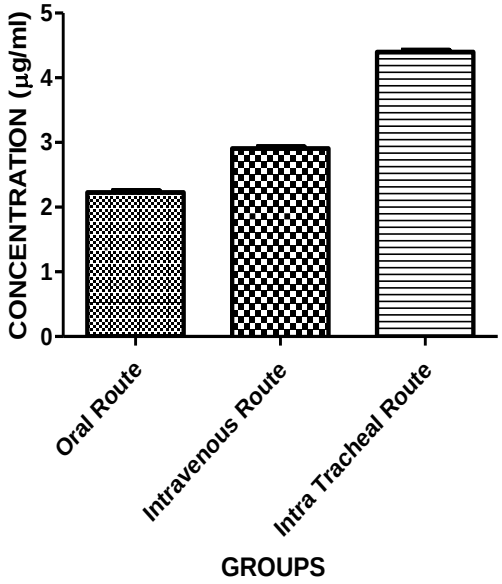


Figure No. 27: Plasma concentration graph for different routes of drug administration at various time intervals

Plasma concentration time profiles for different routes of administration at various time intervals were shown in table 31. Figures 26 depicts that drug concentration decreases with increase in time interval due to elimination. From fig. 27 at 30 minutes time interval, intra venous route attained 10.78µg/ml whereas oral and intra tracheal route attained 5.498 and 6.179 µg/ml respectively indicating that intravenous route had maximum plasma concentration. At 60 minutes time interval the plasma drug concentration was 6.069 µg/ml for iv, 6.042 µg/ml for oral and 7.024 µg/ml for intra tracheal indicating similarity in every routes. Whereas when time interval increases intra tracheal route attained 8.29 µg/ml for 120minutes and 4.39 µg/ml for 240minutes, oral and intravenous administration attained 3.8 µg/ml for 120mins, 2.2 µg/ml for 240mins and 4.34 for 120mins, 2.90 µg/ml for 240mins respectively. This concluded that intratracheal route attained maximum concentration range indicating long duration of action with steady state drug release compared to oral and intravenous route.

Pharmacokinetic Parameters:

Table No. 33: Pharmacokinetic parameters

S.No	Pharmacokinetic parameters	Intravenous	Oral	Intra-Tracheal
1	C_{max} (µg/ml)	10.78	6.042	8.29
2	T_{max} (minutes)	30	60	120
3	AUC (µg/min/ml)	1160.905	914.4	1511.49
4	Relative bioavailability (%)	100	60	80
5	Mean Resident time (MRT) (minutes)	103.276	108.333	115.985

Different pharmacokinetic parameters of DPI in various routes of administration such as intravenous, oral and intra-tracheal of amino acids in rats were collected in Table 34. After intratracheal administration, amino acids has reached its maximum up to 8.29±1.56 µg/ml while after oral administration, reached its maximum up to 6.042 ± 0.10. This indicates that intra tracheal administration of amino acids can provide faster onset of action with longer duration of action over oral administration. Also, DPI formulation has significant p-value(<0.05), higher C_{max} and AUC over oral administration. The higher AUC for the intra tracheal route, which is indicative of enhancement in amino acids

bioavailability can be due to some reasons, including improvement of solubility and dissolution rate of amino acids by nanosizing, the lack of hepatic first-pass elimination, and lower enzymatic activity in the lungs. The bioavailability of DPI via intra tracheal administration relative to its oral administration indicates the ability of the formulation to provide the drug to the lower airways and bronchioles having thin linings, high surface area, and rich vascularization that allows rapid absorption of the medication and makes lungs an excellent portal for DPI medications intended for systemic absorption [103,104] in addition to low local metabolic activity in lungs and avoiding the hepatic first pass effect.[105,106] Relative bioavailability of DPI formulation via intra-tracheal administration was 80% with 1.3 times higher than that of the oral administration. This enhanced bioavailability was obtained through formulating the micronized dry powder of amino acids without further particle modification or engineering. [107]

8.CONCLUSION

All these investigations have brought out crucial factors which lead to the following conclusions:

1. This study targets lungs with the aim of producing an effective, and compliant therapy by the rational use of amino acids which plays an important role in the alteration of mood and behaviour during smoking and other activities.
2. L Tryptophan and L Tyrosine were micronized in the size range of 2-4µm with Lactose as carrier and dry powder inhalation was formulated.
3. The combination of these two amino acids may produce a synergistic effect targeting to minimise the withdrawal symptoms associated with the treatment of smoking cessation.
4. *In vivo* study results showed rapid onset of action, higher local drug concentration, longer retention of drug in the site of administration.
5. Overall this study reports concluded that the formulation of dry powder inhalation offers an effective drug delivery to the lungs when compared with other routes of administration, thus leads to patient compliance and adherence to the therapy.

9. BIBLIOGRAPHY

1. Hickey A, Thompson D, Pharmaceutical Inhalation Aerosol Technology, 2004; 2nd ed. USA: Marcel Dekker.
2. Misra A, Chougule M, Padhi B, Jinturkar K, Development of Dry Powder Inhalers. Journal of Recent Patents on Drug Delivery & Formulation 2007; 1:11-21.
3. Telko M J, Embleton J K. Dry Powder Inhaler Formulation. Respiratory Care 2005; 50(9):1209-27.
4. Kapileshwar Swain, Mahesh Giri, RN Gupta, VK Arora. Dry powder inhaler a Review. Research journal of pharmaceutical, biological and chemical science 2012;3(3): 1346-56.
5. Bissera Pilicheva, Plamen Katsarov, Margarita Kassarova. Flowability Evaluation of Dry Powder Inhalation Formulations Intended for Nasal Delivery of Betahistine Dihydrochloride. Sikkim Manipal University Medical journal 2015;2:77-90.
6. Niti Yadav, Alka Lohani. Dry Powder Inhalers. A Review. Indo Global Journal of Pharmaceutical Sciences 2013;3(2):142-55.
7. Ashurst I, Malton A, Prime D, Sumby B. Latest advances in the development of dry powder inhalers, reviews-research focus 2000;3(7): 246-56.
8. Alagusundaram M, Deepthi N, Angalaparameswari S. R. S, Mohamed Saleem T. S, Gnanaprakash K, Thiruvengadarajan V. S, Madhusudhana C. Dry Powder Inhalers - An Overview. International Journal Research Pharmaceutical Science 2010;1(1):34-42.
9. Malcolmson R. J, Embleton J. K. Dry powder formulations for pulmonary delivery. Reviews research focus 1998;1(9):394-398.
10. Ambikanandan Misra, Kaustubh Jinturkar, Deepa Patel, Jigar R Lalani. Recent advances in liposomal dry powder formulations: Preparation and evaluation. Expert Opinion on Drug Delivery 2009;6(1):71-89.
11. Seville P, Learoyd T, Burrows J, French E. Chitosan based spray dried respirable powders for sustained delivery of Terbutaline sulphate. European Journal of Pharmaceutics and Biopharmaceutics 2008; 68:224-34.
12. Nazrul Islam, Ellen Gldaki. Mini Review Dry Powder Inhalers (DPIs)- A review of device reliability and innovation. International Journal of Pharmaceutics 2008;360:1-11.

13. Dal Negro RW. Dry powder inhalers and the right things to remember: a concept review. Multidisciplinary Respiratory Medicine 2015;10(1):2-4.
14. Swarbrick J, Boylan C. J. Encyclopedia of pharmaceutical technology. New York: Marcel Dekker; Vol .20. 123-126.
15. Martin J Telko, Anthony J Hickey. Dry Powder Inhaler Formulation.Respiratory Care Journal 2005;50(9):1209-1227.
16. SP Sahane, AK Nikhar, S Bhaskaran, DR Mundhada. Dry Powder Inhaler: An Advance Technique for Pulmonary Drug Delivery System. International Journal of Pharmaceutical and Chemical Sciences 2012;1(3):1027-34.
17. Werner Stober. A note on the aerodynamic diameter and the mobility of non-spherical aerosol particles. Journal of Aerosol Science 2003;2(4):453-56.
18. Shaikh S, Nazim S, Khan T, Shaikh A, Zameeruddin M, Quazi A. Recent Advances In Pulmonary Drug Delivery System: A Review.International Journal of Applied Pharmaceutics 2010;2(4): 27-31.
19. Xian Ming Zeng, Gary P. Martin, Christopher Marriott. Particulate Interactions in Dry Powder Formulations for Inhalation, Taylor and Francis, London, First edition 2001.
20. Timsina M P, Martin G P, Marriott C, Ganderton D, Yianneskis, M. Drug delivery to the respiratory tract using dry powder inhalers. Int. J. Pharm 1994;101:1–13.
21. Byron P R, Naini V, Philips E M.Drug carrier selection–important physicochemical characteristics. In: Proc. Respiratory Drug DeliveryJ. 1996;7(9):89-96.
22. Byron P R, Jashnani R and Germain S.Efficiency of aerosolization from dry powder blends of terbutaline sulfate and lactose NF with different particle size distribution. J. Pharmaceut. Res. 1990;7:81-98.
23. Bell J H, Hartley P S, Cox J S G .Dry powder aerosols. I. A new powder inhalation device. J. Pharm. Sci. 1971;60:1559–1564.
24. Kibbe A H (2000) Handbook of Pharmaceutical Excipients, 3rd edn. Washington, American Pharmaceutical Association and Pharmaceutical Press.
25. Kassem N M, Ho K K L and Ganderton D.Effects of air flow and carrier size on the characteristics of an inspirable cloud. J. Pharm. Pharmacol. 1989;41(Suppl.):14.
26. Ganderton D.The generation of respirable cloud from coarse powder aggregates. J. Biopharm. Sci. 1992;3:101–105.

27. Rietema K. (1991) Theoretical derivatives of interparticulate forces. In: The Dynamics of Fine Powders. New York, Elsevier, 65–94.
28. Staniforth J N Ordered mixing or spontaneous granulation? Powder Technol.1985;45:73–77.
29. Bailey A G .Electrostatic phenomena during powder handling. Powder Technol. 1984;37:71–85.
30. Boland D, Geldart D.Electrostatic charging in gas fluidised beds. Powder Technol. 1972;5:289–97.
31. Staniforth J.N. The effect of frictional charges on flow properties of direct compression tableting excipients. Int. J. Pharm.1982a;11:109–117.
32. Ahmed F. Abdel-Magid, and Stéphane Caron (2006). Fundamentals of Early Clinical Drug Development: From Synthesis Design to Formulation. John Wiley and Sons, Inc., Hoboken, New Jersey.
33. Martin J. Rhode (2008). Introduction to Particle Technology. John Wiley and Sons Ltd, Chichester, West Sussex, England.
34. Sarfaraz K. Niazi (2009). Handbook of Pharmaceutical Manufacturing Formulations: Semisolid Products. Informa Healthcare USA, Inc., New York.
35. Shafiur Rahman M. (2007). Handbook of Food Preservation, Second Edition. CRC Press, Taylor and Francis Groups, LLC.
36. Sud Sushant and Kamath Archana.Methods of Size Reduction and Factors Affecting Size Reduction in Pharmaceutics. International Research Journal of Pharmacy 2013;4(8): 57-64.
37. Wen-Ching Yang (2003). Handbook of Fluidization and Fluid-particle Systems. Marcel dekker, Inc., New York.
38. Cordin Arpagaus. Pharmaceutical particle engineering via Nano Spray Drying-Process parameters and application examples on the laboratory-scale. International Journal of Medical Nano Reseach 2018;5(1):1-15.
39. Phillips, E.M. And Stella, V.J.Rapid expansion from supercritical solutions: application to pharmaceutical processes. Int. J. Pharm.1993;94:1–10.
40. Gallagher-Wetmore P, Coffey M P and Krukonis V. (1994) Application of supercritical fluids in recrystallization: Nucleation and gas anti-solvent (GAS) techniques. In: Byron P R, Dalby R N and Farr S J. (eds), Respiratory Drug Delivery IV, Program and Proceedings, Virginia, USA. 303–311.

41. Roberto W, Dal Negro. Dry powder inhalers and the right things to remember: a concept review. Dal Negro Multidisciplinary Respiratory Medicine. 2015;10(13): 2-4.
42. Hamed Hamishehkar, Yahya Rahimpour and Yousef Javadzadeh. The Role of Carrier in Dry Powder Inhaler Recent Advances in Novel Drug Carrier Systems chapter 39-66.
43. Priyanka Kadu, Prakash Kendre, Kanchan Gursal. Dry Powder Inhaler: A Review. Journal of Advanced Drug Delivery 2016; 3(3): 42-52.
44. Nutan Dhanpal Shah, Vishal Vilas Shah, Jyoti Waghamare. Dry Powder for Inhalation (DPI).International Journal of Research and Reviews in Pharmacy and Applied Science;2(3):458-72.
45. Bennett WD. Controlled inhalation of aerosolised therapeutics. Expert Opinion Drug Deliv 2005; 2:763-67
46. G. Scheuch, R. Siekmeier. Novel approaches to enhance Pulmonary Delivery of Proteins And Peptides. Journal of Physiology and Pharmacology 2007;58, Suppl 5: 615-25.
47. Silagy C, Lancaster T, Stead L, et al. Nicotine replacement therapy for smoking cessation (Cochrane Review). In: The Cochrane Library, Issue 4, 2001. Oxford: Update Software.
48. Balfour DJ, Wright AE, Benwell ME, Birrell CE. The putative role of extra-synaptic dopamine in the neurobiology of nicotine dependence. Behav Brain Res 2000;113:73-83.
49. Stauffer HP, Riedwyl H. Interaction and pH dependence of effects of nicotine and carbon monoxide in cigarette smoke inhalation experiments with rats. Agents Actions 1977;5-6:579-88.
50. Zhou FM, Liang Y, Dani JA. Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 2001;4:1224-29.
51. Tomkins DM, Sellers EM. Addiction and the brain: the role of neurotransmitters in the cause and treatment of drug dependence. CMAJ 2001;164:817-21.
52. Pontieri FE, Tanda G, Orzi G, et al. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 1996;382:255-57.

53. West R, Hajek P, Foulds J, et al. A comparison of the abuse liability and dependence potential of nicotine patch, gum, spray and inhaler. Psychopharmacology (Berl) 2000; 149:198-202.
54. Schneider NG, Olmstead RE, Franzon MA, Lunell E. The nicotine inhaler: clinical pharmacokinetics and comparison with other nicotine treatments. J.Clin Pharmacokinet 2001;40:661-84.
55. Benson E. Bupropion induced hypersensitivity reactions. Med J Aust 2001;174:650-51.
56. Bel N, Artigas F. Reduction of serotonergic function in rat brain by tryptophan depletion: effects in control and fluvoxamine-treated rats. J Neurochem. 1996;67(2):669–76.
57. Bongiovanni R, Newbould E, Jaskiw GE. Tyrosine depletion lowers dopamine synthesis and desipramine-induced prefrontal cortex catecholamine levels. Brain Res. 2008;1190:39–48.
58. Leyton M, Young SN, Pihl RO, et al. Effects on mood of acute phenylalanine/tyrosine depletion in healthy women. Journal of Neuropsychopharmacology 2000;22(1):52–63.
59. Oldman AD, Walsh AE, Salkovskis P, Laver DA, Cowen PJ. Effect of acute tryptophan depletion on mood and appetite in healthy female volunteers. J Psychopharmacol. 1994;8(1):8–13.
60. Bowen DJ, Spring B, Fox E.Tryptophan and high carbohydrate diets as adjuncts to smoking cessation therapy; Journal of Behavioural Medicine 1991;14(2):97-110.
61. Yekta Dowlati, Danilo Rde Jesus, Peter Selby, Ian Fan, Jeffrey HMeyer. Depressed mood induction in early cigarette withdrawal is unaffected by acute monoamine precursor supplementation. Journal of Neuropsychiatric Disease and Treatment 2019;15:311–21.
62. Viswanath V Venugopalan, Kevin F Casey, Caitlin O Hara, Jennifer O'Loughlin, Chawki Benkelfat, Lesley K Fellows, Marco Leyton. Acute Phenylalanine/Tyrosine Depletion Reduces Motivation to Smoke Cigarettes Across Stages of Addiction. Journal of Neuropsychopharmacology 2011;36(12):2469-76.

63. John R Hughes, Lindsay F Stead, Tim Lancaster; Antidepressants for smoking cessation: A Review. The cochrane collaboration; Wiley publishers; 2011; (8):1-122.

64. Brian Hitsman, Bonnie Spring, William Wolf, Regina Pingitore, John W Crayton, Donald Hedeker. Effects of Acute Tryptophan Depletion on Negative Symptoms and Smoking Topography in Nicotine- Dependent Schizophrenics and Nonpsychiatric Controls; Journal of Neuropsychopharmacology 2005;30:640-48.

65. Michele Pergadia, Bonnie Spring, Lukasz M Konopka, Barbara Twardowska. Double-blind trial of the effects of tryptophan depletion on depression and cerebral blood flow in smokers.Journal of Addictive Behaviour 2004;29(4):665-71.

66. Mary Perugini, Colleen Mahoney, Vadimllivitsky, Simon N Young, Verner Knott; Effects of tryptophan depletion on acute smoking abstinence symptoms and the acute smoking response.Journal of Pharmacology Biochemistry and Behavior 2003;74(3): 513-22.

67. Verner Knot, Andrea Thompson, Dhrasti Shah, Vadim Ilivitsky. Neural expression of nicotine's antidepressant properties during tryptophan depletion: An EEG study in healthy volunteers at risk for depression. Journal of Biological Psychology 2012; 91(2):190-200.

68. Brian Hitsman, Bonnie Spring, Regina Pingitore, Marcus Munafò, Donald Hedeker. Effect of tryptophan depletion on the attentional salience of smoking cues. Journal of Psychopharmacology 2007;192(3):317-24.

69. Ivan Berlin, Florence Vorspan, Dominique Warot, Benjamin Maneglier, Odile Spreux-Varoquaux; Effect of glucose on tobacco craving. Is it mediated by tryptophan and serotonin?. Journal of Psychopharmacology 2005;178(1):27-34.

70. Marcus R Munafo, Zola N. Mannie, Philip J. Cowen, Catherine J. Harmer, Sarah B. McTavish. Effects of acute tyrosine depletion on subjective craving and selective processing of smoking-related cues in abstinent cigarette smokers. Journal of Psychopharmacology 2007;21(8):805-14.

71. Patrick F. Sullivan, Yuxin Jiang, Michael C. Neale, Kenneth S. Kendler, Richard E. Straub. Association of the tryptophan hydroxylase gene with smoking initiation but not progression to nicotine dependence. American Journal of Medical Genetics 2001;105(5):479-84.

72. Durk Fekkesa, AdriaanTuiten, Isaac Bom, Lolke Pepplinkhuizena. Tryptophan: a precursor for the endogenous synthesis of norharman in man. Journal of Neurosciece Letters 2001;303(3):145-48.
73. Ju Wang, Ming D Li. Common and Unique Biological Pathways Associated with Smoking Initiation/Progression, Nicotine Dependence, and Smoking Cessation. Journal of Neuropsychopharmacology 2010; 35:702–19.
74. Douglas M. Ziedonis, Tony P. George. Schizophrenia and Nicotine Use: Report of a Pilot Smoking Cessation Program and Review of Neurobiological and Clinical Issues. Schizophrenia Bulletin 1997;23(2):247–54.
75. Mark F.McCarty. Nutritional support of central catecholaminergic tone may aid smoking withdrawal**.** Journal of Medical Hypotheses 1982;8(1):95-102.
76. Alice Simon, Maria Ines Amaro, Lucio Mendes Cabral, Anne Marie Healy, Valeria Pereirade Sousa. Development of a novel dry powder inhalation formulation for the delivery of rivastigmine hydrogen tartrate. International Journal of Pharmaceutics 2016; 501(1–2):124-38.
77. Zeng XM, Martin GP, Marriott C, Pritchard J. Lactose as a carrier in dry powder formulations: the influence of surface characteristics on drug delivery. Journal of Pharmaceutical Sciences 2001;90(9):1424-34.
78. Xiao-FeiYang, YingXu, Da-ShengQu, Hao-YingLi. The influence of amino acids on aztreonam spray-dried powders for inhalation. Asian Journal of Pharmaceutical Sciences 2015;10(6):541-48.
79. Yingtong Cui, Xuejuan Zhang, Wen Wang, Zhengwei Huang, Ziyu Zhao, Guanlin Wang, Shihao Cai, Hui Jing, Ying Huang, Xin Pan, Chuanbin Wu. Moisture-Resistant Co-Spray-Dried Netilmicin with L-Leucine as Dry Powder Inhalation for the Treatment of Respiratory Infections. Journal of Pharmaceutics 2018;10(252):1-13.
80. P.C. Seville, T.P. Learoy, H.Y. Lib, I.J. Williamson, J.C. Birchall. Amino acid-modified spray-dried powders with enhanced aerosolisation properties for pulmonary drug delivery. Powder Technology 2007;178:40-50.
81. T E G K Murthy, Bala Vishnu Priya M, Satyanarayana. Formulation and evaluation of dry powder inhaler for tiotropium bromide. International Journal of Innovative Pharmaceutical Research 2010;1(1):14-22.

82. Marcelde Matas, QunShao, Catherine, H.Richardson, Henry Chrystyn. Evaluation of in vitro in vivo correlations for dry powder inhaler delivery using artificial neural networks. European Journal of Pharmaceutical Sciences 2008;33(1):80-90.
83. Joanna Muddle, Stewart B. Kirton, Stewart B. Kirton, Irene Parisini, Andrew Muddle, Darragh Murnane, Jogoth Ali, Marc Brown, Clive Page, Ben Forbes. Predicting the Fine Particle Fraction of Dry Powder Inhalers Using Artificial Neural Networks. Journal of Pharmaceutical Sciences 2017;106(1):313–321
84. Deepak J. Singh, Rajesh R. Jain, P. S. Soni, Samad Abdul, Hegde Darshana, Rajiv V. Gaikwad, Mala D. Menon. Preparation and Evaluation of Surface Modified Lactose Particles for Improved Performance of Fluticasone Propionate Dry Powder Inhaler. Journal of Aerosol Medicine and Pulmonary Drug Delivery 2015;28(4):254-67.
85. P.S. Uttekar and P.D. Chaudhari. Formulation and evaluation of engineered pharmaceutical fine particles of budesonide for dry powder inhalation (DPI) produced by amphiphilic crystallization technique: optimization of process parameters. International journal of pharmaceutical sciences and research 2013;26: 4656-670.
86. Marina Andrade-LimaI, Luiz Fernando Ferreira Pereira, Ana Luisa Godoy Fernandes. Pharmaceutical equivalence of the combination formulation of budesonide and formoterol in a single capsule with a dry powder inhaler. Journal Brasileiro de Pneumologia 2012;38(6):1237-45.
87. Abolghasem Safdari, Mohammad Ali Darbandi. Propranolol particle production by Spray Drying Technique and evaluation of the In Vitro and In Vivo Lung deposition. Interventional Cardiology Journal 2016;2(14):274-85.
88. Rayehe Teymouri Rad , Simin Dadashzadeh , Alireza Vatanara , Sonia Alavi, Elham Ghasemian , Seyed Alireza Mortazavi. Tadalafil Nanocomposites as a Dry Powder Formulation for Inhalation, a New Strategy for Pulmonary Arterial Hypertension Treatment. European Journal of Pharmaceutical Sciences 2019;133:275-86.
89. Birendra Chaurasiya, Muye Zhou, Jiasheng Tu, Chunmeng Sun. Design and validation of a simple device for insufflation of dry powders in amice model. European Journal of Pharmaceutical Sciences 2018;123:495-501.

90. Duret C, Wauthoz N, Merlos R, Goole J, Maris C, Roland I, Sebti T, Vanderbist F, Amighi K. In vitro and in vivo evaluation of a dry powder endotracheal insufflator device for use in dose-dependent preclinical studies in mice. Eur J Pharm Biopharm. 2012;81(3):627-34.
91. L Tryptophan Monograph. Alternative Medicine review 2006;11(1):52-56.
92. L Tryptophan Monograph. Alternative Medicine review 2007;12(4):364-68.
93. Raymond C Rowe, Paul J Sheskey, Marian E Quinn, eds. Handbook of Pharmaceutical Excipients. Sixth edition USA:Pharmaceutical Press and American Pharmacists Association; 2009. 359-361.
94. D.V. Gowda, Vishnu Datta M, Meenakshi S, Siddaramaiah H, Vishal Kumar Gupta N. Formulation and evaluation of dry powders containing anti tuberculosis drugs for pulmonary delivery. Indo American Journal of Pharmaceutical research 2013; 3(12):1239-48.
95. Subrahmanyam CVS. Textbook of Physical Pharmaceutics. Second edition.New Delhi: MK Jain for Vallabh Prakashan;2000;180-228.
96. K Sathishkumar, C Kumaresan. Novel, Sustained-release, dry powder inhalable formulation for monteleucast sodium. Int. Res. J. Pharm. 2016; 7(12):97-103.
97. S. Chennakesavulu, A. Mishra, A. Sudheer, C. Sowmya, C. Suryaprakash Reddy, E. Bhargav. Pulmonary delivery of liposomal dry powder inhaler formulation for effective treatment of idiopathic pulmonary fibrosis. Asian journal of pharmaceutical sciences 2018;13:91–100
98. Caramori G, Adcock I. Pharmacology of airway inflammation in asthma and COPD. Pulmonary Pharmacol.Ther.2003;16:247-77
99. Rayehe Teymouri Rad , Simin Dadashzadeh , Alireza Vatanara , Sonia Alavi, Elham Ghasemian , Seyed Alireza Mortazavi. Tadalafil Nanocomposites as a Dry Powder Formulation for Inhalation, a New Strategy for Pulmonary Arterial Hypertension Treatment. European Journal of Pharmaceutical Sciences 2019; 133: 275-86.
100. Birendra Chaurasiya, Muye Zhou, Jiasheng Tu, Chunmeng Sun. Design and validation of a simple device for insufflation of dry powders in amice model. European Journal of Pharmaceutical Sciences 2018;123:495-501.
101. Wojcieh G Lesniak, Amar Jyoti, Manoj K Mishra, Nicolette Louissaint, Roberto Romero, Diane C Chugani, Sujatha Kannan, Rangaramanujam M Kannan.

Concurrent quantification of tryptophan and its major metabolites. J.Anal Biochem 2013;443(2):222-31.

102. Banan Elshiekh Alsied Basheir, Abdalla A Elbashir. Spectrophotometric methods for the determination of L Tyrosine in pharmaceutical formulations. Journal of ChemXpress 2015;8(2):95-101.
103. Ebtsam M. Abdou, Soha M. Kandil, Amany Morsi, Maysa W. Sleem. In-vitro and in-vivo respiratory deposition of a developed metered dose inhaler formulation of an anti-migraine drug. Journal of drug delivery 2019;26(1):689-99
104. Chua AL, Silberstein S. Inhaled drug therapy development for the treatment of migraine. Expert opinion Pharmacotherapy 2016;17(13):1733-43.
105. Marianecci C, Marzio LD, Rinaldi F, et al.,. Pulmonary delivery:innovative approaches and perspectives. J Biomed Nanobiotechnol 2011;2:567–75.
106. Stank K, Steckel H. Physico-chemical characterisation of surface modified particles for inhalation. Int J Pharm 2013;448:9–18.
107. Patton JS, Byron PR. Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov 2007;6(1):67–74.

10. APPENDICES

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
(Reg.No: 1762/PO/Re/S/14/CPCSEA)
KARPAGAM FACULTY OF MEDICAL SCIENCES & RESEARCH
Coimbatore 641 032, Tamilnadu, India

Date: 27/07/2019

CERTIFICATE

This is to certify that the project title "**Formulation development of dry powder inhalation of amino acids for smoking cessation**" has been approved by the IAEC.

IAEC NO-KFMSR/M. Pharm/01/2019-20

~~Name of Chairman~~/Member Secretary IAEC: T.K. Ponn 27/7/19

Name of CPCSEA nominee: 27/7/2019

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

No.69, ANNA SALAI, GUINDY, CHENNAI - 600 032.

Website : www.tnmgrmu.ac.in
E-mail : mail@tnmgrmu.ac.in
Ph : 22353574, 22353576 - 79, 22301760 - 63, 22353094
Fax : 91-44-22353698

Dr. S.V. SENTHILNATHAN, M.S.,M.Ch.,
DEAN OF STUDENTS.

SW(1)/33738 /2018-18

DATE:19 .12.2018

To

Ms. M. Abinaya,
(Dr. S. Mohan, Principal)
Karpagam College of Pharmacy,
Coimbatore – 641 032

Madam,

Sub.:		Students Welfare Section – The Tamil Nadu Dr.M.G.R Medical University – Project titled **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** – Grants fund for the year 2018-2019 – Intimation – Reg.
Ref.:	1	Your letter dated 26.07.2018
	2	Recommendation of the University Research Council Meeting held on 05.10.2018

I am to inform that your project **"FORMULATION DEVELOPMENT OF DRY POWDER INHALATION OF AMINO ACIDS FOR SMOKING CESSATION"** submitted by you before the University Research Council of this University on 05.10.2018 for seeking Research Grant from the University to do your Research Project Work was approved by the members of the Research Council of this University.

Based on the approval of your Research Project work, I am to enclose herewith a cheque for Rs.25,000/- (Rupees Twenty five thousand only) vide cheque No.995511 dated 29.11.2018 towards the 1st installment of 50 %. Research Grant is hereby released by the University in the name of the Principal Investigator. The said grant will be sanctioned in three (3) installment that is 50 % as 1 st Stage balance 50 % will be released as 25 % each 2 nd and last installment on production of Utilization certificate/ Bills by you.

-2-

Therefore, you are instructed to follow the rules in force as detailed below:

1. Minimum duration of the Project is 2 years. If the project is not completed within the stipulated period, an extrension of 6 months may be allowed with justified reasons.

2. If the project is not completed, within the prescribed time limit granted amount has to be refunded to the University for that you have to submit an Undertaking in a Non Judical Stamp paper for Rs.20/- (Rupees Twenty only) stating that **" I ....., S/o. / D/o. ..... hereby undertake that I will submit my project to the Tamilnadu Dr. M.G.R. Medical University, Chennai with in a period of 2 (Two) years as per the letter dated ..... issued to me and follow the Terms and conditions mentioned therein. If I failed to submit my project to the University within a period of two (2) years, I shall remit the grant received in full to the University by giving a suitable reason for the non uitlization of Grant."**

3. The Utilization certificate along with Expenditutre statement giving the break up is to be provided at the end of the study period.

4. All expenditure should be supported by Official bills / Vouchers. Any expenditutre above Rs.5,000/- (Rupees Five thousand only) should be supported by stamped receipts.

5. At the end of the study period, a detailed report has to be submitted to the University duly signed by the Principal Investigator & Co-Investigator or Guide. Further, any publication arising from the project must acknowledge the role of the University as a funder.

6. An undertaking stating that there is no other source of funding availed for the same project and also if the project is not completed within the prescribed period the entire amont received from the University will be remitted in the University Account.

7. **You are not eligible to apply for further grants for the ensuing three years.**

You are requested to furnish an undertaking in Non Judical stamp paper to the face value of Rs.20/- along with acceptance for the rules and regulation of this University for taking necessary further action.

Kindly acknowledge the cheque by return of post.

19/12/18

DEAN OF STUDENTS

Encl.:
One Cheque No.995511 dated 29.11.2018
for Rs.25,000/-

19/12/18