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FORMULATION OPTIMIZATION AND INVITRO EVALUATION OF ROFLUMILAST LOADED NANOSTRUCTURED LIPID CARRIER FOR PLAQUE PSORIASIS

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ABSTRACT
The present study was primarily aimed at the development and optimization of Roflumilast loaded Nanostructured Lipid Carriers (ROF-NLCs) for the topical management of Psoriasis, a chronic, immune-mediated inflammatory skin disorder requiring long-term therapy. Roflumilast, a selective Phosphodiesterase-4 (PDE4) inhibitor, exhibits therapeutic potential in psoriasis but its clinical use is limited by poor aqueous solubility and inadequate skin penetration. To overcome these limitations, ROF-NLCs were formulated using Glyceryl monostearate, Cetyl palmitate, and Oleic acid as lipid components with Tween 80 as surfactant. A 2^3 factorial design was employed to optimize critical formulation parameters including lipid ratio and surfactant concentration. Pre-formulation compatibility was confirmed through FTIR studies, which indicated no significant drug–excipient interaction. Eight different formulations (F1–F8) were prepared by Hot Homogenization technique and were evaluated for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug content, in vitro drug release, release kinetics, morphological characterization (SEM), and stability studies. Among the prepared formulations, F5 was identified as the optimized batch, showing an average particle size of 210 nm, zeta potential of –20.5 mV, entrapment efficiency of 82.3%, drug content of 96.3% and maximum cumulative drug release of 84.8% at the end of 8 hours. SEM analysis revealed spherical particles with smooth morphology. In vitro release kinetics of F5 followed Zero-order and Hixson–Crowell models, suggesting a controlled release mechanism. Stability studies confirmed the robustness of the formulation under storage conditions. The optimized formulation (F5) of Roflumilast-loaded NLCs demonstrated favourable particle size, stability, high entrapment efficiency, and sustained drug release profile. These findings indicate that NLCs significantly enhance the solubility, stability, and skin penetration of Roflumilast, making them a promising platform for effective topical delivery in the management of psoriasis.

KEYWORDS: Roflumilast, Nanostructured Lipid Carrier, Psoriasis, Hot Homogenization technique, Bioavailability, Phosphodiesterase-4 (PDE4) inhibitor.

1. INTRODUCTION

1.1 NOVEL DRUG DELIVERY SYSTEM[10]

Nanotechnology-based drug delivery systems have emerged as a transformative approach in modern therapeutics, offering significant advantages over conventional drug delivery methods. The aim of Novel Drug Delivery System is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug delivery system should deliver drug at a rate control by the necessarily of the body over a specified term of treatment.

Nano drug delivery systems (NDDS) utilize nanoscale carriers typically ranging from 1 to 1000 nm to improve the solubility, stability, and bioavailability of drugs. These systems enable targeted delivery, controlled release, and reduced side effects by enhancing drug accumulation at the desired site of action while minimizing exposure to healthy tissues. NDDS are especially beneficial for poorly water-soluble drugs and have been extensively explored in areas such as cancer therapy, central nervous system disorders, and topical treatments. As a result, they represent a promising

platform for enhancing therapeutic outcomes and patient compliance in both systemic and localized drug delivery. A number of novel drug delivery system has emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structure is one such system, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity if selective uptake can be achieved. Consequently, number of vesicular drug delivery system such as liposomes, niosomes, transfersomes and pharmacosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of this system that allow drug targeting and sustained or controlled release of conventional drug medicines.

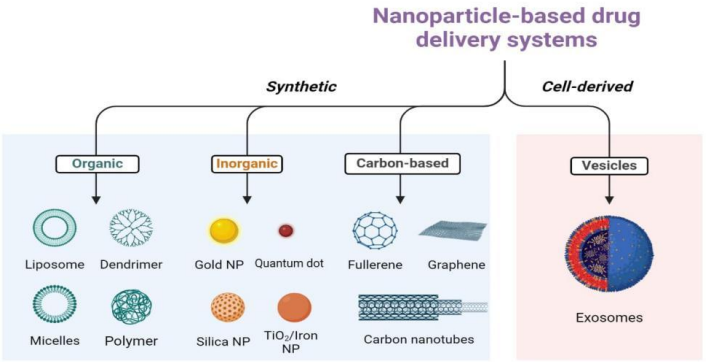

Figure 1.1: Nanoparticle based drug delivery system.

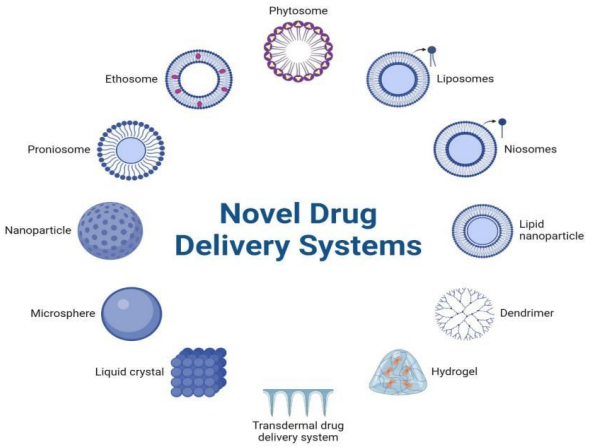

Figure 1.2: Novel drug delivery system.

1.2 NANOTECHNOLOGY[21,30]

Nanotechnology can be defined as the science and engineering involved in the design, characterization, production and applications of structures, devices and systems by controlling shape and size at nanometre scale. Pharmaceutical Nanotechnology has paved the road for disease diagnostics and therapeutics. Nanotechnology is providing solutions for several pharmaceutical drug delivery issues. For over 20 years, researchers have appreciated the potential benefits of, Nanotechnology in providing vast improvements in drug delivery and drug targeting. Improving delivery techniques that minimize toxicity, improve efficacy and offers great potential benefits to patients, and new markets for pharmaceutical and drug delivery companies.

Nanomedicine is the branch of medicine that utilizes the science of nanotechnology in the preclusion and cure of various diseases using the Nanoscale materials, such as biocompatible Nanoparticles and Nanorobots for various applications including, diagnosis, delivery, sensory, or actuation purposes in living organism. Pharmaceutical Nanotechnology based Nanoparticle drug delivery system are widely used nowadays and they can be categorized based on their platform composition into several groups. They are

- Polymeric nanoparticles
- Dentrimers
- Quantum dots
- Carbon nanotubes
- Magnetic nanoparticles
- Inorganic nanoparticles
- Organic nanocrystals
- Nanoparticle albumin bound technology
- Liposomal delivery
- Solid lipid nanoparticles

- Nanostructured lipid carrier.

1.3 TOPICAL DRUG DELIVERY[6,21]

The topical delivery system refers to a method where in the formulation is applied to the superficial areas such as the skin, eyes, nose and vagina for the treatment of local diseases. The drug application to the topical surfaces evades the hepatic first pass metabolism, gastric pH variations and fluctuations in plasma levels, frequently encountered when a drug is administered through the oral route. The stratum corneum is the major barrier to the access of foreign particles through the Skin. The other advantages associated with the topical drug delivery system include the following

- Patient compliance and acceptance,
- Ease and convenience of application,
- Painless and noninvasive technique,
- Improvement in drug bioavailability,
- Better physiological and pharmacological response and
- Minimum systemic toxicity and exposure of drug to non-infectious tissues/ sites

TYPES OF TOPICAL DRUG DELIVERY SYSTEM[47]

Includes two types of topical drug delivery system:

a) **Internal** - that are applied to the mucous membrane of eye (conjunctiva), ear, oropharyngeal cavity, nasal cavity, vagina or anorectal region for local activity.

b) **External** – that are spread or dispersed on the cutaneous surface covering the affected area

SKIN[14,24]

Skin consists of three main parts:

1. Epidermis - outer layer
2. Dermis - beneath the epidermis
3. Hypodermis - inner layer

PATHWAYS OF DRUG ABSORPTION THROUGH THE SKIN[50]

The drug can be absorbed by various pathways through the skin depending on the physicochemical properties of the drug. Both lipophilic and hydrophilic drugs are absorbed from different routes. The upper stratum corneum of the skin opposes the absorption of drug but presence of various absorption routes facilitates the entry of drug and transport of drug to the systemic circulation.

Various drug absorption routes are as follows:

a) Transfollicular route

Transfollicular route is the shortest pathway that drug has to follow to reach the systemic circulation that provides a large area for diffusion of drugs. Skin has various sweat glands, oil glands, hair follicles and pores opening to the outer surface of the skin via their ducts. These ducts offer a continuous channel across the stratum corneum for drug transport but various factors like secretion from glands, content and amount of secretion etc., affect the transport of drugs through this route. However, transappendageal route occupies only 0.1% of total skin surface and therefore contributes a little.

b) Transcellular route

Drug delivering through this route passes from corneocytes which has highly hydrated keratin creating hydrophilic pathway. Corneocytes are surrounded by lipids connecting these cells. So, a drug requires a number of partitioning and diffusion steps. It is the most widely used route by various types of drugs. In transcellular route drug passes through the matrix (cytoplasm) of the cells. This route is suitable for hydrophilic drugs. The drug passes through the corneocytes of stratum corneum. The highly hydrated keratin provides aqueous pathway to the hydrophilic drugs. A number of partitioning and diffusion steps are needed to pass the drug through the cell matrix.

c) Intercellular route

As name indicates in intercellular pathway the drug diffuses through the continuous lipid matrix present between the cells. The barrier property of this route is due tortuous structure formed by corneocytes and the drug has to pass through the alternating lipid and aqueous domain by partitioning into the lipid bilayer and diffusing to the inner side. It has been found that water has to travel 50 times more by this route so; it is suitable mainly for uncharged lipophilic drug.

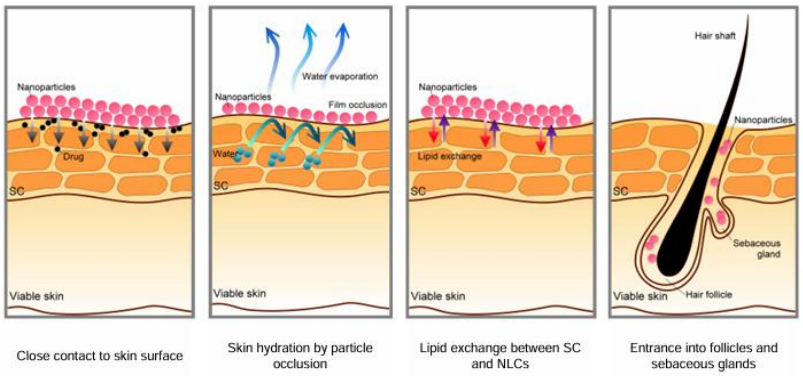

Figure 1.3: Mechanism of Drug Permeation through Skin.

ADVANTAGES OF TOPICAL DRUG DELIVERY SYSTEM

- Avoidance of first pass metabolism.
- Easy application.
- Suitable for self-medication.
- Improved patient compliance.
- Avoidance of gastro- intestinal incompatibility.
- Drug can be delivered more selectively to a specific site.

DISADVANTAGES OF TOPICAL DRUG DELIVERY SYSTEM

- Skin irritation / contact dermatitis due to drug and/or excipients.
- Poor permeability of some drugs through the skin.
- Possibility of allergic reactions.
- Can be used only for those drugs which require low plasma concentration of action.
- Enzymes in epidermis may denature the drugs.
- Drugs with larger particle size are difficult to get absorbed through the skin.

1.4 NANOSTRUCTURED LIPID CARRIER[52]

Lipid nanoparticles as drug delivery systems were considered from the beginning of the 19th century by professor R. H. Muller from Germany and Professor M. Gascon from Italy. In the last decade, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been looked upon as promising carriers for presenting several attractive features for transdermal drug delivery. Nanostructured Lipid Carrier (NLCs) are second generation of lipid-based nanocarriers formed from mixture of solid and liquid lipids and have unstructured-matrix due to the different moieties of the constituents of NLCs. NLCs were designed in order to overcome the Solid Lipid Nanoparticles (SLNs) limitations.

NLCs are colloidal drug delivery system composed of mixture of a solid and liquid lipids, surfactants offering improved drug loading capacity, controlled drug release and enhanced stability. NLCs are suitable for both lipophilic and hydrophilic drugs. These carriers provide several advantages including increased solubility of poorly water-soluble drugs, protecting the sensitive drug molecules and enhanced bioavailability. NLCs can be used for various routes of administration such as oral, parenteral and topical delivery. Due to their biologically non-toxic and non-immunogenic NLCs are going to be widely explored among lipid nanocarrier system.

Many pharmaceutical companies have developed a well-established industrial process for the manufacturing of large-scale batches of nanostructured lipid carriers, but still all major kind of parameters like choice of lipid, surfactants other essential excipients and methods of preparation varies which leads to change in parameters like particle shape and size, phase transition, solubility, bioavailability of drug etc.

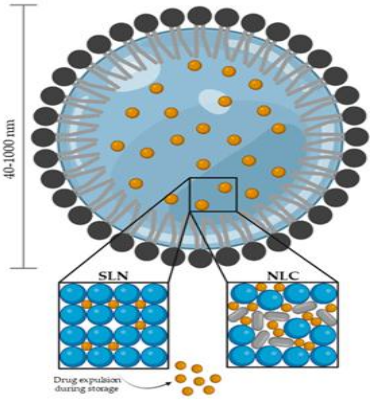

Figure 1.4: Nanostructured Lipid Carrier.

NLCs have higher drug loading capacity because of imperfect crystal structure and could avoid drug expulsion by avoiding lipid crystallization during the manufacturing and storage periods. Due to the presence of liquid lipids in NLCs formulation expulsion of loaded drug after formulation and during the storage period is minimized. NLCs also can increase drug solubility in lipid matrix and they can show more controllable release profiles in comparison to SLNs. Although NLCs are solid in nature even in body temperature but they have low melting point than SLNs and due to their unstructured nature and imperfection in their crystalline behaviours provide more space for drug dissolution and payload in liquid part of the NLCs. So that the loading capacity in NLCs are more than SLNs. Previous researches also confirm on less susceptibility of NLCs than SLNs to gelation during the preparation and storage period, which is another advantage of NLCs.

1.5 TYPES OF NANOSTRUCTURED LIPID CARRIER[42]

Depending on the various production techniques and the composition of the lipid blends, different types of NLCs are obtained. The basic idea is to provide a certain nanostructure for the lipid matrix so as to increase the pay-load for active compounds and reduce the expulsion

of compound during storage. The three types of NLCs can be summarised as,

Types

- NLC Type 1 also known as Imperfect crystals.
- NLC Type 2 also called as Multiple type.
- NLC Type 3 also called as Amorphous type.

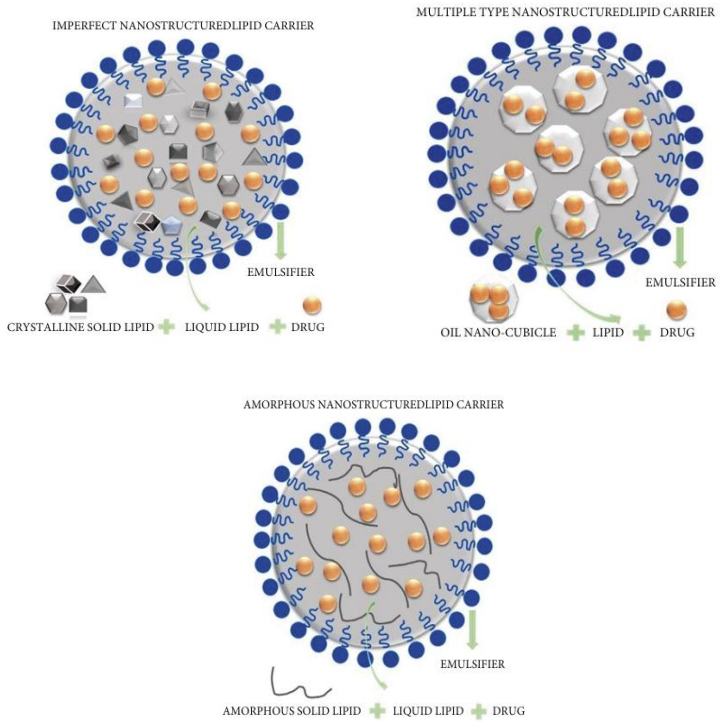

Figure 1.5: Types of Nanostructured Lipid Carrier.

NLC TYPE 1

NLC type 1 also called imperfect crystal types have a badly structured solid matrix. Different fatty acids such as glycerides can be used to improve and modify the structure. The total number of imperfections in the structure are responsible and also helpful for the property of good drug which can be easily increased. The type 1 of NLCs can be prepared by mixing spatially with different lipids which can leads to imperfections in the crystal lattice. The drug molecules lodges extra disorderly crystal as molecular form and amorphous clusters. To avoid this adding to a minor quantity of liquid lipid additional leans to increases the drug-loading. The small quality of the glycerides can be used to overcome this situation.

NLC TYPE 2

The oil-in-lipid-in-water type NLCs is also called as multiple type. In type 2 NLCs the solubility of oil is greater as compare to solubility of solid lipids. In type 2 NLCs high amount of oil are mixed with solid lipids due to this oil molecule can easily spread into the lipid matrix at a low concentration of oil. If the added oil in excess quantity than required of its solubility can lead to separation of different phases, finally produces small oily nano compartments which are bounded by the solid lipid matrix. This kind of formulation permit controlled drug release and leakage of drug from lipid matrix. In this case, lipophilic drugs can be made soluble in oil first and type 2 method can be followed with the cooling procedure of a hot homogenization process.

NLC TYPE 3

This type of NLCs also called as amorphous type. In this technique of preparation of NLC's, the lipids are mixed in such a way that crystallizing can be prevented through mixing procedure. In type 3 method the lipid matrix remains solid but, in an amorphous state the technique and method of crystallization often leads to drug expulsion. To minimize this, NLCs can also be formulated by carefully mixing of solid lipids with special lipids such as hydroxy octacosanyl hydroxyl stearate, isopropyl palmitate or MCT. Solid non-crystalline NLCs are formed.

1.6 COMPONENTS OF NANOSTRUCTURED LIPID CARRIER[41]

The lipid itself is the main ingredient of NLC that influence their drug loading capacity, stability and the sustained release behaviour of the formulations. Lipid nanoparticle dispersions are based upon a variety of lipid materials including fatty acids, glycerides, and waxes. They are approved as generally-recognised-as-safe (GRAS) and are physiologically well-tolerated. Components of NLC includes,

Solid lipid

These solid lipids are chemical compounds which have a

melting point higher than 40˚c and are well tolerated. They are accepted for human use and also available in-vivo biodegradable.

Examples are beeswax, carnauba wax, dynasan, precirol ATO 5, Glyceryl di-stearate, Glyceryl monostearate, Stearic acid, cetyl palmitate, Glyceryl trilaurate etc.

Liquid Lipid
These liquid lipids are well tolerated and accepted for human use.

Examples are Cetiol V, miglyol, castor oil, oleic acid, davana oil, palm oil, olive oil etc.

Emulsifying agents
- Ionic surfactant (Sodium taurodeoxycholate, sodium oleate, sodium dodecyl sulphure)
- Non-ionic surfactant (Span 20, 80,85, Tween 20,80, Poloxamer 188, Poloxamer 407, Solutol HS15)
- Amphoteric surfactant (Egg phospholipid, hydrogenated soy phosphatidyl choline, Phospholipon 80H, Phospholipon 90H)
- Co-surfactant (Butanol, Butyric acid).

Surface modifiers
- Dipalmitoyl-phosphatidyl-ethanolamine conjugated with polyethylene glycol 2000 (DPPE-PEG2000).
- Distearoyl-phosphatidyl-ethanolamine-N-poly (ethylene glycol) 2000 (DSPE-PEG2000)
- Stearic acid-PEG 2000 (SA-PEG2000).
- α-methoxy-PEG 2000-carboxylic acid-α-lipoamino acids (mPEG2000-C-LAA18).

1.7 PREPARATION METHODS FOR NLC[17]
Nanostructured Lipid Carrier can be prepared by various methods. They are as follows
- High shear homogenisation
- Ultrasonification
- Solvent diffusion method
- Solvent emulsification-evaporation technique
- Film-ultrasonification method
- Microemulsion
- Spray drying method
- Double emulsion technique
- Solvent injection
- Hot melt extrusion method
- Supercritical fluid technology

HIGH SHEAR HOMOGENISATION
High pressure homogenization is a well-established and reliable method of lipid nanoparticles preparation. Scale up of lipid NPs preparation can be achieved with the help of HPH. This method is adopted in two ways as follows

a) HOT HOMOGENISATION TECHNIQUE
In this method, the liquid and solid lipids are mixed and heated above the melting temperature of the solid lipid and drug is added to obtain drug dispersed lipid melt. Aqueous phase is prepared separately by adding sufficient quantity of surfactant in deionised water. This phase is also heated at the same temperature as lipid melt. These two phases are mixed and subjected to high shear homogenization at elevated temperature for shorter period of time to obtain pre-emulsion. Immediately, pre-emulsion is passed through HPH at varying pressures for 3–5 cycles. Generally the repetition of number of cycles depends upon the desired average droplet size of nano-emulsion. Then the emulsion is cooled at room temperature while stirring. Here solidification of droplets takes place due to recrystallization of solid lipid. Some reports include the usage of lipophilic emulsifier in lipid melt. This may be to improve the stability of pre-emulsion during homogenization. This method is suitable for the drugs which are not heat sensitive.

b) COLD HOMOGENISATION TECHNIQUE
The well-established hot HPH method has a limitation that the elevated temperature during processing may cause decomposition of hydrophilic and thermo-labile drugs. To avoid this, a simple approach of rapid cooling of nano-emulsion is adopted. The liquid and solid lipids are melted at the temperature higher than melting point of solid lipid. Then drug is dispersed or dissolved in heated lipid melt and subjected to HPH. The emulsion obtained is rapidly cooled by subjecting to liquid nitrogen or dry ice. The solid mass obtained is grounded to obtain microparticles. These are dispersed in the cold aqueous phase containing suitable surfactant and subjected to high shear homogenization or ultrasonication to obtain NLCs.

EMULSION-ULTRASONIFICATION METHOD
This method is somewhat similar to HPH. Drug, liquid and solid lipids are mixed and melted 5–10°C temperature above melting point of solid lipid. The surfactant is dissolved in distilled water and heated at same temperature as lipid melt. Aqueous phase is added in lipid phase and this pre-emulsion is homogenized at high shear by applying re quired rpm for specific time. Then this emulsion is ultrasonicated for specific time and then added to specified volume of distilled water. This is cooled at room temperature to solidify to obtain NLCs. Contamination of formulation due to metal particles may occur during probe sonication.

SOLVENT DIFFUSION METHOD
Solvent diffusion method involves the use of water miscible organic solvents such as methanol, ethanol and acetone, etc. In this method, the drug and lipids are added in the single or a mixture of organic phases. This is sonicated and maintained at elevated temperature to obtain clear lipid phase. Aqueous phase is prepared by adding suitable stabilizer/surfactant and maintained at same temperature as that of lipid phase. The organic-lipid phase is added in the aqueous phase under mechanical stirring at elevated temperature. This dispersion is stirred at room temperature for cooling and evaporation of organic solvent to obtain NLCs.

SOLVENT EMULSIFICATION-EVAPORATION TECHNIQUE

This name is used interchangeably with above method i.e. diffusion. In this method instead of using water miscible organic solvent, water immiscible organic solvents such as chloroform, cyclohexane, etc. are used to dissolve drug and lipids. Use of organic solvents is the prime limitation of solvent diffusion and evaporation method as some traces of it may remain in the formulations.

FILM-ULTRASONIFICATION METHOD

This method is adopted from the preparation methods of vesicular drug delivery systems. Lipids and drug are dissolved in an organic solvent preferably in ethanol. Aqueous phase is prepared by dissolving a surfactant in water and this is kept at elevated temperature. This phase is maintained at elevated temperature with stirring to obtain proper blend of drug-lipids. Organic phase is removed by applying vacuum using rotary evaporator. This will tend to form a thin film of drug-lipid blend which is collected and dispersed in heated aqueous phase under sonication. This dispersion is cooled at room temperature to obtained dispersed solidified NLCs.

MICROEMULSION TECHNIQUE

Melted lipid is added in the preheated oil and drug is dissolved in this mixture. Aqueous phase is prepared by dissolving surfactant in distilled water. Both phases are heated and maintained at elevated temperature. Lipid phase is added in aqueous phase under mechanical stirring at same temperature which allows formation of microemulsion. This warm microemulsion is added in the cold water (temp. 2–4°C) under stirring. Here, dilution with large volume of cold water is required for precipitation of microemulsion globules to form NLCs. Dilution with large volume of water leads to reduction in concentration of actives. So, further concentration of formulation or lyophilization is required.

DOUBLE EMULSION TECHNIQUE

In double emulsion technique the drug (mainly hydrophilic drugs) is dissolved in aqueous solution, and further emulsified in melted lipid. The primary emulsion is stabilised by adding stabiliser that is dispersed in aqueous phase containing hydrophilic emulsifier, which is followed by stirring and filtration. Double emulsion technique avoids the necessity to melt the lipid for the preparation of peptide-loaded lipid nanoparticles and the surface of the nanoparticles could be modified in order to sterically stabilise them by means of the incorporation of lipid-PEG derivatives.

SOLVENT INJECTION METHOD

The basic principle of the solvent injection method is similar to the solvent diffusion method. In case of solvent injection method, lipids are dissolved in a water-miscible solvent (e.g. acetone, isopropanol and methanol) or water-miscible solvent mixture and quickly injected into an aqueous solution of surfactants through an injection needle (Schubert and Müller-Goymann 2003). The advantages of this method are the easy handling and fast production process without technically sophisticated equipment (e.g. high-pressure homogeniser). However, the main disadvantage is the use of organic solvents (Müller *et al*. 2002).

HOT MELT EXTRUSION TECHNOLOGY

Most of the techniques used for the NLC preparation (above methods) are adopted from either the methods of preparation of SLN or other nanocarrier systems. These are multistep methods and their commercialization is difficult except HPH. Hot melt extrusion using twin screw extruder is a newer technology adopted for preparation of NLCs by Bhagurkar et al. There were three feeding ports in extruder in which first for addition of lipid drug mix, second for addition of liquid lipid and third was for addition of aqueous phase. Solid lipid and drug mix was added in first port via volumetric feeder, heated liquid lipid was added through second port by peristaltic pump. As it proceeded in the extrusion barrel, it was subjected to melting and mixing section. Third port was selected for addition of preheated aqueous phase containing suitable surfactant by peristaltic pump and mixed at desired speed to obtain pre-emulsion. That pre-emulsion is extruded into a vessel attached with probe-sonicator to obtain NLCs. The feeding rates were optimized prior to experiment.

SUPERCRITICAL FLUID TECHNOLOGY

Lipids are melted and the solubilization of SCF (preferably carbon dioxide) is carried out in it. This form either gas saturated suspension or solution depending on the solubility of materials in the SCF. Then it is atomized through nozzle and sprayed in a chamber. During this phase, decompression and evaporation of gas occurs forming solid NLCs. A patent survey was carried out by Carbone et al. regarding the methods of production and characterization of lipid nanocarriers. Often patent applications were based on the formulation of hybrid lipid nanoparticles. That is a polymer is added in the lipid mixture while formulation of NP which resulted in the dispersion stability against drug degradation, reduced drug escape in the dispersion media and control on the drug release. Some have suggested the surface coating of lipid NP with the different molecules which improved their targeting potential. For the production of such lipid NP, HPH, microemulsion and nanoprecipitation methods can be employed. Proteins were encapsulated in similar manner using lipid and polymer without using organic solvents. Lipid bilayer NP system showed improved stabilization of lipid carriers in aqueous media. Use of cationic polymers was found to be beneficial in targeting and cellular uptake.

1.8 CHARACTERIZATION AND EVALUATION OF NANOSTRUCTURED LIPID CARRIER[16]

Characterization of NLCs is a key parameter for the successful development of drug delivery. The physiochemical parameters like size, surface charge,

molecular weight and solubility have a profound effect on the uptake and distribution of lipid-based Nanoformulations. So all these parameters need to be critically characterized

- Surface Charge
- Particle size
- Morphological character
- Entrapment efficiency
- Loading capacity
- Stability studies
- Invitro drug release.

ADVANTAGES

- Improved drug loading capacity.
- Enhanced solubility for poorly water-soluble drugs.
- Both hydrophilic and lipophilic drugs are transported at a same time.
- Biocompatibility and safe for use.
- Less risk of toxicity.
- Enhanced bioavailability.
- Reduced drug leakage.
- Enhanced permeation through biological membranes

1.9 APPLICATIONS OF NLC[37]

NLC FOR TOPICAL DELIVERY

Topical route has been greatly exploited for the drug delivery to dermal areas employing lipid-based nanoparticles. In recent years, many studies and experiments have been performed on topical application of NLCs for their unique properties. NLCs can enhance the apparent solubility of entrapped drugs, which can form high concentration gradient on skin to facilitate drug permeation. The nano-sized particles tightly adhere to the skin surface and release the drugs in a more controlled manner. NLCs are used for topical application of various categories of drugs for improvement of penetration along with sustained release. Experimental studies have confirmed the significant improvement in therapeutic response and reduction in local side effects of acitretin NLCs loaded gel indicating its effectiveness in the topical treatment of psoriasis.

NLC FOR ORAL DELIVERY

NLCs have been proved as one of the beneficial systems for oral administration of poorly water-soluble drugs having low bioavailability. Another important feature is the high dispersivity of NLCs due to which they exhibit a high specific surface area for enzymatic attack by intestinal lipases. Other advantages of giving NLC in oral forms include increased drug loading; improved drug inclusion; patient compliance; high particle concentration and cream like consistency of the carrier. The mechanisms involved in the absorption of the NLC from the intestine include direct uptake through the GI tract, increase in permeability by surfactants and decreased degradation and clearance. Besides this, the NLCs can also adhere on to the gut wall prolonging the residence time, and the absorption. Poloxamer is involved in deforming the cell membrane and opening of the tight junction of intestinal epithelial cell, thus facilitating paracellular transport of NLCs.

NLCs FOR PARENTRAL DELIVERY

The nano-drug delivery systems such as nanomicelles, nanoemulsions and nanoparticles has displayed a great potential in improved parenteral delivery of the hydrophobic agents since last two decades. NLC has been considered as an alternative to liposomes and emulsions due to improved properties such as ease in manufacturing, high drug loading, increased flexibility in modulating drug release profile, and along with these, their aqueous nature and biocompatibility of the excipients has enabled. Intravenous delivery of the drug with passive targeting ability and easy abolishment. Another reported example is NLCs of artemether that offers significant improvement in the anti-malarial activity and duration of action as compared to the conventional injectable formulation. Nanoject can be considered as a viable alternative to the current injectable intramuscular (IM) formulation.

NLC FOR OCULAR DRUG DELIVERY

Ophthalmic drug delivery with long pre-corneal retention time and high penetration into aqueous humour and intraocular tissues is the key-limiting factor for the treatment of ocular diseases and disorders. Recent reports indicated that NLC could increase the ocular bioavailability of lipophilic drug, ibuprofen. Previous research showed that NLC could improve the penetration of bioactive compounds into ocular tissues with a good ocular tolerance. Another approach is to increase the trans corneal passage of drugs by incorporating permeation enhancers into formulations. Mucoadhesive nanostructured lipid carrier modified by thiolated agent has also been evaluated as a promising carrier for ocular drug delivery in vitro and in vivo. The in vivo distribution investigation indicated that thiolated NLC could prolong pre-corneal residence time, and deliver high cyclosporine (CyA) level into eye tissues in ocular surface and anterior chamber.

DRUG DELIVERY TO BRAIN

Brain targeting not only increases the CSF concentration of the drug but also reduces the frequency of dosing and side effects. The major advantages of this administration route are avoidance of first pass metabolism and rapid onset of action as compared to oral administration. NLC of this generation are considered to be one of the major strategies for drug delivery without any modification to the drug molecule because of their rapid uptake by the brain, bioacceptability and biodegradability. Further, the feasibility in scale-up and absence of burst effect make them more promising carriers for drug delivery. In addition, NLC further enhanced the intranasal drug delivery of duloxetine in the brain for the treatment of major depressive disorder.

NLC FOR PULMONARY DRUG DELIVERY

Drug delivery via inhalation is also a potential route for the treatment of several pulmonary disorders having

advantages over conventional dosage forms. In pulmonary drug delivery systems, surfactants and co-solvents are also often used to prepare stable formulations of highly lipophilic active ingredients. The lipophilic COX-2 inhibitor, celecoxib, was successfully encapsulated in the NLC nanoparticles using mixture of solid and liquid lipids where most of the nebulised nanoparticles were able to deposit in the alveolar region of the mice lungs and also enhanced the celecoxib lung residence time.

COSMETICS

Recently NLCs have been developed based on the controlled nanostructuring of particle matrix which provides immense advantages with respect to loading capacity and long-term stability. The various forms in which NLC dispersions can be given are gel, cream, lotion, ointment. The beneficial aspects associated with these NLCs in cosmeceuticals are very broad which lies in, enhancing skin bioavailability of active ingredients, film formation and controlled occlusion, UV protection, penetration enhancement and epidermal targeting, enhancement of physical and chemical stability and in vivo skin hydration.

FOOD INDUSTRY

Because of its good stability and high loading capacity, the NLCs are widely applied in the pharmaceutical field. It was seldom reported that the NLC was applied as a nutritional supplement carrier in food industry for the capsule and beverage preparations. However, there are certain difficulties related to the raw material supply, availability and environ mental factors due to which there is still a great risk for food industry to invest in this area. Coenzyme Q10-loaded NLCs for food application were developed to enhance the physicochemical stability and bioavailability.

NUTRACEUTICALS

Nutraceuticals are bioactive compounds, which provide medicinal or health benefits, including the prevention, and treatment of diseases. Among them, the carotenoids are one of the most important groups of natural pigments, because of their wide distribution in plant tissues, structural diversity and numerous functions. Carotene-LNC with highly antioxidant and significant anti-bacterial activities were successfully produced by using natural oils and a versatile high-shear homogenisation technique.

CHEMOTHERAPY

NLCs not only enhanced the efficacy and stability but also reduced side effects of many cytotoxic drugs. Different nanosystems have been developed with anti-cancer drugs, for example, the albumin–paclitaxel nanoparticles were approved in early 2005 in the chemotherapy for metastatic breast cancer; etoposide NLCs were found to be cytotoxic against human epithelial-like lung carcinoma cells; stabilisation and prolonged release of topotecan NLCs in treatment of refractory ovarian and small-cell lung cancer. Advantages of incorporating anti-cancer drugs in NLCs include high drug loading efficiency; prolonged release profile; increased chemical stabilisation; increased cytotoxicity.

GENE DELIVERY AND GENE THERAPY

Transfer of genes to mammalian cells is the most challenging task to achieve efficient and safe gene therapy. Gene delivery systems are basically divided into two types, viz., viral and non-viral vectors. Viral vectors have been extensively investigated because of their high transfection efficiencies while non-viral vectors have the benefits of low immunogenicity and ease of preparation. Colloidal particulate delivery systems like cationic liposomes, SLNs, nanoemulsions, micelles, and some of the polymer-based vectors like poly-L-lysine, poly ethylenimine (PEI), polyamidoamine dendrimer and chitosan, exhibit significant advantages as potential candidates for efficient non-viral gene delivery. Among them, cationic liposomes and PEI are the most extensively investigated where cationic liposomes form a complex with anionic DNA molecules and deliver DNA through endosomes after endo cytosis of the complex (Zhang et al. 2008). Lipopolyplexes are used as nanomedicines for successful and efficient gene delivery. These are prepared by combination of gene (RNA/ DNA), polycations, and lipids. They are mainly preferred for gene delivery in treatment of various cancers.

LIMITATIONS[42]

- Low drug loading for hydrophilic drugs.
- Particle aggregation may occur.
- Scaling up is difficult.
- Drug may get expulsed during storage.
- Little bit complex formulation process.

7. MATERIALS AND METHODS

7.1 RAW MATERIALS

Table 7.1: List of raw materials with name of their suppliers.

S.NO	Name of Materials	Name of the Suppliers
1	Roflumilast	Strides Pharma, Bangalore
2	Glyceryl Monostearate	Mohini Organics Pvt. Ltd., Mumbai
3	Oleic Acid	Loba Chemicals
4	Cetyl Palmitate	Mohini Organics Pvt. Ltd., Mumbai
5	Tween 80	Mohini Organics Pvt. Ltd., Mumbai

8. METHODOLOGY

PREFORMULATION STUDIES

Preformulation testing was an investigation of physical and chemical properties of a drug substance alone. It was the first step in rational development of dosage form.

Organoleptic Properties

The color, odor and taste of the drug were recorded using descriptive terminology.

8.1 IDENTIFICATION OF DRUG

A) Identification of drug by FTIR Spectroscopy[20]

FTIR study was carried out to check identity of drug. Infrared spectrum of drug was determined on Fourier Transform Infrared Spectrometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug by using FT-IR spectrophotometer. The absorption maximum in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

B) Determination of Melting Point[4]

Small amount of drug was loaded in a capillary tube where one of capillary tube was closed and kept in the melting point apparatus and temperature was noted when drug melts.

8.2 Solubility of Roflumilast[3,4]

The solubility of Roflumilast was determined by Ten milligram of the drug was added to the solvent. The drug was added in increments of 10 mg. The quantity after which there is no further solubilization of drug was taken as the end point of drug solubility.

Table 8.1: Values for estimating drug solubility based upon USP definition.

Descriptive Term	Appropriate Volume of solvent in millilitres per gram of solute
Very soluble	Less than 1 part solvent needed to dissolve 1 part solute
Freely soluble	From 1 to 10 parts solvent needed to dissolve 1 part solute
soluble	From 10 to 30 parts solvent needed to dissolve 1 part solute
Sparingly soluble	From 30 to 100 parts solvent needed to dissolve 1 part solute
Slightly soluble	From 100 to 1000 parts solvent needed to dissolve 1 part solute
Very slightly soluble	From 1000 TO 10000 parts solvent needed to dissolve 1 part solute
Practically insoluble	More than 10000 parts solvent needed to dissolve 1 part solute

8.3 Determination of λ_{max}[1]

Weighed 10 mg of Roflumilast in 10 ml of volumetric flask and diluted with methanol to get 1 mg/ml concentration solution. From the stock solution take 1 ml of solution in 100 ml volumetric flask and diluted to get 10 µg/ml solution. This solution was then scanned at 200-400 nm in UV-Visible spectrophotometer to attain the absorption maximum (λ_{max}).

8.4 Development of Standard Curve of Roflumilast

8.4.1 Preparation of pH 7.4 Buffer (Phosphate Buffer)

1.083g of sodium dihydrogen ortho phosphate and 3.258g of disodium hydrogen phosphate is dissolved with 100 ml of distilled water, stirred continuously until its completely dissolved. After dissolved make up with 200 ml of distilled water.

8.4.2 Preparation of Stock Solution of Roflumilast in pH 7.4 Phosphate Buffer[1]

Weighed 10 mg of Roflumilast in 10 ml of volumetric flask and diluted with Methanol to get 1 mg/ml concentration solution. From the stock solution take 1 ml of solution in 10 ml volumetric flask with phosphate buffer (pH 7.4). Further dilution was made by pipetting 0.2, 0.4, 0.6, 0.8, 1 ml into 10ml volumetric flask to acquire solution made up with phosphate buffer pH 7.4 to get 2, 4, 6, 8, 10 µg/ml solution. Scanned in UV spectrometric at 250nm.

8.5 Drug – Excipient Compatibility Study

The possibilities of drug-excipient (lipid and surfactant) interactions were investigated by FT-IR spectrum study. The FT-IR spectrum of pure drug and combination of drug with excipient were recorded using Shimadzu FT-IR spectrophotometer. The spectrum was recorded in the wavelength region of 4000 to 400 cm-1. The IR Spectra of the test sample were obtained by Pressed Pellet Technique using Potassium bromide.

8.6 Solubility of Roflumilast in lipid excipient[43]

The solubility of Roflumilast in solid lipids was determined by a semi quantitative method. 200 mg of lipid was taken in test tube and heated at a temperature of 5 ºC above its melting point. Ten milligrams of the drug was added to the melted lipid. The drug was added in increments of 10 mg. The quantity after which there is no further solubilisation of drug was taken as the end point of drug solubility.

8.7 Optimization of Roflumilast Loaded Nanostructured Lipid Carrier by Factorial Design[1]

A Factorial Design was developed to statistically optimize the formulation factors and evaluate the main effects, interaction effects and linear effects on the independent factors. It was 3 factors, 2 levels Factorial Design was used to explore linear response surfaces with Design Expert (Version 13), and a matrix comprising 3 factors, 2 level and 8 runs is selected for the optimization study. The experimental design is summarized in Table 8.2.

8.8 Validation and data analysis

Statistical validation of the polynomial equation and ANOVA was calculated using Design Expert Software. The resultant experimental values of the responses were quantitatively compared with the predicted values to calculate the prediction error. Factorial Design was used for the optimization of Roflumilast loaded Nanostructured Lipid Carrier formulation. The Concentration of Solid lipid, liquid lipid and Surfactant Concentration were the three factors (independent variables) studied. The responses (dependent variables) studied were Percentage Entrapment efficiency and Percentage Drug release.

Table 8.2: Summary of Experimental Design.

Independent variable	Units	Level	
		Low (-1)	High (+1)
X1 = Concentration of Solid lipid	%	60	70
X2 = Concentration of Liquid lipid	%	30	40
X3 = Concentration of Surfactant	%	1	2
Dependent variable	**Units**	**Constraints**	
R1 = Entrapment Efficiency	%	Maximize	
R2 = Drug Release	%	Maximize	

9. METHOD OF PREPARATION OF ROFLUMILAST LOADED NLCs[24]

Hot Homogenisation Technique was used to formulate Roflumilast loaded Nanostructured Lipid Carrier. A solid lipid (Glyceryl Monostearate) and liquid lipid (Oleic acid) were melted at 70 ± 2˚C continuously stirred to get a transparent mixture and measured roflumilast were added to this lipid mixture. The aqueous phase was prepared by dissolving tween 80 in water and keeping temperature 70 ± 2˚C. Disperse phase was added drop by drop into aqueous phase by stirring on magnetic stirrer at 2000rpm. After it is placed in HPH to form NLCs. The resulted dispersion was allowed to cool for 10 minutes.

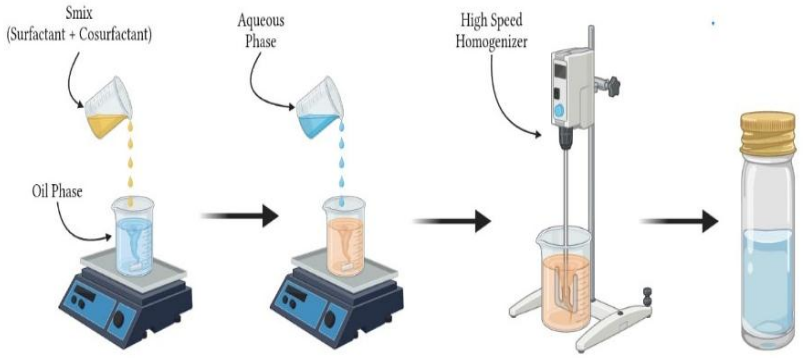

Figure 7.1: Preparation of Roflumilast loaded NLC Dispersion.

FORMULATION OF ROFLUMILAST LOADED NANOSTRUCTURED LIPID CARRIER

Table 9.1: Formulation chart for Roflumilast loaded Nanostructured Lipid Carrier.

Formulation	Roflumilast (%)	GMS (g)	CP (g)	OA (%)	Tween 80 (%)	Distilled water (ml)
F1	0.3	0.490	0.210	3	1	q.s
F2	0.3	0.360	0.240	4	1	q.s
F3	0.3	0.360	0.240	3	2	q.s
F4	0.3	0.490	0.210	4	2	q.s
F5	0.3	0.490	0.210	3	2	q.s
F6	0.3	0.360	0.240	4	2	q.s
F7	0.3	0.490	0.210	4	1	q.s
F8	0.3	0.360	0.240	3	1	q.s

Figure 7.2: Formulation of Roflumilast NLC Dispersion.

9.1 Characterization of Optimized Roflumilast loaded NLC[1,19,41]

a) Entrapment efficiency

The entrapment efficiency of roflumilast NLCs was evaluated indirectly by determining the quantity of free roflumilast in the dispersed aqueous phase. In conclusion, cooling centrifugation (REMI Instruments Ltd., India) was used to centrifuge two millilitres of the roflumilast NLCs dispersion in an Eppendorf tube for 20 min at 12,000 rpm (4˚C). After a sufficient dilution with methanol, the supernatant was filtered, and the quantity of roflumilast was measured by UV-spectroscopy at 250 nm. The following formula was used to get the rate of entrapment %

$$\text{Entrapment efficiency} = \frac{\text{Total drug taken} - \text{Free drug}}{\text{Total drug taken}} \times 100$$

b) Characterization of Optimized Formulation by FTIR Spectroscopy

FTIR study was carried out to identity the drug. Infrared spectrum of optimized formulation was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was performed by using FTIR spectrophotometer. The absorption maximum in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

c) Scanning Electron Microscopy (SEM)

The surface morphology of prepared formulations was examined using scanning electron microscopy.

d) Particle Size and Polydispersity Index

Particle size (z-average diameter) and polydispersity index (as a measure of particle size distribution) of Roflumilast loaded NLC dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 Nano S (Malvern instruments, UK) at 25°C. Prior to measurements all samples were diluted using ultra–purified water to yield a suitable scattering intensity. The diluted NLC dispersion was poured into disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement. Monodisperse samples have a lower PDI value, whereas higher PDI value indicates a wider particle size distribution and the polydisperse nature of the sample can be calculated by following equation.

$$\text{PDI} = \text{d/d avg}$$

Where, d is the width of distribution denoted by SD,
d avg is the average particle size denoted by MV (nm) in particle size data sheet.

Table 9.2: Polydispersity Index.

Polydispersity Index	Type of dispersion
0 – 0.05	Monodisperse stand
0.05 – 0.08	Nearly monodisperse
0.08 – 0.7	Mid range polydisperse
> 0.7	Very polydisperse

e) Zeta Potential

The diameter of the particles was measured using the dynamical light scattering method at 25 $\pm 1^0$C and 90^0 temperatures and scattered angle, respectively, using a Zeta-sizer from Malvern Instrumentation Ltd., UK. The zeta potential at 25^0C was measured using the same apparatus. Three tests were conducted on it.

f) Invitro drug release studies

The drug release profile of Roflumilast NLCs was measured for 8 h at skin temperature and pH using Franz diffusion cells. A 2.4 nm pore-sized, semi-permeable cellulose membrane (MW 12,000-14,000 Da) was attached between the donor and receptor compartments after being soaked in (PBS, pH 7.4) for the entire night. PBS was added to the receptor compartment, which was kept at 37±0.5°C, & swirled at 50 rpm using a magnetic stirrer. The donor section was occupied with 1 milliliter of Roflumilast-NLCs, which contained 3 milligrams of Roflumilast. Methanol was utilized as a co solvent to keep the sink conditions constant during the investigation because it has been discovered that the Roflumilast in PBS varies less. Aliquots (1 mL) were taken out of the receptor compartment at regular intervals. An equivalent volume of PBS was added right away to replace the volume that was removed. The UV-Visible spectrophotometer (UV, Shimadzu) at 248 nm was used to measure the concentration of the medication emitted at various time intervals.

9.2 Preparation of NLC gel[1]

The Nanoemulgel was obtained by addition of weighted amount of carbopol (1% w/w) in distilled water and kept for half day forgetting to swell of carbopol and then add triethanolamine drop by drop up to pH 7. Propylene glycol is added to adjust the consistency. The obtained gel was then diluted with an appropriate amount of NLC dispersion in the ratio between the dispersion and the gel was 1:1 w/w.

9.3 Evaluation of NLC gel[27]

a) Appearance

About 1 week after preparation, the gel was visually assessed for optical appearance (e.g., color, turbidity, homogeneity, presence of macroscopic particles).

b) PH

PH of all formulations is determined by using digital pH meter by immersing the electrode in gel formulation and pH was measured.

c) Drug content

1g of the prepared gel was mixed with 100ml of methanol. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and analysed using UV.

d) In-vitro drug release kinetics

In order to understand the mechanism of drug release, in-vitro drug release data were treated to kinetic model such as zero order, first order and Higuchi model and Korsmeyer- Peppas model.

Zero order

This model can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms. The dosage forms following this profile release the same amount of drug by unit of time and this model can be expressed as,

$$Qt=Qo + Kt$$

Qt=amount of drug dissolved in time "t"
Qo=initial amount of drug in the solution
K=Zero order release constant.

First order

The pharmaceutical dosage forms following this dissolution profile, release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish. The following relation can be used to express this model.

$$\log Qt = \log Qo + Kt / 2.303$$

Qt= amount of drug dissolved in time "t" Qo= initial amount of drug in the solution Kt = first order release constant.

Higuchi model

This model helps to study the release mechanism of water soluble and less water soluble drugs incorporated in semi-solid and solid matrixes. The mathematical expression for drug release is,

$$Q=[D(2C-Cs)\ Cs.t]\ ½$$

Where as,
Q= cumulative % of drug released in time in time "t" per unit area.
C= Initial drug concentration
Cs=Drug solubility in the matrix media
D= diffusion co efficient

Assuming that diffusion co-efficient and other parameter remain constant during release, the above equation reduces to,

$$Q=Kt/2$$

Thus, for diffusion-controlled release mechanism, a plot of cumulative % of drug released vs square root of time should be linear. The linearity of the plots can be checked by carrying out linear regression analysis and determination of regression co efficient of the plot.

Korsmeyer-peppa's model

To verify the fact that whether the diffusion follows ficks law or not, the drug release data can also plotted against log time according to peppa's equation. The drug release can be expressed as,

$$Q=Ktn$$

Taking log on the both sides of equation

$$\text{Log } Q=\text{Log } k + n \log t$$

Where Q is the cumulative % drug release T is the time n is the slope of linear plot of log Q vs log t.

e) Stability Studies

The purpose of stability testing was to provide evidence on how the quality of a drug substance or Nanoparticle varies with time under influence of varies environmental factors such as temperature, humidity and light. The optimized Formulation was kept at room temperature as well as at 4ºc. Upon 30 days of storage, the drug release and entrapment efficiency were determined

10. RESULTS AND DISCUSSION

10.1. PREFORMULATION STUDIES

It is an investigation of the physical, chemical and mechanical properties of a drug substance to develop a safe, effective and stable dosage form. It is the first step in rational development of dosage form.

ORGANOLEPTIC PROPERTIES

Table 10.1: Organoleptic properties of Roflumilast.

S.NO	PROPERTY	SPECIFICATION	OBSERVATION
1	Colour	White to off-white	White colour
2	Odour	Odourless	Odourless
3	Nature	Fine, crystalline or granular	Fine powder

10.2. IDENTIFICATION OF DRUG

10.2.1. MELTING POINT

The melting point of the drug was determined by melting point apparatus which are given in table.

Table 10.2: Melting Point of Roflumilast.

S.NO	Melting Point	Concordant value
1	159 ºC	160 ºC
2	160 ºC	
3	160 ºC	

Inference

Melting point of Roflumilast was observed for quality determination, it matches with standard value.

10.2.2. SOLUBILITY PROFILE

Solubility of Roflumilast pure drug in different solvents was represented in table 10.3.

Table 10.3: Solubility of Roflumilast pure drug in various media.

S No	Solvent	Solubility
1.	Water	Practically Insoluble
2.	Ethanol	Soluble
3.	DMSO	Very soluble
4.	0.1 N HCl	Sparingly soluble
5.	Methanol	Freely soluble

Inference

The solubility of the drug at methanol was significantly higher than that of distilled water. Drug of Roflumilast in distilled water was found to be insoluble.

10.3 FTIR Spectroscopic studies

FTIR spectroscopy gives the possible information about the interaction between the drug and excipients. The results are as follows

10.3.1. FTIR Spectrum of Roflumilast Drug

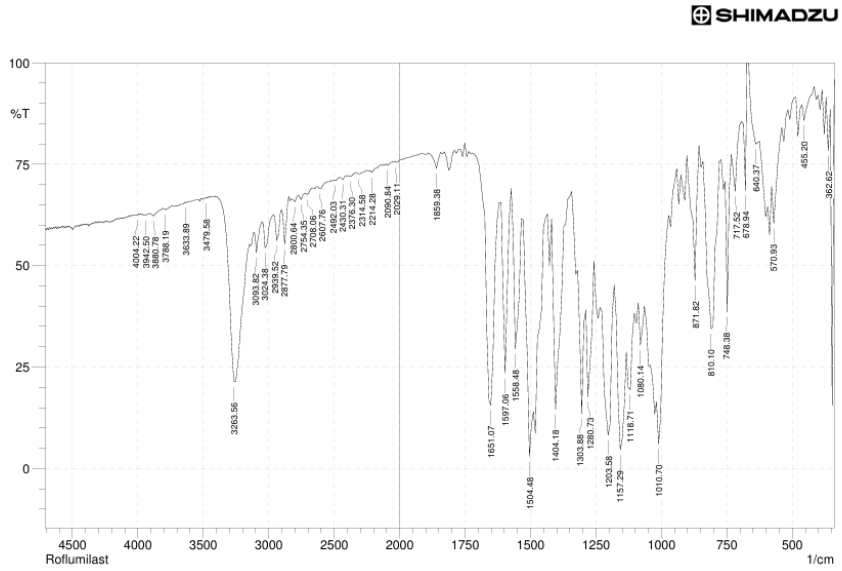

Figure 10.1: FTIR Spectrum of Roflumilast (Pure drug)

Table 10.4: FTIR Spectral Interpretation of Roflumilast

Wave number (cm^{-1})	Types of vibrations
1651	C=O Stretching
1558	C=N Stretching
1118.7	C-N Stretching
1203	C-O Stretching
3263	N-H Stretching

10.4. Physical compatibility study

Table 10.5: Physical compatibility study of drugs and excipients.

S. No	Drugs and Excipients	Description and conditions						
		Initial	At room temperature (in days)			At 40ºC ± 2º and 75%RH ±5% (in days)		
			10	20	30	10	20	30
1.	Roflumilast	White powder	NC	NC	NC	NC	NC	NC
2.	Glyceryl Monostearate	White solid flakes	NC	NC	NC	NC	NC	NC
3.	Cetyl Palmitate	White solid flakes	NC	NC	NC	NC	NC	NC
4.	Roflumilast + Glyceryl Monostearate	White colour	NC	NC	NC	NC	NC	NC
5.	Roflumilast + Cetyl Palmitate	White colour	NC	NC	NC	NC	NC	NC
6.	Roflumilast + Glyceryl Monosterate + Cetyl palmitate	White colour	NC	NC	NC	NC	NC	NC

*NC-No Change

Inference

The physical compatibility is shown in Table. They were evaluated for 10,20 and 30 days at room temperature. There was no change in colour. Therefore the drug and excipients are physically compatible with each other. The excipients which are compatible with the drug are selected for formulation.

10.5. DETERMINATION OF λ MAX FOR ROFLUMILAST

The maximum absorbance of the Roflumilast was studied. The maximum absorbance of the drug dolutegravir sodium was found to be 250 nm. Hence the wavelength of 250 nm. was selected for analysis of drug in dissolution media.

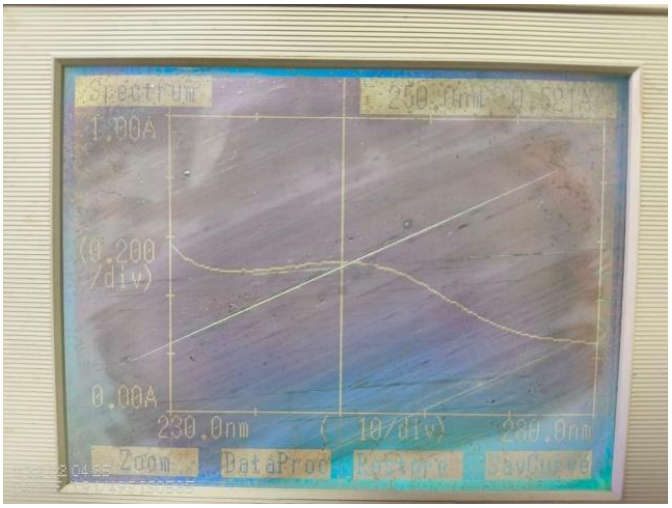

Figure 10.2: UV spectrum of Roflumilast.

10.6. STANDARD CALIBRATION CURVE OF ROFLUMILAST IN PHOSPHATE BUFFER pH 7.4

The UV Spectrophotometric method was used to analyze the calibration curve of Roflumilast. The absorbance of the drug in of concentration ranging from 2-10 µg/ml was measured at a wavelength of 250 nm against blank.

Table 10.6: Data for calibration curve of Roflumilast in Phosphate buffer pH 7.4.

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE
1.	2	0.165
2.	4	0.332
3.	6	0.492
4.	8	0.670
5.	10	0.834

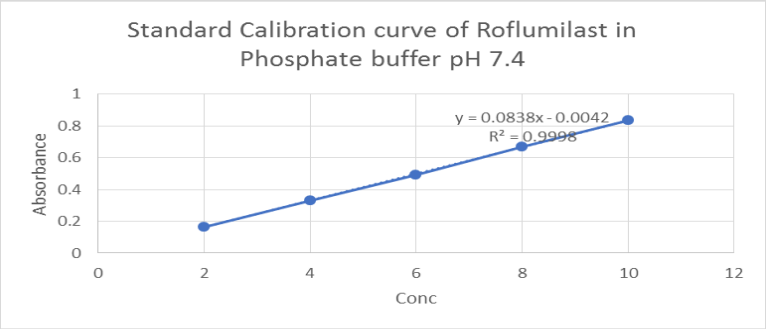

Figure 10.3: Standard curve of Roflumilast using Phosphate buffer pH 7.4

Inference

It was found that the solutions show linearity (R2 = 0.9998) at a concentration of 2-10 µg/ml and obeys Beer Lambert's law.

10.7. DRUG EXCIPIENT COMPATIBILITY STUDY

10.7.1. FTIR SPECTRUM OF DRUG AND GLYCERYL MONOSTEARATE

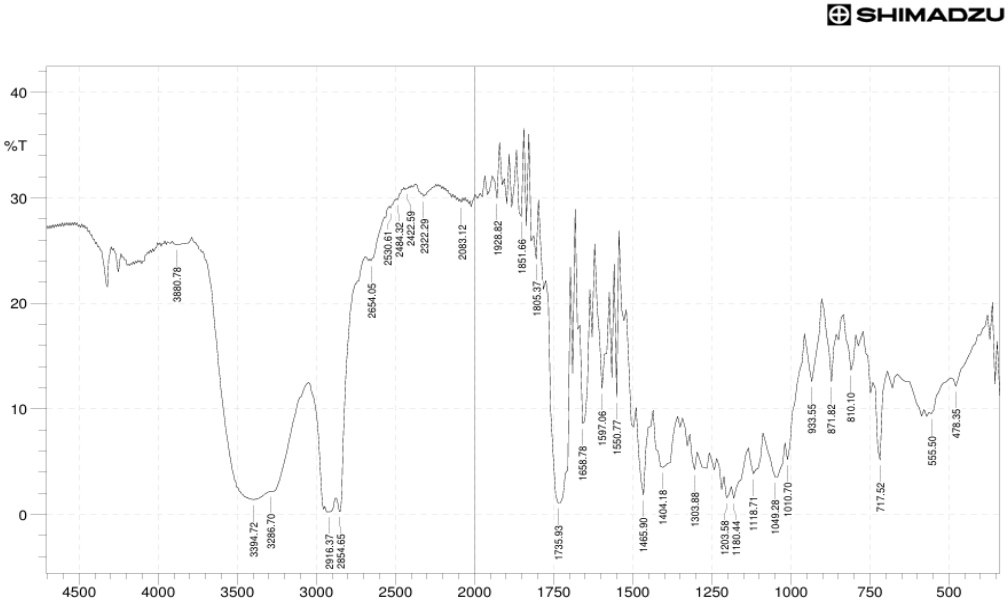

Figure 10.4: FTIR Spectrum of Roflumilast and Glyceryl Monostearate.

Table 10.7: FTIR spectral interpretation of Roflumilast and Glyceryl Monostearate.

Wave number(cm^{-1})	Types of Vibrations
1735	C=O Stretching
1550	C=N Stretching
1118	C-N Stretching
1203	C-O Stretching
3286	N-H Stretching

10.7.2. FTIR SPECTRUM OF DRUG AND CETYL PALMITATE

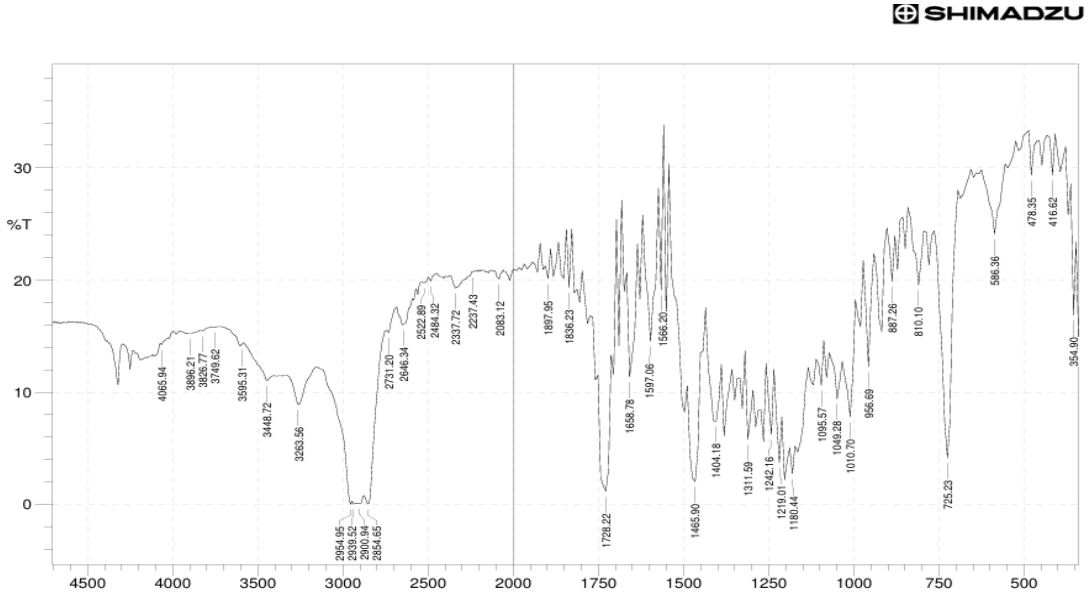

Figure 10.5: FTIR Spectrum of Roflumilast and Cetyl Palmitate.

Table 10.8: FTIR spectral interpretation of Roflumilast and Cetyl Palmitate.

Wave number(cm^{-1})	Types of Vibrations
1728	C=O Stretching
1566	C=N Stretching
1118	C-N Stretching
1219	C-O Stretching
3263	N-H Stretching

10.7.3. FTIR OF DRUG, GLYCERYL MONOSTEARATE AND CETYL PALMITATE

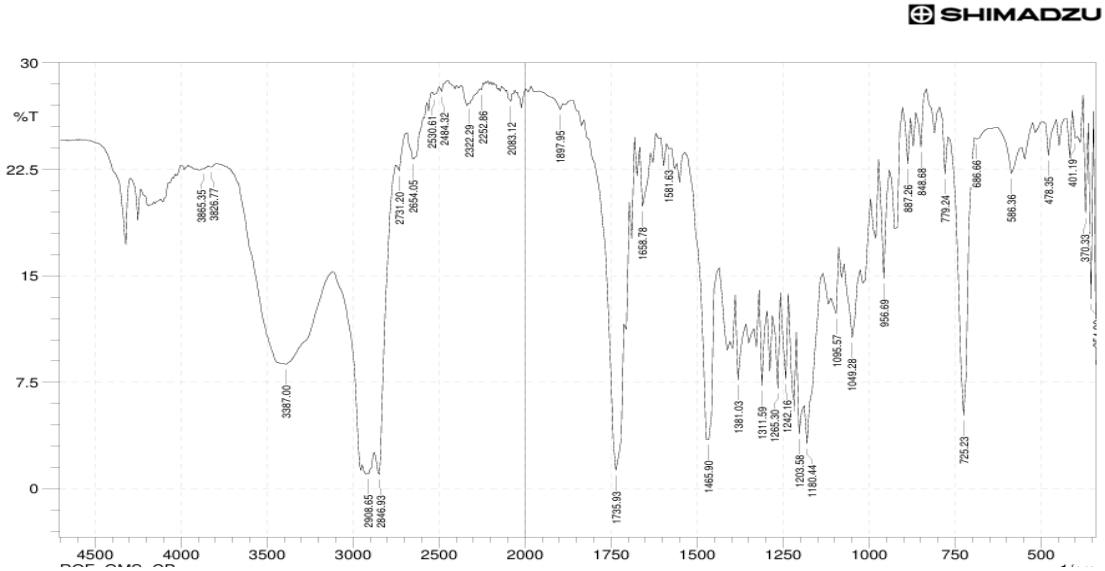

Figure 10.6: FTIR Spectrum of Roflumilast, Glyceryl Monostearate and Cetyl Palmitate.

Table 10.9: FTIR spectral interpretation of Roflumilast, Glyceryl Monostearate and Cetyl Palmitate.

Wave number(cm^{-1})	Types of Vibrations
1735	C=O Stretching
1581	C=N Stretching
1118	C-N Stretching
1203	C-O Stretching
3357	N-H Stretching

0.8. OPTIMIZATION OF ROFLUMILAST LOADED NANOSTRUCTURED LIPID CARRIER BY 2^3 FACTORIAL DESIGN

A 2^3 factorial design was used to optimize the Roflumilast loaded formulation. At three factors and two levels, factorial design requires 8 experimental runs to determine the optimized formulation. A total of 8 experimental runs were generated and evaluated using Design Expert Software. The significant response factors were used to assess the quality of the formulation including Percentage Entrapment Efficiency and Cumulative Percentage Drug Release.

Table 10.10: Variables used in Factorial Design.

VARIABLE	LOW	HIGH
A: SOLID LIPID	60	70
: LIQUID LIPID	30	40
C: CONC OF SURFACTANT	1	2

10.9. FORMULATION OF ROFLUMILAST LOADED NANOSTRUCTURED LIPID CARRIER

Eight different formulations of Roflumilast NLC dispersion were prepared by Hot Homogenisation method, by using a drug and with excipients in different concentration. Glyceryl Monostearate, Cetyl Palmitate and Oleic acid were used in formulation. The formulation was designed as F1, F2, F3, F4, F5, F6, F7 and F8 respectively. All the formulated dispersion were taken for further evaluation.

Table 10.11: Formulation Chart of Roflumilast loaded NLC.

RUNS	SOLID LIPID (%)	LIQUID LIPID (%)	CONC OF SURFACTANT (%)
1	70	30	1
2	60	40	1
3	60	30	2
4	70	40	2
5	70	40	1
6	60	30	1
7	70	30	2
8	60	40	2

10.10. CHARECTERIZATION OF ROFLUMILAST LOADED NLCs

a) Determination of Entrapment Efficiency

Table 10.12: Entrapment efficiency of Roflumilast Loaded NLCs.

Formulation Code	Percentage Entrapment Efficiency
F1	72.6
F2	64.3
F3	69.4
F4	66.8
F5	**82.3**
F6	70.2
F7	75.3
F8	71.8

Figure 10.7: Graphical Representation of Percentage Entrapment Efficiency.

Inference
The percentage entrapment efficiency was found to be in the range of 64.3 to 82.3%.

b) Determination of Drug Content
Table 10.13: % Drug content of NLC.

FORMULATION CODE	% DRUG CONTENT
F1	94.1
F2	92.6
F3	86.3
F4	88.8
F5	**96.3**
F6	93.7
F7	85.7
F8	92.2

Inference
The percentage drug content was found to be in the range from 85.7 to 96.3%.

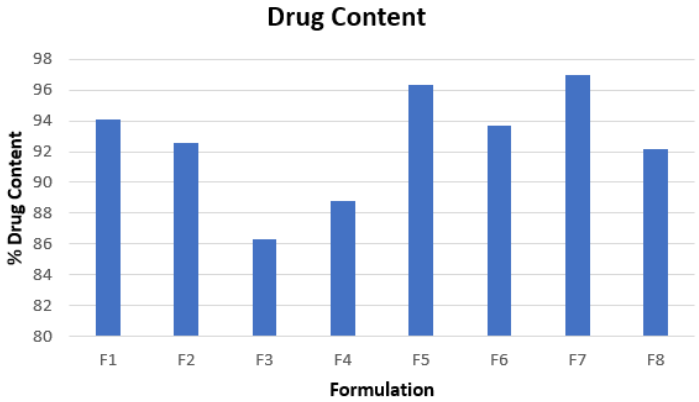

Figure 10.8: Graphical Representation of Percentage Drug Content.

c) Determination of *in vitro* Drug release of Roflumilast NLCs
Table 10.14: Determination of *in vitro* Drug release of Roflumilast NLCs.

Time (hours)	Cumulative Percentage Drug Release							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	**0**	0	0	0
1	4.14	5.5	9.9	4.93	**11.3**	7.9	11.5	10.7
2	11.5	13.29	17.6	14.6	**23.03**	16.8	19.6	18.6
3	22.4	24.2	28.2	25.4	**35.9**	28	30.1	26
4	30.8	31.3	33.1	37.75	**47.7**	36.7	41.3	37.15
5	41.3	43.32	45.7	42.9	**59.4**	43.7	50.6	46.76
6	53.06	51.07	52.2	51.6	**67.7**	50.6	62.2	55.05
7	64	63.8	60.8	62.4	**75.14**	63.4	73.5	61.6
8	77.1	74.3	71.6	71.3	**84.8**	75.5	80.9	74.3

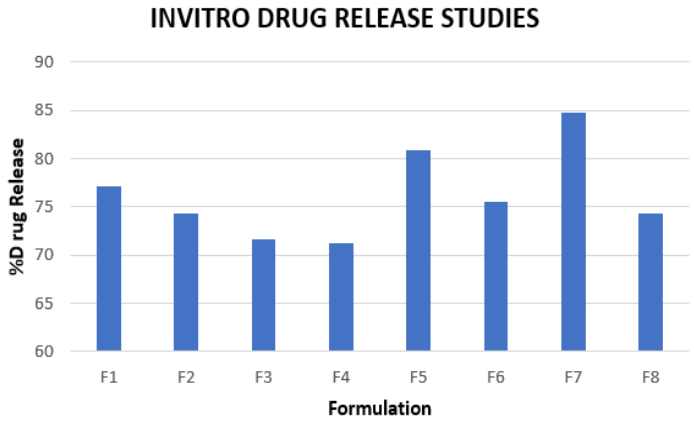

Figure 10.9: Graphical Representation of Cumulative Percentage Drug Release.

10.11. OPTIMIZATION OF ROFLUMILAST LOADED NLCs

Table 10.15: Summary of 2^3 factorial design for Roflumilast loaded Nanostructured Lipid carrier.

Runs	Factor 1	Factor 2	Factor 3	Response 1	Response 2
	Solid Lipid Concentration (g)	Liquid Lipid Concentration (g)	Surfactant Concentration (g)	Entrapment Efficiency (%)	Cumulative Percentage Drug Release
1	70	30	1	72.6	77.1
2	60	40	1	64.3	74.3
3	60	30	2	69.4	71.6
4	70	40	2	66.8	71.3
5	70	30	2	82.3	84.8
6	60	40	2	70.2	75.5
7	70	40	1	75.3	80.9
8	60	30	1	71.8	74.3

Table 10.16: Design summary.

Factor	Name	Unit	Type	Minimum	Maximum	Coded low	Coded high	Mean
A	Solid Lipid	%	Numeric	60	70	-1	+1	65
B	Liquid Lipid	%	Numeric	30	40	-1	+1	35
C	Conc of Surfactant	%	Numeric	1	2	-1	+1	1.5

Table 10.17: Polynomial analysis.

Response	Name	Units	Minimum	Maximum	Mean	Ratio
R1	Entrapment efficiency	%	64.2	82.3	71.59	1.28
R2	Cumulative percentage drug release	%	71.3	84.8	77.86	1.29

Response 1: Entrapment Efficiency

ANOVA for linear model

Table 10.18: Analysis of variance table (Partial sum of squares – Type III).

Source	Sum of Squares	Df	Mean Square	F- value	p-value	
Model	**177.70**	**3**	**59.23**	**6.84**	**0.0471**	**Significant**
A-Solid Lipid	56.71	1	56.71	6.55	0.0627	
AC	33.21	1	33.21	3.83	0.1218	
ABC	87.78	1	87.78	10.13	0.0334	
Residual	34.65	4	8.66			
Cor Total	212.35	7				

The **Model F-value** of 7.96 implies the model is significant. There is only a 4.71% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case ABC is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 10.19: Fit statistics.

User standard deviation	0.0000	**R^2**	0.8368
Standard deviation	2.94	**Adjusted R^2**	0.7145
Mean	71.59	**Predicted R^2**	0.3474
C.V.%	4.11	**Adequate Precision**	7.7006

The **Predicted R^2** of 0.3474 is not as close to the **Adjusted R^2** of 0.7145 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.701 indicates an adequate signal. This model can be used to navigate the design space.

Table 10.20: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Df	Standard error	95% CI Low	95% CI High	VIF
Intercept	71.59	1	1.04	68.70	74.48	
A-Solid Lipid	2.66	1	1.04	-0.2264	5.55	1.0000
AC	-2.04	1	1.04	-4.93	0.8514	1.0000
ABC	-3.31	1	1.04	-6.20	-0.4236	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1: VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Normal plot of residuals for Y1 – Percentage Entrapment Efficiency

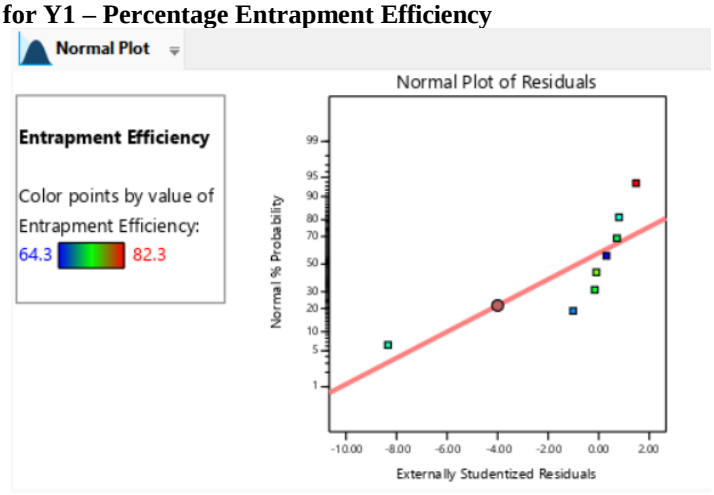

Figure 10.10: Normal plot of residuals for Y1.

The normal % probability vs. externally studentized residuals designed that the maximum of the colored points demonstrating the entrapment efficiency was seen around the normal probability line.

The normal plot of residual shows satisfaction. Since, the residuals are move near to the straight line.

Box – cox plot for Y1 – Percentage Entrapment Efficiency

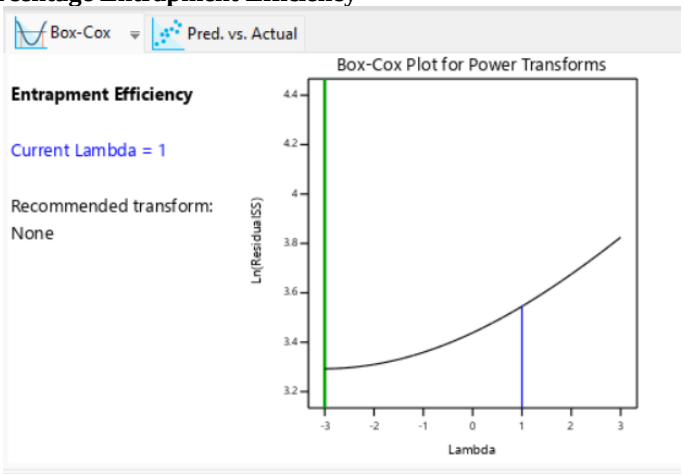

Figure 10.11: Box – cox plot for Y1.

The Box — Cox plot for power exposed a linear relationship given in fig 10.11.

Residual vs. Predicted plots for Y1 – Percentage Entrapment Efficiency

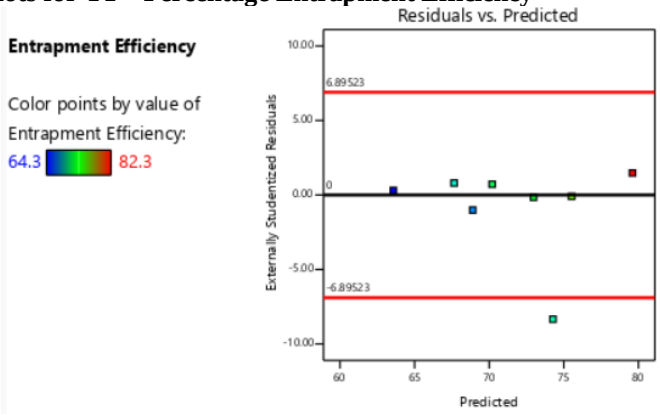

Figure 10.12: Residual vs. Predicted plots.

The externally studentized residuals vs. predicted tenets plot signifies that the colored points of Entrapment efficiency was privileged the limits.

Predicted vs. Actual graph for Y1 – Percentage Entrapment Efficiency

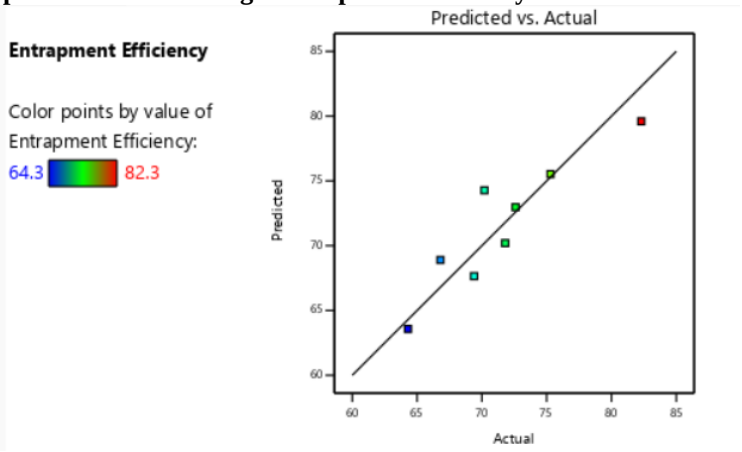

Figure 10.13: Predicted vs. Actual graph for Y1.

The predicted vs. actual plot exposed linear relationship. The color point located near to the straight line. So, this plot indicates pass the desirability limits.

Contour Plot for Y1- Entrapment Efficiency

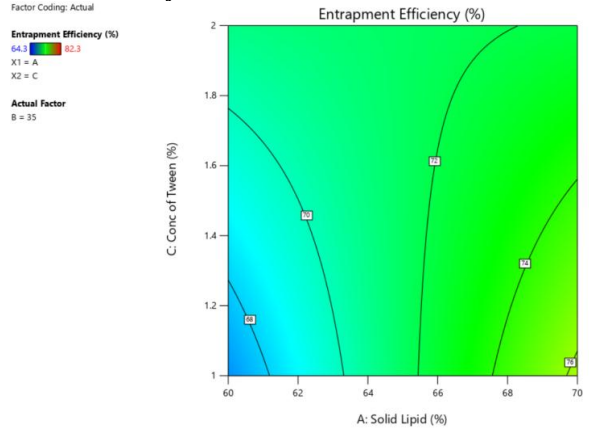

Figure 10.14: Contour Plot for Y1.

3D Response Plots of Y1 – Entrapment Efficiency

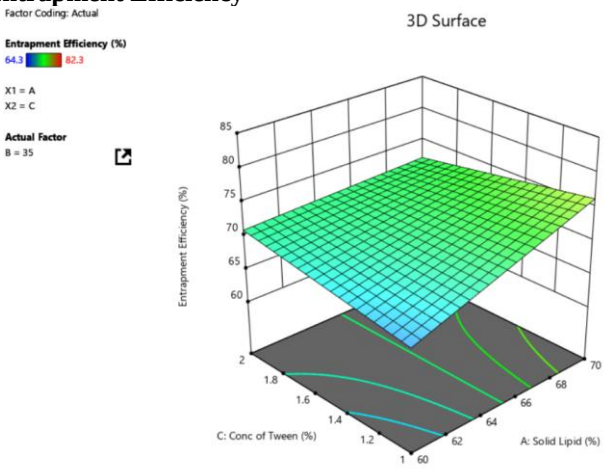

Figure 10.15: 3D Response Plots of Y1.

Response 2: Cumulative percentage drug release

ANOVA for Linear Model

Table 10.21: Analysis of variance table [Partial sum of squares - Type III].

Source	Sum of squares	Df	Mean Square	F-Value	p-Value	
Model	**105.48**	**2**	**52.74**	**13.42**	**0.0098**	**Significant**
AC	17.70	**1**	**17.70**	**4.50**	**0.0873**	
BC	87.78	**1**	**87.78**	22.33	**0.0052**	
Residual	19.66	5	3.93			
Cor Total	125.14	7				

The **Model F-value** of 13.42 implies the model is significant. There is only a 0.98 % chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 10.22: Fit statistics.

Standard deviation	1.98	**R^2**	0.8429
Mean	77.86	**Adjusted R^2**	0.7801
C.V.%	2.55	**Predicted R^2**	0.5979
		Adequate Precision	7.9066

The **Predicted R²** of 0.5979 is in reasonable agreement with the Adjusted R² of 0.7801.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of indicates an adequate signal. This model can be used to navigate the design space.

Table 10.23: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Df	Standard error	95% CI Low	95% CI High	VIF
Intercept	77.86	1	0.7010	76.06	79.66	
AC	-1.49	1	0.7010	-3.29	0.3145	1.0000
BC	-3.31	1	0.7010	-5.11	-1.51	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1: VIFs greater than 1 indicate multi — co linearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Normal Plots of Residuals for Y2 – Cumulative Percentage drug release

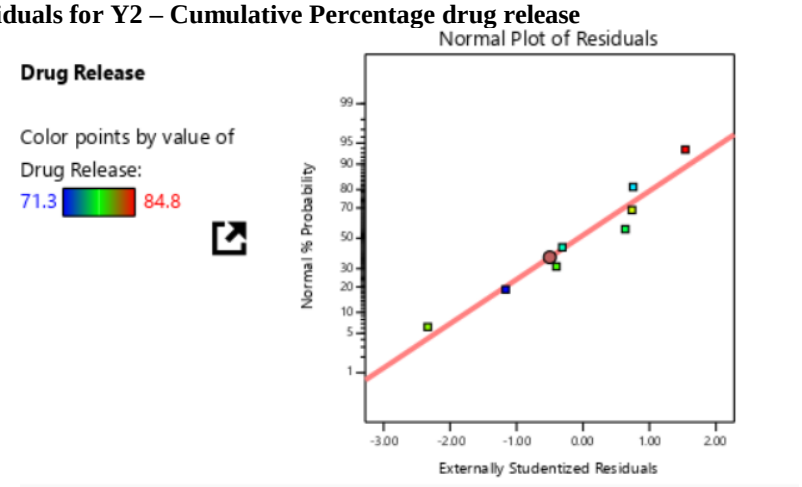

Figure 10.16: Normal Plots of Residuals for Y2.

The normal % probability vs. externally studentized residuals designed that the maximum of the colored points demonstrating the DR at the end of 12 hr was seen around the normal probability line.

The normal plot of residual shows satisfaction. Since, the residuals are move near to the straight line.

Residual vs. Predicted plots for Y2 – Cumulative Percentage drug release

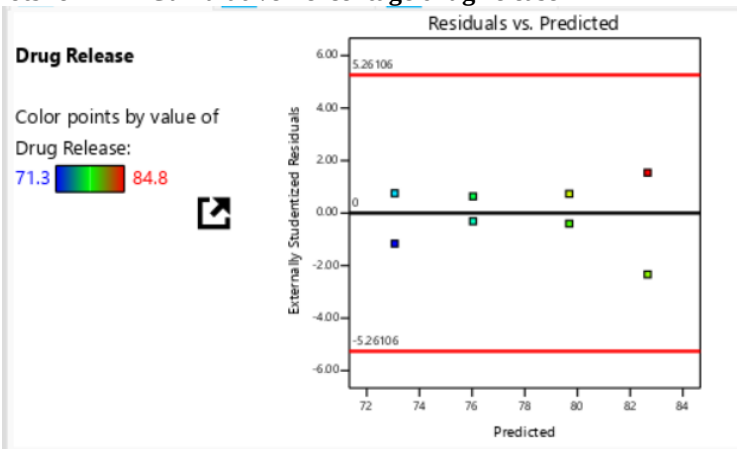

Figure 10.17: Residual vs. Predicted plots for Y2.

Contour Plot for Y2- Drug Release

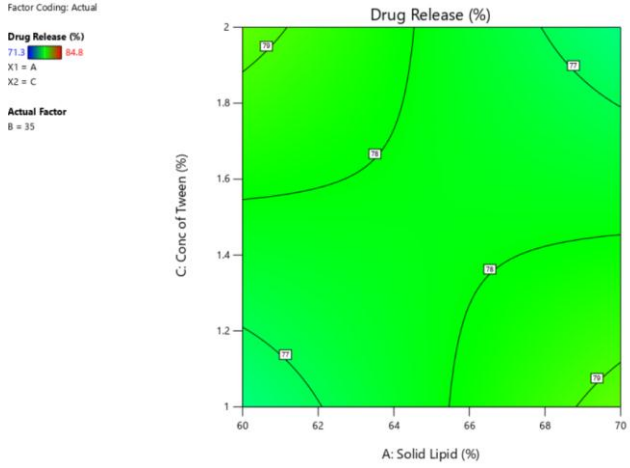

Figure 10.18: Contour Plot for Y2.

3D Response Plots of Y2 – Cumulative Percentage drug release

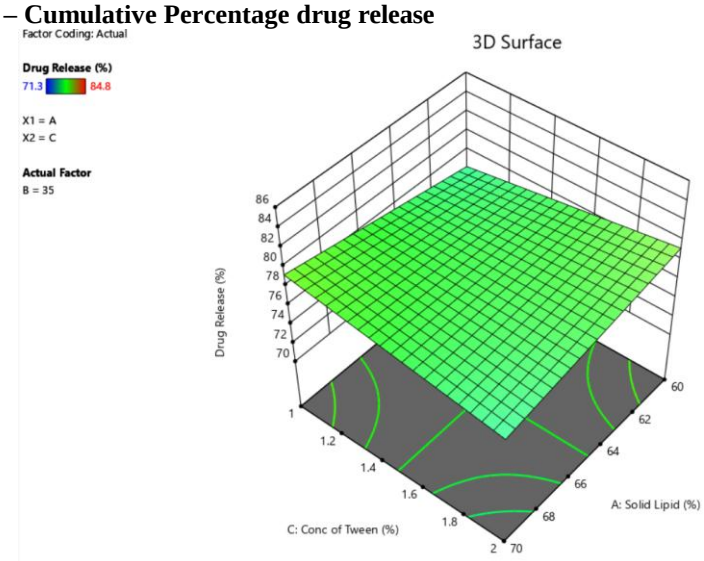

Figure 10.19: 3D Response Plots of Y2.

Cook's Distance Plot for Y2 – Cumulative Percentage Drug Release

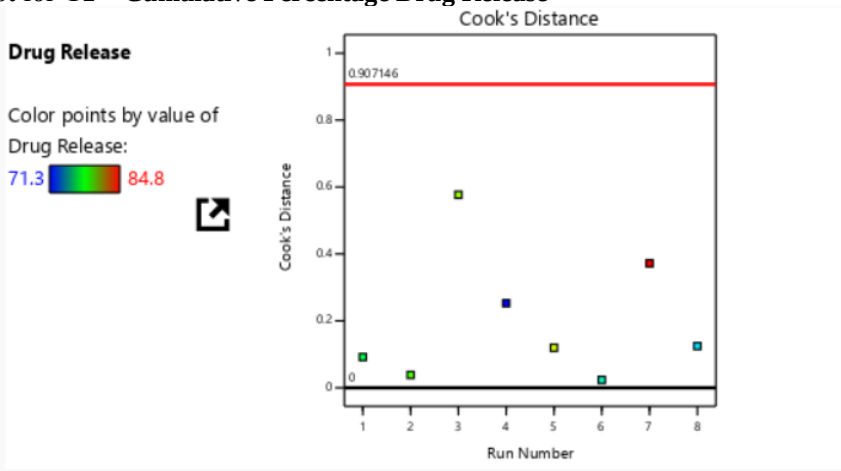

Figure 10.20: Cook's Distance Plot for Y2.

The cook's distances were plotted and the red line represents DR at the end of 12 hr was in close contact with the predicted values.

Predicted vs. Actual graph for Y2 – Cumulative Percentage Drug Release

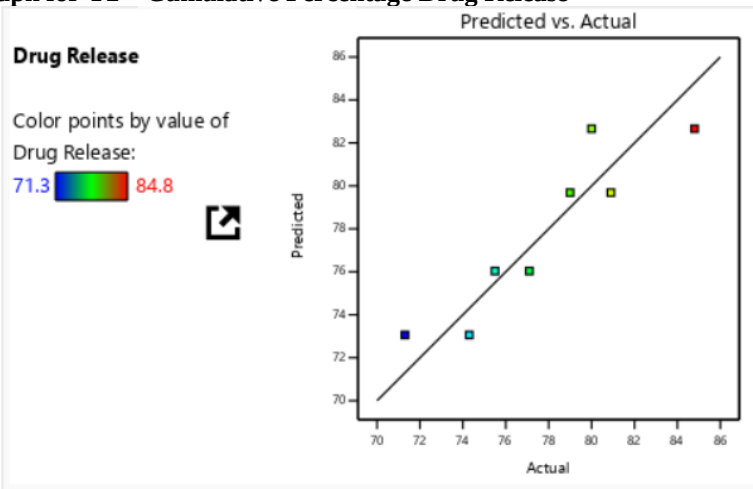

Figure 10.21: Predicted vs. Actual graph for Y2.

The predicted vs. actual plot exposed linear relationship. The color point located near to the straight line. So, this plot indicates pass the desirability limit.

10.12. CHARACTERIZATION OF OPTIMIZED FORMULATION OF ROFLUMILAST LOADED NANOSTRUCTURED LIPID CARRIER

a) Percentage entrapment efficiency of optimized formulation

The Percentage Entrapment Efficiency of Optimized Formulation was determined and their Percentage Entrapment efficiency was found to be 82.3%.

b) Cumulative Drug Release of Optimized Formulation

The UV- Visible spectrophotometric method was used to determine the Cumulative Drug Release of optimized formulation. The Cumulative Drug Release was found to be 84.8%.

c) Morphology of Roflumilast by Scanning Electron Microscopy Analysis (SEM)

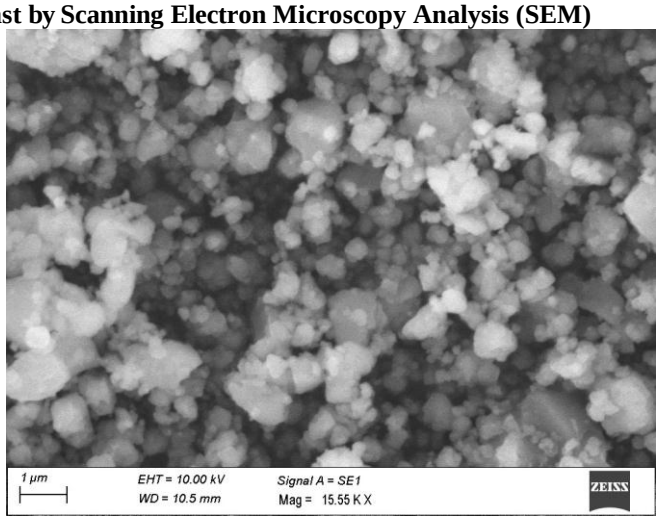

Figure 10.22: SEM image of Optimized Roflumilast NLCs.

Inference

The SEM micrographs of prepared nanoparticle showed that the nanoparticles synthesized were spherical in shape.

d) Determination of Particle Size and Polydispersity of Optimized Formulation

PDI indicates the particle size distribution, which ranges from 0 to 1. Theoretically, a monodisperse population indicates PDI equal to zero. The low value of PDI signifies the uniformity of particle size within the formulations.

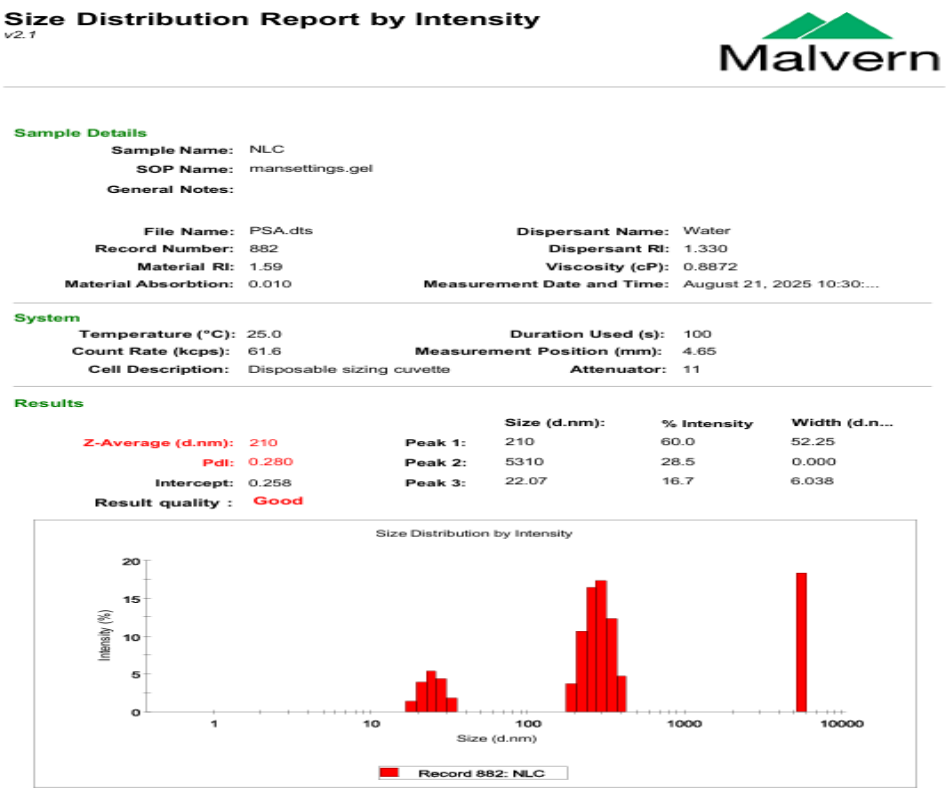

Figure 10.23: Particle Size and Polydispersity of Optimized Formulation Inference.

The Particle Size and Polydispersity of Optimized Formulation was found to be 210nm and 0.280.

e) Determination of Zeta potential of Optimized Formulation

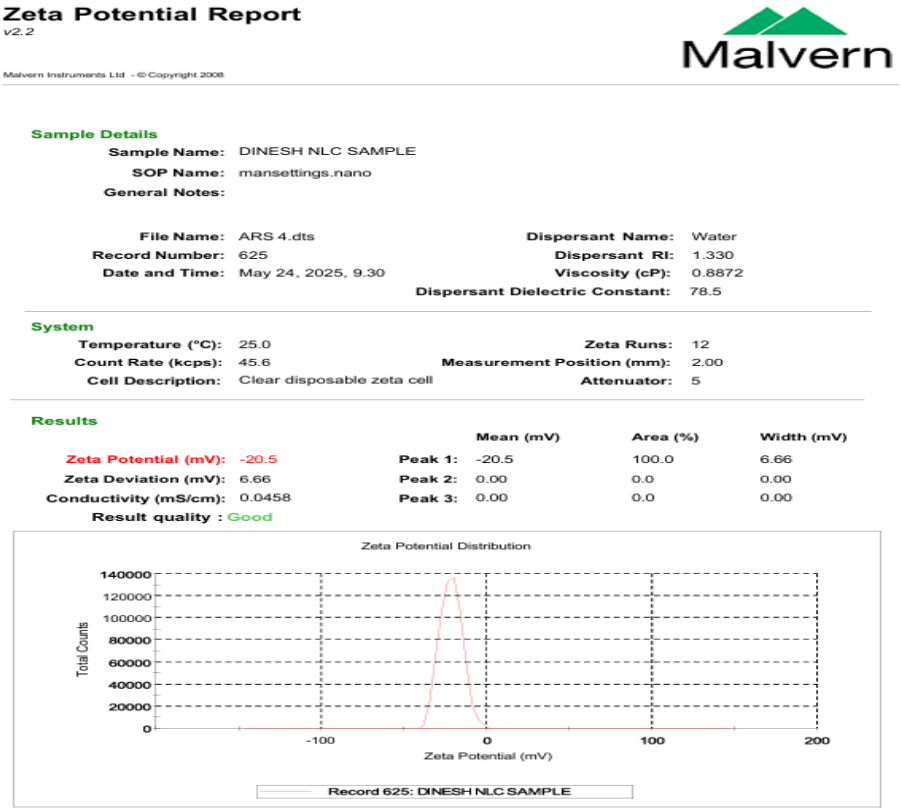

Figure 10.24: Zeta potential of Optimized Formulation.

Inference

The Zeta potential for the optimized formulation was found to be -20.5 mv and shows that the formulation is stable.

10.13. Formulation of Optimized Roflumilast loaded NLC gel

From the Cumulative drug release, entrapment efficiency and drug content it is found that F5 is the best formulation. So it was selected and formulated to gel.

Figure 10.25: Formulation of Roflumilast NLC gel.

10.14. EVALUATION OF NLC GEL

A] Appearance

It was determined by visual inspection. All the formulations were found to be homogenous.

B] pH

The pH was found to be 6.4, which was close to skin pH.

C] Drug Content Estimation of NLC Gel

Drug content of the gel formulations was found to be 96 %. (The reading is an average of 3 determinations).

Table 10.24: Drug Content of NLC Gel.

S.NO	% DRUG CONTENT	AVERAGE
1	96.32	96.31
2	96.34	
3	96.27	

10.15. RELEASE KINETICS OF OPTIMIZED FORMULATION OF ROFLUMILAST LOADED NANOSTRUCTURED LIPID CARRIER

Table 10.25: Release kinetics of Optimized Roflumilast loaded NLC.

Time (hours)	Log time	Square root of time	Cumulative % drug release	Cumulative % drug remaining	Log cumulative % drug release	Log cumulative % drug remaining	Cube root of %drug Remaining
0	∞	0	0	100	∞	2	4.641
1	0	1	11.3	88.7	1.053	1.947	4.459
2	0.301	1.414	23.03	76.97	1.362	1.886	4.253
3	0.477	1.732	35.9	64.1	1.555	1.806	4.002
4	0.602	2	47.7	52.3	1.678	1.718	3.739
5	0.698	2.236	59.4	40.6	1.773	1.608	3.436
6	0.778	2.449	67.7	32.2	1.830	1.507	3.181
7	0.845	2.645	75.14	24.86	1.875	1.395	2.918
8	0.903	2.828	84.8	15.2	1.928	1.181	2.477

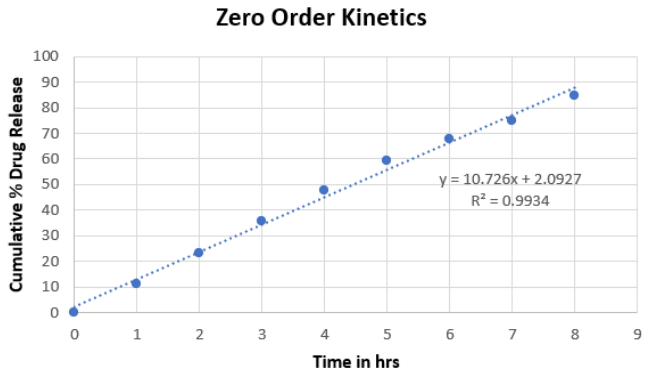

Figure 10.26: A plot for zero order kinetics of optimized Roflumilast loaded NLC.

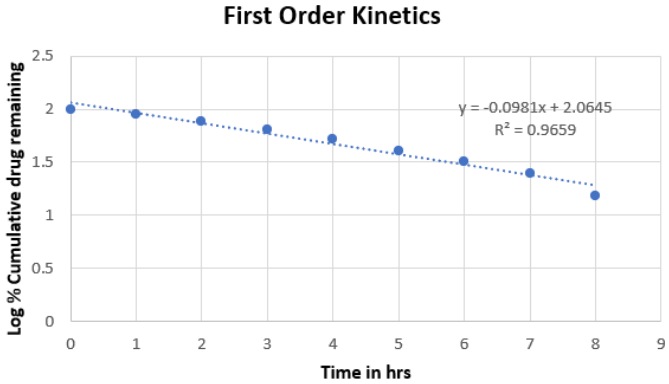

Figure 10.27: A plot for first order kinetics of optimized of Roflumilast loaded NLC.

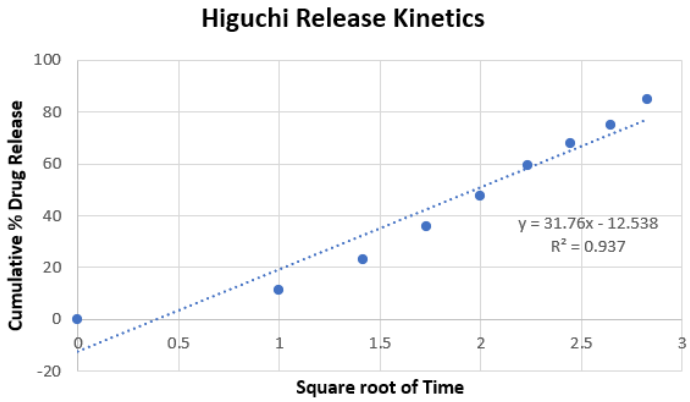

Figure 10.28: Higuchi release kinetics of optimized Roflumilast loaded NLC.

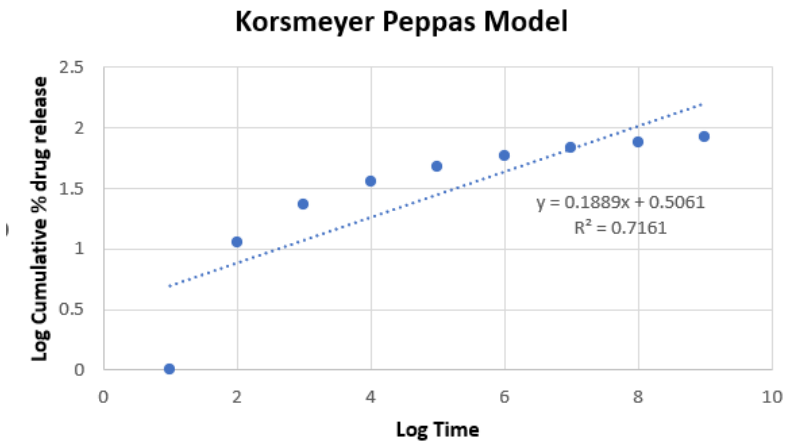

Figure 10.29: Korsmeyer- Peppas kinetics of optimized Roflumilast loaded NLC.

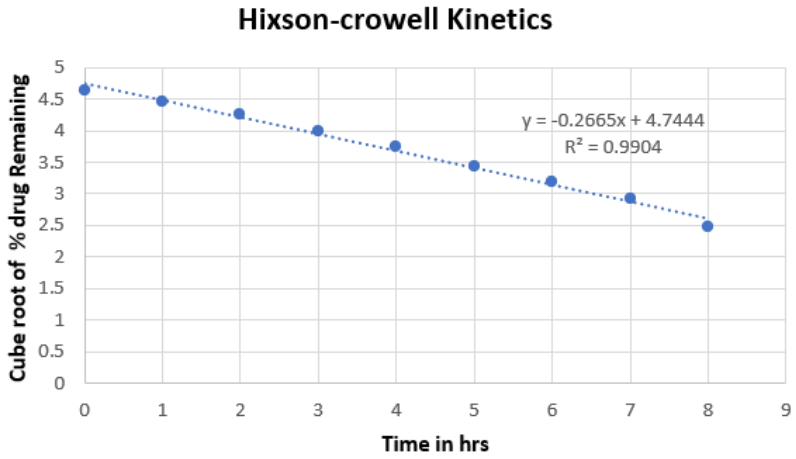

Figure 10.30: Hixson – crowell kinetics of optimized Roflumilast loaded NLC.

The coefficient of determination (R^2) was taken as criteria for choosing the most appropriate model.

Table 10.26: kinetics Model and its R^2 value.

S.No	Kinetics models	Coefficient of determination (R^2) of optimized formulation
1	Zero order	0.9934
2	First order	0.9659
3	Higuchi	0.937
4	Korsmeyer and Peppas	0.7161
5	Hixson Crowell	0.9904

The data from *in vitro* release of optimized formulation was fit into various kinetic models.

Good linearity was observed with the Zero order and Hixson Crowell (R2=0.9934). Hence the result indicating that the drug release was based on their change in surface area and diameter of the particles and release of drug in sustained release pattern.

10.16. STABILITY STUDIES

The optimized formulation (F5) subjected to stability studies as per ICH guidelines. The results are shown in table 10.27.

Table No. 10.27. Stability data for Optimized Roflumilast NLC gel.

Stability condition		Physical appearance			Percentage Entrapment Efficiency			Percentage drug release		
		Initial	After 15 days	After 30 days	Initial	After 15 days	After 30 days	Initial	After 15 days	After 30 days
F5	4±2°C	NC	NC	NC	82.3	80	80	84.5	83.2	83.2
F5	Room temperature (20±5°C)	NC	NC	NC	82	81	80	84.8	83.6	83.4

*NC-No change

Inference

The results show No Significant Change in Appearance, Entrapment Efficiency and Drug release of optimized formulation after one month.

11. SUMMARY AND CONCLUSION

The purpose of this research was to prepare Roflumilast loaded Nanostructured Lipid Carrier for increasing the bio-availability of Roflumilast. Roflumilast is a poorly soluble drug and thus selected as a model drug for Nanostructured Lipid Carrier.

Hot Homogenisation technique was employed to produce Nanostructured Lipid Carrier using Glyceryl Monostearate, Cetyl Palmitate and tween 80.

The compatibility study of Roflumilast with excipients was carried out using FTIR Spectroscopy. It revealed no interaction between the drug and excipients.

Calibration curve was plotted for Roflumilast and it was found that the solutions show linearity (0.998) and obeyed Beer Lambert's law.

Formulation study design was developed by using 2^3 Factorial Design (Design Expert, version 13, Stat Ease., Inc), the formulation design was predicted and statistically analysed. The independent variables selected were Solid Lipid (A), Liquid Lipid (B) and concentration of Tween 80 (C) and the dependent variable chosen were Percentage Entrapment efficiency (R1) and Cumulative % drug release (R2). The formulations were optimized using Factorial design by comparing the predicted values with the observed values. The design predicted the values of optimized formulation which was then formulated and evaluated.

The optimized Roflumilast loaded Nanostructured Lipid Carrier was evaluated for FT-IR study and it is clearly evident that the optimized Roflumilast loaded Nanostructured Lipid Carrier showed the presence of characteristics bands of Roflumilast. This indicates the absence of chemical interaction between the drug and the excipients.

The entrapment efficiency of the optimized Roflumilast loaded Nanostructured Lipid Carrier was determined and its entrapment efficiency was observed to be 82.3%.

The UV-visible Spectrophotometric method was used to determine the drug content of optimized Roflumilast loaded Nanostructured Lipid Carrier. The drug content was found to be 96.3%.

The optimized Roflumilast loaded Nanostructured Lipid Carrier was characterized for surface morphology, particle size analysis and zeta potential. It showed that the NLC was spherical and discrete in morphology. The particle size distribution and polydispersity study were carried out using particle size analyser. The mean particle size of optimized Roflumilast loaded Nanostructured Lipid Carrier was found to be 210 nm. Polydispersity of optimized Roflumilast loaded Nanostructured Lipid Carrier was found out to be 0.212, indicating uniformity of particle size within formulation. The zeta potential study was done by zeta sizer. The zeta potential for the optimized Roflumilast loaded Nanostructured Lipid Carrier was found to be-20.5 mV which showed that the formulation is stable.

The optimized Nanostructured Lipid Carrier was further formulated as NLC gel.

The formulated optimized Roflumilast loaded Nanostructured Lipid Carrier filled in was characterized. Drug release kinetics study and Stability studies. In vitro release of optimized Roflumilast loaded Nanostructured Lipid Carrier gel showed a rapid initial burst, followed by a very slow drug release. The cumulative % drug release for the optimized Roflumilast loaded NLC Gel was 84.8% at 8 hrs. The release kinetics of the optimized Roflumilast NLC gel was fitted to various kinetic models and the optimized formulation was best fitted to zero order kinetics (R2=0.9934). It indicating that the drug release was based on their change in surface area and diameter of the particles in a controlled release manner.

The optimized formulation was subjected to room temperature and refrigerator temperature (4±2ºC). The results show no significant change in appearance, drug entrapment efficiency and drug content of optimized formulation after one month. It is concluded High Pressure Homogenisation method was a best method for the successful incorporation of poorly water-soluble drug Roflumilast into Nanostructured Lipid Carrier with high entrapment efficiency.

FUTURE SCOPE

- *In vivo* Study

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