# OPTIMIZATION OF IBUPROFEN LOADED NANOSTRUCTURED LIPID CARRIER (NLC) USING RESPONSE SURFACE METHODOLOGY (RSM): PREPARATION AND *INVITRO* EVALUATION

**A Dissertation submitted to**

**THE TAMILNADU 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-I PHARMACEUTICS**

**submitted by**

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# DECLARATION

I KAUSALYA.S (**261710008**) hereby declare that the thesis entitled, "**OPTIMIZATION OF IBUPROFEN LOADED NANOSTRUCTURED LIPID CARRIER (NLC) USING RESPONSE SURFACE METHODOLOGY (RSM): PREPARATION AND *INVITRO* EVALUATION**" has been originally carried out under the guidance and supervision of **Prof. Dr. UBAIDULLA, M. Pharm,Ph. D.,** Department of Pharmaceutics, **C.L. Baid Metha College Of Pharmacy,** Chennai 97 and **Dr. V.BAVITHRAN B Pharm, MBA, Ph.D.**, Managing Director , Mastrowin pharmaceuticals, Chennai -600092. during the academic year 2018-2019 . This work has not been submitted in any other degree at any other university and that all other sources we have used or quoted have been indicated and acknowledged by complete reference.

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## LIST OF ABBREVIATIONS

| Abbreviated as | Expanded form |
|---|---|
| NLC | Nanostructured lipid carrier |
| SLN | Solid lipid nanoparticles |
| GMS | Glyceryl monostearate |
| BCS | Biopharmaceutical classification system |
| BDDCS | Biopharmaceutics drug disposition classification system |
| PC | Phosphatidyl choline |
| IBU NLC | Ibuprofen loaded nano structured lipid carrier |
| TEWL | Trans epidermal water loss |
| COX | Cyclo oxygenase |
| NSAIDS | Non steroidal anti inflammatory drug |
| HME | Hot melt extrusion technique |
| SCF | Super critical fluid |
| PVA | Poly vinyl alcohol |
| FTIR | Fourier transform infrared spectrophotometer |
| SDIB | Solid dispersion Ibuprofen |
| SDIBM | Solid dispersion Ibuprofen with menthol |
| DOE | Design of experiments |
| AUC | Area under curve |
| mcg | Microgram |
| Rpm | Revolutions per minute |
| Hrs | Hours |
| U-V | Ultra violet |
| P-gp | P -glycol protein |
| CYP | Cytochrome p 450 enzymes |
| ABTS | 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| PBS | Phosphate buffer solution |

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# 1.INTRODUCTION

## LIPID BASED DRUG DELIVERY SYSTEMS

Drugs which are poorly water solubility are made well suitable for lipid-based formulation. Water insoluble and weakly basic drugs require special care in the design and development of lipid based formulation. These drugs administered in the solubilised form in the lipid vehicle may come out of the formulation due to solubilisation in the gastric fluid and may precipitate in the intestinal fluid on gastric emptying. The bioavailability of this system would depend on how rapidly the precipitates can be resolubilized by the formulation.

The percentage of new chemical entities synthesized with low aqueous solubility and high therapeutic efficacy is growing, this presents a major challenge for the drug delivery. To overcome the above challenge different methods were developed for the enhancement of bioavailability.

Lipid based formulations are more effective delivery system for oral route and improve bioavailability because of its proven safety and efficacy. Lipid Formulation Classification System was established by Pouton . It aims to enable in vivo studies for interpreting and for the identification of the most appropriate formulations for specific drugs, their physiochemical properties are taken into consideration.

## SOLID LIPID NANOPARTICLES (SLNs):

SLNs are particulate system with particle diameters ranging 50-1000nm. They are derived from oil-in-water emulsions, by replacing the liquid oil by a solid lipid. Particle size of SLN is in submicron range, ranging from 40 to 1000 nm. They have several advantages that the lipid matrix is generally made from physiologically well-tolerated lipid components, which decreases the toxicity. They have a stability of around 3 years and can easily be manufactured at industrial scales. SLNs, lipid micro particles and lipospheres have been used as alternative carriers for therapeutic peptides, proteins and antigens. Formulation as SLNs confers improved protein stability, avoids proteolysis, as well as providing sustained release of the incorporated molecules. Well-known peptides such as cyclosporine A, insulin, calcitonin and somatostatin have been incorporated into solid lipid particles.

## NANO-STRUCTURED LIPID CARRIER (NLC)

The nano-structured lipid carriers (NLCs) were obtained by the modification of nanostructured that hold the qualities of the SLN, increases the stability of the drug, and prevents drug leakage. The limitations of the drugs that are delivered in the body using different routes are poor solvency, first pass metabolism, and poor bioavailability hence to overcome these limitations NLCs were prepared. Oral, topical, transdermal, ocular, and parenteral are the different routes through which nano based systems are delivered. Spatially incompatible liquid lipids and solid lipids are blended and NLCs were prepared. It stays solid at room temperature. The advantages of drug therapy over conventional carriers NLCs are increased solubility, improved bioavailability[1].

Nanostructured lipid carriers (NLCs) are drug-delivery systems composed of both solid and liquid lipids as a core matrix. It was shown that NLCs reveal some advantages for drug therapy over conventional carriers, including increased solubility, the ability to enhance storage stability, improved permeability and bioavailability, reduced adverse effect, prolonged half-life, and tissue-targeted delivery. NLCs have attracted increasing attention in recent years. Special attention is paid to parenteral injection and topical delivery since these are the most common routes for investigating NLCs. Relevant issues for the introduction of NLCs to market, including pharmaceutical and cosmetic applications[2].

These systems are basically divided into two groups: polymeric nanoparticles and lipid nanoparticles[3]. Polymeric nanosystems are solid colloidal particles consisting of non-biodegradable synthetic polymers or biodegradable macromolecular materials from synthetic, semisynthetic or natural resources. The drawbacks of polymeric nanoparticles are the cytotoxicity of polymers and the lack of suitable large-scale production techniques. Owing to the natural and biological origins of the materials, the toxicological risk associated with lipid nanoparticles is much less than the risk associated with polymeric nanoparticles. Lipid nanoparticles made with a solid matrix (solid lipid nanoparticles, SLNs) are derived from oil-in-water nanoemulsions formed by replacing liquid oil with a solid lipid .The first generation of SLNs was developed at the beginning of 1990[4].

Over the last 20 years, nanotechnology has practically made its influence in all technical fields, including pharmaceutics. Industry estimates suggest that approximately 40% of lipophilic drug candidates fail due to solubility and formulation stability issues, which has been solved by various novel and advanced lipophilic drug delivery technologies . The lipids employed to

prepare lipid nanoparticles are usually physiological lipids (biocompatible and biodegradable) with low acute and chronic toxicity [5].

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 SLNs limitations. 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. In this regard, 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, NLCs can facilitate separation of nanoparticle from the rest of the medium and dosage form preparation for parenteral administration[6].

**ADVANTAGES OF NLC's**

• Better physical stability,

• Ease of preparation and scale-up,

• Increased dispersability in an aqueous medium,

• High entrapment of lipophilic drugs and hydrophilic drugs,

• Controlled particle size,

• An advanced and efficient carrier system in particular for substances,

• Increase of skin occlusion,

• Extended release of the drug,

•One of the carriers of choice for topically applied drugs because their lipid components have an approved status or are excipients used in commercially available topical cosmetic or pharmaceutical preparations,

•Small size of the lipid particles ensures close contact to the stratum corneum thus enhancing drug penetration into the mucosa or skin,

• Improve benefit/risk ratio,

• Increase of skin hydration and elasticity and

•These carriers are highly efficient systems due to their solid lipid matrices, which are also generally recognized as safe or have a regulatory accepted status[7].

**Drug candidates for nanostructured lipid carriers formulation**

In general, the criterion utilized to classify the drugs includes BCS, which implies that aqueous solubility and membrane permeability are two major factors limiting drug absorption. Considering the biopharmaceutical obstacles in oral absorption of lipophilic drugs such as UWL, P-gp efflux, intra-enterocyte, and hepatic metabolism, BCS alone is not a satisfactory tool for selecting drug candidates for advanced lipid-based formulations. There is a great need of a modified classification system, which also takes into account attributes such as drug metabolism, disposition, and the role of transporters as they affect the absorption process to a very large extent.

A review of the drugs, done by Wu and Benet, classified the drugs in Classes I-IV of BCS such that drugs in Classes I and II were metabolized and eliminated, in contrast, Classes III and IV drugs were eliminated unchanged . This serves as a core criterion for the customized classification system, namely the biopharmaceutics drug disposition classification system (BDDCS). According to this system, the extent of metabolism (or major route of drug elimination) substitutes membrane permeability as classification condition. Importantly, this system takes into account the knowledge of efflux transporters and presystemic metabolism. Lipophilic and poorly water soluble drugs, which are classified as Class II or IV have been known to be potential substrates for intestinal efflux transporters such as P-gp and are also known to be metabolized by intestinal CYP enzymes. Consequently, BDDCS could play an essential role in identifying suitable drug candidates which are expected to benefit from NLC formulations. As per this classification, absorption of Class II drugs could be greatly enhanced possibly by selection of those lipids in the formulations, which influence metabolism and/or efflux. The understanding of a particular transporter(s) in the disposition of a specific drug will guide appropriate lipidic excipient selection, with intent of modulating this effect and improving bioavailability. Hence, BDDCS helps in choosing the appropriate drug candidate

for apt lipid carrier and maximizes the benefits from co-administration of suitable lipids in NLC[8].

**Drug release** :The release of the drugs from a matrix is depends upon the rate of degradation and diffusion in case of NLC's. It is well documented in literature that it is compulsory to have exact and controlled release going beyond diffusion and degradation. The particle should be triggered by an impulse when a particle is administered the release . The drug will have to trapped in NLC's because of their unordered and unorganized lipid structure. By applying different methods and techniques the structure of the lipid can be modified, which leads to convert the structure of lipid molecule and hence ongoing drug release can be initiated . It was observed that this method is essential in case of NLC's are incorporated in cream for use in the skin as well as for the treatment of different dermatological problems like psoriasis, eczema. These type of NLC's are useful and have very advantageous properties if used by rubbing it increases the temperature and water evaporation from the formulation, based upon this method cyclosporine-lipid particles are under development to treat psoriasis .The drug release from NLC by initiating the alteration from a extremely disordered lipid structure to more ordered stable modifications . It was observed that in case of SLNs particle aggregation can occur during long-term storage of dispersions . The collision of the particle can cause perkinetic flocculation in the very concentrated NLC dispersions the particles form a pearl-like network, so particle required be in a fixed position to avoid a collision and perikinetic flocculation . Aggregates formation from lipid particle are storage and pearl-like network in NLC's dispersions [9].

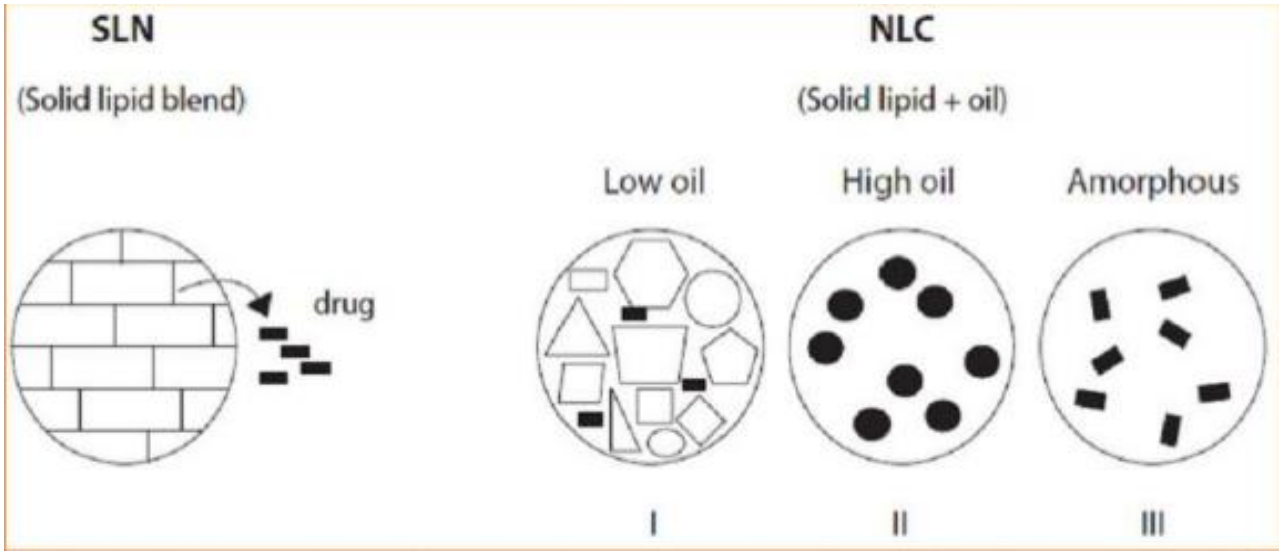



**Figure 1:** Different types of NLC

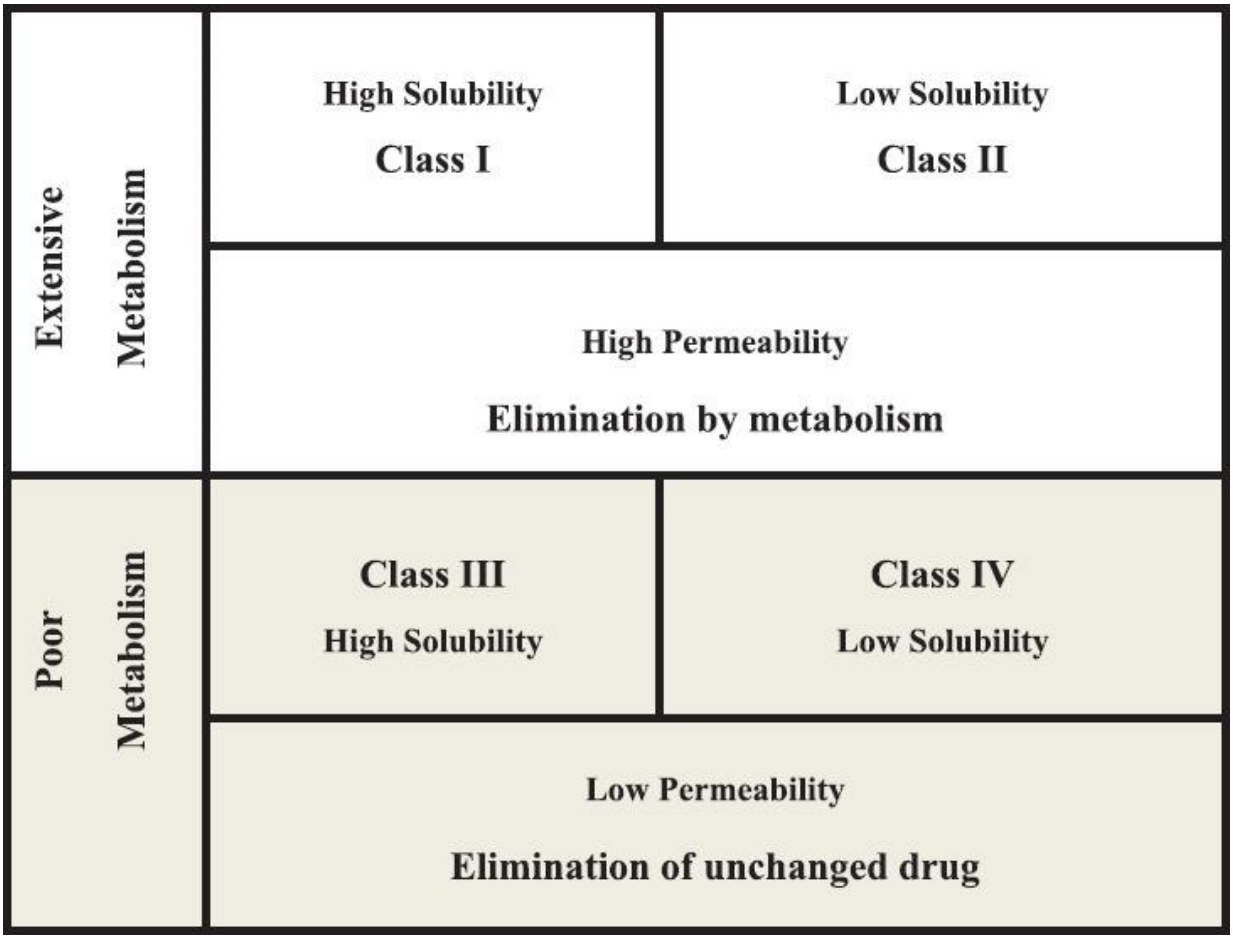



**Figure 2:** Biopharmaceutics classification system

NLC can be classified into three different types based on the nano-structure, composition and ratios of solid and liquid lipids

a. The imperfect type

b. The multiple O/F/W type

c. The amorphous type

I. **The imperfect type**: This is produced by mixing chemically different solid and liquid lipids which gives rise to imperfections and hence enhanced drug loading.

II. **The multiple O/F/W type**: This contains oil nanocompartments encapsulated with solid lipid. The drug is loaded/ dissolved in the oil compartments. It was prepared by lipid- lipid precipitation technique.

III. **The amorphous type**: This is prepared by controlled mixture of special types of solid and liquid lipids (eg, Isopropylmyristate) such that the NLC obtained are in amorphous state[10].

**Table 1:** Process variables and their role in the preparation of NLCs.

| Process Variables | Step involved | Process Responses |
|---|---|---|
| Speed and Time | Mixing | Particle shape, Particle size |
| Temperature | Melting | Phase transition, Solubility |
| Speed and Time | Stirring | Particle shape, Particle size |
| Speed, Temperature, Pressure | Homogenization | Particle size, Particle shape |

## INGREDIENTS USED IN FORMULATION OF NANO STRUCTURED LIPID CARRIER

### SOLID LIPIDS:

**Fatty acids:** Dodecanoic acid, Myristic acid, Palmitic acid and Stearic acid.

**Monoglycerides:** Glyceryl monostearate, and Glyceryl behenate.

**Diglycerides:** Glyceryl palmitostearate and Glyceryl dibehenate.

**Triglycerides:** Caprylate triglyceride, Caprate triglyceride, Glyceryl and tribehenate/Tribehenin.

**Waxes:** Cetyl Palmitate, Carnauba, and wax Beeswax.

### LIQUID LIPIDS:

Soya bean oil, Oleic acid, Medium chain triglycerides (MCT)/caprylic- and capric triglycerides, α-tocopherol/Vitamin E, Squalene

Hydroxyoctacosanyl hydroxystearate and Isopropyl myristate.

**Cationic lipids** Cetyl pyridinium chloride (hexadecyl pyridinium chloride, CPC), Cetrimide tetradecyl trimethyl ammonium bromide (CTAB).

**SURFACTANTS**

**Ionic surfactants**

Sodium taurodeoxycholate, Sodium oleate, Sodium dodecyl sulphure.

**Non-ionic surfactants**

Span 20, 80, 85, Tween 20, 80, Tyloxapol, Poloxamer 188 Poloxamer 407, Solutol HS15.

**Amphoteric surfactants**

Egg phospholipid (Lipoid E 80, Lipoid E 80 S) Soy

Hydrogenated soy phosphatidylcholine (Lipoid S PC-3,

Hydrogenated egg phosphatidylcholine (Lipoid E PC-3)

Phospholipon 80 H, Phospholipon 90 H)

**Co-surfactants**

Butanol, Butyric acid [11].

**NLC AS COMPARED TO SLN**

Nano lipid carrier, the next generation state of the art lipid nanoparticle which as a fictive carrier system has been prepared to swipe some demerits of the solid lipid nanoparticle. To overthrow this drug exclusion at the time of storage, lipid fusions were preferred because they don't cast a notably organized crystalline arrangement which is desired. Matrixes of NLCs are prepared by blending spatially organized varied lipid molecules, typically a fusion of solid and liquid lipid, presents deformities in the matrix to aggregate more drug molecules than SLN. Alternative to the existence of liquid lipid, NLC matrix is solid at room temperature. NLCs are nothing but a blend of solid lipid and liquid lipid and reside in the solid state by regulating the content of liquid lipid. NLCs can thoroughly paralyse the drugs and prohibit the particles from coagulating by means of the solid matrix correlated to emulsions. NLC has enhanced scientific and commercial attention midst of the last few years due to the decreased risk of systemic side effects. Also, the exclusion of drug entrapped in NLC during storage is decreased or avoided.

This comprises of high amounts of drug payload, enhanced drug stability, the chances to control drug release and targeting and avoidance of organic solvents.

NLCs are made up of biocompatible solid lipid matrices and liquid lipid which have varied chemical structure than that of the solid lipid. Furthermore, NLCs have the usual particle diameter ranging 10–1000 nm. Nano lipid carriers (NLC) are the next generation SLN comprised of solid lipid matrix which is integrated with liquid lipids. Amidst the nano lipid carriers that comprises of solid lipids together with liquid oils are, Miglyol®, α-tocopherol, etc. The existence of liquid lipids with varied fatty acid C-chains yields NLC with less classified crystalline structure and thus provides better loading capacity for drug. Liquid lipids are said to be the good solubilizers of drugs than solid lipids. These carriers comprises of physiological and biodegradable lipids showing less systemic toxicity and less cytotoxicity.

Most of the lipids have a suggested status or are excipients used in commercially available pharmaceutical preparations. The small size of the lipid particles ensures close contact to stratum corneum and can enhance the amount of drug penetrating into mucosa or skin. Due to their solid lipid matrix, a controlled release from these carriers is possible. This becomes an important tool when it is necessary to supply the drug over a long period of time, to reduce systemic absorption, and when drug produces irritation in high concentrations. NLC have been shown to exhibit a controlled release behaviour for various active ingredients such as ascorbyl palmitate, clotrimazole, ketoconazole and other antifungal agents[12].

**Methods for Preparing NLCs**

Many types of protocols are available for the preparation of NLCs. The most commonly used approach is the high-pressure homogenization method, which utilizes both high temperature and high pressure. Another is the low-temperature, high-pressure homogenization method. The techniques for preparing SLNs can also be employed for NLC preparation. These approaches include high-pressure homogenization, ultrasonic emulsion evaporation, solvent dispersion, the film-ultrasonic method, high-temperature emulsion evaporation–low temperature curing, microemulsion, emulsion, supercritical fluid (SCF), membrane contactor, microchannel, and microtube methods.

**High-Pressure Homogenization Method**

Methods for preparing NLCs are relatively mature and can be used in mass production and dispersing techniques that do not involve organic solvents. These methods can be divided into

high-temperature, high-pressure and low-temperature, high-pressure homogenization protocols. The high-temperature, high-pressure homogenization method is the more commonly adopted and involves the melting of solid lipid materials first before mixing them with liquid lipid and drugs. After mixing, the molten liquid is scattered throughout the aqueous phase, which contains surfactants. The mixture is stirred to form the beginning of an emulsion. Then, by high-speed impact and decompression expansion under an extremely high shear force, fluid droplets are gradually broken into nanoparticles. Generally, high temperatures reduce the viscosity of the mixed liquid, decreasing the particle size but increasing the probability of degrading the drug and the carrier. This method can be successfully used for insoluble drugs and lipophilic ones, but is not entirely suitable for hydrophilic drugs. The advantages are avoidance of organic solvents and large-scale production.

**Ultrasonic Emulsion Evaporation Method**

In this method, the solid lipid, the liquid lipid, and the drug mixture as oil phase are added and dispersed in an aqueous surfactant solution by probe ultrasonication. The sample was then cooled down and solidified to form NLCs. When a stable emulsion is formed, the oil phase is evaporated by heating under reduced pressure, or by evaporation while stirring continuously. Avoidance of heat during the preparation is the most important advantage of this method. Toxicological problems may result from solvent residues from the product obtained by this method.

**Solvent Dispersion**

In the solvent dispersion method, solid lipid, liquid lipid, and the drug are dissolved in a water miscible organic solvent (ethanol, acetone, or isopropanol).Then, the organic solution is slowly added to the water containing the emulsifier, and the NLC is obtained by centrifugation. The drug loading of NLCs prepared by this method generally increases with the mass of the liquid. To further increase the drug loading of NLC, the dispersed phase is usually employed to enclose a saturated drug solution. The advantages of this method are speed, simplicity, and the low requirements of the instrument. The disadvantages of this method are that it is not entirely suitable for industrial production, and there is residual organic solvent.

**Film-Ultrasonic Method**

In the film-ultrasonic method, the solid lipids, liquid lipids, and drugs are dissolved in an appropriate organic solvent, which is later removed by vacuum evaporation. To form a layer

of mixed lipid films, a surfactant aqueous solution is added. Small and uniform NLCs are then produced using an ultrasound probe for ultrasonic dispersion. This method is most often used due to its simplicity and practicality, and its yield of small, uniform particles. However, toxicological problems may result from solvent residues from the product obtained by this method.

**High-Temperature Emulsion Evaporation—Low-Temperature Curing**

This method involves independently heating the organic and aqueous phases to the same temperature and then adding the organic phase to the aqueous phase containing an emulsifier so that an emulsion is produced. The volatile organic solvent is then evaporated from the system by heating, and the resulting concentrated liquid is quickly dispersed in ice water (0–4 °C). In this way, a NLC dispersion solution is obtained. The advantages of this method include its simplicity and speed. The disadvantages of this method are that it is not entirely suitable for industrial production, and there is residual organic solvent.

**Microemulsion Method**

Using a microemulsion approach, the lipid carrier is heated and melted, and then drugs, emulsifier, auxiliary emulsifier, and deionized water are added to yield a mixture with a transparent appearance and a thermodynamic stability similar to that of oil-in-water (O/W)-type microemulsion. The microemulsion is quickly dispersed in ice water (0–4 °C), forming an NLC dispersion system. The sizes of the nanoparticles and particles from microemulsion and dilution are extremely close to the temperature difference between the cold water and the microemulsion, which is a key factor in preparing small-particle-sized NLCs. Rapid cooling and solidification can prevent the aggregation of some particles. The advantages of this method include its low drug content and simplicity, while the disadvantages are the abundance of auxiliary emulsifier and emulsifier required.

**Melt Emulsification Method**

In this protocol, the solid and liquid lipids are heated and mixed. Then the drugs are added to form an organic phase. The organic phase is added to a water phase containing the surfactant and stirred to form a coarse emulsion. High-pressure homogenization is subsequently applied to form the NLCs. This is advantageous because there is no organic solvent residue, no burst release at the initial time, and dispersions with high lipid concentration. The disadvantages of

this method are that it is not entirely suitable for industrial production and there is residual organic solvent.

**Emulsion Evaporation Method**

This method involves the dissolution of a polymer in methylene chloride, chloroform, ethyl acetate, or another organic solvent. The drug is dissolved or dispersed in the polymer solution to form an organic phase. The organic phase is then uniformly and slowly added to the water phase and emulsified to form an O/W system. The emulsifier or other surface active agents used in this system include gelatin and polyvinyl alcohol (PVA), Span 80, and Poloxamer188. After a stable emulsion is formed, the organic solvent is evaporated by heating under reduced pressure. Avoidance of heat during the preparation is the most important advantage of this method. Toxicological problems may result from solvent residues from the product obtained by this method.

**Double-Emulsion Evaporation Method**

The method involves water-soluble drugs as the internal phase dispersed throughout an organic phase containing PLGA or other carriers to form a W/O as the start of the emulsion. The start of the emulsion is then dispersed in an external aqueous phase to form a W/O/W type double emulsion, removing the organic solvent during preparation. W/O/W double emulsion preparation technology can be applied to preparing water-soluble drug nanoparticles. Toxicological problems may result from solvent residues from the product obtained by this method.[13]

## COMMERCIALLY AVAILABLE PRODUCTS FROM LIPID NANOPARTICLES IN MARKET

Today, most of the commercially available products from lipid nanoparticles are cosmetic products such as

Cutanova Cream Nano Repair Q10,

Intensive Serum Nano Repair Q10,

Cutanova Cream Nano Vital Q10,

SURMER Crème Legère Nano-Protection,

SURMER Crème Riche Nano-Restructurant,

SURMER Elixir du Beauté Nano-Vitalisant,

SURMER Masque Crème Nano-Hydratant,

NanoLipid Restore CLR,

NanoLipid Q10 CLR,

NanoLipid Basic CLR,

NanoLipid Repair CLR,

IOPE SuperVital cream, serum, eye cream, extra moist softener and extra moist emulsion,

NLC Deep Effect Eye Serum,

NLC Deep Effect Repair Cream,

NLC Deep Effect Reconstruction Cream[14].

**APPLICATIONS OF NANO STRUCTURED LIPID CARRIER:**

**1. Topical delivery:**

Tacrolimus (TL), which is currently the main stay of immunosuppressive therapy faces significant hurdles subsequent to its oral administration attributable to its poor aqueous solubility and extensive intestinal and hepatic first pass metabolism. Therefore, the present study aimed to design the stable nanostructured lipid carrier (NLC) of TL that would be able to overcome such hurdles. Capmul MCMC8 and Compritol 888ATO in 3:2 were selected as binary lipid phase on the basis of solubility study. An exhaustive screening of surfactants is done by aqueous titration to select the surfactant with best emulsifying potential and to optimize the concentration of lipids and surfactants in NLC. Different methods of preparation were explored and compared to optimize NLC which could have the best characteristic properties. TL-NLC was characterized for particle size, drug entrapment efficiency, crystal state, surface morphology and drug release. The obtained particle size, PDI and % drug entrapment efficiency of optimized formulations i.e., NLC-C2 and NLC-N2 were 70±5.42nm, 98±7.52nm; 0.43±0.081, 0.2±0.029 and 87±2.34%, 94±3.18%, respectively. The results of in vitro release studies showed significantly increased ($^{***}p<0.001$) and sustained release of TL from NLC dispersions as compared to drug suspension (95.73% from NLC-C2, 99.86% from NLC-N2 and 9.27% drug suspension in pH 1.2 in 24h; 93.11% from NLC-C2, 96.65% from NLC-N2 and 10.2% drug suspension in pH 6.8 in 24h). The study demonstrated that proper

selection of excipients (by aqueous titration) and modification of method of preparation (by inclusion of cold step) would lead to production of NLC with best characteristic properties.[15]

**2. Oral delivery:**

The oral antidiabetic Repaglinide (RPG) was loaded into NLC using emulsification–ultrasonification technique. A design of experiment was constructed to study the formulation variables. The influence of the liquid lipid to the solid lipid ratio and the concentration of the surfactant on mean particle size, zeta potential, and drug entrapment efficiency was demonstrated. The mean particle size ranged from 182 ± 7.9 nm to 452 ± 66.1 nm. All particles were negatively charged and the zeta potential values ranged from −7.9 ± 0.9 mV to −44.4 ± 6.2 mV. The highest entrapment efficiency was obtained with the minimum solid lipid to liquid lipid ratio and lowest surfactant concentration. All RPG–NLC formulae showed biphasic time-dependent in vitro release and the studied factors were optimized and the optimum formula was evaluated for in vitro release and crystallinity. The in vitro release of the optimized formula fitted to the Higuchi diffusion model. In conclusion, this study showed the potential of NLC as a carrier for controlled release of RPG.[16]

**3. Ocular administration:**

To develop a thiolated non-ionic surfactant, cysteine-polyethylene glycol stearate (Cys-PEG-SA), for the assembling of nanoparticulate ocular drug delivery system with mucoadhesive property. Cys-PEG-SA was synthesized in two steps reaction involving a new derivative intermediate formation of p-nitrophenylcarbonyl-PEG-SA (pNP-PEG-SA). Up to 369.43+/-25.54 mumol free thiol groups per gram of the conjugates was reached. The nanostructured lipid carrier (NLC) loaded cyclosporine A (CyA) was prepared by melt-emulsification method. The mucoadhesive NLC (Cys-NLC) was obtained by incubating NLC emulsion with Cys-PEG-SA. The mucoadhesive properties of these nanocarriers were examined by using mucin particles method. The particle size or zeta potential of the porcine mucin particles were changed with the added concentration of Cys-PEG-SA, and the disulphide bond breaker cysteine significantly reduced the adhesion of Cys-NLC to mucin particles ($P<0.05$), whereas PEG-SA and NLC did not alternate the properties of the mucin particles. When Cys-NLC was administered topically to the rabbit eye, the encapsulated cyclosporine was found to remain on the ocular surface in the cul-de-sac for up to 6 h, both precorneal retention time and concentration were dramatically increased ($P<0.05$), compared with the NLC without thiomer modification.[17]

**4. Parenteral administration:**

To formulate nanostructured lipid carriers (NLC) for the parenteral delivery of an anticancer drug, all-*trans* retinoic acid (ATRA). The ATRA was incorporated into NLC by the de novo emulsification method. The effect of the formulation factor, i.e., type and oil ratio, initial ATRA concentration on physicochemical properties was determined. The anticancer efficacy of ATRA-loaded NLC on HL-60 and HepG2 cells was also studied. NLC was formulated using a blend of solid lipids (cetyl palmitate) and liquid lipids (soybean oil (S), medium-chain triglyceride (M), S/oleic acid (O; 3:1) and M/O (3:1)) at a weight ratio of 1:1. ATRA-loaded NLC had an average size of less than 200 nm (141.80 to 172.95 nm) with a narrow PDI and negative zeta potential that was within an acceptable range for intravenous injection. The results indicated that oleic acid enhanced the ATRA-loading capacity of NLC. *In vitro* ATRA release was only approximately 4.06% to 4.34% for 48 h, and no significant difference in ATRA release rate from all NLC formulations in accordance with the composition of the oil phase. Moreover, no burst release of the drug was observed, indicating that NLC could prolong the release of ATRA. The initial drug concentration affected the photodegradation rate but did not affect the release rate. All ATRA-loaded NLC formulations exhibited the photoprotective property. The cytotoxicity results showed that all ATRA-loaded NLC had higher cytotoxicity than the free drug and HL-60 cells were more sensitive to ATRA than HepG2 cells[18].

**5. Intranasal delivery:**

To utilize potential of nanostructured lipid carriers of the quercetin for direct nose to brain delivery of drug as tool for the targeted delivery. The aim of this study was to prepare and characterize quercetin loaded NLC and to study its brain distribution. Novel QUE-NLCs were formulated. NLC Formulation was evaluated for various physicochemical properties such as particle size, zeta potential, drug loading, percent entrapment efficiency, morphology, in vitro drug release, and histopathology. The size of the QUE-NLC was about 118.2nm with poly dispersity index of 0.220 and zeta potential of -20.1 mV. Nose to brain distribution studies were performed using wistar rats. QUE-NLC exhibited sustained delivery of drug. Significant targeting to brain was achieved when compare to quercetin.The result showed that NLCs might be the promising approach for the nose to brain delivery of quercetin[19].

**6. Brain delivery:**

A transferrin-conjugated nanostructured lipid carrier (TF-NLCs) for brain delivery of artemisinin (ART) was developed. ART-loaded NLCs (ART-NLCs) were prepared using

solvent evaporation method and the impact of various formulation or process variables on the responses were assessed using a Taguchi design. Optimized ART-NLC was then coupled with transferrin as targeting ligand and its in vitro cytotoxicity was investigated against U-87MG brain cancer cell line. As a result, the following values are suggested by the software to prepare the optimized formulation: 20 mg Compritol®, 0.25% Tween 80, 5 mg oleic acid, 2.5 mL dichloromethane and 4 min sonication. Mean particle size (PS), zeta potential (ZP), polydispersity index (PDI), entrapment efficiency (EE), mean release time (MRT) of adopted formulation were confirmed to be 145 ± 12.5 nm, 24.3 ± 1.5 mV, 0.513 ± 0.021, 82.3 ± 7.3 % and 24.0 ± 1.1 h, respectively. Following conjugation of optimized ART-NLCs with TF, PS and MRT were increased, while ZP, and EE were decreased significantly. TF-ART-NLCs showed higher cytotoxic activity compared to non-targeted NLCs and free drug. These results indicated that the TF-ART-NLCs could potentially be exploited as a delivery system for anticancer and antimalarial drug ART in brain tumors and malaria[20].

**MECHANISM OF PERMEATION OF NANO STRUCTURED LIPID CARRIER:**

Topical drug application has been introduced since long time to achieve several purposes on different levels (skin surface, epidermis, dermis and hypodermis). However, several problems have been reported with the conventional topical preparations e.g. low uptake due to the barrier function of the stratum corneum and absorption to the systemic circulation. The scientific literature today provides several systems that can deliver active pharmaceutical ingredients (APIs) across the skin. These include reservoir matrices, matrix diffusion-controlled devices, multiple polymer devices and multilayer matrix assemblies. Among these, topical application of the Solid lipid nanoparticles(SLN) and composed of physiological lipid materials suitable for topical, dermal and transdermal administration. Many features, which these carrier systems exhibit for dermal application of cosmetics and pharmaceutics, have been pointed out. SLN and NLC are composed of physiological and biodegradable lipids that show low toxicity. The small size ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin. Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis.

1. **Increase of skin occlusion**: The lipid film formation on the top of the skin and the subsequent occlusion effect was reported for lipid nanoparticles. By using very small lipid particles, which are produced from highly crystalline and low melting point lipids, the

highest occlusion will be reached. Particles smaller than 400 nm containing at least 35% lipid of high cystallinity have been most effective . Souto et al. found a higher occlusive factor for SLN in comparison to NLC of the same lipid content. Comparing NLC with different oil content showed that an increase in oil content leads to a decrease of the occlusive factor.

2. **Increase of skin hydration and elasticity**: The reduction of transepidermal water loss (TEWL) caused by occlusion leads to an increase in skin hydration after dermal application of SLN, NLC or formulations containing them. An in vivo study showed that the SLN-containing o/w cream increased the skin hydration significantly more than the conventional o/w cream. In study shows that the skin hydration effect after repetitive application of an o/w cream containing SLN and a conventional o/w cream was investigated for 28 days . A significant higher increase in skin hydration was found by Müller et al. for an NLC-containing cream compared to conventional cream .
3. **Enhancement of skin permeation and drug targeting:** The stratum corneum in healthy skin has typically a water content of 20% and provides relatively an effective barrier against percutaneous absorption of exogenous substances. Skin hydration after applying SLN or NLC leads to a reduction of corneocytes packing and an increase in the size of the corneocytes gaps. This will facilitate the percutanious absorption and drug penetration to the deeper skin layers .
4. **Improve benefit/risk ratio:** Skin atrophy and systemic side effect occurred after applying conventional prednicarbate cream could be avoided when this drug was formulated as SLN. Predinicarbate uptake was enhanced and it was accumulated in the epidermis with a low concentration in the dermis. In another study Joshi et al. compared a valdecoxib loaded NLC carbopol gel with a valdecoxib market product. The NLC containing gel showed no skin irritation while the market gel showed slight irritation after 48 hrs. Moreover, the NLC based gel showed prolonged activity up to 24 hrs while the activity of the market gel was shorter. This indicates a better skin tolerability and a longer activity of the NLC formulation compared to the marketed formulation . Tretinoin loaded-SLN formulation was studied by Shah et al. concerning skin irritation. One of the major disadvantages associated with the topical application of tretinoin is the local skin irritation such as erythema, peeling and burning as well as increased sensitivity to sunlight. In the in vitro permeation studies through rat skin they found that SLN based tretinoin gel has a permeation profile comparable to that of the market tretinoin cream. But on the other hand, Draize patch test showed that SLN based tretinoin gel resulted in remarkably less erythemic episodes

compared to the currently marketed tretinoin cream and hence, a better benefit/risk ratio is expected for the formulations containing tretinoin-loaded SLN. Conclusively, applying SLN or NLC can enhance skin penetration of incorporated actives, promote the epidermal targeting and minimize the systemic side effects and therefore, the benefit/risk ratio is improved.

5. **Enhancement of UV blocking activity**: Some side effects of organic UV blockers were reported due to the penetration of these compounds into the skin causing skin irritation and allergic reaction. This penetration can be reduced by incorporating these compounds in lipid nanoparticles. It was found that incorporating benzophenone in SLN not only improves the UV blocking activity evaluated using in vitro photoprotection assay but also reduces the absorption of the benzophenone into the skin in comparison to a conventional nanoemulsion. Improving the UV blocking activity allows the reduction of the concentration of the UV blocker while maintaining the protective level of the conventional formulation. These findings were confirmed by Song and Lui comparing UV absorption properties of 3,4,5- tri methoxy benzo chitin-loaded SLN and SLN free system . Furthermore, a significant increase in SPF up to about 50 was reported after the encapsulation of titanium dioxide into NLC. Encapsulation of inorganic sunscreens into NLC is therefore a promising approach to obtain well tolerable sunscreens with high SPF.13,14
6. **Enhancement of chemical stability of chemically labile compounds**: Enhancement of chemical stability after incorporation into lipid nanocarriers was proven for many cosmetic actives e.g. coenzyme Q 10, ascorbyl palmitate, tocopherol(vitamin E) and retinol (Vitamin A)[21].

**IBUPROFEN:**

Ibuprofen [2-(4-isobutylphenyl) propionic acid] is a potent non steroidal anti-inflammatory(NSAID) drug commonly indicated for the treatment of acute and chronic arthritic conditions trauma, swelling of soft tissues and other forms of pain due to its good analgesic properties. It also has antipyretic properties.[22]

**Uses**

Ibuprofen is available as gels, sprays, tablets or mousses, and it is used to relieve a variety of symptoms. The short term use of ibuprofen can help to resolve headaches. For anti-inflammatory effects related to chronic conditions, such as arthritis, long-term use is necessary.

- fever
- inflammation
- headache
- toothache
- back pain
- arthritis, including juvenile arthritis
- menstrual main
- minor injuries[23.]

**Mechanism of action:**

Ibuprofen is a non-selective inhibitor of an enzyme called cyclooxygenase (COX), which is required for the synthesis of prostaglandins via the arachidonic acid pathway. COX is needed to convert arachidonic acid to prostaglandin H2 (PGH2) in the body. This PGH2 is then converted to prostaglandins. The inhibition of COX by ibuprofen therefore lowers the level of prostaglandins made by the body. The prostaglandins that are formed from PGH2 are important mediators of sensations such as pain and processes such as fever and inflammation. The antipyretic effects may arise as a result of action on the hypothalamus leading to vasodilation, an increased peripheral blood flow and subsequent heat dissipation. Anticoagulant effects are also mediated through inhibition of COX, which converts arachidonic acid into thromboxane A2, a vital component in platelet aggregation that leads to the formation of blood clots. There are two forms of COX in the body - COX-1 and COX-2. The pain and inflammation reducing effects of NSAIDs are mediated through the inhibition of COX-2, while COX-1 inhibition

blocks the formation of thromboxane. The long-term blockage of COX-1 with chronic use of NSAID, however, may cause gastric toxicity, as COX-1 usually maintains the gastric mucosa[24].

Ibuprofen is an over-the counter nonsteroidal anti-inflammatory drug (OTC NSAID) used to treat inflammation, fever, and mild pain. The medication is used by millions to treat:

- headaches
- back pain
- toothaches
- arthritis
- menstrual cramps
- fevers

Some brand names for ibuprofen are:

- Motrin
- Advil
- Midol
- Nuprin
- Pamprin IB

**Recommended dosage**

The recommended dose of ibuprofen depends on a person's age.

**For adults**

The recommended dosage for adults is one or two 200 milligram (mg) tablets every four to six hours. Adults should not exceed 800 mg at once or 3,200 mg per day.

Adults over the age of 60 should take as little ibuprofen as possible to manage their symptoms. Older adults have a higher risk of kidney and gastrointestinal side effects.

**For children**

To determine the safe dosage for children, need to know the child's weight and the formulation of ibuprofen . Ibuprofen for children is available in infant drops, liquids, and chewable tablets.

Liquid measurements are given in milliliters (mL). It should not be given more than four doses in one day.

**Table 2**: Ibuprofen dosage for children

| **Weight** | **50 mg/1.25 mL infant drops dosage** | **100 mg/5 mL liquid dosage** | **50 mg/1 chewable tablet dosage** |
|---|---|---|---|
| 12 to 17 pounds | 1.25 mL (50 mg) | As recommended by physician. | As recommended by physician |
| 18 to 23 pounds | 1.875 mL (75 mg) | As recommended by physician. | As recommended by physician |
| 24 to 35 pounds | 2.5 mL (100 mg) | 5 mL (100 mg) | 2 tablets (100 mg) |
| 36 to 47 pounds | 3.75 mL (150 mg) | 7.5 mL (150 mg) | 3 tablets (150 mg) |
| 48 to 59 pounds | 5 mL (200 mg) | 10 mL (200 mg) | 4 tablets (200 mg) |
| 60 to 71 pounds | n/a | 12.5 mL (250 mg) | 5 tablets (250 mg) |
| 72 to 95 pounds | n/a | 15 mL (300 mg) | 6 tablets (300 mg) |
| over 95 pounds | n/a | 20 mL (400 mg) | 8 tablets (400 mg) |

**For babies**

Ibuprofen should not be given to children under six months of age. For infants of age six months to a year, the safe dose of infants' formulation depends on their weight.

**Table 3**: Ibuprofen dosage for babies

| **Weight** | **50 mg/1.25 mL infant drops dosage** |
|---|---|
| under 12 pounds | As recommended by physician |
| 12 to 17 pounds | 1.25 mL (50 mg) |
| 18 to 23 pounds | 1.875 mL (75 mg) |

**Drug interactions**

Certain medications can increases the risk of having an overdose of ibuprofen. Not to take any of the following medications with ibuprofen without first consulting the physician:

- Aspirin, because it may increase the risk of serious side effects
- Diuretics (water pills), due to an increased risk of kidney failure
- Lithium, due to an increased risk of toxicity
- Methotrexate, due to an increased risk of toxicity
- Anticoagulants (blood thinners), such as warfarin, because it can increase the risk of serious gastrointestinal bleeding

Mixing ibuprofen with alcohol can also increase the risk of having serious side effects, like stomach or intestinal bleeding.

**Symptoms of an ibuprofen overdose**

Mild symptoms may include:

- Tinnitus (Ringing In The Ears)
- Heartburn
- Nausea
- Vomiting
- Stomach Pain
- Diarrhoea
- Dizziness
- Blurred Vision
- Rash
- Sweating

Severe symptoms can include:

- Difficult Or Slow Breathing
- Convulsions
- Hypotension (Low Blood Pressure)
- Seizures

- Little To No Urine Production
- Severe Headache
- Coma

Infants who overdose may show signs of lethargy (unresponsiveness) or apnoea (temporary cessation of breathing) following a more serious overdose of ibuprofen.

**Treating an overdose**

To monitor breathing, heart rate, and other vital signs. A physician may insert a tube through the mouth to look for internal bleeding. To receive the following treatments:

- Medications that make you throw up
- Gastric lavage (stomach pumping), only if the drug was ingested within the last hour
- Activated charcoal
- Laxatives
- Breathing support, such as oxygen or a breathing machine (ventilator)
- Intravenous fluids

**Complications of an ibuprofen overdose**

An overdose of ibuprofen can cause severe problems in the gastrointestinal tract. These include:

- Inflammation
- Bleeding
- Ulcers
- Stomach or intestinal perforation, which can be fatal
- Liver or kidney failure[25.]

## 2.LITERATURE REVIEW:

1. **Fang CL et al[26] ., (2013)** reviewed that Nanostructured lipid carriers (NLCs) for drug delivery and targeting . It was shown that NLCs reveal some advantages for drug therapy over conventional carriers, including increased solubility, the ability to enhance storage stability, improved permeability and bioavailability, reduced adverse effect, prolonged half-life, and tissue-targeted delivery. NLCs have attracted increasing attention in recent years. This review describes recent developments in drug delivery using NLCs strategies. The structures, preparation techniques, and physicochemical characterization of NLCs are systematically elucidated in this review. The potential of NLCs to be used for different administration routes is highlighted. Special attention is paid to parenteral injection and topical delivery since these are the most common routes for investigating NLCs. Relevant issues for the introduction of NLCs to market, including pharmaceutical and cosmetic applications, are discussed. The related patents of NLCs for drug delivery are also reviewed.

2. **Jessie sofia pamudji et al[27]., (2016)** developed a nanostructured lipid carrier formulation containing of retinyl palmitate. The formulas of NLC were developed by using virgin coconut oil and oleic acid as a liquid lipids, cetyl palmitate, and stearic acid as solid lipids, Tween 80 and Poloxamer as a surfactant and glycerine as co-surfactant. Characterization of NLC consisted of visual appearance, morphology, particle size, polydispersity index (PI), and physical stability test using freeze-thaw, centrifugation, and accelerate stability test method. NLC formulations with 7.2% cetyl palmitate, 4.8% of oleic acid, 10% of Tween 80, 10% of glycerol, and 2% of retinyl palmitate is the most optimal formula that showed a good characteristic. Stability study revealed that NLC provided better stability than macroemulsion.

3. **P.K. Lakshmi et al[28]., (2011)** studied the formulation and evaluation of ibuprofen topical gel. Eight solid dispersion formulations of ibuprofen were prepared using different drug: polymer ratios viz. 1:0.5, 1:1, 1:2, and 1:3 for 2-HPβ-CD and β cyclodextrin using the co-evaporation method, and were evaluated for partition coefficient, dissolution studies, and Fourier Transform Infra Red (FTIR) spectrophotometer. The optimized solid dispersion of ibuprofen was incorporated into gel and was compared with penetration enhancers. The formulations were analysed to determine their pH, spreadability, viscosity, and in vitro drug release. The absence of extraneous interactions among ingredients was confirmed by FTIR,

and differential scanning calorimetry (DSC) . The formulation with 1:0.5 SDIB (drug: HPβCD) with a partition coefficient of 1.28 was incorporated in carbopol gel, and produced 98.21% drug release compared to solid dispersion of ibuprofen with menthol (SDIBM5%), which produced 96.5% drug release. In ex vivo studies, SDIB and SDIBM5% formulations gave 94.3% and 92.36% drug release within 24h.Stability studies conducted for SDIB incorporated gel according to International Conference on Harmonization guidelines showed it to be stable for two months.

4. **Bazigha K Abdul Rasool et al[29]., (2010)** developed and evaluated the Ibuprofen Transdermal Gel Formulations . Ibuprofen gel formulations, incorporating various permeation enhancers, were prepared using chitosan as a gelling agent. The formulations were examined for their in vitro characteristics including viscosity, pH and drug release as well as in vivo pharmacological activities. Carrageenan-induced rat paw oedema model was used for the evaluation of their analgesic and anti-inflammatory activities. Ibuprofen gel preparations containing 5 % menthol and 20 % propylene glycol, respectively, exhibited pronounced analgesic activity and could be further developed for topical and systemic delivery of ibuprofen.

5. **Petersen-Braun et al[30].,(2013)** investigated the Topical gel formulation of ibuprofen in the treatment of acute and chronic joint and soft tissue pain. The therapeutic effect of a topical gel formulation of Ibuprofen was investigated in an observational trial. A total of 170 patients suffering either from pain, inflammation or swelling of soft tissues or joints or having experienced a blunt trauma were enrolled into this study. Physicians and patients evaluated typical symptoms at study entry and after 1 week at the final visit. Further, the patients were asked and instructed to monitor their symptoms in a daily diary over this week. The results of this study confirm findings from existing clinical trials data. In acute trauma a faster symptom relief is found, in chronic conditions the product leads to symptom reduction , in case seven where pretreatment has shown insufficient results. Thus this topical treatment provides a treatment option either alone or in combination with orally taken NSAIDS.

6. **Arvind Bagde et al[31]., (2019)** studied Formulation of topical ibuprofen solid lipid nanoparticle (SLN) gel using hot melt extrusion technique (HME) and determining its anti-inflammatory strength. Solid lipid nanoparticles(SLN) have been formulated using various batch processes, e.g., solvent diffusion evaporation , emulsification solvent

evaporation followed by size reduction using high-pressure homogenization (HPH) or ultrasonication. However , for the manufacturing of formulations, continuous processes are always preferred over batch processes since they are more efficient and offer better quality of the end product. Hence , we developed topical SLN of ibuprofen (IBU) using hot melt extrusion (HME), prepared a gel formulation, and performed its in vitro and in vivo evaluation. Effect of different variables of HME equipment and materials used in SLN was optimized using design of experiment (DoE) approach. In conclusion, HME offers a single step process for manufacturing for SLN which avoids high stress particle size reduction techniques used for SLN preparation.

7. **Hadgraft et al[32]., (2003)** studied the skin penetration of topical formulations of ibuprofen 5%: an invitro comparative study. An in vitro isolated human skin technique with known reliable predictive value for in vivo performance was used to compare the skin penetration of the proprietary ibuprofen gel formulation, Ibugel™, with five other commercially available topical formulations containing ibuprofen 5%: Ibuspray™, Ibumousse™, Proflex Cream™, Fenbid Gel™ and Deep Relief Gel™. There was a marked difference between some formulations in the percentage of applied ibuprofen penetrating the skin samples, with Ibuspray™, Ibugel™ and Ibumousse™ showing the most efficient penetration. The percentage of applied ibuprofen penetrating the skin samples from these formulations was significantly greater ($p < 0.05$) at all sampling intervals when compared with Proflex Cream™, Fenbid Gel™ or Deep Relief Gel™. By 48 h, the percentage of applied ibuprofen that had penetrated through the skin samples from Ibuspray™, Ibugel™ and Ibumousse™ was approximately 2.5 times greater than that from Deep Relief Gel™, 3 times greater than that from Proflex Cream™ and 5 times greater than that from Fenbid Gel™. The data demonstrate that, with topically applied preparations, the composition of the vehicle can have a significant impact on the percutaneous penetration of the active medicament. The possible reasons for this are discussed in terms of partition and diffusion phenomena.

8. **Van-An Duong et al[33]., (2019)** Nanostructured lipid carriers (NLCs), the second generation of lipid nanoparticles could enhance the drug loading capacity and minimize the drug expulsion during storage . They are prepared from mixtures of solid and liquid lipids . The article described the data for the preparation, optimization, and drug release studies of NLCs loaded with ondansetron hydrochloride (OSH), a water-soluble drug. The OSH-loaded NLCs were prepared using a modified cold high-pressure homogenization method.

The NLCs were optimized for various parameters of formulation and preparation process on the basis of particle size (PS), polydispersity index (PI), entrapment efficiency (EE), and drug loading (DL). The dataset presented here supports "Nanostructured lipid carriers containing ondansetron hydrochloride by cold high-pressure homogenization method: Preparation, characterization, and pharmacokinetic evaluation

9. **S. Manchun et al[34]., (2019)** Although caffeine was suggested as one of the pharmacological agents for the cellulite treatment, its skin permeation restricted. The present work was aimed at formulating caffeine loaded nanostructured lipid carriers (CAF-NLCs) containing coconut oil as a topical delivery system. CAF-NLCs were prepared by the ultrasonic emulsification method, using coconut oil as a liquid lipid. The proper selection of solid lipid and surfactants for these formulations were investigated. Subsequently, physicochemical properties, entrapment efficacy, stability, and *in vitro* drug release were evaluated. The CAF-NLCs containing coconut oil was successfully prepared using glyceryl behenate as a solid lipid and showed an interesting entrapment efficiency (62-99%). The obtained CAF-NLCs presented the nanosized range (≈ 60-390 nm), with a low polydispersity index and high negative zeta potential values (over -30 mV). However, the type and concentration of surfactant also affected these properties. These results suggested that CAF-NLCs containing coconut oil are the promising carrier for delivery of caffeine following topical application.

10. **Ashwini M.et al[35]., (2019)** Lipid based carriers (solid lipid nanoparticles-SLN and nanostructured lipid carriers-NLC) were developed at the beginning of the 90s and has been extensively used for topical and transdermal delivery of pharmaceuticals and cosmeceuticals. Among them, NLC's are widely accepted for maintaining drug stability, improving drug therapy, solubilizing poorly water soluble drugs, achieving controlled and sustained drug delivery and reduced toxicity. This review article discusses different formulations and characterization techniques and discusses how NLCs can penetrate the skin barrier. Further, overview on the current state of the art of NLCs as therapeutic and cosmetic formulations are also discussed in detail. The study highlights the reported data on oral bioavailability and toxicological studies and how these NLC's can be employed as promising drug delivery systems for novel treatments in the near future.

11. **QianKang et al[36]., (2019)** This paper aimed to formulate and optimize nanostructured lipid carriers (NLCs) formulation loaded with tripterine (TRI) for transdermal

administration by employing Quality by Design (QbD) approach. NLCs loaded with TRI (TRI-NLCs) were prepared by emulsification evaporation method. The effect of critical parameters on critical quality attributes of NLCs was studied. Design space with favourable response values was defined. The optimized TRI-NLCs were characterized by morphology, powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) analysis and *in vitro* properties. Further, a gel (TRI-NLCs-gel) was made by dispersing TRI-NLCs into carbopol 980 for transdermal delivery. Different drug administration methods and dermato kinetic study of TRI-NLCs-gel were carried out. The optimized TRI-NLCs possessed nanometric size and high entrapment efficiency. DSC and PXRD results showed TRI was completely incorporated in lipids. *In vitro* drug release result revealed the sustained release characteristics of TRI-NLCs. Different drug administration methods and dermato kinetic study revealed the rapid lose water of gel could enhance NLCs into deep skin layer. Histopathology study showed intact skin treated with TRI-NLCs-gel, indicating good biocompatibility of TRI-NLCs-gel. This investigation revealed that QbD design was crucial to understand the formulation process of NLCs, which was of great importance to guide the preparation of nano-scale pharmaceutical product.

12. **Chun-Yu, Chen;et al [37] ., (2019)** This paper reports on the incorporation of oleic acid (OA) within nanostructured lipid carriers (OA-NLC) to improve the anti-inflammatory effects in the presence of albumin. NLCs produced via hot high-shear homogenization/ultrasonication were characterized in terms of particle size, zeta potential, and toxicity. We examined the effects of OA-NLC on neutrophil activities. Dermatologic therapeutic potential was also elucidated by using a murine model of leukotriene B4-induced skin inflammation. In the presence of albumin, OA-NLC but not free OA inhibited superoxide generation and elastase release. Topical administration of OA-NLC alleviated neutrophil infiltration and severity of skin inflammation. OA incorporated within NLC can overcome the interference of albumin, which would undermine the anti-inflammatory effects of OA. OA-NLC has potential therapeutic effects in topical ointments.

13. **Chee Chin Chu et al[38]., (2019)** Both pumpkin and kenaf seed oil with carnauba wax (CW) and beeswax (BW) are used to develop nanostructured lipid carriers (NLCs) loaded with Uvinul T150 and Uvinul A Plus Granular for a UV protection formulation. The study aims to optimize the concentration and the type of seed oil in order to develop a stable NLC formulation with high entrapment efficiency, drug loading, antioxidant activities, and UV absorbing properties. The physical properties of the NLCs are analyzed based on the mean

particle size, polydispersity index (PDI), long-term storage stability, Fourier-transform infrared spectroscopy (FT-IR), and differential scanning calorimetry (DSC). They are also compared for their entrapment efficiency, drug loading, in vitro antioxidant activities, and in vitro UV absorbing properties. The optimized NLC consists of 10% lipid phase and 1% Uvinul T150 and Uvinul A Plus Granular, respectively. It has mean particle size of $238.20 \pm 3.61$ nm and remains physically stable on storage at both $25 \pm 2$ and $40 \pm 2$ °C. Spherical amorphous NLC structure with encapsulated UV filters is observed by transmission electron microscopy (TEM). Besides, it shows high entrapment efficiency (≥95%) for both Uvinul T150 and Uvinul A Plus Granular in addition to its antioxidant activities as indicated by both DPPH and ABTS radical scavenging activities. In addition, the formulation had high UV absorbing properties, showing its potential to be utilized in the formation of sunscreen prototype.

14. **Mariam Zewail et al[39]., (2019)** Oral treatment of rheumatoid arthritis (RA) with the immunomodulator, leflunomide (LEF), is associated with systemic side effects namely immunosuppression and hepatotoxicity. Herein, attempts to improve LEF therapeutic outcomes via nanostructured lipid carriers (NLCs) targeting inflamed rheumatic joints were executed. LEF-NLCs coated with either chondroitin sulphate (CHS) or chitosan (CS) were around 250 nm in size with negative or positive charge, respectively. Particle coating was evidenced by TEM and FTIR analysis. NLCs generally ensured sustained release profile up to 21 days, particularly extended in coated formulations. *In vivo* pharmacokinetic study of LEF suspension, uncoated NLCs, CHS- and CS-coated NLCs was carried out. Following oral administration in RA-induced rat model, joint diameter, paw inflammation, liver functions were measured, in addition to histological examination of liver, kidney and joints. Results revealed improved joint healing and reduced hepatotoxicity following treatment with nanoencapsulated LEF compared to LEF suspension, whereby CHS–NLCs ensued the highest $C_{max}$, AUC and lowest TNF-α level. The dual potential of CHS to achieve active targeting to CD44-receptor and hence maximize LEF concentration at the target site in addition to its synergistic effect in joint healing endow promises for a competent oral nanosystem for targeted drug delivery to the joints.

15. **Akram Pezeshki et al[40]., (2019)** Encapsulation using nano lipid carriers (NLC) is an effective way of protecting sensitive nutraceutical compounds from adverse environmental condition during production and storage. The objectives of the present study were to prepare β-carotene-loaded NLC using hot-high shear homogenization (Hot-HSH) and

investigate their particle size, % encapsulation efficiency (%EE), stability and rheology. Poloxamer 407 was used as the surfactant and octyl octanoate and Precirol ATO5 were used as liquid oil and solid lipid, respectively. The optimum formulation was determined using the results of particle size obtained using different surfactant concentration (1%, 2%, 3% and 4% w/v) and solid lipid: liquid oil ratios (2:1, 4:1 and 10:1). Fourier transform infrared spectra (FTIR) were used to detect any possible bioactive-lipid complex formation and the results showed that there were no chemical interactions between β-carotene and NLC components and β-carotene loaded NLC was simply a physical mixture. The smallest particle size was observed in the formulation containing 2% Poloxamer 407 and solid lipid/liquid oil ratio of 10:1. The %EE of optimal sample was 97.7% ($p < 0.05$) and remained stable for 14 days at 25 °C. Results showed that production of β-carotene-loaded NLC gave nanoscale particles which were stable over time and the established NLC could offer a system for new functional foods based on nanocarriers.

16. **Naglakshmi Sethuraman et al[41]., (2018)** Aceclofenac is non steroidal anti inflammatory drug (NSAID) and is considered to be first line drug in treatment of rheumatoid arthritis, osteo arthritis and ankylosing spondylitis. Aceclofenac undergoes first pass metabolism when taken orally and it also produces some GI problems. The limitations of oral administration have been overcome by topical route. Drug aceclofenac has been loaded with lipid carriers and then formulated in to topical formulation with the objective of prolonging its action and avoiding its most side effects by incorporation of solid lipid carriers which is achieved by Nanostructured lipid carrier (NLC). Nanostructured lipid carrier was prepared by Ultra sonication or High Speed Homogenization method. Results and Discussion: Characterization of nanostructured lipid carrier was performed by measuring particle size, drug entrapment efficiency and in-vitro drug release. Spherical uniform particles (size below 500 nm), Drug entrapment efficiency was found to be in the range of about 75-85%. The drug release profile of all the formulations after 8 h study was found to be in the range of 40%- 78 %. Formulation showing sustained release profile at the end of the study was found to be the best formulation. Optimized formulation was converted in to Topical gel with Carbopol as gelling agent was formulated and characterized for its physical appearance, pH, viscosity, spreadability, homogeneity studies. The result concludes that aceclofenac loaded nanostructured lipid carrier could be a potential drug delivery system for topical applications.

17. **Arun sharma et al[42]., (2016)**The purpose of this research was to formulate nanostructured lipid carrier system (NLCs) in such a way that they can be applied for bacterial as well as fungal infectious diseases. Methods: To achieve the prime objective, varying concentrations of clotrimazole (CLZ) and ciprofloxacin (CIPRO) were selected for formulations. Stearic acid (solid lipid polymer), oleic acid (liquid lipid polymer), and polyvinyl alcohol (surfactant) were utilized for formulating NLCs through solvent diffusion technique. NLCs were characterized for their surface morphology, Fourier transform infrared spectroscopy (FTIR) drug-polymer interaction, particle size distribution, zeta potential, loading capacity, drug entrapment efficacy (EE), and in vitro drug release profile. Results: NLCs were fabricated with size range varying from 276 nm to 564 nm, possessing smooth spherical morphology. No drug-polymer interaction was observed through FTIR analysis. The highest drug EE for CLZ and CIPRO was found to be 78.6% and 65.8%, respectively. Formulated NLCs depict the biphasic release profile with initial burst release of 40% within 2 hrs followed by controlled release. Conclusion: Better homing of drug molecules and controlled drug release through formulated NLCs makes them suitable carrier system for various anti-microbial and anti-fungal applications.
18. **Kheradmandnia .S et al [43]., (2010)** Solid lipid nanoparticles (SLNs) have been proposed as suitable colloidal carriers for delivery of drugs with limited solubility. Ketoprofen as a model drug was incorporated into SLNs prepared from a mixture of beeswax and carnauba wax using Tween 80 and egg lecithin as emulsifiers. The characteristics of the SLNs with various lipid and surfactant composition were investigated. The mean particle size of drug-loaded SLNs decreased upon mixing with Tween 80 and egg lecithin as well as upon increasing total surfactant concentration. SLNs of 75 ± 4 nm with a polydispersity index of 0.2 ± 0.02 were obtained using 1% (vol/vol) mixed surfactant at a ratio of 60:40 Tween 80 to egg lecithin. The zeta potential of these SLNs varied in the range of -15 to -17 (mV), suggesting the presence of similar interface properties. High drug entrapment efficiency of 97% revealed the ability of SLNs to incorporate a poorly water-soluble drug such as ketoprofen. Differential scanning calorimetry thermograms and high-performance liquid chromatographic analysis indicated the stability of nanoparticles with negligible drug leakage after 45 days of storage. It was also found that nanoparticles with more beeswax content in their core exhibited faster drug release as compared with those containing more carnauba wax in their structure.

## 3.SCOPE AND PLAN OF WORK

Osteoarthritis (OA), one the most prevalent chronic joint diseases, is accompanied by considerable pain. Since pain and inflammation are among the most important causes of a decline in the life quality, the primary aim of the currently available treatments is to relieve these pain. The American College of Rheumatology has published recommendations for the use of nonpharmacologic and pharmacologic therapy in OA. The use of nonsteroidal anti-inflammatory drugs is highly recommended. The low bioavailability of oral and systemic forms of ibuprofen coupled with side effects necessitates the need to explore topical administration.

The Ibuprofen drug, usually administered in its racemic form, is a first line anti-inflammatory drug , is relatively lipophilic (log P=4.0) with low water solubility (21 mg/L at 25°C). [32]Earlier studies revealed that the topical therapeutic effectiveness of a drug is a function both of its penetration through the skin and of its potency. The aforementioned physicochemical properties of IBU have hampered the preparation of a formulation satisfying the requirements of a long-lasting treatment for a chronic disease such as OA.

Topical deliveries provide an increased bioavailability by avoiding first pass metabolism by the liver and a consistent delivery for an extended period. Topical delivery vehicles (creams, gels) can improve patient compliance due to decrease in the dosage frequency. The advantages of its local application over their systemic use include the avoidance of adverse effects and to provide relatively consistent drug levels at the application site for prolonged periods.

In turn solid lipid nanoparticles(SLN) of ibuprofen shows drug leakage after 45 days of storage. Nanostructured lipid carriers (NLCs) may serve as a solution to overcome the limitations of the SLN. NLCs can comprise physiological and biodegradable lipids, which were earlier reported to possess low systemic toxicity and low cytotoxicity[2]. The small size of the lipid nanoparticles ensures close contact between the lipid particles and the lipid bilayer of the stratum corneum, resulting in the penetration of an increased amount of drug into the skin.

The solid lipid, when used alone for preparing SLNs, forms a perfect crystal lattice with limited space for accommodating the drugs. In NLC both solid lipid and liquid lipid was included in formulation which allows more space to accommodate drug and increases the solubility of drug. So, Nano structured lipid carrier could be a great way to ensure that the compounds are delivered efficiently and effectively to the desired area of the body. The size of the carrier depends on the desired route of administration and ranges from few nanometres to the micro

meter. Nano structured lipid carriers are proved to be suitable carriers with various advantages like

(i) Controlled release of the drug
(ii) Burst release of drug
(iii) Increased drug stability
(iv) High drug loading
(v) Skin occlusion properties due to film formation
(vi) Avoidance of organic solvents and
(vii) No problems with respect to large scale production and sterilization
(viii) Encapsulation of unstable compounds can be protected and shield their functionality, protect from oxidation and degradation.
(ix) Targeted release of drug
(x) Prevents drug leakage.

**Objective:**

- The specific objective of the present work is to develop nano structured lipid carriers (NLC's) loaded with ibuprofen using high speed homogenization technique.
- To characterize the prepared NLC formulations.
- To perform in vitro diffusion study for NLC formulation using dialysis membrane method.
- To develop NLC loaded ibuprofen into gel formulation.
- To evaluate the physical properties of the gel formulations.
- To evaluate in-vitro skin diffusion study for gel formulations.

**Plan of work:**

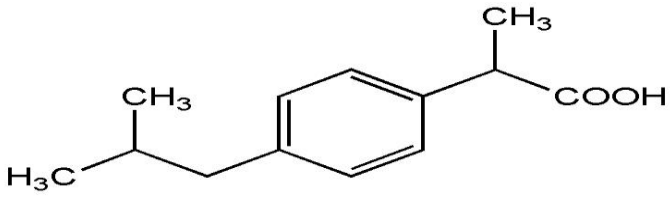
- Preformulation studies
    - Solubility studies
    - Determination of $\lambda$-max
    - Partition coefficient
    - Determination of calibration curve of drug
    - FTIR compatibility study
    - Optimization of formulation parameter using response surface methodology.
- Formulation development
    - Formulation of nano structured lipid carrier using high speed homogenization method.
    - Formulation of gel and incorporation of NLC into gel.
- Characterization studies of the Ibuprofen loaded NLCs
    - Particle size determination
    - Entrapment efficiency
    - Scanning electron microscopy
    - In vitro diffusion study
- Characterization studies of Ibuprofen loaded NLC gel
    - pH
    - Spreadability
    - Drug content
    - In vitro permeation study
    - Release kinetics

## 4.DRUG AND EXCIPIENTS PROFILE [44]

**Ibuprofen[24]**

| | | |
|---|---|---|
| Derivative | : | propionic acid derivatives |
| IUPAC name | : | 2-[4-(2-methylpropyl) phenyl] propanoacid, |
| Formula | : | C13H18O2, |
| Molecular mass | : | 206.28, |
| Melting point | : | 76 °C (1. 69 °F) |
| Bioavailability | : | 49-73, |
| Protein binding | : | 99%, |
| Metabolism | : | Hepatic, |
| Half life | : | 1.8-2 hours, |
| Appearance | : | White crystals |
| Density | : | 0.853 g/cm3 at 62 °C |
| Boiling point | : | 351-352°C (215 °C at 15 mmHg) |
| Solubility in water | : | Insoluble |
| Log p | : | 3.97 |
| Log S | : | -3.99 |

**Structure :**



Uses

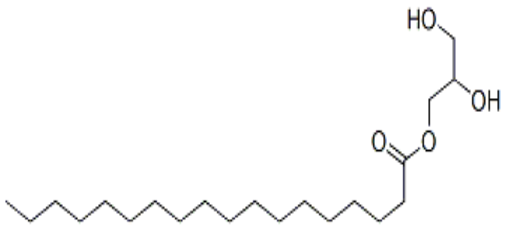
1. Ibuprofen is used as a simple analgesic and antipyretic in the same way as low dose of aspirin. It is particularly effective in dysmenorrhoea in which the action is clearly due to PG synthesis inhibition. It is available as an 'over-the-counter' drug.

2. Ibuprofen and its congeners are widely used in rheumatoid arthritis, osteoarthritis and other musculoskeletal disorders, especially where pain is more prominent than inflammation.

3. They are indicated in soft tissue injuries, fractures, vasectomy, tooth extraction, postpartum and postoperatively: suppress swelling and inflammation.

**Glyceryl monostearate:**

| | | |
|---|---|---|
| Synonyms | : | 2,3-dihydroxypropyl octa decanoate; glycerol stearate; |
| Chemical Name | : | Octadecanoic acid, monoester with 1,2,3-propanetriol |
| CAS Registry Number | : | 31566-31-1 |
| Empirical Formula | : | C21H42O4 |
| Molecular Weight | : | 358.6 |
| Melting point | : | 78-81 °C |
| Boiling point | : | 410.96°C (rough estimate) |
| Density | : | 0.9700 |
| Storage Temp. | : | -20°C |
| Refractive index | : | 1.4400 (estimate) |
| Structural Formula | : | |



Functional Category : Emollient; emulsifying agent; solubilizing agent; stabilizing agent; sustained-release ingredient; tablet and capsule lubricant.

Solubility : Soluble in hot ethanol, ether, chloroform, hot acetone, mineral oil, and fixed oils. Practically insoluble in water, but may be dispersed in water with the aid of a small amount of soap or other surfactant.

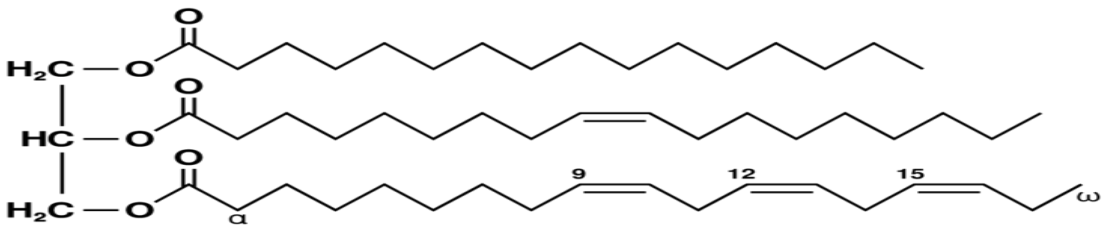
Applications in Pharmaceutical Formulation or Technology:

The many varieties of glyceryl monostearate are used as non-ionic emulsifiers, stabilizers, emollients and plasticizers in a variety of food, pharmaceutical, and cosmetic applications. It acts as an effective stabilizer, that is, as a mutual solvent for polar and non polar compounds that may form water-in-oil or oil-in-water emulsions. These properties also make it useful as a dispersing agent for pigments in oils or solids in fats or as a solvent for phospholipids, such as lecithin. Glyceryl monostearate has also been used in a novel fluidized hot-melt granulation technique for the production of granules and tablets.

**Coconut oil**

Synonyms : Virgin coconut oil, medium chain triglycerides.

Structure:




Palmitic acid 16:0 : 7.5%

C24–C30 content : <4%

Caprylic acid 8:0 : 9%

Capric acid 10 : 7%

Mean MW of triglycerides : 638

| | | |
|---|---|---|
| Melting point ℃ | : | 24 |
| Moisture (%) | : | <0.1 |
| Iodine value (cg I2/g) | : | 12-15 |
| Peroxide value (meq. O2/kg) | : | 0-1 |

Uses: Coconut oil is good for the skin, especially in the treatment of wounds, burns, and dermatitis. It also acts as sunblock, and as a moisturizer for the skin, thanks to the two primary fatty acids in unrefined coconut oil, caprylic and lauric, and to its antioxidant component, which team up to reduce inflammation under the skin and promote better healing.

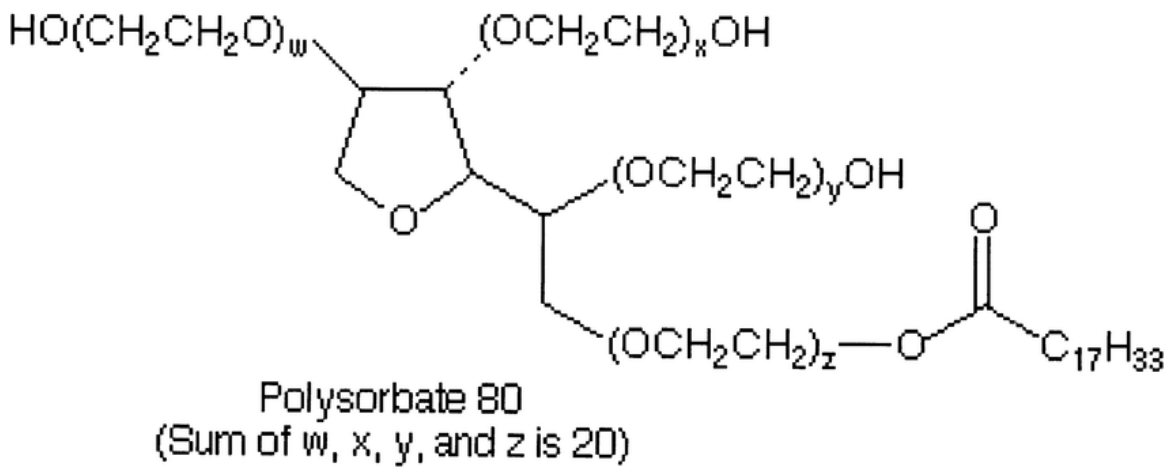
**Tween 80**

| | | |
|---|---|---|
| Chemical formula | : | $C_{64}H_{124}O_{26}$ |
| Molar mass | : | 1310 g/mol |
| Appearance | : | Amber colored viscous liquid |
| Density | : | 1.06–1.09 g/mL, oily liquid |
| Boiling point | : | > 100°C |
| Solubility in water | : | Very soluble |
| Solubility in other solvents | : | soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene[3] |
| Viscosity | : | 300–500 centistokes (@25°C |

Storage/Stability :

Aqueous solutions of polysorbates as well as the neat liquid will undergo autoxidation over time, with changes being catalyzed by light, increased temperature, and copper sulfate. Solutions are reasonably stable at 2 - 8 ℃ for short periods. For special applications, storage under argon or nitrogen may be preferred. The product is not sterile. Autoclaving of solutions is generally not advised. Sterile filtration is more easily done if the liquid is warmed to about 40 ℃ and alternate portions of hot distilled water and tween 80 are poured through the 0.22 mm filter. The tween 80 will blend and remain in solution.

Structure:




Polysorbate 80
(Sum of w, x, y, and z is 20)

Uses **:**

Polysorbate, a substance formulated by the reaction of sorbitan fatty acid ester (a nonionic surfactant with ethylene oxide), is used in many foreign countries, including the U.S. and the EU, where it acts as an emulsifier, a solubilizer in many foods, including bread, cake mix, salad dressing, shortening oil and chocolate .

Polysorbate 80 is a hydrophilic nonionic surfactant. It is utilized as a surfactant in soaps and cosmetics and also as a lubricant in eye drops. In food or pharmaceutical products, it can act as an emulsifier. Polysorbate 80 is an excipient that is used to stabilize aqueous formulations of medications for parenteral administration or vaccinations .

**Carbomer**

Synonyms : Carbopol; carboxy polymethylene, polyacrylic acid.

Chemical formula : $(C_3H_4O_2)_n$

Molar mass : variable

Log P : 0.25700

Functional Category : Bioadhesive; emulsifying agent; release-modifying agent ; suspending agent; tablet binder; viscosity-increasing agent.

Acidity : pH - 2.7–3.5 for a 0.5% w/v aqueous dispersion;

Alkalinity : pH - 2.5–3.0 for a 1% w/v aqueous dispersion.

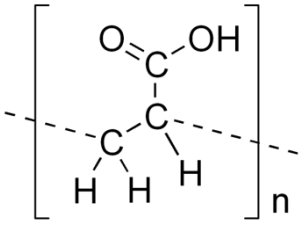
Density (bulk) : 1.76–2.08g/cm3

Density (tapped) : 1.4g/cm3

Glass transition temperature : 100–105$^0$C

Melting point : decomposition occurs within 30 minutes at 26$^0$C.

Structure




Description : Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a slight characteristic odor.

Solubility : soluble in water and, after neutralization, in ethanol (95%) and glycerine.

Stability and Storage Conditions : Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104$^0$C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Complete decomposition occurs with heating for 30 minutes at 260$^0$C.

Application

**Table 4**: Uses of carbomer and their concentration

| Use | Concentration (%) |
|---|---|
| Emulsifying agent | 0.1–0.5 |
| Gelling agent | 0.5–2.0 |
| Suspending agent | 0.5–1.0 |
| Tablet binder | 5.0–10.0 |

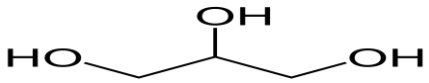
**Glycerine**

Chemical Name : Propane-1,2,3-triol

CAS Registry Number : 56-81-5

Empirical Formula : C3H8O3

Molecular Weight : 92.09

Structural Formula : 

Functional Category : Antimicrobial preservative; emollient; humectant; plasticizer; solvent; sweetening agent; tonicity agent.

Description : Glycerine is a clear, colourless, odourless, viscous, hygroscopic liquid; it has a sweet taste, approximately 0.6 times as sweet as sucrose.

Boiling point : $290^0$C (with decomposition)

Density : 1.2656g/cm3 at $15^0$C

Flash point : $176^0$C (open cup)

Melting point : $17.8^0$C

Osmolarity : 2.6% v/v aqueous solution is isosmotic with serum.

Stability and Storage Conditions : Glycerine is hygroscopic. Pure glycerine is not prone to oxidation by the atmosphere under ordinary storage conditions but it decomposes on heating, with the evolution of toxic acrolein. Mixtures of glycerine with water, ethanol (95%), and propylene glycol are chemically stable. Glycerine may crystallize if stored at low temperatures; the crystals do not melt until warmed to $20^0$C. Glycerine should be stored in an airtight container, in a cool, dry place.

Uses : Antimicrobial preservative ,Emollient , Humectant , Ophthalmic formulations , Plasticizer in tablet film coating ,Variable Solvent for parenteral formulations , Sweetening agent in alcoholic elixirs.

**Triethanolamine**

Synonyms : TEA; Tealan; triethylolamine; trihydroxytriethylamine;

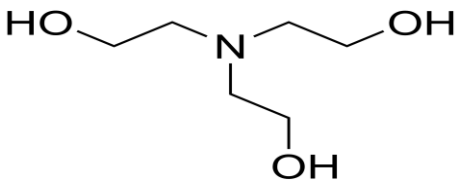
Chemical Names : 2,20,200-Nitrilotriethanol

CAS Registry Number : 102-71-6

Empirical Formula : $C_6H_{15}NO_3$

Molecular Weight : 149.19

Structural Formula




Acidity/alkalinity : pH = 10.5 (0.1N solution)

Boiling point : $335^0$C

Flash point : $208^0$C

Freezing point : $21.6^0$C

Hygroscopicity : very hygroscopic.

Melting point : $20–21^0$C

Moisture content : 0.09%

Stability and Storage Conditions : Triethanolamine may turn brown on exposure to air and light. The 85% grade of triethanolamine tends to stratify below $15^0$C; homogeneity can be restored by warming and mixing before use. Triethanolamine should be stored in an airtight container protected from light, in a cool, dry place.

Uses :

Triethanolamine is used as an intermediate in the manufacturing of surfactants, textile specialties, waxes, polishes, herbicides, petroleum demulsifiers, toilet goods, cement additives, and cutting oils. Triethanolamine is also claimed to be used for the production of lubricants for the rubber gloves and textile industries. Other general uses are as buffers, solvents, and polymer plasticizers, and as a humectant.

## 5.MATERIALS

The following materials were used from the indicated sources , without further purification .

Materials used in formulation ( Drug and excipients)

**Table : 5** List of materials used in the formulation and their manufacturer

| S.NO | INGREDIENTS | USES | MANUFACTURER |
|---|---|---|---|
| 1 | Ibuprofen | Anti-inflammatory agent | Kaushik Therapeutics |
| 2 | Glyceryl monostearate | Solid lipid | Saloni Enterprise (Ahmedabad) |
| 3 | Virgin Coconut oil | Liquid lipid | Marketing Plants and seeds Private limited (New Delhi) |
| 4 | Tween 80 | Emulsifying agent | Jain Industrial Chemical (Chennai) |
| 5 | Carbomer | Gelling agent | Chimica Pomponesco S.P.A. (Italy) |
| 6 | Glycerine | Moisturizer | Sigma Inc (Mumbai) |
| 7 | Triethanolamine | pH adjuster | Navakar Biochemical (Chennai) |

**Table 6**: List of chemicals and reagents used in evaluation studies

| SL.NO | INGREDIENTS | MANUFACTURER |
|---|---|---|
| 1 | Potassium dihydrogen phosphate | Loba Chemicals Private Limited |
| 2 | Disodium hydrogen phosphate | Fischer scientific ,Mumbai |
| 3 | Sodium chloride | Loba Chemicals Private Limited |
| 4 | Acetone | Fisher scientific, Mumbai |
| 5 | Octanol | Merck Limited, Mumbai |
| 6 | Chloroform | Thomas Baker, Mumbai |
| 7 | Methanol | Loba Chemicals Private Limited |
| 8 | Ethanol | Loba Chemicals Private Limited |

**Table 7** : List of Instrumentation used in formulation

| S.NO | INSTRUMENTS | MANUFACTURER |
|---|---|---|
| 1 | Weighing balance | Wensar Electronics-PGB200 |
| 2 | Stirrer (5000 rpm) | Remi Elektrotechnik Limited (Vasai -Mumbai) |
| 3 | U-V spectroscopy | Shimandu , Japan |
| 4 | Fourrier transform infrared spectroscopy | Avatar, 370 FT-IR, Thermo Nicolet, USA |
| 5 | Franz diffusion cell apparatus (jacketed) | Orchid Scientific , Nashik |
| 6 | Laboratory cenrifuges | Remi Elektrotechnik Limited (Vasai-Mumbai) Model - C854/4 |
| 7 | pH meter | Digisun Electronics - Hyderabad |

## 6.METHODS

**Preformulation studies :**

**1. Solubility :**

The solubility of ibuprofen drug can be identified by dissolving the small amount of drug to fixed volume of solvents such as acetone, water, chloroform, methanol, ethanol, phosphate buffer (pH 7.4) and shake vigorously until a clear solution formed and examined visually for undissolved solute particles .

**2. λ-max**

**Preparation of standard stock solution (1000 mcg/ml)**

Accurately weighed quantity of 100 mg of ibuprofen was transferred into 100 ml volumetric flask which was dissolved and diluted up to the mark with Phosphate buffer solution (pH 7.4). The solution was sonicated on bath sonicator to get a clear standard stock solution (1000 mcg/ml).

**Determination of λ-max:** The standard stock solution of ibuprofen having the concentration 1000 mcg/ml was further diluted to 100 mcg/ml with Phosphate buffer solution -pH 7.4 (buffer). The absorbance of resultant solution was scanned in U-V Spectrophotometer ranging from 200-400 nm.

**3. Partition coefficient**

The water phase was adjusted to pH 7.4 with phosphate buffer and saturated with octanol . The octanol phase was saturated with phosphate buffer both phases were left for separation for 2 hours. The drug were weighed and dissolved in separating funnel containing octanol (50 ml) and phosphate buffer (50 ml). The phases were shaken and left for separation for 2 hours. A solution of 1 ml of phosphate buffer and 1 ml of octanol phase was transferred into a 1.5 ml of vial and analysed using UV-Visible Spectrophotometer at 264 nm.

**4. Calibration curve**

The stock solution was prepared by taking 10 mg of ibuprofen in 100 ml of PH 7.4 phosphate buffer. The drug was dissolved in a solvent by using sonicator. Four different dilutions were prepared from stock solution having concentration as 4 mcg/ml, 6 mcg/ml ,8 mcg/ml, 12 mcg/ml and 16 mcg/ml respectively. These prepared dilutions were then analysed by UV-Visible Spectrophotometer (Shimadzu, Japan) at 264 nm.

## 5. Drug excipient compatibility (FTIR)

In order to identify possible interactions that may be occur during production of nano structured lipid carrier, the IR spectrum of nano structured lipid carrier and pure ingredients was measured by Fourier Transform Infrared (FTIR) spectrometer (Avatar, 370 FT-IR, Thermo Nicolet, USA) using KBr pellets technique at room temperature. In this technique, samples were mixed with KBr and compressed to form a thin pellet that was used for testing. The measurements were recorded in the frequency range of 4000–400 $cm^{-1}$.

## 6. Optimization - experimental design

Box-behnken statistical design with 3 factors, 3 levels and 17 runs was employed for the optimization study using design-expert software version 11.0 The variables stirring speed, lipid concentration and drug concentration were main factors that impacted the particle size, entrapment efficiency and drug release of NLCs in our preliminary experiments. To investigate the impact of stirring speed (A) , lipid concentration (B) and drug concentration (C) were selected as independent variables and they were set at high , medium and low level on the basis of the results of initial trials. In accordance with the design , 17 NLC formulations were prepared and characterized for particle size (R1) , entrapment efficiency (R2) and drug release (R3) . As such, we conducted preliminary experiments with various experimental ranges. From these results, we adjusted the values of the range for each factor in our study. This design explicates the Main effects, interaction effects and quadratic effects of the independent variables on the formulation characteristics.

**Table 8:** Independent variables and their constraints of Ibuprofen-loaded NLCs prepared for Box-Behnken design response surface methodology.

| **Variables** (Independent variables) | **Constraints** | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| A-Stirring speed (rpm) | 5000 | 7500 | 10000 |
| B-Lipid concentration (%) | 70 | 80 | 90 |
| C-Drug concentration (%) | 2.5 | 5 | 7.5 |
| **Response** (dependent variables) | **Goal** | | |
| R1-Particle size (nm) | Minimize | | |
| R2-Entrapment efficiency (%) | Maximize | | |
| R3-Drug release (%) | Minimize | | |

**IBU-NLC preparation:**

The IBU-NLC was prepared by using high speed homogenization method (**table 9)** . Firstly, the lipid phase were form by mixing GMS-**glyceryl mono stearate** (solid lipid) and virgin coconut oil (**Unrefined coconut oil or pure coconut oil**) at $75^0$C. The lipid phase mixture were melted to form a uniform and clear mixture of lipid. As lipid mixture was completely melted, Ibuprofen were added to the mixture. On the other hand, distilled water, tween 80 were thoroughly mixed to form an aqueous phase. The aqueous mixture was then heated to $75^0$C and subsequently added to lipid mixture to form a pre-emulsion mixture. Both aqueous phase and lipid phase should be maintained at same temperature ($75^0$C). A pre-emulsion obtained was prone to homogenization using an (**Remi motor RQ 134H**), at 5000-7000 rpm for 10 minutes to prevent the crystallization of lipids. The O/W emulsion obtained was subsequently cooled down to room temperature with continuous stirring, and the lipid was recrystallized to form NLC. The obtained NLC dispersions were used for further studies.

**Table 9.** NLC encapsulates Ibuprofen: preparation matrix

| Formulations | Amount of Ibuprofen (%w/w) | Amount of lipid | | Amount of tween 80 (%w/w) | Distilled water (%w/w) | Stirring speed (Rpm) |
|---|---|---|---|---|---|---|
| | | GMS (%w/w) | Coconut oil (%w/w) | | | |
| F1 | 7.5 | 65 | 15 | 10 | 2.5 | 7000 |
| F2 | 7.5 | 60 | 20 | 10 | 2.5 | 7000 |
| F3 | 7.5 | 55 | 25 | 10 | 2.5 | 7000 |
| F4 | 7.5 | 50 | 30 | 10 | 2.5 | 7000 |
| F5 | 7.5 | 45 | 35 | 10 | 2.5 | 7000 |
| F6 | 7.5 | 40 | 40 | 10 | 2.5 | 7000 |

An IBU suspension containing 7.5% of IBU dispersed in purified water was prepared as a reference for comparative in vitro diffusion studies of IBU-NLC

**Preparation of ibuprofen loaded NLC gel:**

Carbopol was selected as the gelling agent. The composition of gel formulation are mentioned in **Table 10**. The required amount of Carbopol was dispersed in the water and allowed to hydrate for 4 to 5 hour. Glycerine (10% w/w) used as humectants was added subsequently to the aqueous dispersion using a mechanical stirrer (Remi, Mumbai, India) at a speed of 1200 rpm for 1 hour. The dispersion was neutralized using triethanolamine. The gel was allowed to stand overnight to remove entrapped air.

**Table 10:** Composition of NLC based gel formulations

| **Sl .no** | **Ingredients (%w/w)** | **Formulation** |
|---|---|---|
| | | **IBU- NLC gel (%w/w)** |
| 1 | Carbopol 940P | 1 |
| 2 | Glycerine | 10 |
| 3 | Triethanolamine | 0.5 |
| 4 | Distilled water | 88.5 |
| 5 | Methyl paraben | 0.002 |

The gel was formulated as given in above table 10 . IBU-NLC (equivalent to ibuprofen 5%w/w) was dispersed into the gel. All NLC formulations were formulated as gel in the same composition as given in **table 10** and evaluated.

**Characterization of NLC**:

**1. Particle size and zeta potential**

The mean particle size and zeta potential of the IBU-loaded NLC formulations were determined using Malvern® Zetasizer Nano ZS90 (Malvern® Instruments Limited, Worcestershire, UK). All the measurements were made in triplicate after dilution (1:200) with distilled water at room temperature using 90° scattering angle[45].

## 2. Scanning electron microscopy

The shape and surface characteristics of NLCs were determined by SEM using gold sputter technique (ZEISS EV40, Carl Zeiss NTS, North America). Samples of NLC were dusted onto a double-sided tape on an aluminum stub. The stubs containing the sample were coated with gold using a cool sputter coater (Polaran E 5100) to a thickness of 400 $A^0$. Photomicrographs were taken at the accelerated voltage of 20 kV and chamber pressure of 0.6 mmHg[46].

## 3. Drug encapsulation efficiency

Drug encapsulation efficiency (EE) was determined through indirect method where an aliquot of Ibuprofen-loaded NLCs (1gm) was dissolved in methanol and centrifuged at 10,000 g for 2 h at 4 °C . The proportion of unencapsulated ibuprofen in the clear supernatant fluid was measured spectrophotometrically at 264 nm against blank. Calibration curve for the validated UV assay of Ibuprofen was performed on six solutions in the concentration ranges of 4–16 μg/ml. Correlation coefficient was >0.999. Each point represents the mean of three measurements and standard deviation (±SD) was calculated. The encapsulation efficiency of Ibuprofen was then calculated according to the following equation

$$\mathbf{EE\% = (Da\text{-}Df / Da)x\ 100}$$

where EE%= the percentage of encapsulation efficiency, Da = the amount of added drug during preparation of NLCs and Df = the amount of free drug in the clear supernatant fluid after centrifugation[45].

## 4. In vitro drug diffusion study

In vitro release studies were performed using a modified Franz diffusion cell. Dialysis membrane having pore size 2.4 nm and molecular weight cut-off between 12,000 and 14,000 was used. The membrane was soaked in double distilled water for 12 h before mounting in a Franz diffusion cell. About 0.5 g of NLC formulation was applied to the donor compartment, and the receptor compartment was filled with phosphate buffer, pH 7.4 (5 mL). During the experiments, the solution in the receptor side was maintained at 37 ± 0.5 °C and stirred at 100 rpm with Teflon-coated magnetic stirring bars . At various time intervals such as 1,2,3,4,5,6,7,8,9,10,11,12 hour , 1 mL of the sample was withdrawn from the receiver compartment through a side tube and analyzed spectrophotometrically at 264 nm[47].

**Characterization of NLC dispersed into gel:**

**1. pH**

The pH of the gel was determined by using a digital pH meter Model EQ 610, standardized using pH 4.0 and 7.0 standard buffers before use. One gram of gel was dispersed in 100 mL of distilled water and immersed into the electrode.

**2. Drug Content**

About 1.5g of the gel was weighed in a 100 mL volumetric flask and dissolved in 50 mL of phosphate buffer of pH 7.4. The volumetric flask was kept for 2 h and shaken well in a shaker to mix it properly. It was diluted appropriately and analyzed in a UV spectrophotometer at 264 nm[47].

**3. Spreadability[48]**

Two sets of glass slides of standard dimensions were taken. The gel formulation (1.5g) was placed over one of the slides. The other slide was placed on the top of the gel, 100gm weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. A 20 gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time taken for calculation.

Spreadability was calculated by using the following formula:

$$S=M \times L/T$$

Where S is spreadability,

M is weight tied on upper slide(20g).

L is the length of glass slide(6 cm),

t is time taken.

**4. In vitro diffusion[49]**

2.4 $cm^2$ area of the excised rat skin samples was clamped between the receptor and the donor chamber of a Franz diffusion cell with the stratum corneum facing the donor chamber. Then, 1.5 g of the IBU-NLC gel was placed into the donor chamber. The receptor chamber was filled with 5 mL of phosphate-buffered saline (PBS), pH 7.4. The receptor medium was maintained at 37°C±0.5°C and stirred at 100 rpm throughout the experiment. Subsequently, 1 mL of the sample was collected from the receptor medium every hour for 12 hours and then immediately replaced by the same amount of phosphate buffer solution. The collected samples were filtered through a filter paper and analyzed using UV spectrophotometrically at 264 nm.

**5. Release kinetics:**

In order to investigate the mode of drug diffusion from the NLC formulations, the in vitro permeation data were analyzed using the following mathematical models:

Zero order equation…….$Q = K_0t$

First order equation……..$In\ (100- Q) = InQ-K_1t$

Korsmeyer and Peppas equation …….. $Q = Kp\ tn$

Hixson crowell release equation ……$Q_0^{1/3}-Q_t^{1/3}= K_{hc}\ t$

Where Q, is the percent of the drug release at time t and $K_0$ and Kt are the constants of the equations. Kp is the constant incorporating structural and geometric characteristic of the release device, Ks is a constant incorporating the surface volume relation and n is the release exponent indicative of mechanism of release. $K_{hc}$ as Hixson crowell release constant.

Fickian diffusion when $n<0.5$,

Non-Fickian transport when $0.45<n<0.89$,

Case II transport when $n=0.89$, and

Super case II transport when $n>0.89$.

## 7.RESULTS & DISCUSSION

**Results for preformulation studies**:

### 1. Solubility studies:

The solubility shows that the drug was lipophilic in nature and the drug was freely soluble in organic solvents and soluble in phosphate buffer (7.4).

**Table 11: Result of solubility study**

| Sl no | Solvents | Results |
|---|---|---|
| 1 | Acetone | +++ |
| 2 | Phosphate buffer solution | +++ |
| 3 | Water | -- |
| 4 | Chloroform | +++ |
| 5 | Methanol | +++ |
| 6 | Ethanol | +++ |

Insoluble = --

Freely soluble = +++

### 2. *λ*-max

The ***λ*-max** value was found as 264 nm when scanned in U-V Spectrophotometer in the range of 200-400 nm.

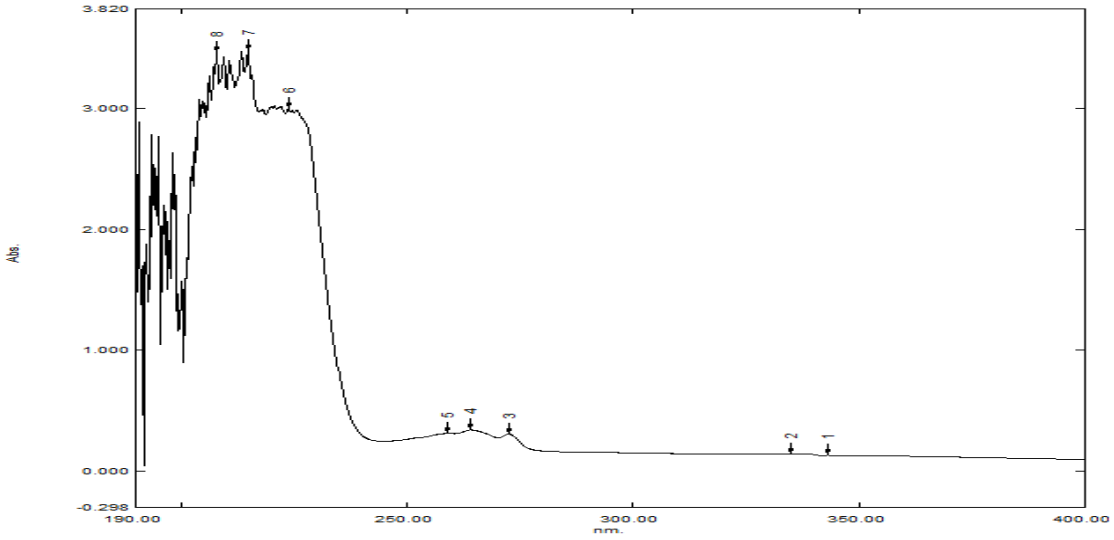



**Figure 3**: *λ*-max curve of Ibuprofen.

### 3. Partition coefficient :

The log P value was found as 3.227

The log S value was found as -3.287 when calculated using yalkowsky and banerjee equation.

$$\textbf{Log S= 0.8-Log } P_{o/w} \textbf{-0.01(MP-25)}$$

Where,

S-solubility

Log $P_{o/w}$ -octanol/water partition coefficient

MP-melting point ($76^0$C)

The log p and log S value for ibuprofen drug was found to be 3.227 and -3.287 . A positive value for log P denotes a higher concentration in the lipid phase (i.e., the compound is more lipophilic). The negative value for log S indicates poor solubility in polar phase.

### 4. Calibration curve

The calibration curve of drug obeyed Beer Lambert's law in the concentration range of 4-16 µg/ml ($R^2$ = 0.999) and result shown in figure 4.

**Table 12 :** Calibration curve of Ibuprofen

| **Concentration (mcg)** | **Absorbance (nm)** |
|---|---|
| 4 | 0.045 |
| 6 | 0.058 |
| 8 | 0.071 |
| 12 | 0.098 |
| 16 | 0.125 |

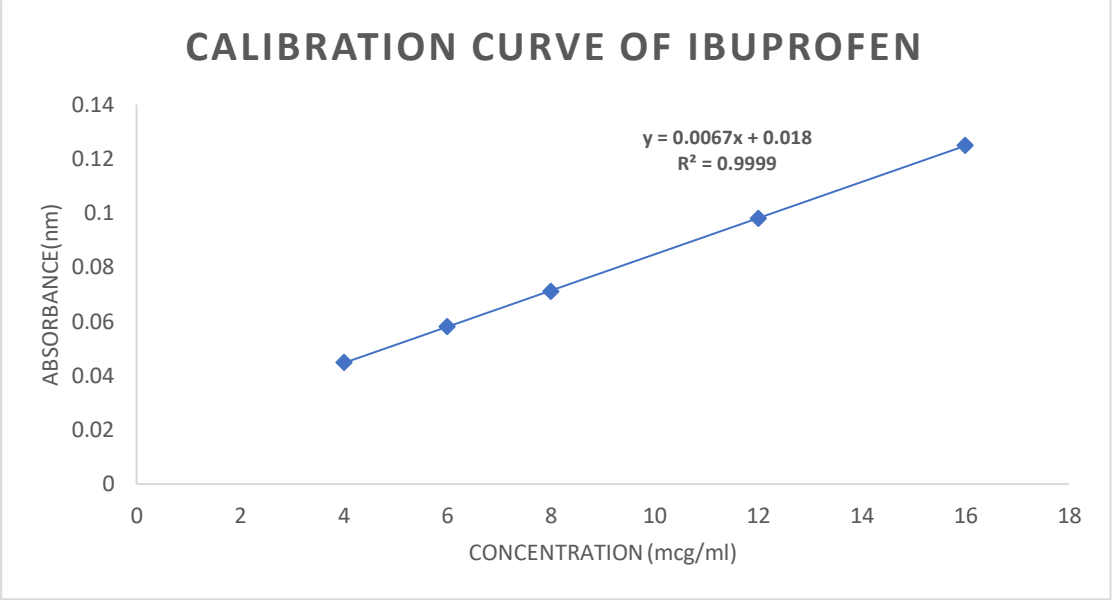



**Figure 4:** Calibration curve of Ibuprofen

## 5. Compatibility study by FTIR

The FTIR spectra of pure ibuprofen ,solid lipid (glyceryl monostearate) and the NLC formulation shown in Fig. 5, 6, 7 respectively. The IR spectra of the pure drug show principle peaks at 1721.21 cm-1 (C= O stretching Vibrations of - COOH group), 866.21, 779.58 cm-1 (Aromatic stretching bending vibration). The formulation shows peaks at 1740.49 and 2955.96. Likewise, the solid lipid shows peak at 2955.89 and 1739.62. Thus it concluded that the physical mixture of the drug ibuprofen does not show any major interactions with formulation components like solid lipid (glyceryl monostearate).

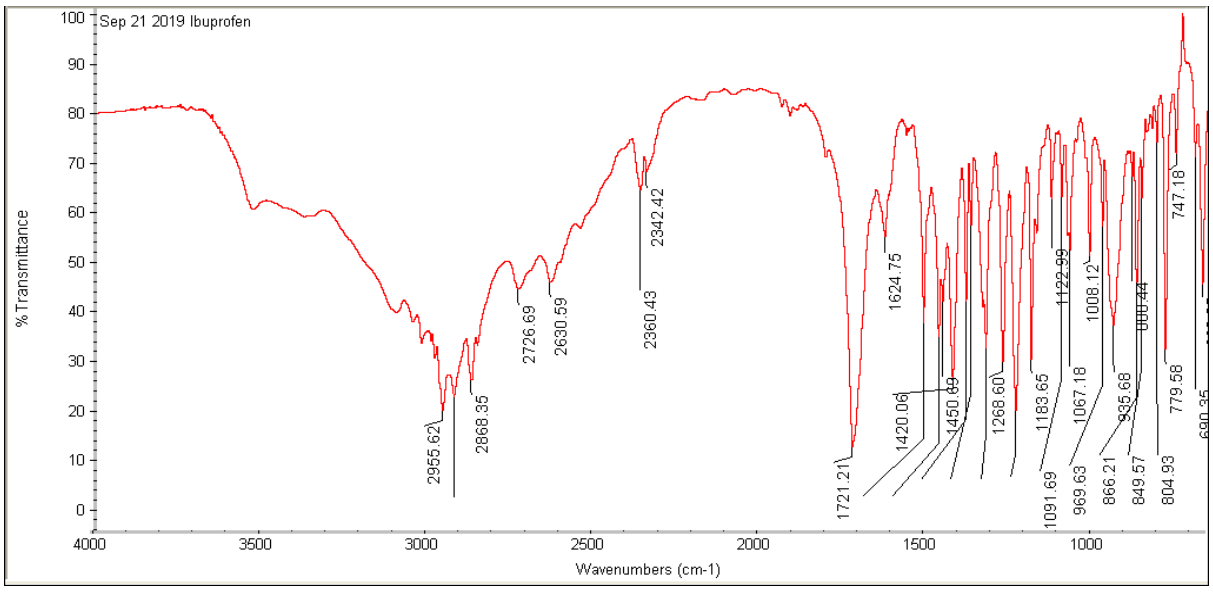


**Figure 5:** FTIR graph of pure drug (Ibuprofen)

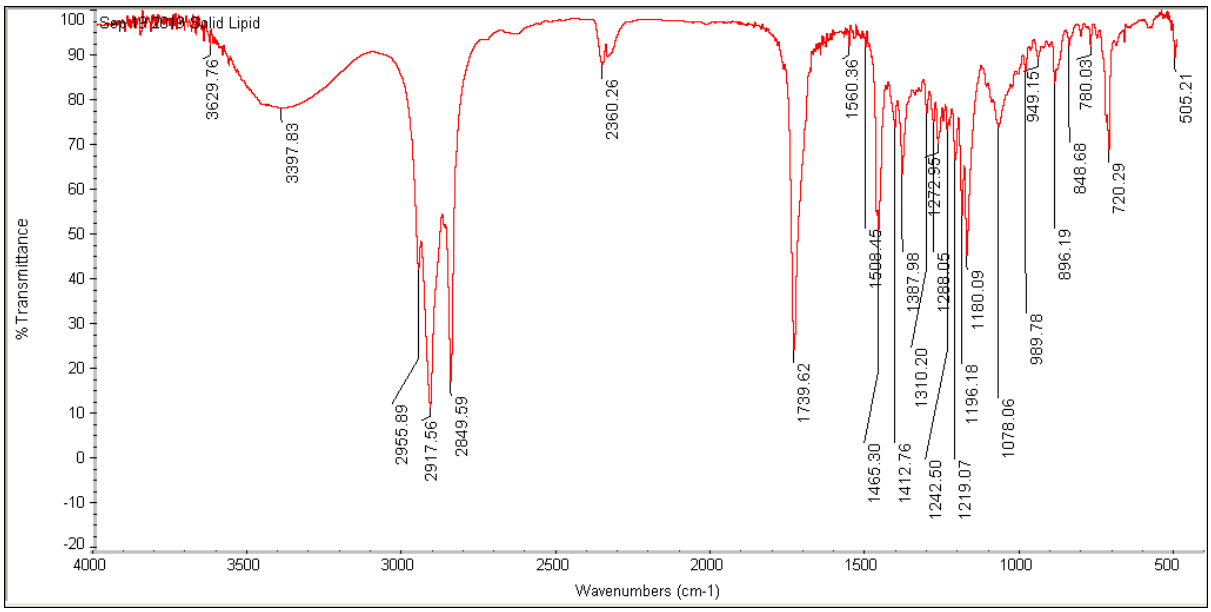



**Figure 6:**FTIR graph of solid lipid (glyceryl monostearate)

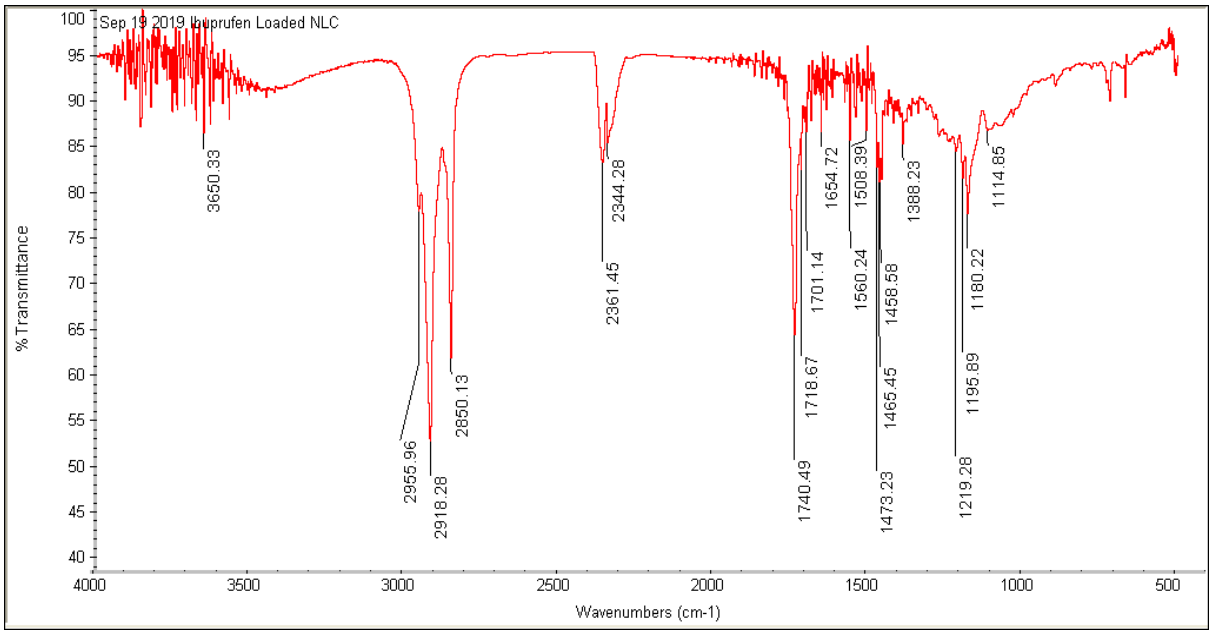



**Figure 7:**FTIR graph of ibuprofen loaded NLC (F1)

## 6. Results of optimization study :

On the basis of defined constraints for each independent variable, the design expert software version 11 automatically generated the optimized formula. The experiments performed and the responses obtained are detailed in **Table 13**. It was observed that the best fitted model for all

the three dependent variables was the quadratic model with coefficient of correlation ($R^2$) nearly equal to 1.The quadratic mathematical model for three independent factors is provided in below equation:

$$\mathbf{Y= \beta_0 + \beta_1X1+ \beta_2X2+ \beta_3X3+ \beta_{12}X1X2+ \beta_{23}X2X3+ \beta_{13}X1X3+ \beta_{11}X1^2+ \beta_{22}X2^2+ \beta_{33}X3^2}$$

where Y is the response to be modelled, β is the regression coefficient, and X1, X2 and X3 represent factors A, B, and C, respectively. **Figure 8 ,9, 10** illustrates the response surface plots for particle size, entrapment efficiency and drug release. The analysis of perturbation plots and response plots of the optimisation models revealed that stirring speed influenced all three responses of particle size, entrapment efficiency and drug release. The investigated procedure was well explained through the perturbation plots presented in **Figure 11 , 12, 13**. Particle size was highly influenced by stirring speed, and entrapment efficiency was influenced by stirring speed, followed by lipid concentration , and then drug concentration. **Table 19** demonstrates that the experimental values were very close to the predicted values for NLCs prepared under optimal assay conditions for the ibuprofen NLC formulation.

**Table 13 :** Three level three-factorial Box-Behnken experimental design

| **Run** | **Factor 1** | **Factor 2** | **Factor 3** | **Response 1** | **Response 2** | **Response 3** |
|---|---|---|---|---|---|---|
| | **A:Stirring Speed Rpm** | **B:Lipid %** | **C:Drug %** | **Particle Size Nm** | **Entrapment Efficiency %** | **Drug Release ($Q_{6h}$) %** |
| 1 | 10000 | 80 | 2.5 | 150 | 81.4 | 53 |
| 2 | 7500 | 80 | 5 | 210 | 48.5 | 50 |
| 3 | 7500 | 80 | 5 | 230 | 49.51 | 50 |
| 4 | 5000 | 80 | 2.5 | 300 | 76.44 | 32 |
| 5 | 7500 | 90 | 7.5 | 195 | 69.9 | 41 |
| 6 | 7500 | 80 | 5 | 200 | 42.66 | 39 |
| 7 | 7500 | 90 | 2.5 | 185 | 72 | 30 |
| 8 | 5000 | 80 | 7.5 | 260 | 72.56 | 29 |
| 9 | 10000 | 80 | 7.5 | 185 | 92.5 | 65 |
| 10 | 7500 | 70 | 7.5 | 198 | 65.82 | 50 |
| 11 | 7500 | 80 | 5 | 174 | 47.21 | 48 |
| 12 | 5000 | 90 | 5 | 310 | 79.21 | 18 |
| 13 | 10000 | 90 | 5 | 185 | 90.1 | 43 |
| 14 | 10000 | 70 | 5 | 167 | 75.32 | 61 |
| 15 | 7500 | 80 | 5 | 167 | 47.52 | 56 |
| 16 | 5000 | 70 | 5 | 290 | 69.32 | 25 |
| 17 | 7500 | 70 | 2.5 | 170 | 46.52 | 59 |

**Response surface methodology:**

The figure 8 ,9 and 10 shows the three-dimensional surface plots indicating the effects of the interactions between three different parameters on the particle size, entrapment efficiency and drug release of nano structured lipid carrier .

**Particle size:**

The figure 8A,8B and 8C depicts the three dimensional (3D) response plots showing the impact of three variables on the response- Particle size

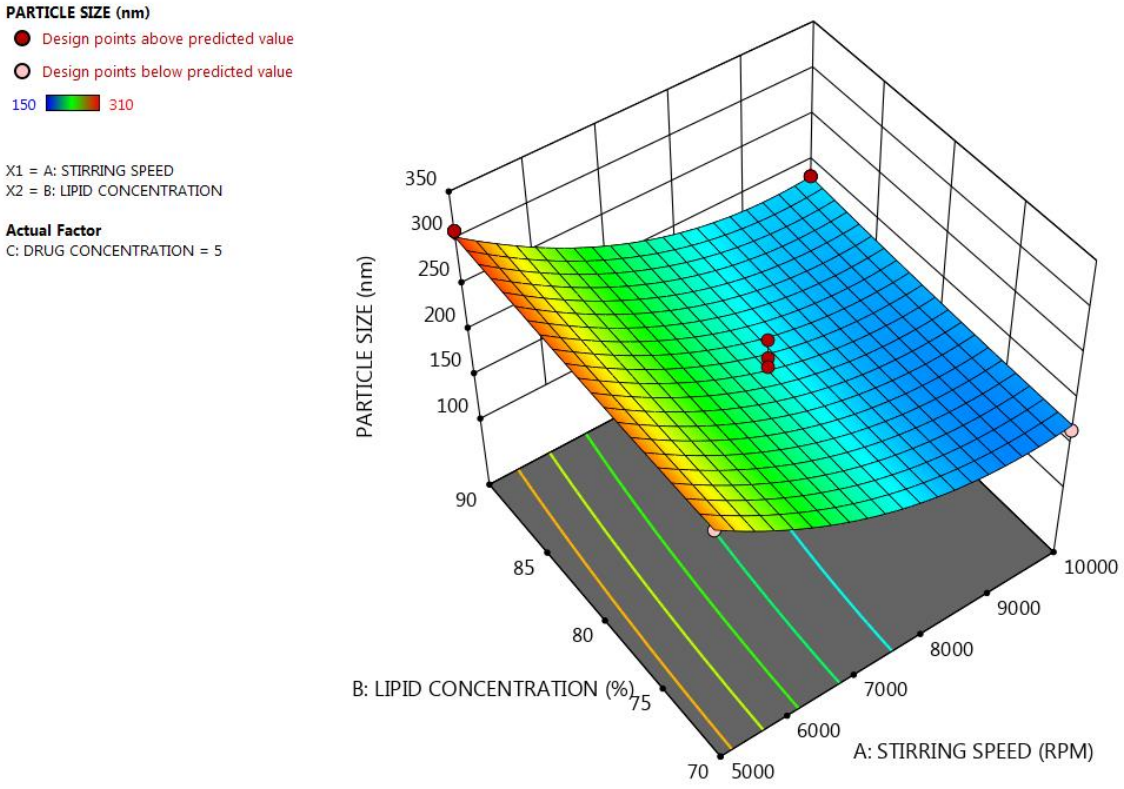



**Figure 8A**

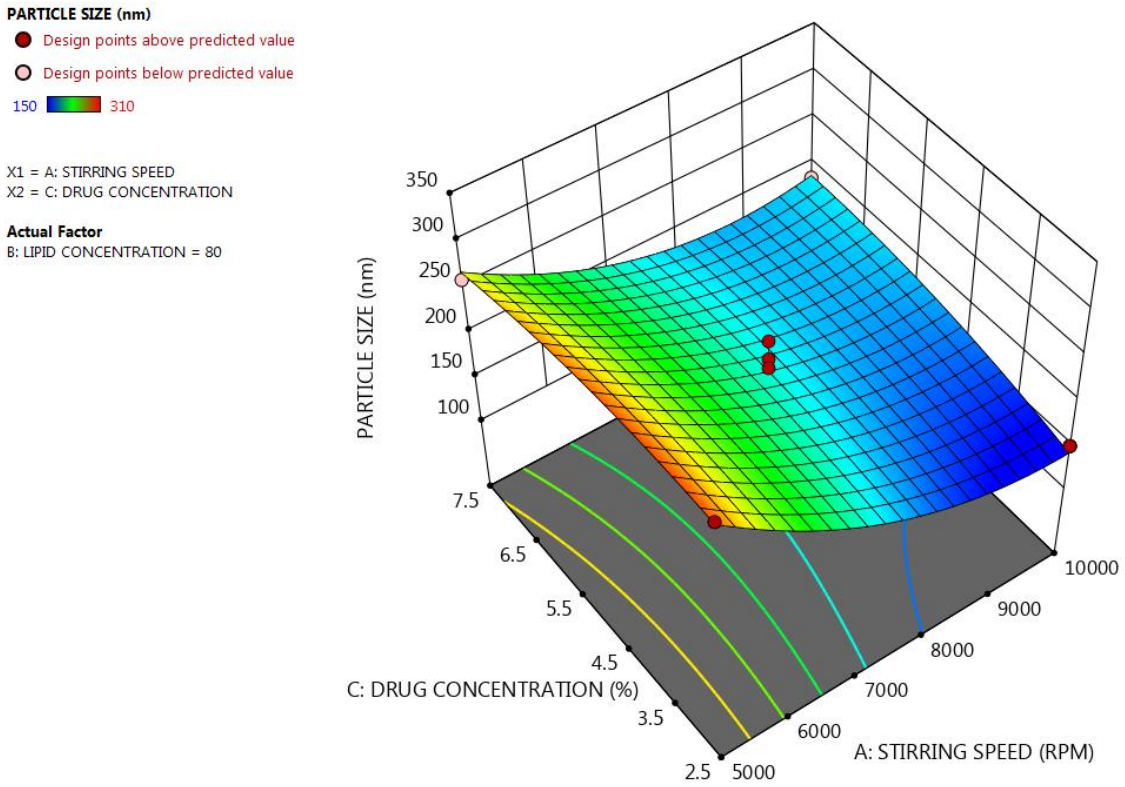



**Figure 8B**

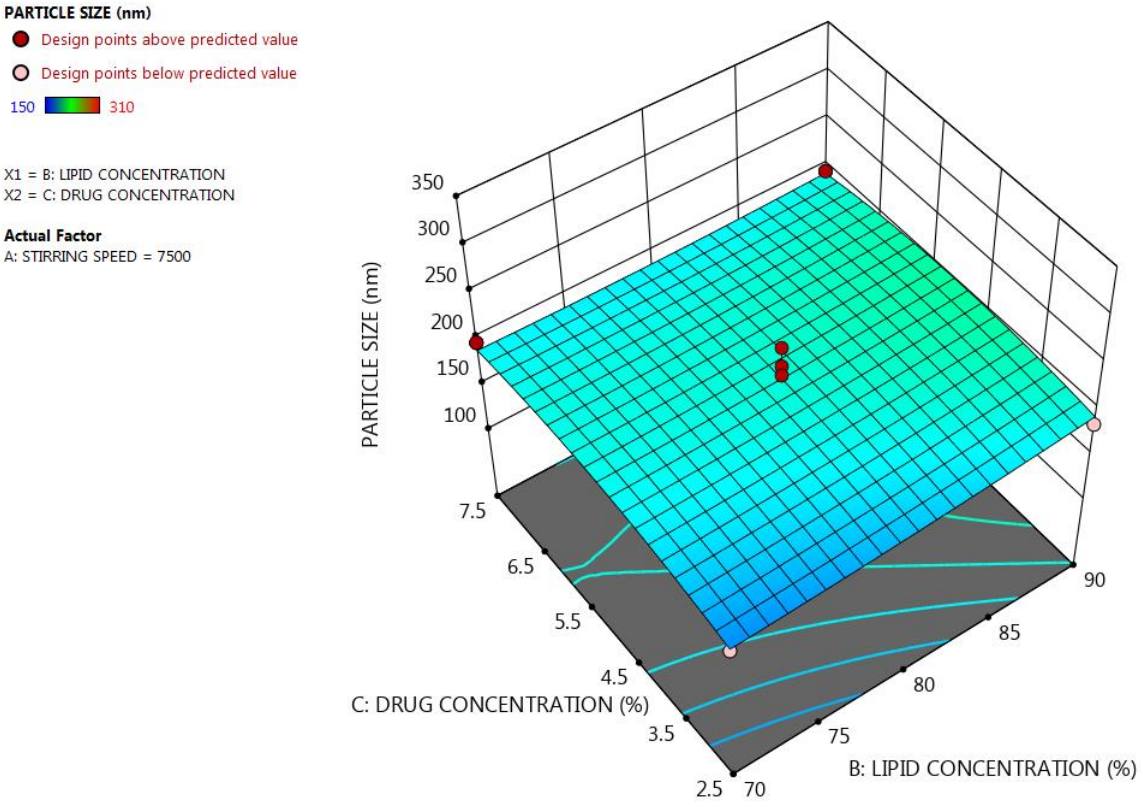



**Figure 8C**

Figure 8A,8B and 8C shows that the particle size value decreased toward the higher value of stirring speed where maximum values in particle size could be seen (red color area); however, the lowest particle size values of nanostructured lipid were observed at higher stirring speed. This is due to a sufficient amount of stirring speed available to emulsify the oil and the aqueous phase and to break the coarse emulsion into small particles. The plotted model depicted a linear increase in particle size when the stirring speed was increased. It was observed that on increasing the lipid concentration, the size of the NLCs increased. This effect could be attributed to the fact that during NLCs formulation, by increasing the solid content, the dispersion viscosity also increases that result into higher surface tension and thus higher particle size. This effect was also promoted by increasing the concentration of surfactant in the formulation, which is required to stabilize the higher % of solid lipid . This result could be attributed to the accumulation of excess surfactant molecules on the NLCs surface probably due to a hydrophobic interaction, in which nonpolar groups such as alkyl chains of the surfactant and solid lipid molecules could interact with each other. The effects of factors on particle size could be further justified by the perturbation plot shown in the figure 11,12,13.

**Entrapment efficiency:**

The figure 9A,9B and 9C depicts three dimensional (3D) response plots showing the impact of three variables on the response-Entrapment efficiency

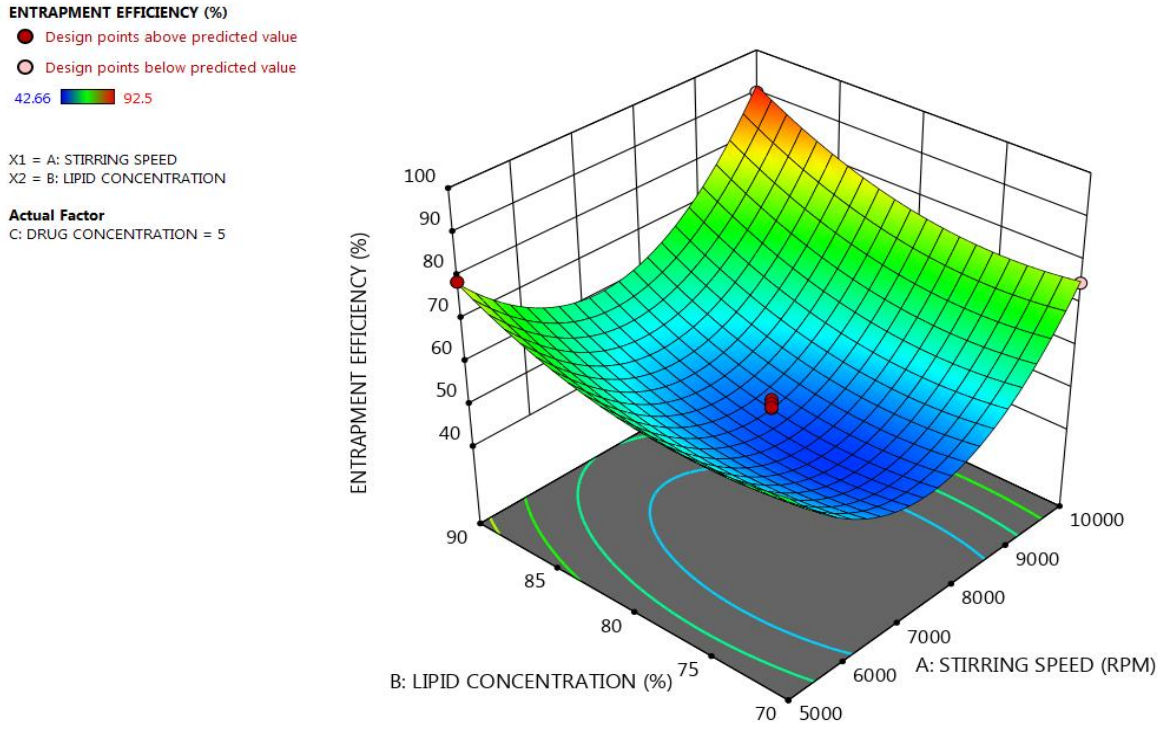



**Figure 9A**

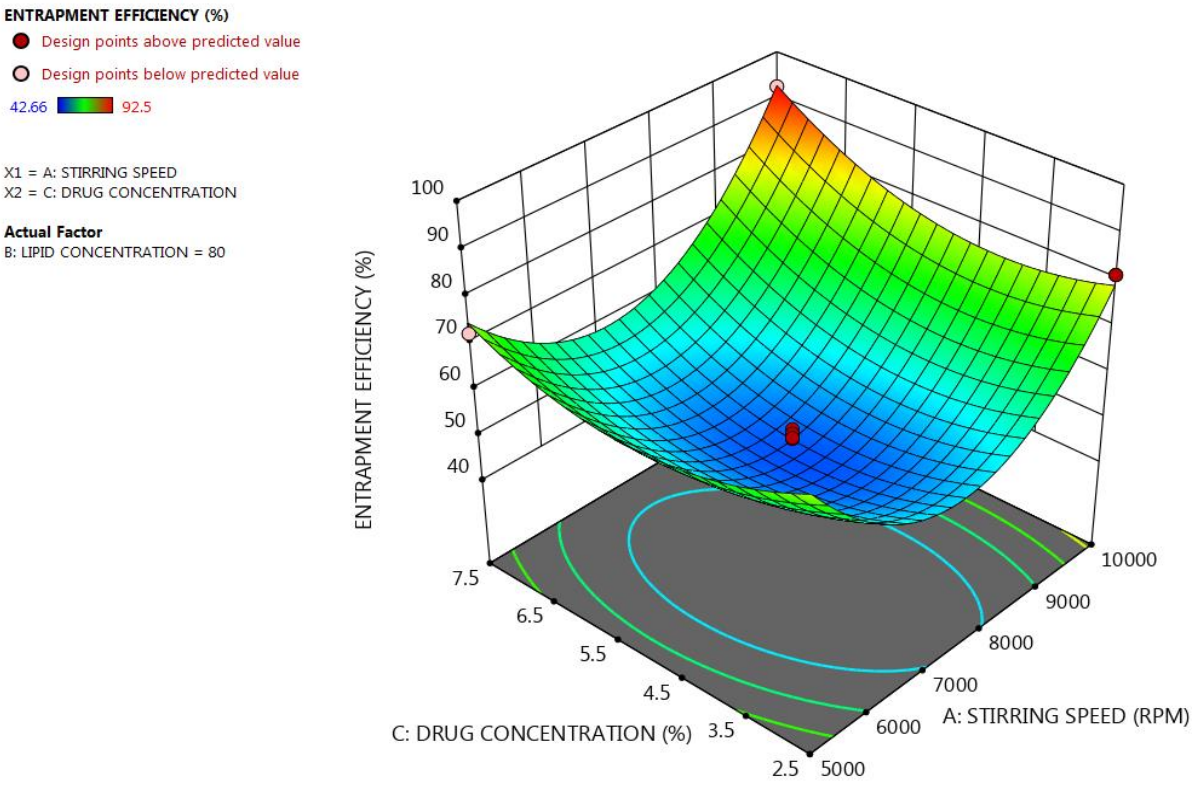



**Figure 9B**

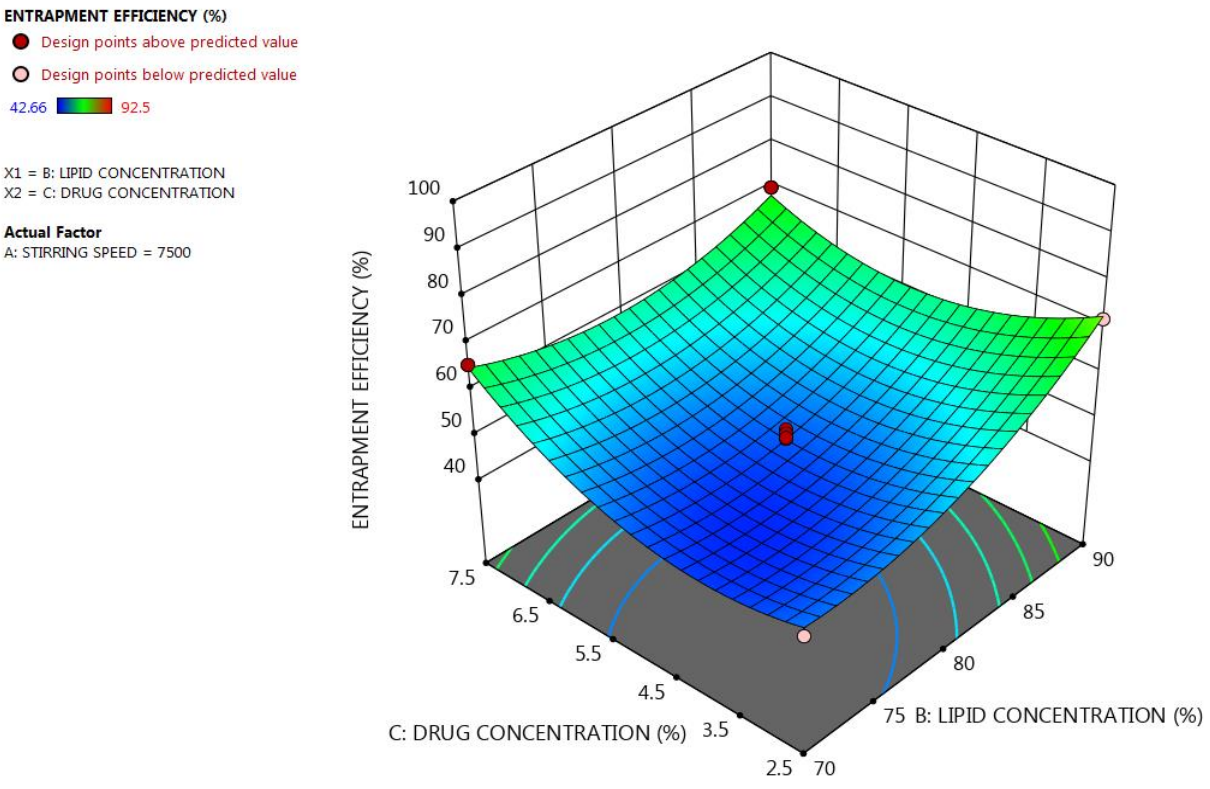



**Figure 9C**

Figure 9A ,9B and 9C shows response surface curve of entrapment efficiency the value increased toward the higher amount of lipid concentration where maximum values in entrapment efficiency could be seen (red colour area); however, the lowest entrapment efficiency values of nanostructured lipid were observed at lower lipid concentration. This is due to a sufficient amount of lipid concentration available to entrap the drug particle. The plotted model depicted a linear increase in entrapment value when the lipid concentration was increased.

**Drug release:**

The figure 10A , 10B and 10C depicts the three dimensional (3D) response plots showing the impact of three variables on the response-Drug release

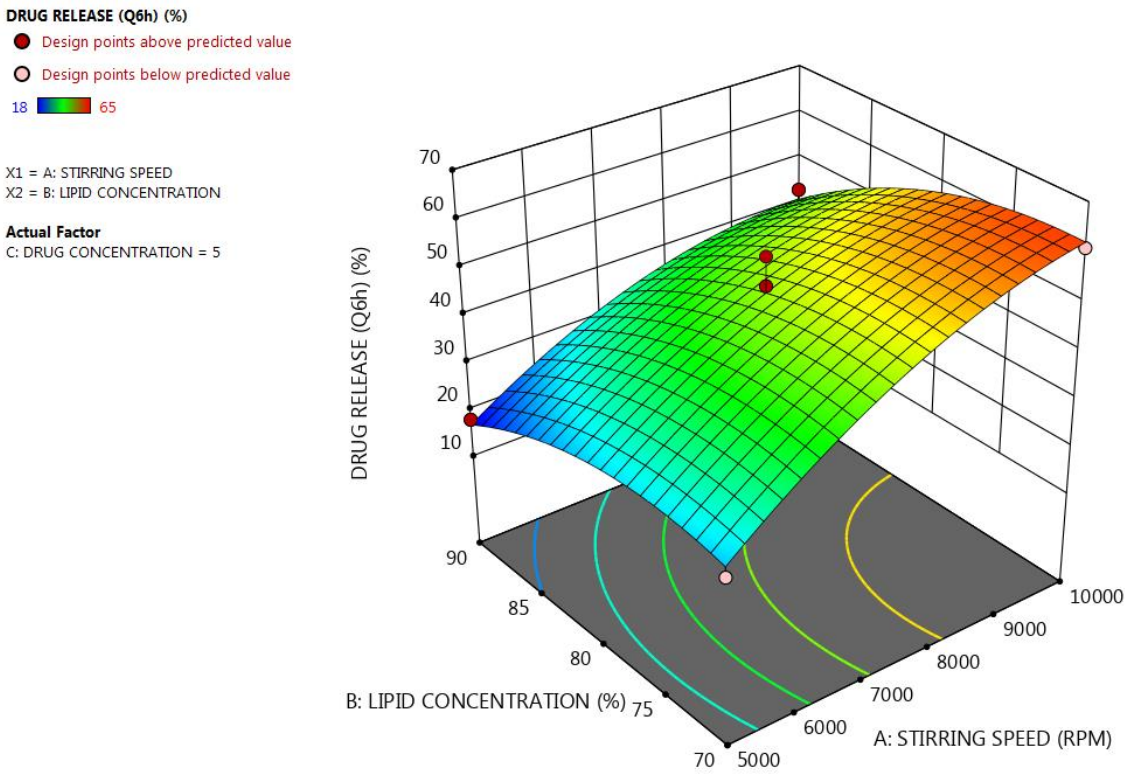



**Figure 10A**

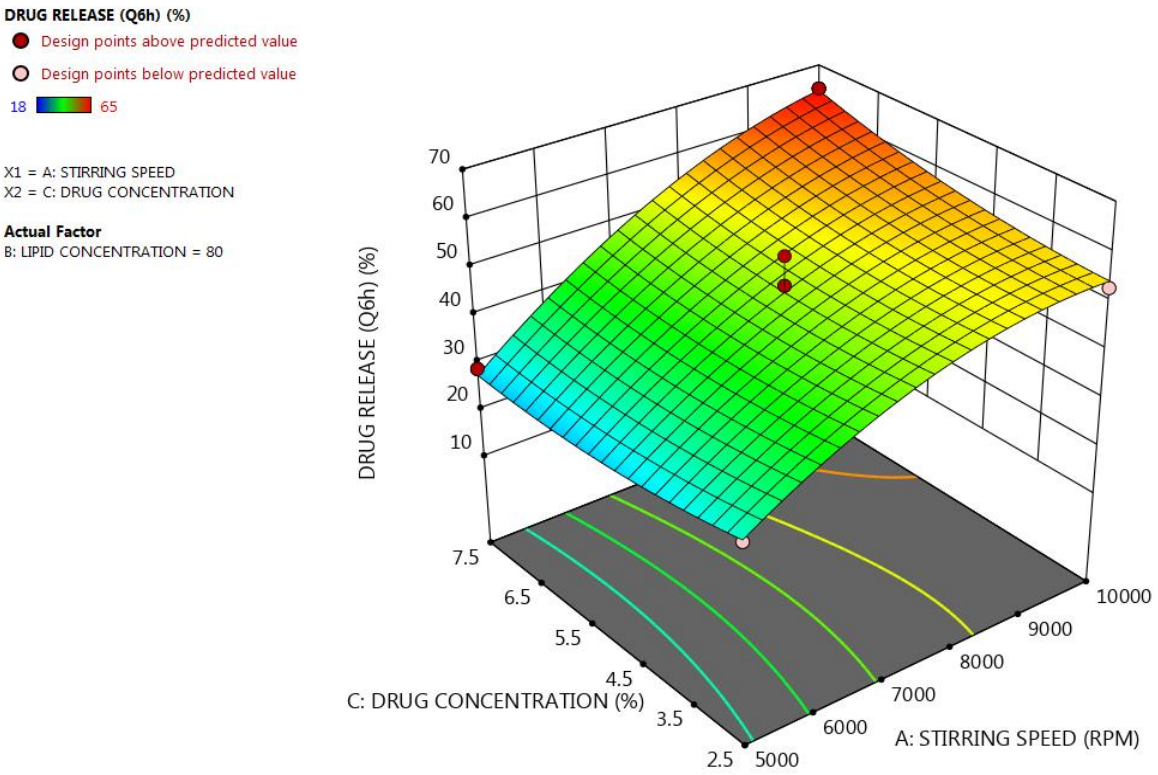



**Figure 10B**

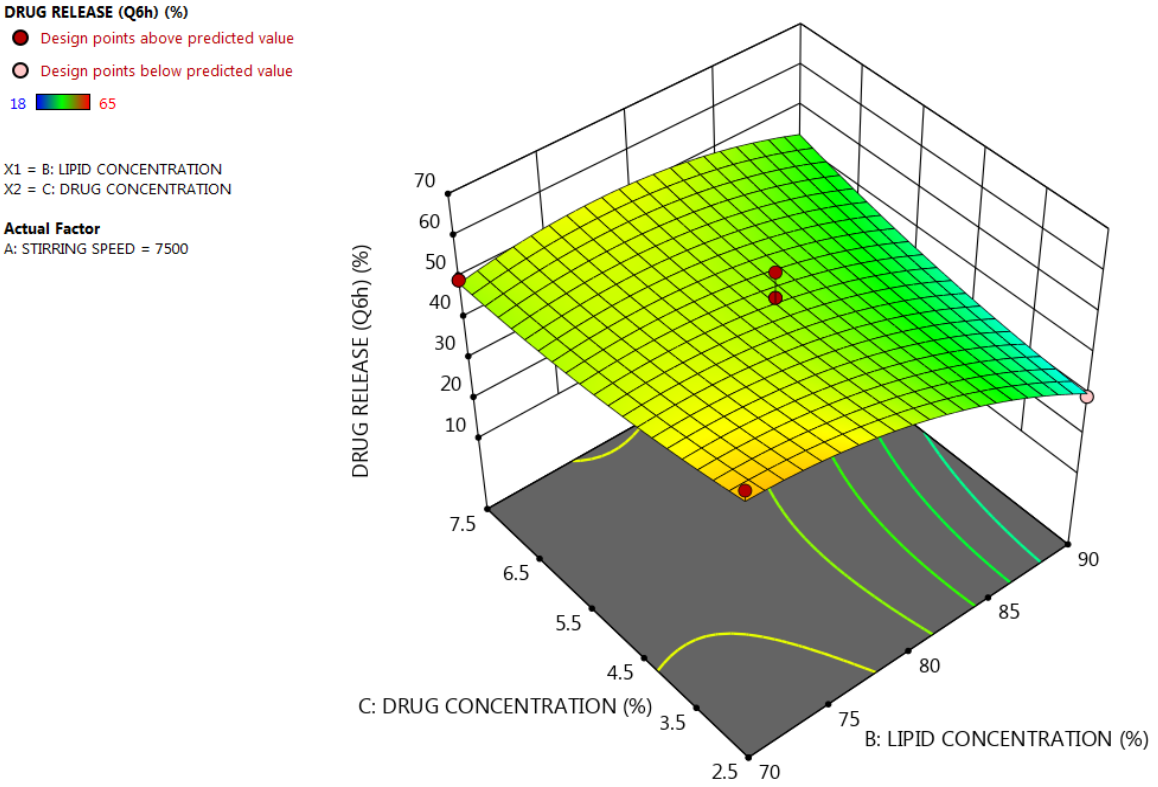



**Figure 10C**

Figure 10A, 10B and 10C shows response surface curve of drug release the value increased toward the higher amount of drug concentration where maximum values in drug release could be seen (red color area); however, the lowest drug release values of nanostructured lipid were observed at lower drug concentration and high lipid concentration. The more lipid encapsulation will increases the particle size and retards the drug release. This is due to initial burst release of drug followed by controlled release. The drug present in the outer core follows burst release. The controlled release was due to more lipid concentration.

Perturbation plots showing the impact of each of the independent variables on particle size, entrapment efficiency and drug release (figure 11,12,13). These plots were used to study the interaction effects of two independent variables on the responses while holding the third factor at a constant level. Moreover, the perturbation plots for each responses were plotted that helped to compare the effects of all three factors at any particular point in the design space. The responses were plotted by changing only one factor in its constrained range while keeping other two factors constant. A steep slope or curvature in a factor shows that the response is sensitive to that factor.

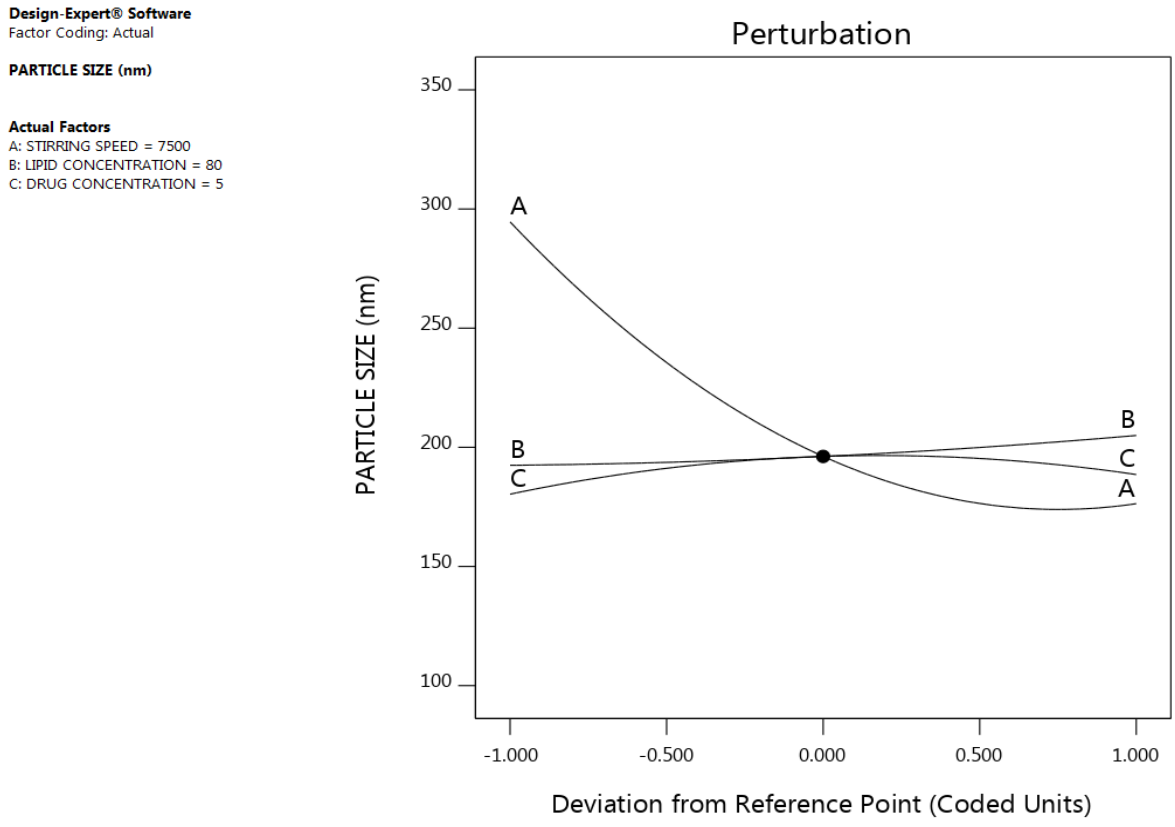



**Figure 11:** Perturbation plots on particle size

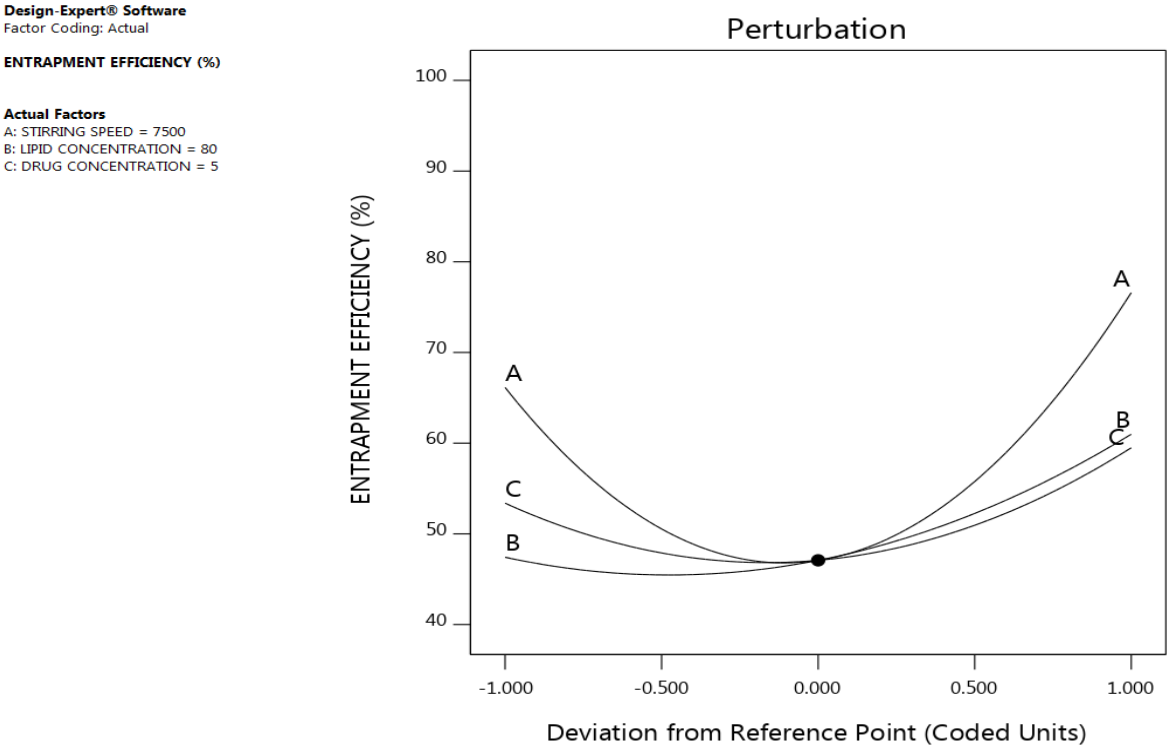



**Figure 12:** Perturbation plots on entrapment efficiency

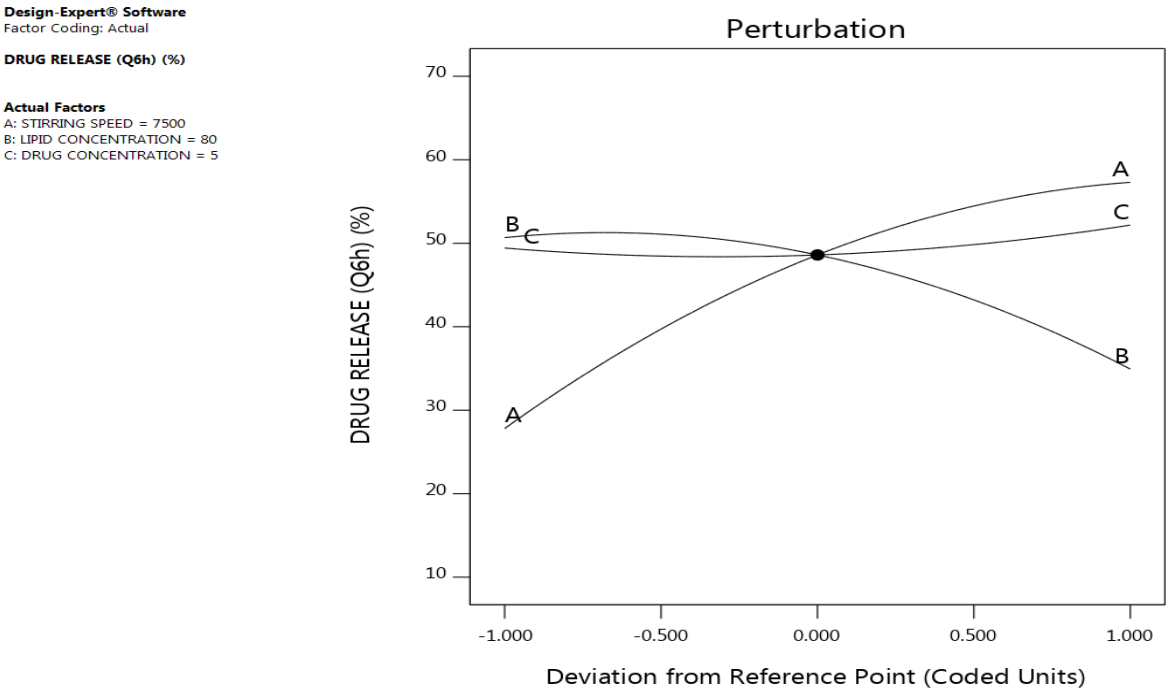



**Figure 13:** Perturbation plots on drug release

**Table 14 :** Response models and statistical parameters obtained from ANOVA for box-behnken design

| **Responses** | **Adjusted $R^2$** | **Predicted $R^2$** | **Model P values** | **Adequate precision** | **% CV** | **Model F values** |
|---|---|---|---|---|---|---|
| Particle size (Y1) | 0.8240 | 0.7417 | <0.0038 | 10.0863 | 9.96 | 9.32 |
| Entrapment efficiency(Y2) | 0.9721 | 0.9001 | <0.0001 | 22.0314 | 4.08 | 62.99 |
| Drug release (Y3) | 0.8598 | 0.7605 | <0.0018 | 12.2622 | 11.52 | 11.90 |

**Table 15 :** Regression model equations.

| Regression model | |
|---|---|
| Particle size (Y1) | +196.20-59.125*A+6.25 *B+4.125*C-0.5*AB +18.75*AC-4.5*BC+39.275*$A^2$+2.525*$B^2$-11.725*$C^2$ |
| Entrapment efficiency(Y2) | +47.08+5.22375*A+6.7787*B+3.0525*C+1.22*AB+3.475*AC-5.35*BC+ 24.286*$A^2$+7.121*$B^2$+9.3582.89*$C^2$ |
| Drug release (Y3) | +48.6+14.75*A-7.875*B+1.375*C-2.75*AB+3.75*AC+5*BC-6.05*$A^2$-5.8*$B^2$+2.2*$C^2$ |

The response surface analysis plots in three-dimensional model graphs were constructed using the design expert software version 11. The equation obtained for responses Y1, Y2 and Y3 are shown in the **table 15** . The effect of independent variables on particle size could be quantified by the quadratic equation. The positive values before a factor in the above regression equation indicate that the response increases with the factor and vice versa. As the lipid portion was

increased relative to drug, more amount of drug could be entrapped into the lipid matrix and hence entrapment efficiency also increased. Moreover, increasing amount of liquid lipids lead to increased solubility of drugs and hence, entrapment efficiency increased.

Table 14 represents the statistical parameters, such as adjusted $R^2$ , model p value, adequate precision, and % CV obtained from ANOVA for the reduced models. Based on **table 14** , the response particle size, entrapment efficiency and drug release was well fitted to the quadratic model with P-value of <0.0001. According to the values of linear terms in table 14 it can be said that all the linear mixture components (A, B and C) were effective on the response according to their coefficients and P-value of mixture. A response was investigated regarding outliers, and it was found that all points were placed in a normal distribution. Table 14 shows that the "adjusted $R^2$" for Y1, Y2 and Y3 is in reasonable agreement with the "predicted $R^2$". "Adequate precision" measures the signal-to-noise ratio. A ratio >4 is desirable. The ratio of 10.0863 for Y1, 22.0314 for Y2 and 12.2622 for Y3 indicates an adequate signal. This model can be used to navigate the design space. The results show that 90% of the response variations in the particle size, entrapment efficiency and drug release could be described by box-behnken design model as a function of the main composition. So it can be concluded that the quadratic model was a suitable model for analysis and could show the trends very good, the interaction between the parameters was more effective on particle size, entrapment efficiency and drug release of nanostructured lipid carrier.

**1.Particle size**

Predicted and actual values of particle size of nanostructured lipid carrier obtained from Box-Behnken experimental design.

**Table 16:** Predicted and actual value for particle size

| Run Order | Actual Value | Predicted Value | Residual |
|---|---|---|---|
| 1 | 150.00 | 141.75 | 8.25 |
| 2 | 210.00 | 196.20 | 13.80 |
| 3 | 230.00 | 196.20 | 33.80 |
| 4 | 300.00 | 297.50 | 2.50 |
| 5 | 195.00 | 192.88 | 2.12 |
| 6 | 200.00 | 196.20 | 3.80 |
| 7 | 185.00 | 193.63 | -8.63 |
| 8 | 260.00 | 268.25 | -8.25 |
| 9 | 185.00 | 187.50 | -2.50 |
| 10 | 198.00 | 189.38 | 8.62 |
| 11 | 174.00 | 196.20 | -22.20 |
| 12 | 310.00 | 303.88 | 6.12 |
| 13 | 185.00 | 184.63 | 0.3750 |
| 14 | 167.00 | 173.13 | -6.13 |
| 15 | 167.00 | 196.20 | -29.20 |
| 16 | 290.00 | 290.38 | -0.3750 |
| 17 | 170.00 | 172.13 | -2.13 |

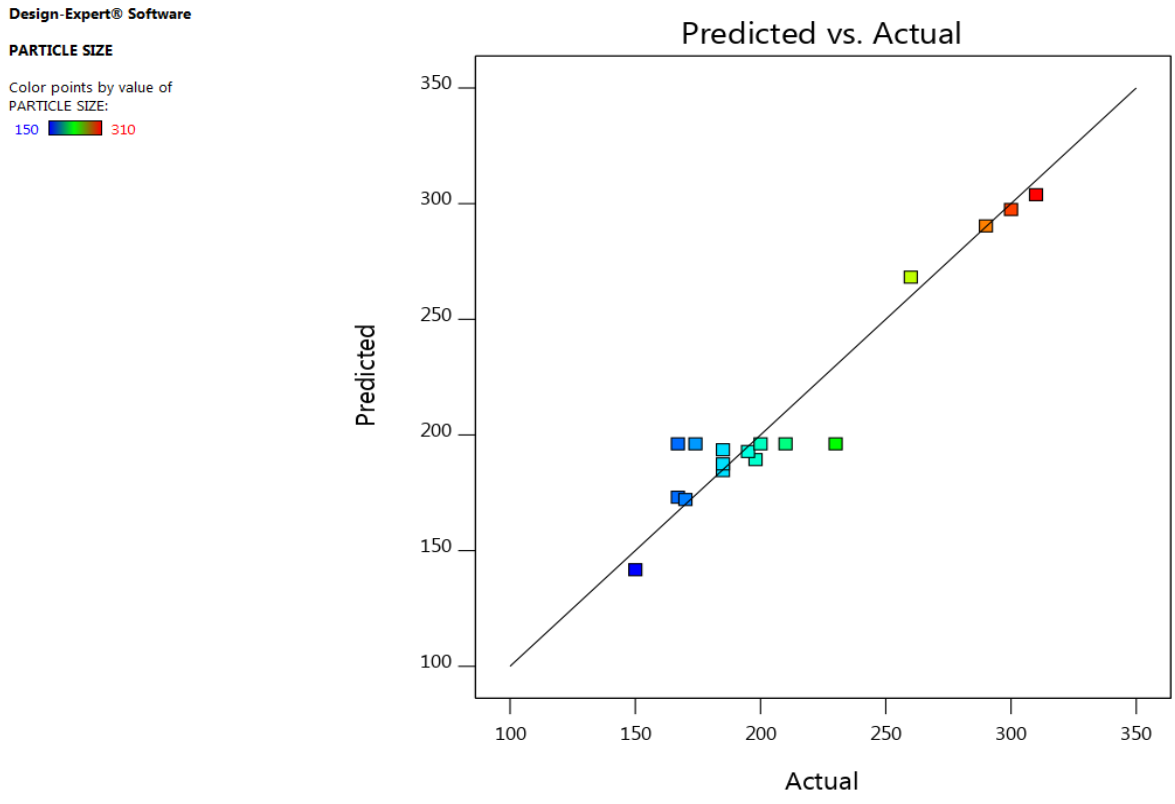



**Figure 14**: Predicted vs Actual plot for particle size

2.**Entrapment efficiency:**

Predicted and actual values of entrapment efficiency of nanostructured lipid carrier obtained from Box-Behnken experimental design.

**Table 17:** Predicted and actual value for entrapment efficiency

| Run Order | Actual Value | Predicted Value | Residual |
|---|---|---|---|
| 1 | 81.40 | 79.15 | 2.25 |
| 2 | 48.50 | 47.08 | 1.42 |
| 3 | 49.51 | 47.08 | 2.43 |
| 4 | 76.44 | 76.19 | 0.2463 |
| 5 | 69.90 | 68.04 | 1.86 |
| 6 | 42.66 | 47.08 | -4.42 |
| 7 | 72.00 | 72.64 | -0.6362 |
| 8 | 72.56 | 74.81 | -2.25 |
| 9 | 92.50 | 92.75 | -0.2463 |
| 10 | 65.82 | 65.18 | 0.6363 |
| 11 | 47.21 | 47.08 | 0.1300 |
| 12 | 79.21 | 78.82 | 0.3900 |
| 13 | 90.10 | 91.71 | -1.61 |
| 14 | 75.32 | 75.71 | -0.3900 |
| 15 | 47.52 | 47.08 | 0.4400 |
| 16 | 69.32 | 67.71 | 1.61 |
| 17 | 46.52 | 48.38 | -1.86 |

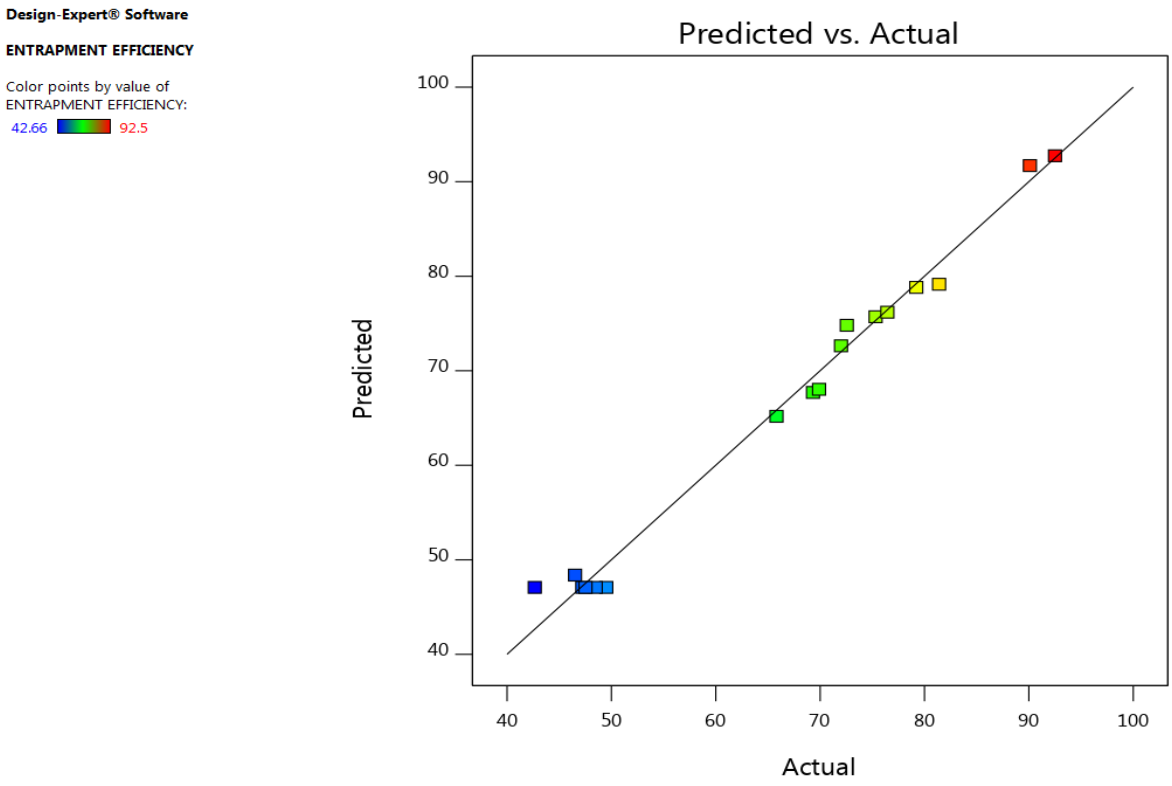



**Figure 15**: Predicted vs Actual plot for entrapment efficiency

### 3.Drug release

Predicted and actual values of drug release of nanostructured lipid carrier obtained from Box-Behnken experimental design.

**Table 18:**Predicted and actual value for drug release

| Run Order | Actual Value | Predicted Value | Residual |
|---|---|---|---|
| 1 | 53.00 | 54.38 | -1.38 |
| 2 | 50.00 | 48.60 | 1.40 |
| 3 | 50.00 | 48.60 | 1.40 |
| 4 | 32.00 | 32.38 | -0.3750 |
| 5 | 41.00 | 43.50 | -2.50 |
| 6 | 39.00 | 48.60 | -9.60 |
| 7 | 30.00 | 30.75 | -0.7500 |
| 8 | 29.00 | 27.63 | 1.37 |
| 9 | 65.00 | 64.62 | 0.3750 |
| 10 | 50.00 | 49.25 | 0.7500 |
| 11 | 48.00 | 48.60 | -0.6000 |
| 12 | 18.00 | 16.88 | 1.12 |
| 13 | 43.00 | 40.88 | 2.13 |
| 14 | 61.00 | 62.12 | -1.12 |
| 15 | 56.00 | 48.60 | 7.40 |
| 16 | 25.00 | 27.13 | -2.13 |
| 17 | 59.00 | 56.50 | 2.50 |

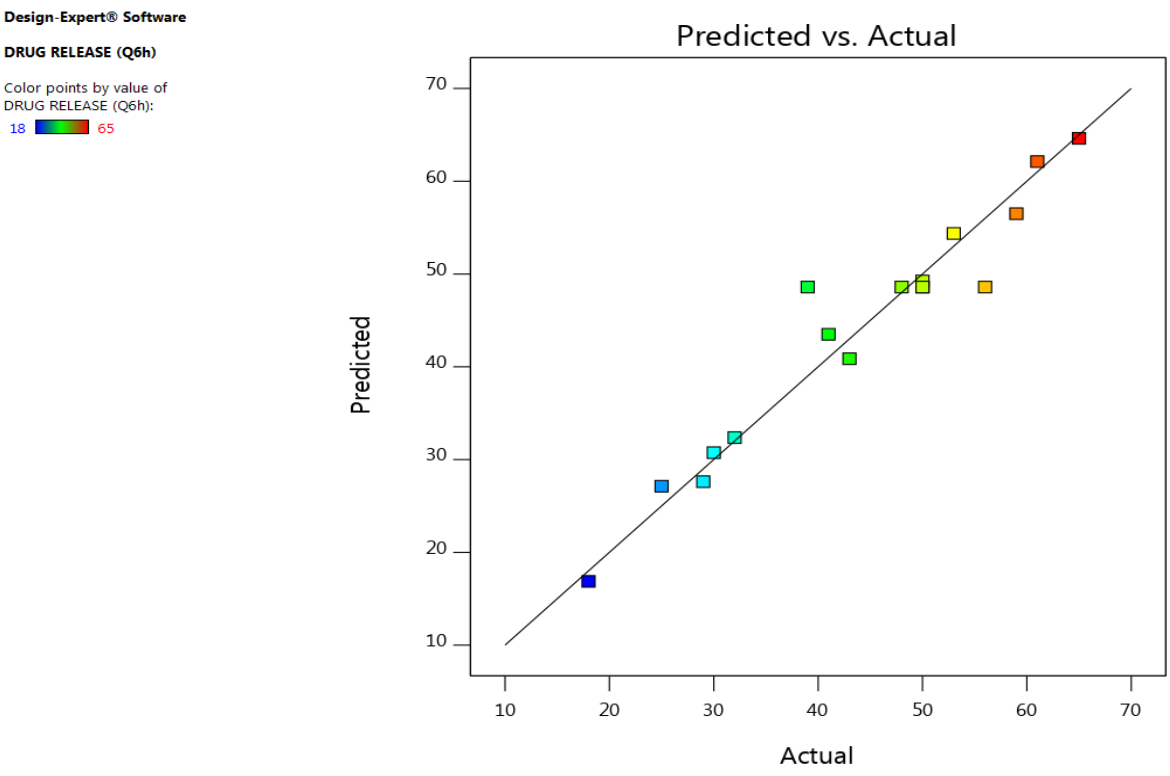



**Figure 16**: Predicted vs Actual plot for drug release

**Optimization:**

**Table 19 :** Experimental and predicted values under optimal assay conditions for ibuprofen – loaded NLC formulation

| | **Particle size (nm)** | **Entrapment efficiency(%)** | **Drug release Q6h(%)** |
|---|---|---|---|
| Predicted | 183.92±3.65 | 88.906±1.22 | 65.23±2.6 |
| Experimental | 193.6±1.52 | 89±2.1 | 68.32±2.8 |
| % error | 5.26 | 0.105 | 4.737 |

**%error = (observed value – predicted value)/predicted value × 100**

The NLCs was formulated and responses were measured. The observed value of responses were compared to the predicted values and % error was calculated **[Table 19]** to validate the method. The observed value of Y1, Y2 and Y3 were in a very close agreement to the predicted ones. By this the validity of the optimization procedure was proven.

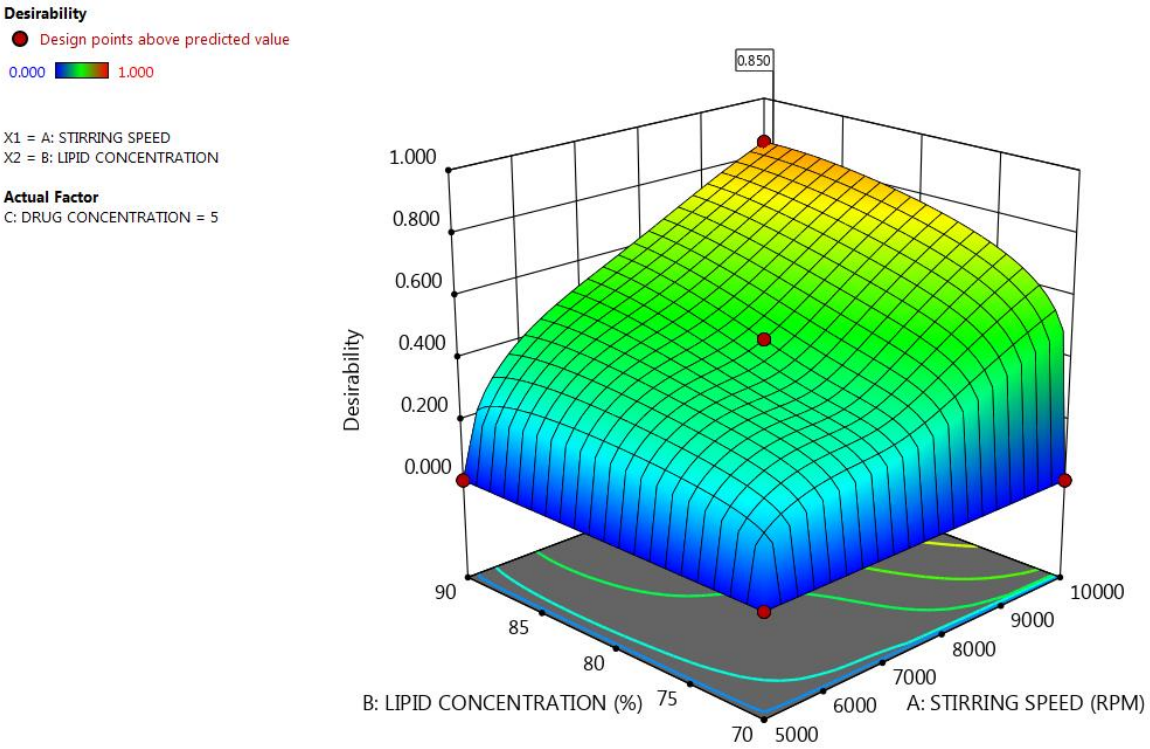



**Figure 17:** Three dimensional response plot representing overall desirability function.

**Table 20 :**Optimum formulation derived by Box-Behnken experimental design

| **Factors** | **Stirring speed (rpm)** | **Lipid concentration (%)** | **Drug concentration (%)** | **Desirability** |
|---|---|---|---|---|
| Optimal formulation | 9999.99 | 89.337 | 5 | 0.850 |

An optimum and stable formulation has the smallest particle size, the entrapment efficiency of >80% and the drug release of >50%. Optimum formulation was obtained based on minimum particle size of nanostructured lipid carrier and optimum lipid concentration . Using this approach, a set of components was found. A composition of 9999.99 rpm stirring speed, 89.337% of lipid concentration and 5% of drug concentration was predicted that the nano structured lipid carrier would have particle size, entrapment efficiency and drug release of 183.92 nm, 88.906 % and 42.323 % respectively.

Desirability of optimum formulation was 0.850. When desirability value was between 0.8 and 1, the formulation quality was regarded to be acceptable and excellent. When this value was <0.63, the formulation quality was regarded as poor. Formulation performance was considered unacceptable when the desirability value was <0.37[50].

**Evaluation of Ibuprofen loaded NLC :**

**1. Particle size:**

The particle size and zeta potential was determined using Malvern Mastersizer .The particle sizes of formulation, increases when the concentration of solid lipid increases. The particle size of sample was found to be 193.6 nm. Zeta potential was found to be -44.4 , it indicates that the surface charge was negative and obtained results shown in figure 18. It is known that ZP values of more than ±30 are considered as a good indication for the stability of the nanostructured lipid carrier[49]. The small particle size indicates the formulation contain greater interfacial area, which will provide better drug partitioning and absorption at the skin surface.

# Size Distribution Report by Intensity

*v2.2*

Malvern

## Sample Details

**Sample Name:** W Gel 1

**SOP Name:** mansettings.nano

**General Notes:**

| | | | |
|---|---|---|---|
| **File Name:** | DLS RESULTS 2019.dts | **Dispersant Name:** | Water |
| **Record Number:** | 464 | **Dispersant RI:** | 1.330 |
| **Material RI:** | 1.33 | **Viscosity (cP):** | 0.8872 |
| **Material Absorbtion:** | 0.101 | **Measurement Date and Time:** | Monday, September 30, 20... |

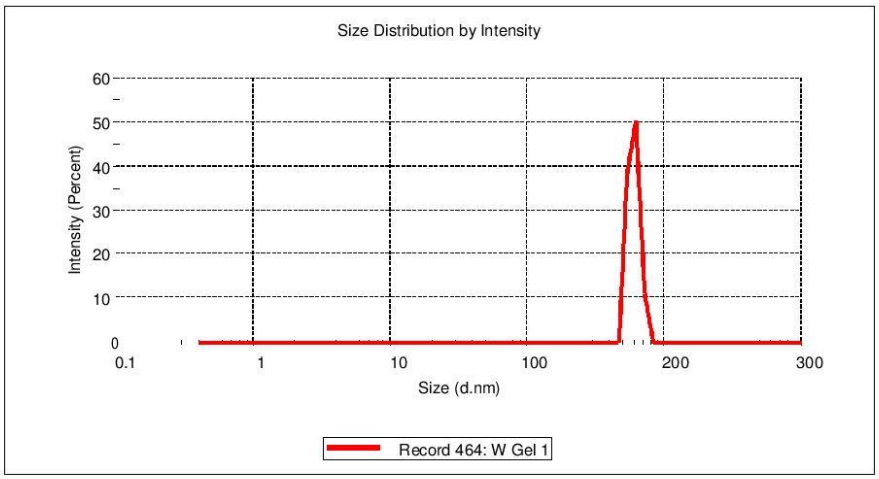
## System

| | | | |
|---|---|---|---|
| **Temperature (°C):** | 25.0 | **Duration Used (s):** | 80 |
| **Count Rate (kcps):** | 145.0 | **Measurement Position (mm):** | 4.65 |
| **Cell Description:** | Disposable sizing cuvette | **Attenuator:** | 7 |

## Results

| | | Size (d.nm... | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| **Z-Average (d.nm):** 163.8 | **Peak 1:** | 193.6 | 100.0 | 57.41 |
| **PdI:** 0.461 | **Peak 2:** | 0.000 | 0.0 | 0.000 |
| **Intercept:** 0.989 | **Peak 3:** | 0.000 | 0.0 | 0.000 |

**Result quality** **Refer to quality report**



# Zeta Potential Report

*v2.3*

Malvern



## Sample Details

**Sample Name:** w gel 1

**SOP Name:** mansettings.nano

**General Notes:**

| | | | |
|---|---|---|---|
| **File Name:** | DLS RESULTS 2019.dts | **Dispersant Name:** | Water |
| **Record Number:** | 465 | **Dispersant RI:** | 1.330 |
| **Date and Time:** | Monday, September 30, 2019 ... | **Viscosity (cP):** | 0.8872 |
| | | **Dispersant Dielectric Constant:** | 78.5 |

## System

| | | | |
|---|---|---|---|
| **Temperature (°C):** | 25.0 | **Zeta Runs:** | 10 |
| **Count Rate (kcps):** | 173.1 | **Measurement Position (mm):** | 2.00 |
| **Cell Description:** | Clear disposable zeta cell | **Attenuator:** | 6 |

## Results

| | | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|---|
| **Zeta Potential (mV):** | -41.6 | **Peak 1:** | -44.4 | 84.5 | 7.69 |
| **Zeta Deviation (mV):** | 10.4 | **Peak 2:** | -23.5 | 15.5 | 4.08 |
| **Conductivity (mS/cm):** | 0.0108 | **Peak 3:** | 0.00 | 0.0 | 0.00 |
| **Result quality** | See result quality report | | | | |

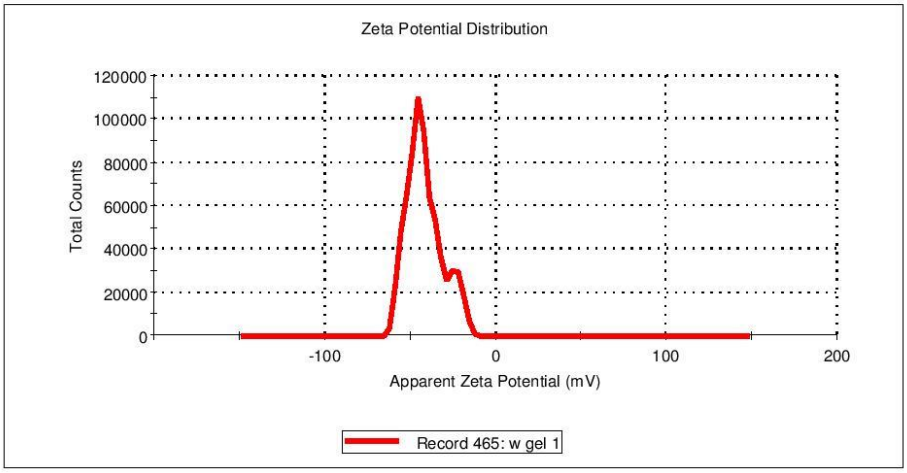
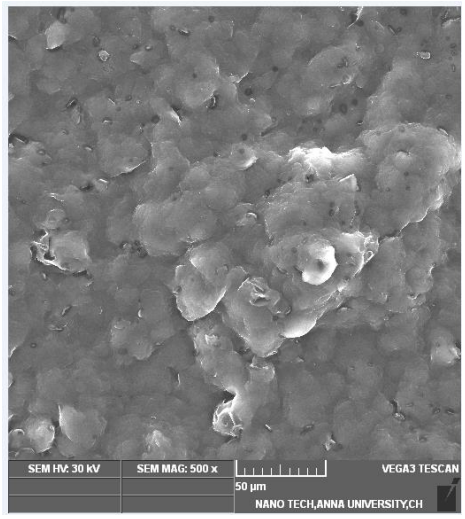




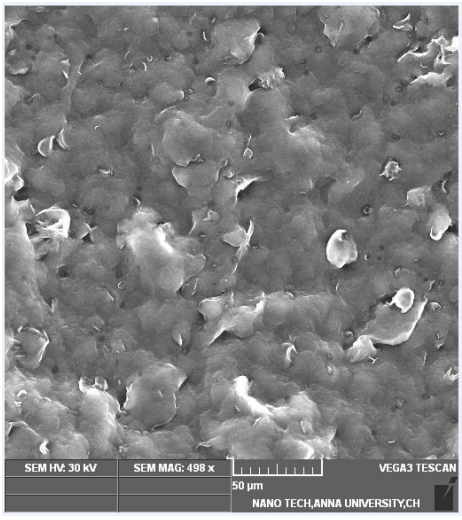

**Figure 18**: Particle size distribution and zeta potential

## 2. Scanning electron microscopy:

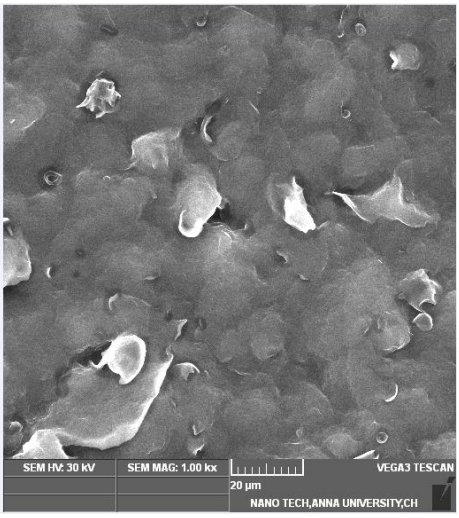
Surface analysis of Ibuprofen loaded nano structured lipid carrier was carried out by Scanning Electron Microscopy. Images obtained after SEM are shown in **Figures 19** for F1, F3 and F6. Among all the formulations, F1 was spherical- shape, and fine- smooth surface because of optimum concentration of solid and liquid lipid.




F1




F3




F6

**Figure 19:** SEM images of NLC formulations

### 3. Percent entrapment efficiency:

The nanostructured lipid carrier were prepared by different proportions of Drug, glyceryl monostearate , and surfactant. The entrapment efficiency of nanostructured lipid carrier loaded Ibuprofen increases with increase in the concentration of glyceryl monostearate and stirring speed. The entrapment efficiency found in the range between 73.9% to 89.00% . Formulation (F1 and F3) of NLC has shown maximum entrapment ( 89.00% and 85.50%) as the concentration of lipid increases, while Formulation (F4 and F6) of NLC has shown lowest entrapment that is (74.00% and 73.90%) as the concentration of solid lipid decreases. High lipophilicity of Ibuprofen resulted in high entrapment efficiency of drug in triglyceride lipids . This might be because of the long-chain fatty acids attached to the glyceride resulting in increased accommodation of lipophilic drugs . The less ordered lipid matrix created imperfections leading to void spaces in which drug molecules could be entrapped . In the method of preparation, drug was dissolved in molten lipid at temperature above the melting point of lipid and there was no drug leakage or precipitation of drug during the preparation. High encapsulation efficiency of drug in NLC can cause high amount of drug to pass through the stratum corneum[51].

**Table 21:** Experimental values for entrapment efficiency

| Sl.no | Entrapment efficiency (%) |
|---|---|
| F1 | 89 ±2.1 |
| F2 | 84.6 ±1.66 |
| F3 | 85.5 ±1.56 |
| F4 | 74 ±2.44 |
| F5 | 76.5 ±5.2 |
| F6 | 73.9 ±2.6 |

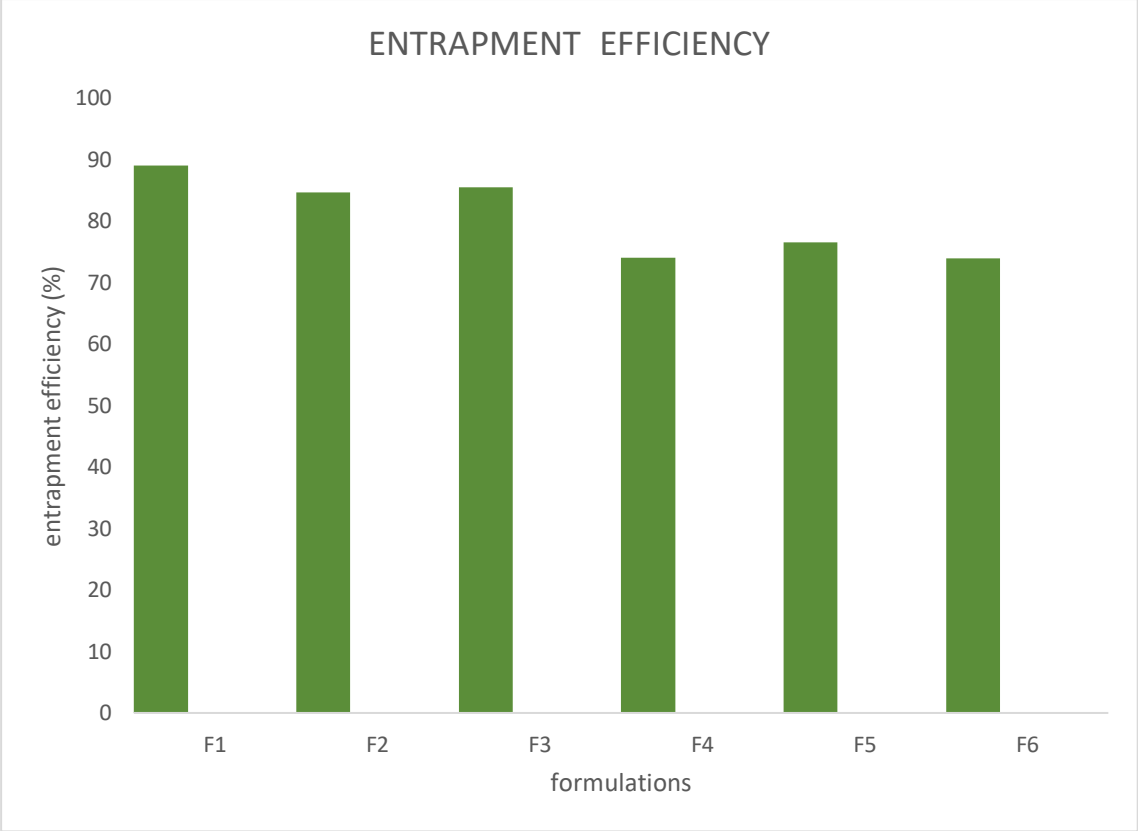



**Figure 20:** Graph indicating entrapment efficiency

## 4. In vitro diffusion study:

The curve for percentage release for formulations F1,F2,F3,F4,F5,F6 shown in the graph (figure 21, 22, 23, 24, 25, 26) given below by taking time on x-axis and percentage drug diffused on y-axis.

In vitro diffusion profile F1:

**Table 22:** In vitro drug diffusion data for formulation F 1

| Time | % Drug Diffused |
|---|---|
| 0 | 0 |
| 1 | 29.6 ±1.33 |
| 2 | 39.64 ±2.55 |
| 3 | 49.25 ±3.88 |
| 4 | 65.23 ±1.5 |
| 5 | 68.32 ±1.6 |
| 6 | 72.53 ±2.8 |
| 7 | 79.62 ±1.4 |
| 8 | 84.26±1.21 |
| 9 | 85.62±0.25 |
| 10 | 89.35±2.5 |
| 11 | 92.35±3.25 |
| 12 | 98.63 ±2.2 |

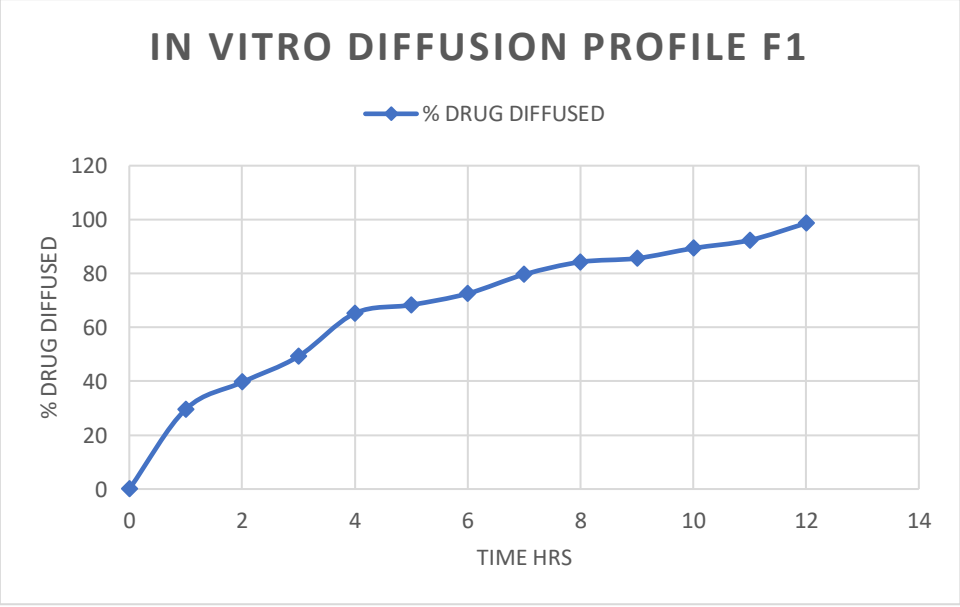



**Figure 21:** In vitro diffusion graph of F1

In vitro diffusion  profile F2:

**Table 23:** In vitro drug diffusion data for formulation F 2

| Time | % Drug diffused |
|---|---|
| 0 | 0 |
| 1 | 19.35 ±1.2 |
| 2 | 36.85±2.5 |
| 3 | 45.32±3.2 |
| 4 | 49.63±0.1 |
| 5 | 58.32±0.5 |
| 6 | 61.25±1.7 |
| 7 | 69.45±0.6 |
| 8 | 73.02±0.7 |
| 9 | 78.24±0.55 |
| 10 | 82.65 ±1.3 |
| 11 | 84.22 ±0.2 |
| 12 | 85.24 ±3.2 |

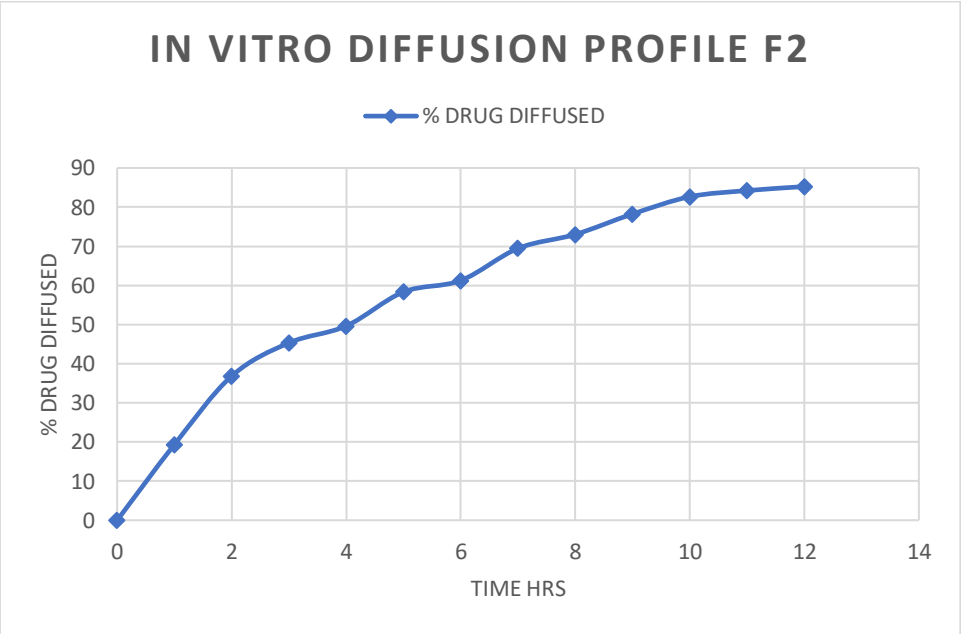



**Figure 22:** In vitro diffusion graph of F2

In vitro diffusion profile F3:

**Table 24 :** In vitro drug diffusion data for formulation F 3

| Time | % Drug diffused |
|---|---|
| 0 | 0 |
| 1 | 14.87 ±1.22 |
| 2 | 37.54 ±1.55 |
| 3 | 42.32 ±1.5 |
| 4 | 48.65 ±2.4 |
| 5 | 57.32 ±3.6 |
| 6 | 60.25 ±2.6 |
| 7 | 68.45±1.55 |
| 8 | 70.25 ±2.65 |
| 9 | 71.01 ±5.4 |
| 10 | 74.98±1.3 |
| 11 | 78.65±0.025 |
| 12 | 80.26 ±0.5 |

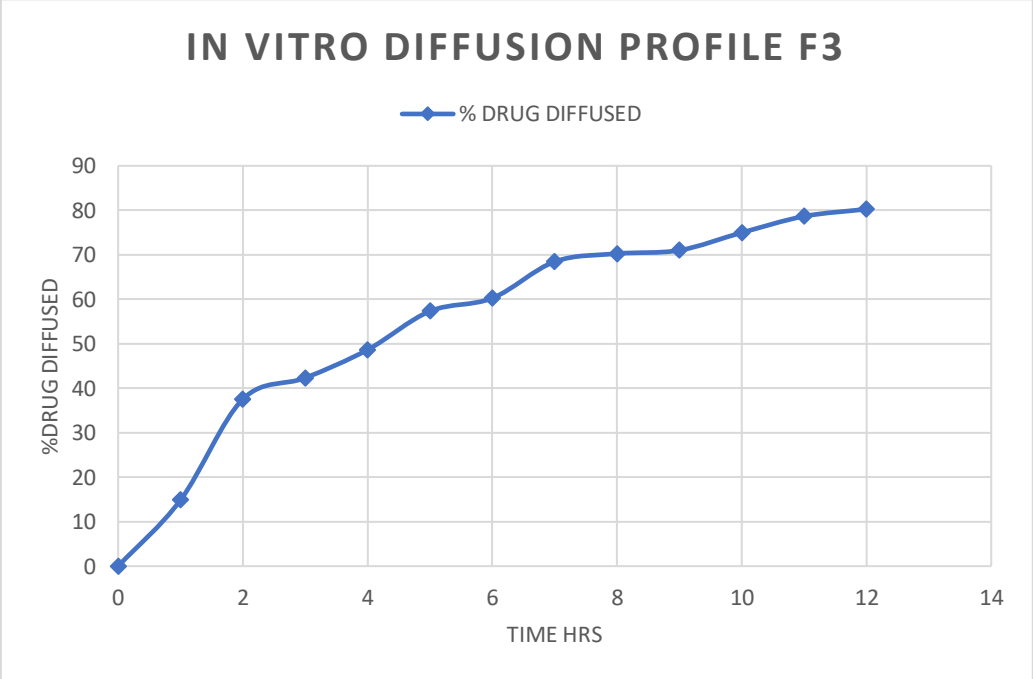



**Figure 23:** In vitro diffusion graph of F3

In vitro diffusion profile F4

**Table 25:** In vitro drug diffusion data for formulation F 4

| Time | % Drug diffused |
|---|---|
| 0 | 0 |
| 1 | 19.58±0.7 |
| 2 | 29.54±2.12 |
| 3 | 35.82±0.023 |
| 4 | 49.63±1.23 |
| 5 | 58.32±1.1 |
| 6 | 60.25±2.11 |
| 7 | 63.45±0.015 |
| 8 | 65.32±2.9 |
| 9 | 69.54±1.55 |
| 10 | 71.25±0.099 |
| 11 | 75.23±1.52 |
| 12 | 79.63±2.5 |

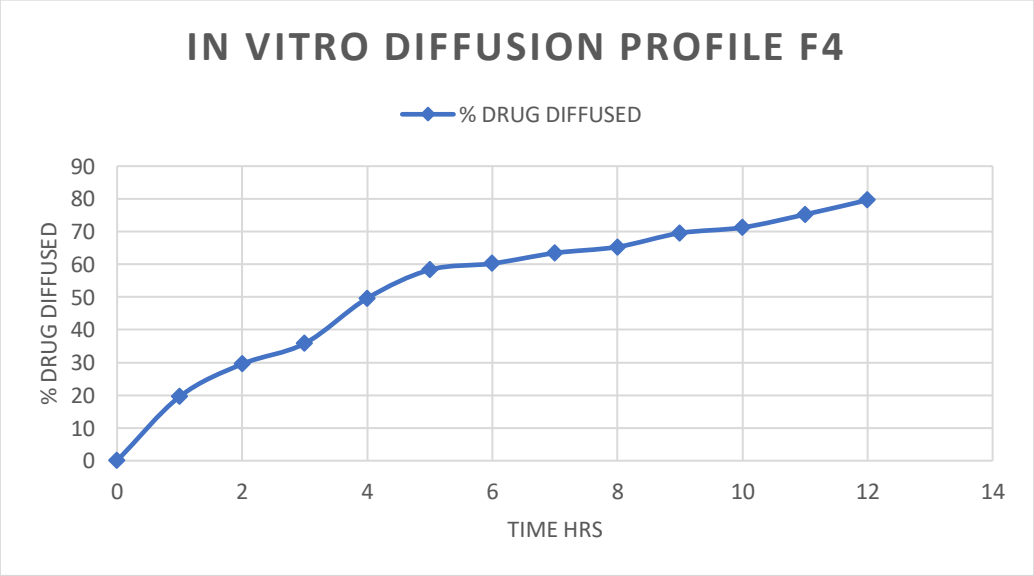



**Figure 24:** In vitro diffusion graph of F4

In vitro diffusion profile F5

**Table 26:** In vitro drug diffusion data for formulation F 5

| Time | % Drug Diffused |
|---|---|
| 0 | 0 |
| 1 | 14.35±1.1 |
| 2 | 26.85±0.8 |
| 3 | 34.25±0.018 |
| 4 | 47.88±0.69 |
| 5 | 55.21±1.29 |
| 6 | 59.62±0.25 |
| 7 | 63.55±1.43 |
| 8 | 69.54±1.94 |
| 9 | 75.24±1.7 |
| 10 | 76.85±1.88 |
| 11 | 78.55±1.3 |
| 12 | 80.01±0.08 |

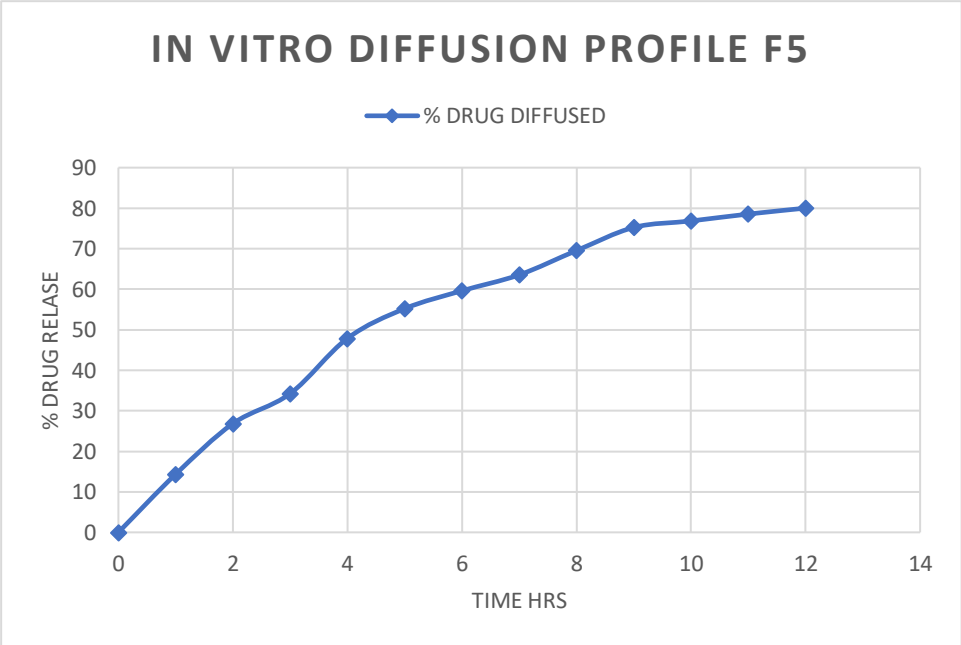



**Figure 25:** In vitro diffusion graph of F5

In  vitro diffusion profile F6

**Table 27:** In vitro drug diffusion data for formulation F 6

| Time | % Drug Diffused |
|---|---|
| 0 | 0 |
| 1 | 15.54±0.85 |
| 2 | 28.99±0.39 |
| 3 | 44.22±1.2 |
| 4 | 47.36±2.1 |
| 5 | 58.32±0.007 |
| 6 | 59.26±0.05 |
| 7 | 63.33±1.2 |
| 8 | 65.02±3.4 |
| 9 | 68.24±0.005 |
| 10 | 70.54±0.6 |
| 11 | 75.32±1.8 |
| 12 | 76.45±2.3 |

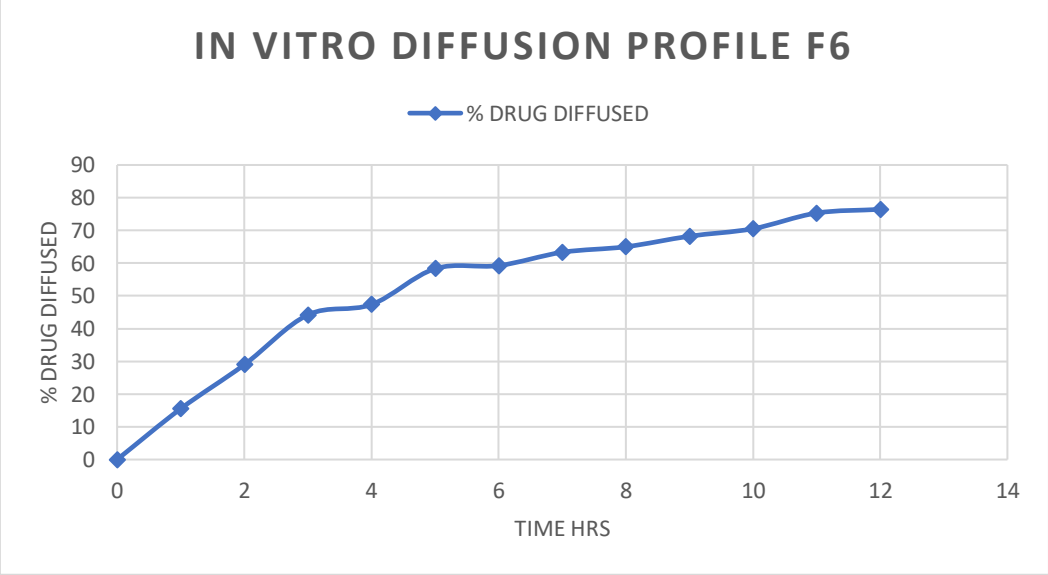



**Figure 26:** In vitro diffusion graph of F6

In vitro release of Ibuprofen from IBU-NLCs was studied in pH 7.4 phosphate buffer by dialysis membrane method . In pH 7.4 phosphate buffer, the cumulative % of release from

formulations F1-F6 was 98.63%, 85.24%, 80.26%, 79.63%, 80.01% and 76.45% respectively in 12 hours (table 22,23,24,25,26,27) . The release profiles of NLC formulations exhibited a typical biphasic pattern with an initial rapid phase followed by a slow phase in phosphate buffer. The initial rapid phase could be due to the burst release of drug. A possible explanation is a short diffusion path due to enrichment of drug in the outer region of NLC or drug deposition on the solid surface. Among other formulation, F1 was obtained as best formulation because it contains 65% solid lipid and 15% of liquid lipid makes a perfect nanostructured lipid carrier. Formulation F1 showed maximum release of 98.63% in pH 7.4 phosphate buffer during 12 hours diffusion study. In comparison with other formulations, F1 exhibited reasonably good particle size, high zeta potential value, and the higher entrapment efficiency with release of drug from the lipid matrix in pH 7.4 phosphate buffer, hence it was considered as the optimized formulation.

During the formulation, the solubility of the drug is increased when the temperature increases (70°C–80°C) in the presence of a surfactant in the aqueous phase. During the cooling phase, the drug is repartitioned into the lipid phase, and the solid lipid recrystallizes and forms a solid lipid core. Greater amounts of the drug are entrapped in the core lipid matrix and lower amounts of the drug are deposited at the shell or surface of nano structured lipid carrier. Therefore, the formulation contains less amount of drug on the surface and the outer shell of the IBU-NLC contributes to the initial fast release; moreover, the drug present in the core of the lipid matrix contributes to the second slow release phase. During the solidification at a low temperature, due to the solid lipid (GMS) owning a higher melting point, it would rapidly solidify to form a solid lipid core in which liquid lipid was randomly distributed. When the liquid lipid (Coconut oil) content is higher, liquid lipids would be located at the outer shell of the NLC besides being distributed in the solid lipid core, which led to drug-enriched shell related with drug burst release at the initial stage. In addition, as liquid lipid was distributed in solid lipid, the crystalline structure of NLC became more imperfect and allowed drugs loaded to release more easily, thus increasing the rate of drug release. [49]

**Comparative in vitro diffusion study of ibuprofen NLC and Ibuprofen suspension**

In vitro release of Ibuprofen from IBU-NLCs formulations F1,F2,F3,F4,F5,F6 and Ibuprofen suspension was studied in pH 7.4 phosphate buffer by dialysis membrane method. In pH 7.4 phosphate buffer, the cumulative % of release from ibuprofen suspension was found to be 29.66 % respectively in 12 hours. The amount of ibuprofen diffused from the IBU NLC after 12 hours

was significantly higher (98%) than the IBU suspension. IBU-NLCs showed a biphasic drug release pattern was observed, that was drug burst release at the initial stage and followed by sustained release at a constant rate.

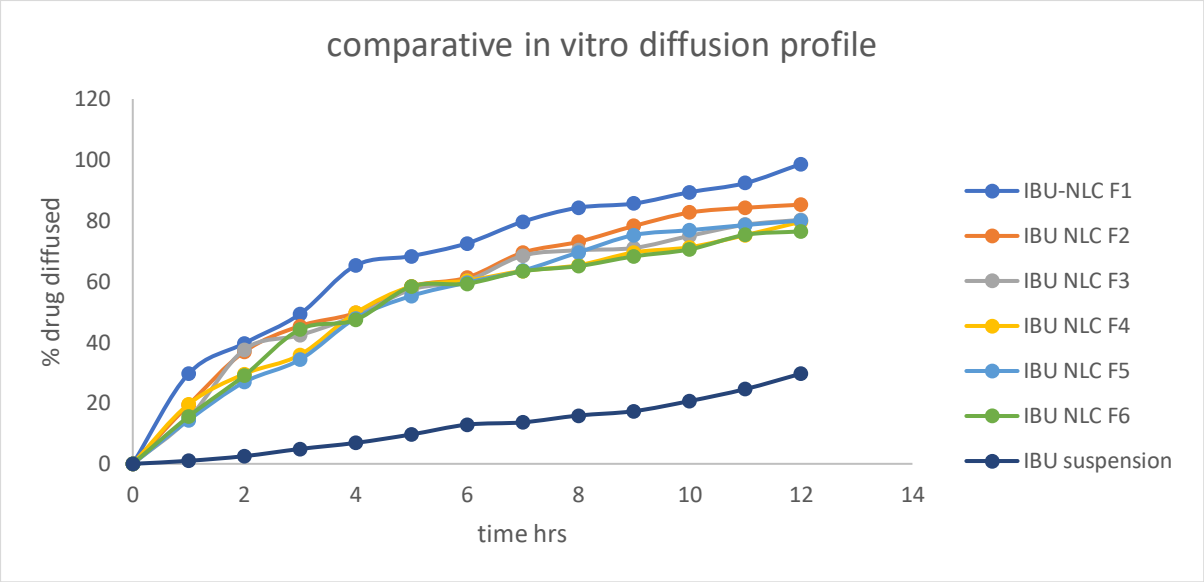



**Figure 27** : Comparative in vitro study of suspension and IBU-NLC formulations.

**IBU NLC dispersed Gel evaluation:**

**1. Physical evaluation :**

The Gel shows white color and Translucent appearance.

**2. pH:**

pH values were found out to be complacent as a skin preparation.. The pH of the gel formulations is within range of 6.8 to 7.2, which lies in the normal pH range of the skin and would not produce any irritation to the skin.

**Table 28:** Experimental values of pH for NLC-IBU gel formulations

| **Formulation** | **pH** |
|---|---|
| F1 | 7.0±0.09 |
| F2 | 6.9±0.11 |
| F3 | 7.1±0.52 |
| F4 | 6.8±0.33 |
| F5 | 6.5±0.52 |
| F6 | 6.9±0.115 |

### 3. Spredability :

Spreadability of the preparation was also found out to be satisfying and assures the suitability. The results show good spreadability for F1 as 5.5 gm-cm/sec. Spreadability is an important property of topical formulation from a patient's compliance point of view. Application of the formulation to inflamed skin is more comfortable if the base spreads easily, exhibiting maximum slip and drag. The prepared gel produces excellent spreadability.

**Table 29:** Experimental values of spreadability for NLC-IBU gel formulations

| **Formulation** | **Spreadability (gm-cm/sec)** |
|---|---|
| F1 | 5.5±0.9 |
| F2 | 4.3±2.5 |
| F3 | 5.2±1.3 |
| F4 | 5.1±2.5 |
| F5 | 4.9±1.5 |
| F6 | 5.2±2.66 |

### 4. Drug Content

The percentage of drug present in gel formulation was found to be above 98% for formulation F1.The formulation F2 to F5 shows drug content in the range of 90-94%.

**Table 30:** Experimental values of drug content for NLC-IBU gel formulations

| **Formulation** | **Drug content (%)** |
|---|---|
| F1 | 98.25±1.63 |
| F2 | 92.6±3.2 |
| F3 | 94.55±3.6 |
| F4 | 90.25±2.5 |
| F5 | 93.33±0.09 |
| F6 | 90.2±0.54 |

## 5. Comparative invitro study of NLC gel with the marketed gel :

The cumulative % release of gel from formulations F1 ,F2, F3 ,F4,F5 and F6 was compared to marketed Ibuprofen gel formulation. The release of the NLC gel was sustained over a period of 12 Hours whereas marketed IBU gel showed poor permeation of the drug. The results of this study signify that topical administration of gel in nano structured lipid carrier is compared to marketed IBU-Gel.(table 31)

**Table 31 :** Comparative in vitro diffusion study of IBU-NLC gel and marketed gel formulation

| Time | IBU Gel (Marketed) | F1 Gel | F2 Gel | F3 Gel | F4 Gel | F5 Gel | F6 Gel |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 2.45±0.06 | 28.66±1.2 | 15.56±1.55 | 14.88±1.2 | 18.57±0.3 | 13.99±0.06 | 15.24±1.5 |
| 2 | 4.9±2.12 | 39.54±0.05 | 35.42±0.41 | 35.62±0.1 | 28.66±0.05 | 27.88±0.06 | 29.9±0.06 |
| 3 | 5.5±0.36 | 58.65±0.01 | 44.32±0.02 | 43.25±0.06 | 33.69±0.04 | 35.41±0.04 | 41.52±0.01 |
| 4 | 10.9±0.04 | 66.85±1.5 | 48.65±0.5 | 47.55±0.5 | 48.75±0.2 | 48.57±0.12 | 47.65±0.06 |
| 5 | 12.9±1.25 | 67.58±0.004 | 57.21±2.56 | 55.32±0.6 | 57.22±0.1 | 54.04±0.05 | 59.22±0.04 |
| 6 | 19.9±0.09 | 71.55±2.8 | 60.5±3.22 | 60.62±0.65 | 60.88±0.32 | 59.33±0.05 | 60.25±0.6 |
| 7 | 23.6±0.25 | 78.64±3.66 | 65.22±2.15 | 65.24±0.06 | 62.52±0.18 | 64.58±0.06 | 62.55±0.5 |
| 8 | 26.98±1.55 | 83.62±0.52 | 72.01±1.6 | 70.55±0.5 | 69.5±0.69 | 70.54±0.01 | 65.29±0.6 |
| 9 | 35.28±0.008 | 85.32±0.96 | 78.22±0.005 | 72.04±0.05 | 70.12±0.17 | 76.25±1.2 | 69.44±0.01 |
| 10 | 40.58±0.01 | 88.31±0.25 | 80.55±0.56 | 73.55±0.01 | 73.25±0.06 | 77.44±0.06 | 71.54±1.23 |
| 11 | 45.69±0.005 | 93.99±0.32 | 81.22±12 | 77.65±0.3 | 76.22±0.09 | 79.54±0.2 | 75.09±0.9 |
| 12 | 50.36±0.54 | 98.65±0.44 | 85.32±0.07 | 80.24±0.05 | 79.24±0.06 | 80.55±0.03 | 78.25±0.01 |

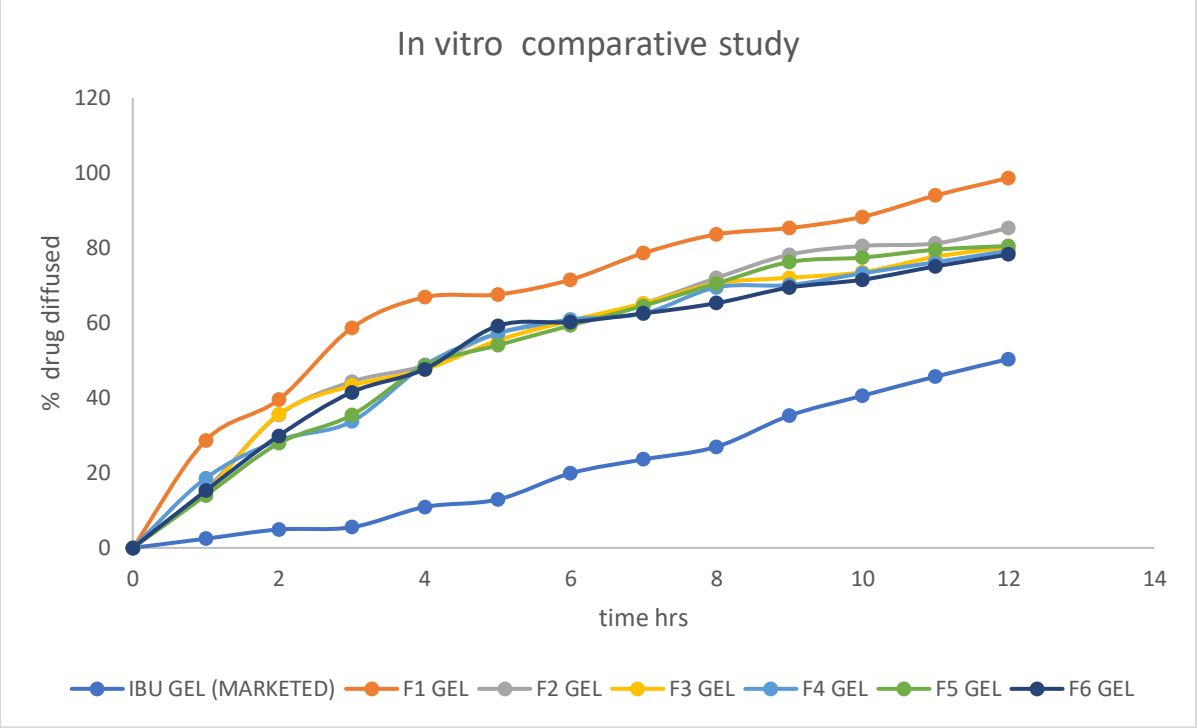



**Figure 28:** In vitro diffusion graph of IBU-NLC gel and marketed ibuprofen gel formulation.

The drug diffusion through the skin was high in the case of NLC based gel than the marketed formulation indicated its skin targeting ability , which is desirable for effective therapy . By formulating NLC based drug delivery system , the drug can be targeted to the skin with its reduced systemic access and reduced systemic side effects. While application of NLCs incorporated into gel on skin may induce structural change of particle structure due to evaporation of water resulting in the transition of lipid matrix into a highly ordered structure causing drug permeation[49]. NLC-based gel formulation of Ibuprofen remained superior to the marketed product in its ability to suppress pain and sustain the anti-inflammatory activity for 12 hrs.

**RELEASE KINETICS STUDY:**

The diffusion data were examined for first order, zero order, Higuchi, Korsmeyer-Peppas and Hixson crowell models.

**Table 32:** Zero order plot of optimized formulation IBU-NLC F1 gel

| Time | % Drug diffused |
|---|---|
| 1 | 28.66 |
| 2 | 39.54 |
| 3 | 58.65 |
| 4 | 66.85 |
| 5 | 67.58 |
| 6 | 71.55 |
| 7 | 78.64 |
| 8 | 83.62 |
| 9 | 85.32 |
| 10 | 88.31 |
| 11 | 93.99 |
| 12 | 98.65 |

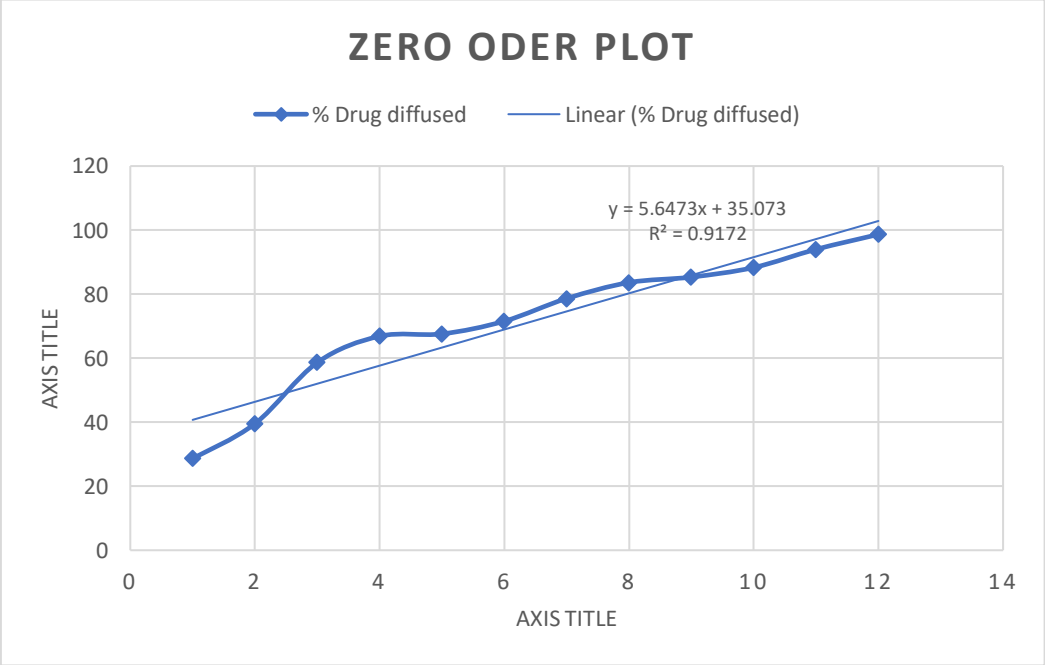



**Figure 29:** Zero order plot

**Table 33 :**First order plot of optimized formulation IBU-NLC F1 gel

| Time | log cumulative % drug remaining |
|---|---|
| 0 | 2 |
| 1 | 1.853 |
| 2 | 1.781 |
| 3 | 1.616 |
| 4 | 1.520 |
| 5 | 1.511 |
| 6 | 1.454 |
| 7 | 1.330 |
| 8 | 1.214 |
| 9 | 1.167 |
| 10 | 1.068 |
| 11 | 0.779 |
| 12 | 0.130 |

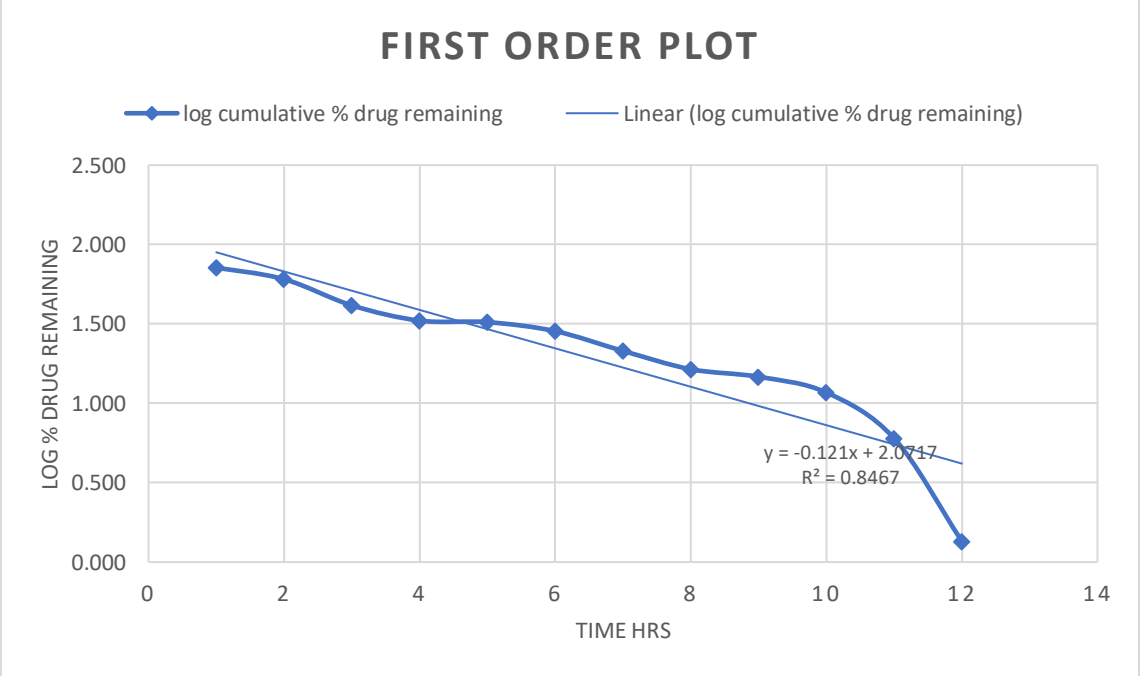



**Figure 30:** First order plot

**Table 34:** Higuchi (diffusion)co-efficient plot of optimized formulation IBU-NLC F1 gel

| SQUARE ROOT OF TIME | % drug diffused |
|---|---|
| 1 | 28.66 |
| 1.414 | 39.54 |
| 1.732 | 58.65 |
| 2 | 66.85 |
| 2.236 | 67.58 |
| 2.449 | 71.55 |
| 2.645 | 78.64 |
| 2.828 | 83.62 |
| 3 | 85.32 |
| 3.162 | 88.31 |
| 3.316 | 93.99 |
| 3.464 | 98.65 |

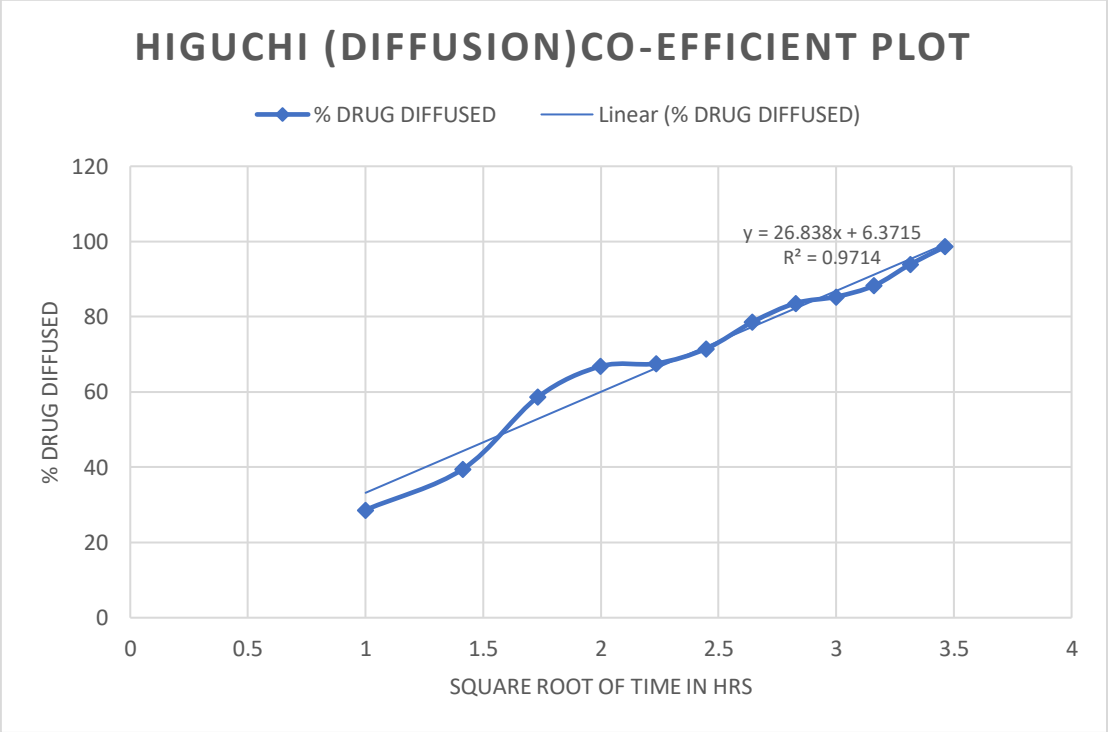



**Figure 31:** Higuchi (diffusion)co-efficient plot

**Table 35 :**Korsmeyer Peppas plot of optimized formulation IBU-NLC F1 gel

| Log time | Log cumulative % drug diffused |
|---|---|
| 0.000 | 1.457 |
| 0.301 | 1.597 |
| 0.477 | 1.768 |
| 0.602 | 1.825 |
| 0.699 | 1.830 |
| 0.778 | 1.855 |
| 0.845 | 1.896 |
| 0.903 | 1.922 |
| 0.954 | 1.931 |
| 1.000 | 1.946 |
| 1.041 | 1.973 |
| 1.079 | 1.994 |

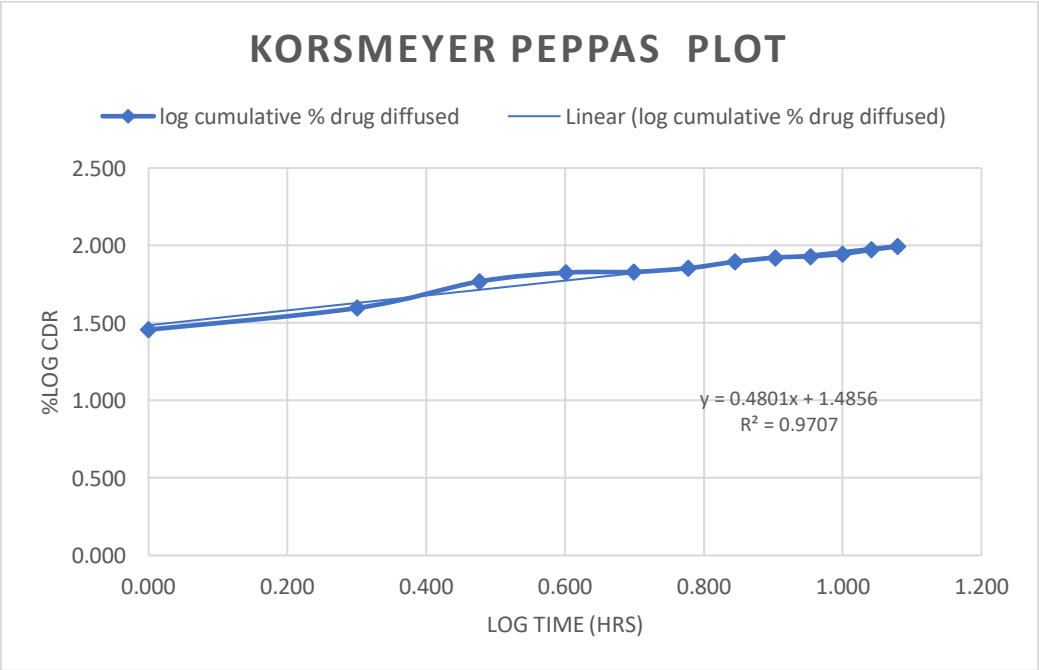



**Figure 32:** Korsmeyer Peppas plot

Further **'n'** value of peppas model was found 0.4801. It indicates the drug release follows fickian diffusion mechanism.

**Table 36 :** Hixon-crowell model optimized formulation IBU-NLC F1 gel

| Time | cube root of percentage drug diffused |
|---|---|
| 1 | 3.06 |
| 2 | 3.406 |
| 3 | 3.885 |
| 4 | 4.058 |
| 5 | 4.073 |
| 6 | 4.151 |
| 7 | 4.284 |
| 8 | 4.372 |
| 9 | 4.402 |
| 10 | 4.453 |
| 11 | 4.546 |
| 12 | 4.62 |

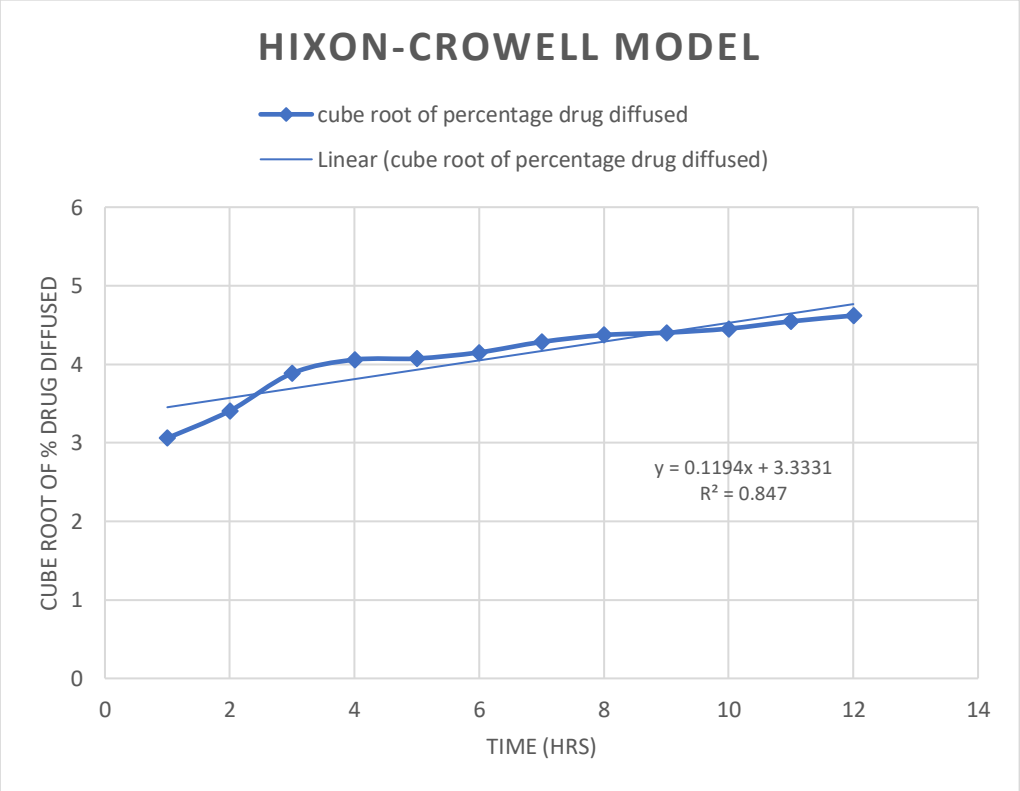



**Figure 33:** Hixon-crowell model

**Table 37:** Correlation coefficient ($R^2$) values obtained for all models.

| **Release kinetics** | **Correlation coefficient ($R^2$)** | |
|---|---|---|
| Zero order equation | 0.9172 | |
| First order equation | 0.8467 | |
| Higuchi(diffusion)co-efficient | 0.9714 | |
| Korsmeyer Peppas equation | 0.9707 | 'n' value 0.4801 |
| Hixson crowell release equation | 0.847 | |

Examination of the correlation coefficient ($R^2$) value indicated that the drug permeation followed a diffusion-controlled mechanism from the NLC gel, as the $R^2$ value for zero order plot (0.9172) , first-order (0.8467) , the higuchi plot (0.9714), korsmeyer peppas plot (0.9707) and hixson crowell plot (0.847) kinetic models, was shown in Table 37. The $R^2$ value was found to be highest for Higuchi square root of time as 0.9924 indicates that topical NLC gel formulations follows Higuchi model. Further **'n'** value of korsmeyer peppas model was found to be 0.4801. It indicates the drug release follows fickian diffusion mechanism. Fick's diffusion refers to the transport process in which the polymer(solid lipid) relaxation time is much greater than the characteristic solvent diffusion time. The solid lipid melts and form as a thin lipid film on skin, occlusion occurs lead to drug permeation[21].

## 8.SUMMARY AND CONCLUSION

The present work describes a study on “optimization of ibuprofen loaded nano structured lipid carrier (NLC) using response surface methodology (RSM): preparation and in vitro evaluation”

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID). It works by reducing hormones that cause inflammation and pain in the body. This drug was selected for the study because it has good percutaneous absorption and appears to be more active as anti inflammatory activity and is well tolerated.

Nano structured lipid carrier was formulated by high speed homogenization method which was found to be simple and economic. Excipients (lipids) used in the study was economic and safe.

Effect of various factors on the formulation can be studied by the Box-Behnken design. With the use of desirability plots minor change in the formulation is possible for the required response.

It is evidence from the FTIR spectrum shows that the lipids ( solid lipid ) used in the NLC formulations were compatible with the drug Ibuprofen.

In vitro release studies of the formulations were carried out across the dialysis membrane using a diffusion cell. Among all the formulations, the release was highest for the formulation NLC F1 as 98.63% in 12 hours diffusion study. It also shows lowest particle size , better entrapment efficiency and high zeta potential value, hence NLC-IBU F1 was concluded as optimized formulation.

The optimized nano structured lipid carrier IBU-NLC formulations was dispersed into gel. The polymers namely Carbopol-940 were used as gelling agent for formulation of gels and studied for their drug permeation from the NLC- gel formulations. Carbopol gels were transparent, non-greasy and smooth on application.

The pH of the formulations ranged from 6.5 to 7.1. The spreadability data ranges from 4.3-5.5 gm-cm/sec. The drug content was found to be 90-94%. The pH, spreadability and drug content were good and up to the acceptable range.

The in vitro diffusion study of NLC gel formulation were carried out across the skin membrane using Franz diffusion cell. It shows good permeation into skin for prolonged release of 12 hours than the marketed gel formulation .

From this investigation, it was concluded that formulation F1 was concluded as best formulation . It could be concluded that NLCs may play an important role in controlling the release of Ibuprofen from NLCs as well as targeting of drug to the skin. The amount of drug retained in the skin for NLC based gel was found to be significantly higher as compared to marketed formulation. The dermal retention of Ibuprofen was attributed to the increased contact with corneocytes, skin occlusion and sustained release owing to the properties of NLCs. Due to their small particle size, NLC make closer contact with the superficial junctions of corneocytes clusters and furrows present between corneocyte islands and favour accumulation for several hours , allowing sustained drug release. Therefore, it can be concluded that the Ibuprofen NLCs gel formulation can be used to extend the duration of drug release and as an efficient topical drug delivery carrier for chronic treatment of inflammation .

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