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## LIST OF ABBREVIATIONS

|  |  |
| --- | --- |
| BCS = Biopharmaceutical classification system | °C = Degree Centigrade |
| NNRTI = non-nucleoside reverse transcriptase inhibitor | % = Percentage |
| Etravirine= ETR | %E= Encapsulation |
| SLN = Solid lipid nanoparticles | hrs. = Hours |
| COM= Compritol 888 ATO | mg = Milligrams |
| GEL= Gelucire 50/13 | gm = Gram |
| POL= Poloxamer 188 | μg/ml = Microgram per  milliliter |
| DMF = Dimethyl formamide | Min = Minute |
| nm = Nanometers | pH = Negative logarithm of  hydrogen ion concentration |
| rpm = Revolution per minute | IP = Indian pharmacopoeia |
| sec = Second | EP= European pharmacopeia |
| M.P = Melting point | mm = Mill molar |
| Da = Daltons | U.V = Ultraviolet |

|  |  |
| --- | --- |
| F= Formulation | XRD = X- ray diffraction |
| PDI = Poly dispersity index | Fig = Figure |
| FT-IR = Fourier transmission infraredspectroscopy | Log = Logarithm |
| DSC = differential scanning  calorimetry | %CDR = % Cumulative drug  release |

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**ABSTRACT**

Etravirine (ETR) is an antiviral drug belongs to BCS class IV drugs due to low solubility in aqueous medium and poor permeability. In this study, to augment the dissolution profile of ETR, solid lipid nanoparticles (SLN) has been build up by utilizing lipid mixture Compritol 888 ATO and Gelucire 50/13 and surfactant Poloxamer 188. SLN formulations were made by hot homogenization technique using probe sonicator. Effect of variables like ratio of lipids, concentration of surfactant, and sonication time were studies at 2 different levels and by 23 full factorial statistical design to analyze the effect on response variables % yield, % encapsulation (%E), and % drug release. Formulations were evaluated for all the responses and the effect of factors on responses was well explained by a significant linear model. The optimum formulation was selected and found to be significantly equivalent to the predicted response values from the optimum design space. The optimized SLN preparation was evaluated for zeta potential of dispersed, size of the particle and (poly dispersibility index) PDI. Zeta potential of -36.2 mV particle size of 178 nm and PDI value 0.075 indicates SLN particles are Nano size which is monodispersed. Absence of interaction of drug with excipients was confirmed with differential scanning calorimetry (DSC). The release kinetics for optimum preparation is explained by first-order kinetics. In aqueous buffer *In*-*vitro* drug profile of selected optimized formulation was observed to be 43.68% in comparison with pure drug release 19.99%. Hence, it is concluded that SLN of Etravirine can be formulated using lipid mixture COM: GEL and Poloxamer as a surfactant to increase drug release and thereby can enhance oral bioavailability.

Keywords: Etravirine ETR, solid lipid nanoparticles SLN, 23 full factorial design, BCS class IV, antiviral drug.

# INTRODUCTION

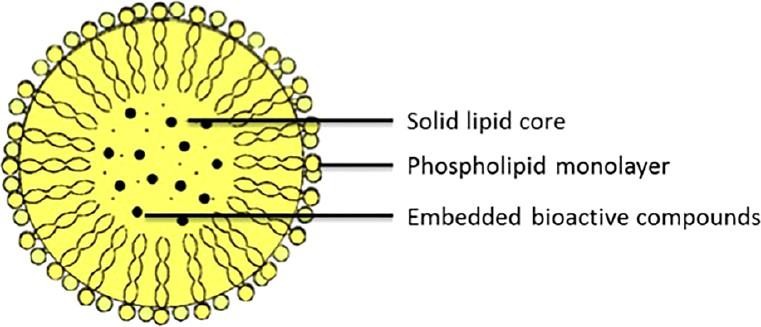
Nanotechnology is a trade working system on a molecular or submicron scale. It has many advantages with respect to targeted delivery and controlled free of therapeutic complexes for improved drug pharmacokinetics and pharmacodynamics activity. It can solve vigorous solubility difficulties of insoluble drugs and used to affect mononuclear phagocyte organisms to permit specific regions for distribution and reduce adverse effects in other body parts. To explain the problems of solubility and bioavailability enhancement nanotechnology method has been discussed. Nanoparticles are within the range 10-1000nm. Nanoparticles may be combined name for both Nano spheres and Nano capsules. Nanoparticles are prepared which are insoluble in water and soluble in oil with high log P, melting point, and high doses. There are many methods to increase the solubility of insoluble drugs such as micronization and solubilization using diluents, salts, surfactant dispersion, precipitation procedure, and oil mixture (1,2).

Oral delivery is a favored route of drug administration for better patient convenience, infections, and wounds. Non-invasive delivery has numerous results for the aspects like low solubility of the drug, weak gastrointestinal absorption, sudden metabolism, a high flux in plasma level, and variability due to food effects. These factors may have some disadvantages, In-vivo which ends in the breakdown of conventional delivery systems. Therefore, these systems have several drawbacks, like physical stability, accumulation, leakage of the drug on storage, presence of organic solvent residues in the final product, cytotoxicity, etc. oral delivery has taken a new step, to rise in functions of carrier lipids and for low water-soluble drugs. Functional lipids are biocompatible and biodegradable with low acute and chronic toxicity are selected to make nanoparticles. Lipid nanoparticles have the best qualities than other colloidal methods like polymeric nanoparticles, liposomal systems, oil-in-water emulsions, and Nano emulsions. Therefore, lipid nanoparticles made a solid medium that has strong potential for oral delivery(3).

Lipid-based delivery methods involve emollients, emulsifiers, and diluents. It is one of the most explained factors to control the absorption barrier and to increase the bioavailability of low water-soluble drugs. The aspects of lipid-based include are the lipid absorption, with of emulsion droplet, drug lipophilicity, and lipid type. They can adjust the slow and incomplete dissolution of drugs and facilitate the growth of solubilized stages from the absorption. The administration of lipids with drugs might affect their absorption tract. Oral administered drugscan pass from systemic circulation to hepatic portal vein and a few highly lipophilic drugs canbe transported into systemic circulation in the intestinal lymphatic’s, which gives better oral bioavailability(5).

Solid lipid nanoparticles (SLN) initiated in 1991. It is dissolved in water or in aqueous surfactant solution and made of spherical solid lipid molecule in the range of 50-1000 nm. They made up of hydrophobic core and covers with a phospholipid monolayer. It holds the drug molecules in the lipid medium. The hydrophobic chains of phospholipids are occupied in the lipid matrix. Hence, both physicochemical and pharmacological properties of lipids can be used (4).

### Figure 1: Structure of SLN



* 1. **Advantages(5)**
     1. Combined effect of Controlled and targeted release of the drug have accomplished.
     2. Increase the drug targeting by coating the ligands to SLN.
     3. SLNs can enhance the bioavailability.
     4. Because of the preparation methods they have good reproducibility.
     5. Bothhydrophilic and hydrophobic drugs are used.
  2. **Preparation methods(5)**

SLNs are prepared by solid lipids, emulsifiers, and water or solvent. The lipids are tri and partial glycerides, fatty acids (stearic acid, palmitic acid), and steroids (cholesterol) and waxes (cetyl palmitate). To stabilize the lipid dispersion several emulsifiers and its combination are used. The mixtures of emulsifiers can prevent particle agglomeration. The preparation methods are as follows:

* Hot homogenization
* Cold homogenization
* micro emulsion
* Solvent emulsification diffusion
* Precipitation
* W/O/W Double emulsion
* Spray drying

### Lipid materials in oral administration

Based on the evaluation of their Polymorphism, solid structure, miscibility, and physical and chemical arrangement the lipids are selected. It is essential to take care of the matrix at a solid-state temperature. Compared to pure lipids like monoacid triglycerides, mono and diglycerides as lipid matrix mixtures might increase in drug solubility. Oil and lipids are made with mono, di, and triglycerides with fatty acids that differ in chain length and degree of unsaturation. Stable lipid

nanoparticles have an advantage in providing suitable surfactants and their concentration. In the hot homogenization method, the result indicates the quality of SLN dispersions, with a higher melting point. The results in the theory of high- pressure homogenization are termed as the upper viscosity of the dispersed stage. Differ in the lipid composition (e.g. impurities) have an effect on the quality of SLN dispersion. Increase in lipid 5–10% it shows up greater particles and wider particle size distributions (6).

### Effect of surfactants in lipid nanoparticles

The surfactant and its concentration have an effect on the quality of SLN dispersion. In the homogenization process, high surfactant concentration reduces normal phenomena and helps in the separation of particles. It covers the new surfaces compares with the set of uncovered surfaces. To cover up the new surfaces the main dispersion must hold excessive surfactant molecules. The excessive surfactant molecules may vary in various forms, for e.g. molecular solubilized, micelles (SDS), or liposomes (lecithin). It has studied that SLNs are stable with surfactants like Lipoid S 75 or tyloxapol with smaller particle size and better storage stability (6).

According to World health organization, nearly 35 million global people and less than 15 years’ age of 3.2 million children are suffering from HIV/AIDS. Mostly

2.1 million people worldwide are ill yearly. From the early stage of the widespread, nearly 78 million people are infected and died around 39 million people. AIDS is the prominent reason among 25–44 aged people death in the United States. Antiretroviral drugs are effective in decreasing plasma levels and ineffective in eliminating the virus from other body parts in recent treatments. It is mainly effective in treating abnormalities, mental, mild neurocognitive disorder, dementia and encephalitis (7).

### 1.5 Mechanisms of action (8)

**1.5 (A)Non-nucleoside reverse transcriptase inhibitors (NNRTIS)**

It is inhibiting by binding to the reverse transcriptase enzyme. It blocks the polymerization in allosteric regulation by changing the position of its modules within the catalytic region of the transcriptase enzyme. It is an important step in viral replication. It does not inhibit the reverse transcriptase of other lentiviruses, such as simian immunodeficiency virus (SIV) and these molecules do not need any metabolic activation for the phosphorylation of 5’ nucleoside hydroxyl group to form an active nucleotide. The current approved NNRTIs are Etravirine, Delavirdine, Efavirenz, and Nevirapine.

Antiretroviral (ARV) drugs have the power to control the effect of HIV and AIDS and its symptoms. Etravirine is a NNRTI used to cure HIV-1 infection because it has activity against viruses with mutations (particularly the common K103N mutation), Etravirine is a Biopharmaceutics Classification System Class IV compound (low solubility and permeability). It is insoluble in water over a wide pH range, slightly soluble in propylene glycol, ethanol, and freely soluble in organic solvents. It is yellowish-brown powder. Half-life is 30–40 hours. A dose of Etravirine 200 mg twice daily. Based on an amorphous drug substance and dispersions of the compound in a glassy carrier, the enhancement of GI uptake was offered by systems (9,10).

Solid lipid nanoparticles (SLNs) based systems are mainly for the delivery of hydrophobic drugs. Low stability and burst release in acidic pH conditions, results particles aggregation in GI. SLNs are mainly used for the controlled and site- specific targeting delivery systems. Transfer of hydrophilic molecules to lipophilic molecules by conjugation or the presence of lipids in the absorption can increase the oral absorption. Lipids can help oral absorption of the encapsulated drug from lymphatic uptake. The type of surfactant and its concentration will affect its structure, and it is a very important factor to discuss in SLN. Due to low surfactant concentration, the results may show sustained drug release and burst release. The

particle size will affect the drug release rate and depends on the composition of SLN formulation such as surfactant concentration, structural properties of the lipids and drugs, and conditions such as time preparation equipment, sterilization, and lyophilization) (11,12).

Because of the surfactant effect, there will be an improved BA, with the solubilized endothelial cells and membrane fluidity(13). There is a huge capability in the anti- HIV drug-usingSLNas a carrier(14).

Novel drug targets may lead to redefining the goals of antiretroviral therapy: with an attempt achieve the ultimate objective is the eradication of infections. The aim is to prepare solid lipid nanoparticles to increase the bioavailability and drug stability. These solid lipid nanoparticles have potential to meet all the criteria like controlled and site-specific drug delivery and well characterization of excipients. Hence in the present study, we have aimed at the preparation of SLNs of class IV drug Etravirine for enhancing the oral dissolution.

## AIM

**AIM AND OBJECTIVES**

To design and evaluate the solid lipid nanoparticles of antiviral drug for oral delivery.

## OBJECTIVE

* To select suitable lipid and surfactants for preparation of SLN.
* To carry out drug excipient compatibility studies.
* To formulate SLN by Hot homogenization / probe sonication method
* To characterize SLN

1. Particle size
2. Surface morphology
3. Entrapment efficiency and drug loading

* To study *In-vitro* drug release.
* To carry out stability study of optimized formulation.

# 3.REVIEW OF LITERATURE

**Monika Scholler - Gyure. *et al.,* (2009),** studied Etravirine, an NNRTI in treatment of HIV – I by generating a barrier and sustaining its antiviral activity given 200mg twice a day, validates modest intersubjective variability and no time dependency. Etravirine has possible interactions effect by inducing and inhibiting CYP3A, CYP2C9 and 2C19 respectively. It’s a minor P-glycoprotein inhibitor. Dose adjusting is not a requirement for the patients having impaired i.e. mild - moderate renal (10).

**Jawahar. N, *et al..,* (2012),** reported solid lipid nanoparticles have formulated with physiologically well-known lipids. To prepare nanoparticles some materials have been captured, varying such as water insoluble as well as soluble drugs, proteins along peptides. The advantage of using this technique mainly to enhance the therapeutic action of poor in-soluble drugs. The present work reviewed and discussed on the properties such as physical and chemical of solid lipid nanoparticles, mechanism in lymph’s, preparation techniques, lipids *in-vivo* outcome with possible medicinal uses (5).

**Surajit Das, *et al..,* (2010),** have described on the latest improvements in nanoparticles with lipids for the oral delivery. Mainly for the lipophilic molecules lipid nanoparticles are used as solid matrix to prolong gastrointestinal absorption also oral bioavailability. Different preparation and evaluation parameters, drug

incorporation types, how well the preparation is stable and stored in suitable condition of the preparations will be studied (3).

**Khaled M Hosny*et al*., (2014),** have discussed the sildenafil citrate as SLN through hot homogenization by way of ultra-sonication method. Solubility studies were screened for various lipids and studied its effect like type and concentration of surfactant, homogenization time, ultra-sonication time. Particle size, zeta potential and encapsulation efficiency were characterized. *In-vitro* drug release, stability and In-vivo pharmacokinetics were discussed (15).

**VivekMakwana*et al*., (2015),** have performed SLN formulation. The Efavarinz SLN preparation was formulated with Gelucire 44/14, Compritol 888 ATO, Lipoid S 75 and Poloxamer188 by a hot homogenization technique and ultra-sonication process. No change was observed in physical properties of product with respect to particles after storage (16).

**M.R.Ajialex*et al*.,(2010),** have demonstrated a Lopinavir loaded solid lipid nanoparticles. SLN with mean particle size, polydispersity index and surface electrical charge were prepared by hot homogenization process and by ultra- sonication. The percentage bioavailability was enhanced. The accelerated stability studies states that there was no change in the mean particle size and PDI even after storage (17).

**Heba a. Hazzah*et al..,* (2015),** have developed Curcumin (Cur) solid lipid nanoparticles. Cur SLN are prepared via method of hot homogenization, by usage

of different types of lipids such as, Gelucire 39 / 01, Gelucire 50 / 13, Precirol, Compritol, and Poloxamer as a surfactant. The microbiological studies were performed for the preparations (18).

**H. R. Kelidari. *et al..,* (2016),** have described, Solid lipid nanoparticles and Nano structured lipid carriers (NLC s) formulations are examined for rise in dissolution paces of poorly water soluble drug spironolactone. SLN and NLC are prepared by Probe ultra-sonication method. All NLC s have solid and liquid lipids such as stearic acid and oleic acid prepared and optimized. And characterized. NLC s preparation is suitable formulation compared to all nanoparticle preparations (19).

**Anand Kumar Kushwaha, *et al*.., (2013)** have described solid lipid loaded Raloxifene. An enhancement in the bioavailability, nanoparticles prepared using solvent emulsification with Compritol as carrier also using Pluronic as surfactant and altered the surfactant concentration, and homogenization speed were preferred as process variables for optimization and characterization for physical state. *In- vitro* are biphasic following Higuchi model kinetics. By studying pharmacokinetics, the SLN loaded Raloxifiene had 5 times higher release than pure Raloxifene (20).

**Makarand Suresh Gambhire*et al..,* (2011),** discussed on loaded solid lipid nanoparticles with dithranol (DTH) were prepared via pre-emulsion along with ultra-sonication using 32 optimizations and evaluated. physical characteristics for DTH-loaded SLN characterized. *Ex-vivo* studies indicated a twice increase in optimized formulation compared to marketed preparation (21).

**R. Gardouh*et al..,* (2013),** prepared solid nanoparticles by ‘high - shear hot homogenization’. The physicochemical properties were characterized. The stability studies were discussed by thermal measures and infrared spectroscopy. Thermal analysis results signify the solubilization of drugs within lipid matrix. particle size of Nano range has excellent encapsulation effect, also great loading capacity to model drugs as Erythromycin base, Tri amcinoloneacetonide, Di benzoyl peroxide is used for the method (22).

**MangeshBhalekar*et al..,* (2017),** performed on the bioavailability problems, in related with solubilization effect of drug and steady dispersal outcomes, surfactants and lipids are being selected and formulated viz, hot-homogenization. Optimization of variables like the concentration of lipid, surfactant oil, time of homogenization effecting on entrapment, and particle size studied and characterized by SEM, DSC, and PXRD and there are no changes in stability (23).

# DRUG PROFILE

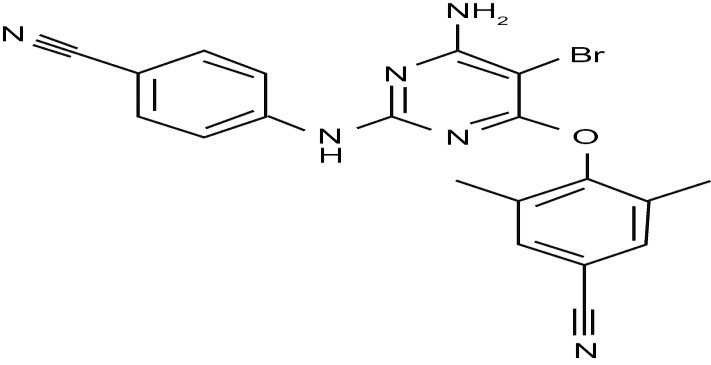
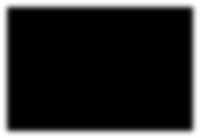
## ETRAVIRINE (24)

**Category:** Antipsychotic agent

**Description:** Etravirine is an antiretroviral agent characterized as NNRTI to treat HIV – I i.e. Human Immune Deficiency virus infection.

**Molecular formula:** C20 H15 Br N6 O

### Chemical structure:



**Solubility:**water – insoluble, propylene glycol and ethanol – slightly soluble,tetra hydro furan and dimethyl form amide – soluble.

### PHYSICAL PROPERTIES:

**Color:** off white, non-hygroscopic, crystal- line powder.

**Melting point:** 260 °C

**Log p:** >5

**Pka:** <3

**Molecular weight:** 435 Da

### Mechanism of action:

Etravirine is a diary pyrimidine (DAPY) that binds to the reverse transcriptase enzyme by its conformational isomerism property allows great interaction even there is a mutation.

### PHARMACOKINETICS

**Bioavailability:**90 **Absorption**: Oral 2.5-4hrs

**Metabolism:** Hepatic system by enzymes CYP3A4, CYP2C9 and CYP2C19. **Protein binding:** 99.9% in vitro, 99.6% - albumin, 97.66% - 99.02% - 1-α glycoprotein.

### Adverse effects:

Chronic reactions in skin and hypersensitivity, obesity, diarrhea, no feeling on hands and feet, rashes.

### Uses:

Control of HIV infection with supplement of other medications of HIV, betterment in the immune of body by reducing the amount of virus.

**EXCIPIENT PROFILE (25-27)**

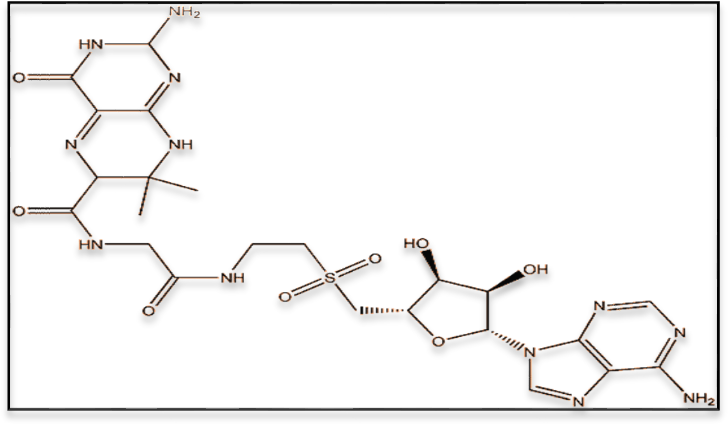
## GELUCIRE 50/13

**Chemical name:** Stearoyl macrogol-32 glycerides.

**Description:** water dispersible surfactant, non-ionic made up of esters of PEG i.e. a fraction of glyceride and free form of PEG, which self emulsifies in aqueous developing dispersion of fine solution forming a micro emulsion also has property of increasing the surface wettability and enhance the solubility of the drug in-vivo and in-vitro.

**Chemical formula:** C6H14O6

### Chemical structure:



**PHYSICAL PROPERTIES:**

**Molecular weight: -** 636.64078

### Physical description Melting point: 50 °C HLB value: 13 Applications:

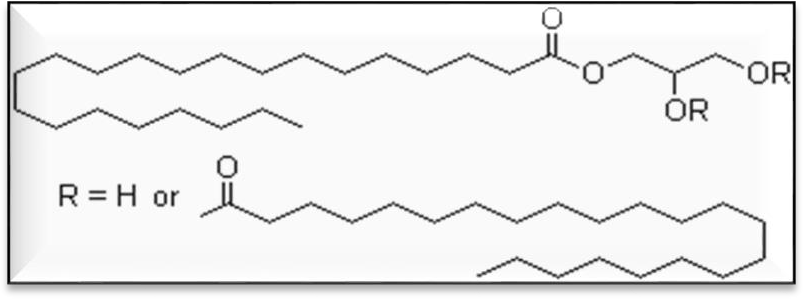
Binder Substance for melt process method, melt granulating and extrusion method, for molding in the capsules made up of hard gelatin, as carrier in tablets, capsule filling, preparing SMEDDS. Bioavailability enhancer: increases solubility of and enables absorption. According to the data of toxicological studies it is safe to use inPharmaceuticalproducts.

## COMPRITOL 888 ATO

**Description:** According to the European Pharmacopeia, compritol is combination of diacylglycerols, the glycerin is esterified through behenic acid in absence of catalyst gives compritol. According to European Pharmacopeia it is also known as glyceryl di behenate made up of the combination of 40 – 60 % of di acyl glycerols along 13 to 21 % of mono acyl glycerols with 21 to 35 % of tri acyl glycerols and the content is 12 – 18 % of mono glycerides.

**Formula:** c69h134o6

### Structure:



**PHYSICAL PROPERTIES:**

**Colour**: white – half white powder with free flowing property, else as hard wax type mass, faint odour, tasteless, does not interact with other excipients used for formulation.

**Molecular weight:** 1059.8 g/m **Melting point:** 65–77 ° C **Water content:** > 0.5%

**Solubility**: dichloromethane and chloroform – soluble by heating. hexane, mineral oil, water and 95% ethanol-insoluble.

### Pharmaceutical applications:

It is used in cosmeceuticals, foods and in medicinal preparations. Lubricant for capsules and tablets, lipid coat excipient in hot melt method, encapsulation, in the formulation including sustain release tablets as a matrix formulator in controlled release of hydrophilic drugs, solid lipid micro particles (SLMs), solid lipid Nanoparticles, carrier for entrapment of water insoluble drugs.

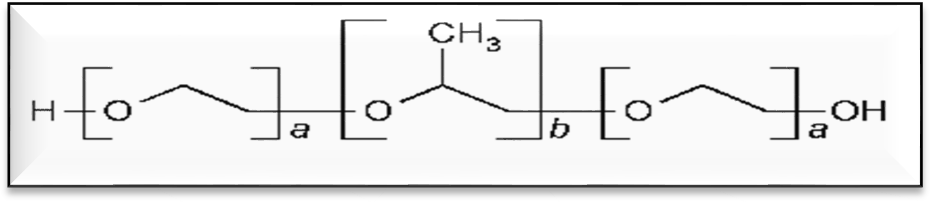
### Storage conditions:

Under 35° C in a tight container and sealed.

### POLOXAMER 188

**Description**: synthetic non-ionic triblock co-polymers, fabricated with hydrophobic chain in Central of Poly oxy propylene linked with two hydrophobic chains of polyoxyethylene.

### Structure:



**PHYSICAL PROPERTIES:**

**Color:** white to half-white, waxy particles, microbeads or flecks.

**Solubility**: 96% Ethanol and water - soluble

### pH (aqueous solution): 5.0-7.5 Melting point: 52-57 °C Pharmaceutical applications:

Emulsifier, solubilize, stabilizer, wetting agents in semi solid as well as binders and Coating material for tablets, improves therapeutically in the functioning of heart in the cardiac disease, to cure constipation as lubricant. Increase the water solubility of lipophilic substances in cosmetics and pharmaceuticals.

### Storage and stability

In closed container and store in a cool and dry place.

### Incompatibility

Poloxamers are incompatible in nature- based on their concentration.

## METHODOLOGY

### Table 1: List of chemicals used

|  |  |  |
| --- | --- | --- |
| **Sl.no** | **List of chemicals** | **Source** |
| **1** | Etravirine | Apotex Research Pvt Ltd |
| **2** | Gelucire 50/13 pellets | Gattefosse SAS |
| **3** | Compritol 888 ATO | Gattefosse SAS |
| **4** | Polaxamer 188 | SdfcPvt Ltd |
| **5** | Concentrated Hydrochloric acid | Sisco laboratories Pvt Ltd, Mumbai |
| **6** | Sodium lauryl sulphate | SdfcPvt Ltd |
| **7** | Dimethyl formamide | Sisco laboratories Pvt Ltd, Mumbai |
| **8** | Dimethane sulfoxide | Sisco laboratories Pvt Ltd, Mumbia |
| **9** | Methanol | Sisco laboratories Pvt Ltd, Mumbai |

**Table 2: List of equipment’s**

|  |  |
| --- | --- |
| **Equipment’s** | **Manufacturers** |
| Digital Analytical Balance | Wensar weighing scales limited, Bengaluru |
| UV Spectrophotometer | Shimadzu UV-1700 PC, Shimadzu corporation, Japan |
| FTIR Spectrophotometer | Jasco 460 plus FTIR Spectrophotometer |
| USP II Dissolution Tester | TDT-08L, Electrolab, India |
| Digital pH meter | Digisum Electronics, Hyderabad |
| Magnetic Stirrer | Remi EquipmentsPvt Ltd, Mumbai |
| Differential Scanning Calorimeter | DSC 60;Shimadzu |
| Homogenizer | Remi EquipmentsPvt Ltd, Mumbai |
| Cooling Centrifuge | Remi Instruments Ltd, Mumbai |
| Membrane Filter | HIMEDIA laboratories Pvt, Ltd, Mumbai |
| Dhona balance | Servewell instruments Pvt Ltd, Bengaluru |
| Poly dispersibility and Zeta Potential analyzer | Malvern zeta sizer instrument |
| Differential scanning Calorimetry | DSC-60, Shimadzu |

**5. METHODOLOGY**

**5.1 PREFORMULATION STUDIES**

* 1. **(a) Melting point determination**

Seal capillary tube on one side and then add the sample in capillary tube tied to the thermometer and place in the Thiele tube which is immersed in liquid paraffin. Slightly heat the Thiele tube with stirring and maintain the temperature. Note the temperature when the sample starts melting and at the temperature at which sample gets melted.

### Determination of λ max of Etravirine 0.01M HCl with 1% SLS

For stock solution (1000 μg/ml) dissolve accurately weighed 10 mg of Etravirine in

10 ml of organic solvent. An aliquot sample was pipetted out and diluted approximately. The standards solutions were scanned using UV visible spectrometer over the wavelength between 200-400 nm and the maximum absorption was recorded.

### Calibration curve of Etravirine

Accurately weigh 10 mg of Etravirine and dissolve in methanol and sonicated till drug is dissolved and make up the volume with methanol to obtain a primary stock of 1000μg/ml. From stock solution pipette 1ml and made up to 10ml with methanol to produce a secondary concentration of 100μg/ml. From secondary stock aliquots of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml were diluted to 10 ml using HCl containing 1% SLS to get resultant concentrations of 3-10μg/ml respectively. The dilutions were

measured at 315 nm, UV spectrometer, using 0.01M HCl with SLS as a blank. The standard graph was obtained by plotting the absorbance against concentration.

### Solubility study of Etravirine in lipids (18,28)

For saturation solubility study of ETR solid lipids such as Compritol 888 ATO (COM), Gelucire 50/13, stearic acid, and glyceryl monostearate (GMS) were used to determine the Etravirine in different lipids. The specific amount of lipid was taken in a vial and melted at temperature greater its melting point on a hot plate. Increments of 10 mg ETR were added to the melted lipid and vortexed until the complete Etravirine was solubilized in the molten lipid. Amount of drug solubilized in the lipid was noted.

### Fourier Transform Infrared Spectroscopy (29)

Drug excipient interactions were carried out by FT IR spectroscopy. A small amount of the sample was mixed with the KBr and triturated for uniform mixing. The blank was done using dried KBr. The prepared mixture was placed and spectra were determined in the range of 400 to 4000 cm-1by using FT IR.

### FACTORIAL DESIGN BY 23 FULL FACTORIAL DESIGN

As per 23 Eight formulations ET1 to ET8 as shown in table 4 were formulated. Two dependent variables, and three independent variables were selected, as shown in table

3. And formulations with and two levels of each factor were prepared according to a 23full factorial experimental table 5.

### Table 3: Designing the formulation using 23 factorial design

|  |  |  |  |
| --- | --- | --- | --- |
| **Independent variables**  **(factors)** | | **Dependent variables**  **(responses)** | |
| A | Effect of lipid ratio (COM: GEL), gms | Y1 | Percentage yield (%) |
| B | Effect of surfactant  concentration (Poloxamer 188), % | Y2 | Encapsulation (%) |
| C | Effect of sonication time, min | Y3 | *In-vitro* cumulative release (%) |

* 1. **(a)FORMULATION OF SOLID LIPID NANOPARTICLES (15,17,19)**

Hot homogenization technique was followed for preparation of SLN and by probe ultra-sonication. The SLN formulation was prepared using Gelucire 50/13 and Compritol 888 ATO as lipids and Poloxamer 188 as Surfactant. A weighed amount of solid lipids was taken and melted at 70°C on a hot plate and followed by the addition of drugs until drug does not go into lipid solution. An aqueous phase was prepared separately in a beaker by dissolving Poloxamer 188 in distilled water and heated up to 70°C. Once the lipid mixture melts the hot surfactant aqueous solution was added to the molten lipid and homogenized at 15000 rpm for 10 min by using IKA ultra-turrax, during this homogenization process temperature remain constantat70°C.The o/w emulsion was ultra-sonicated by probe sonicator at 100W for 5 and 10 min. The obtained milky Nano emulsion was cooled down to room temperature to form SLN suspension. The Nano suspension product was centrifuged at 14000rpm and the supernatant solution was separated. After centrifugation, the dispersion of SLN is stored in an airtight container and dried in a vacuum desiccator.

### Table no 4: List of formulation

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Formulation** | **ET1** | **ET 2** | **ET 3** | **ET 4** | **ET 5** | **ET 6** | **ET 7** | **ET 8** |
| **Drug**  **Etravirine (mg)** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **COM:GEL (gm)** | 1:1 | 1:1 | 1:2 | 1:2 | 1:1 | 1:2 | 1:1 | 1:2 |
| **Poloxamer 188**  **(%)** | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 1 |
| **Distilled water**  **(ml)** | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| **Sonication time**  **(min)** | 5 | 10 | 10 | 5 | 10 | 5 | 5 | 10 |

As per 23 design eight formulations were prepared. The high and low design points in coded and un-coded standards for the three factors are shown in Table 5 & 6. The code plus and minus 1 indicates high and value of the independent variables.

### Table no 5: coded levels for the formulation used by 23 factorial design

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Formulation** | **Drug Etravirine** | **Lipid ratio COM:GEL** | **Surfactant**  **Poloxamer 188** | **Distilled water** | **Sonication time** |
| **ET 1** | 1 | -1 | -1 | 1 | -1 |
| **ET 2** | 1 | -1 | -1 | 1 | +1 |
| **ET 3** | 1 | +1 | +1 | 1 | +1 |
| **ET 4** | 1 | + | +1 | 1 | -1 |
| **ET 5** | 1 | -1 | +1 | 1 | +1 |
| **ET 6** | 1 | +1 | -1 | 1 | -1 |
| **ET 7** | 1 | -1 | +1 | 1 | -1 |
| **ET 8** | 1 | +1 | -1 | 1 | +1 |

**Table 6: Independent variables and levels**

|  |  |  |
| --- | --- | --- |
| **Factor** | **Coded value** | |
| **-1** | **+1** |
| **Lipid ratio (Gms)** | 1:1 | 1:2 |
| **Surfactant concentration** | 1% | 2% |
| **Sonication time** | 5 min | 10 min |

**(b) Statistical study of responses by Design expert (7,30)** Factorial design allows all factors to altered at a time, permitting quantification of the effects affected by independent variables and interactions between them. 23 factorial design was used for was used for the analysis of effect of each variable on the designated response (Design Expert 12.0) software was used for statistical analysis for ANOVA. Pareto charts were made for the analysis of each response coefficient for its statistical significance. The suggestive response linear equations were created by Design Experts, and treated to justify the statistical data. Response surface plots were generated to imagine simultaneous effect of each variable on each response parameter.

### PHYSICOCHEMICAL CHARACTERIZATION OF SLN

* 1. **(a) Percentage yield (31)**

Percent yield is the fraction of actual yield to the theoretical yield. The percentage yield was calculated using the formula:

**% Yield=** Actualyield

Theoretical yield

× 100

Where,

Actual yield= Actual amount recovered after preparation.

Theoretical yield= It is the yield calculated based on ingredients added in preparation.

### b) Drug content (32)

The prepared SLNs were dissolved in DMF in a 10 ml volumetric flask and make up the volume. From this solution, 1 ml was taken and diluted with 0.01 M HCl with SLS in a volumetric flask and then sonicated for 30 min then filtered using a 0.45 m membrane filter. The absorbance of solution was measured at 315nm using appropriate blank. The drug content of SLNs was calculated using a calibration curve.

Concentration = 𝐴𝑏𝑠𝑜𝑟𝑏𝑠𝑛𝑐𝑒 𝜇𝑔⁄𝑚𝑙

𝑆𝑙𝑜𝑝𝑒

Amount of drug = 𝑐𝑜𝑛𝑐𝑒𝑛𝑡𝑟𝑎𝑡𝑖𝑜𝑛×𝑑𝑖𝑙𝑢𝑡𝑖𝑜𝑛𝑓𝑎𝑐𝑡𝑜𝑟 𝑚𝑔⁄𝑚𝑙

1000

### (C) Percentage encapsulation (33)

The % encapsulation of SLN was determined by the centrifugation method. A fixed quantity of SLN dispersion was centrifuged at 20000 rpm, 25 min. 1ml sample from supernatant solution was diluted using DMSO and further quantified using UV spectrophotometer at 315 nm.

% EE= 𝑤𝑒𝑖𝑔ℎ𝑡 𝑜𝑓 𝑖𝑛𝑡𝑖𝑎𝑙 𝑑𝑟𝑢𝑔−𝑤𝑒𝑖𝑔ℎ𝑡 𝑜𝑓 𝑓𝑟𝑒𝑒 𝑑𝑟𝑢𝑔 × 100

weight of initial drug

* 1. ***IN-VITRO* RELEASE (19)**

Pure drug and formulation *in-vitro* release was performed by USP Apparatus II (Paddle type). using 900 ml of 0.01 M HCl with 1%SLS at 37 °C, 50 rpm. Aliquots (1ml) were withdrawn at regular time intervals and the volume is replaced with fresh

media and filtered by 0.45 45 𝜇m membrane filter. Amount of drug released was analyzed using a UV-visible spectrophotometer λ 315nm.

## CHARACTERIZATION OF SLN

1. **Particle size (34)**

SLNs preparations were dispersed in distilled water and stirred for 5 min to form a dispersion and then measure polydispersity index (PDI) and mean particle size by dynamic light scattering using zeta sizer (Malvern Zetasizer).

1. **Zeta potential (35,36)**

Prepared SLNs are appropriately diluted with double-distilled water. Zeta potential measure-ments (Malvern Zetasizer) were done at 25°C and the electric field strength was approximately 23 V/cm. The zeta potential was calculated from electrophoretic mobility.

### Differential scanning calorimetry (29)

Differential scanning calorimetry (DSC) was analyzed for dry samples of the solid lipid nanoparticles using DSC 60- shimadzu. Samples are accurately weighed and placed on the aluminum pan and heated at 25-300 °C below nitrogen flow. The flow of heat was measured as a sample temperature and used to study the thermal behavior ofnanoparticles.

### Release kinetics (37)

*In-vitro* drug release data obtained from release studies were subjected to various kinetic models like zero order, first order, Higuchi model, korsmeyer- peppas model and Hixson Crowell model. Drug release mechanism is explained in terms of highest correlation coefficient; the highest values of correlation coefficient suggest the release mechanism.

### Table 7: Drug transport mechanism

|  |  |
| --- | --- |
| **slope (n)** | **Transport mechanism** |
| **0.5** | Fickian Diffusion |
| **0.5<n<1** | Non-Fickian Diffusion |
| **1** | Case II Transport |
| **n>1** | Super case II Transport |

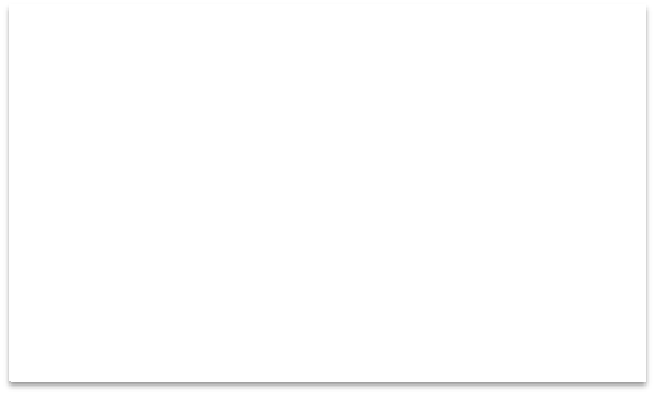
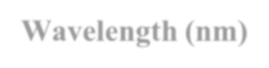
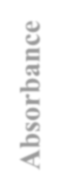
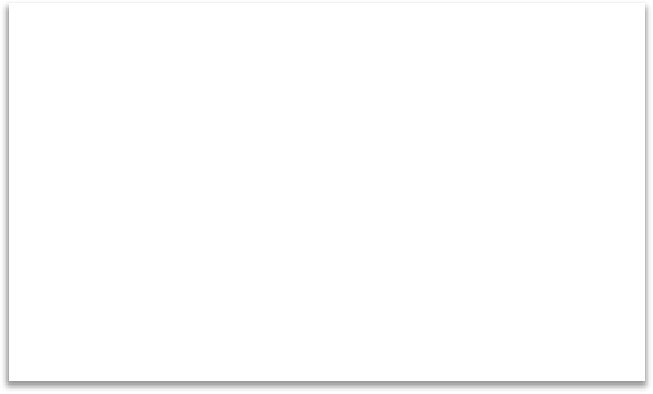
1. **RESULTS AND DISCUSSION**
   1. **PREFORMULATION STUDIES**
   2. **(a) Determination of melting point (9)**

The melting point was observed at 250 ±1.0°𝐶 and it was similar to the reported value 260 °𝐶.

### λ max of Etravirine

Peak was observed at 315 nm in 0.01M HCl as shown in fig.2

### Figure 2: U V spectrum of Etravirine in 0.01M HCl with 1% SLS



0.25

0.2

0.15

0.1

0.05

315nm

0

0

100

200

300

400

500

**Wavelength (nm)**

**Absorbance**

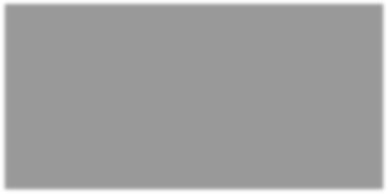
1. **Standard curve of Etravirine**

The standard curve was observed to be linear over a conc. 3-10 μg/ml as shown in table 8, with a regression value of 0.995 as shown in fig 3.

### Table 8: Etravirine calibration curve data

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl.no** | **Conc. (μg/ml)** | **ABSORBANCE** | | | **Average Absorbance** | **STD**  **Dev** | **Error** |
| **Trail 1** | **Trail 2** | **Trail 3** |
| 1 | 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2 | 0.3 | 0.214 | 0.264 | 0.248 | 0.242 | 0.026 | 0.015 |
| 3 | 0.4 | 0.226 | 0.302 | 0.322 | 0.283 | 0.051 | 0.029 |
| 4 | 0.5 | 0.354 | 0.387 | 0.379 | 0.373 | 0.017 | 0.010 |
| 5 | 0.6 | 0.439 | 0.454 | 0.455 | 0.449 | 0.009 | 0.005 |
| 6 | 0.7 | 0.532 | 0.541 | 0.558 | 0.544 | 0.013 | 0.008 |
| 7 | 0.8 | 0.645 | 0.652 | 0.658 | 0.652 | 0.007 | 0.004 |
| 8 | 0.9 | 0.719 | 0.707 | 0.732 | 0.719 | 0.013 | 0.007 |
| 9 | 1 | 0.793 | 0.785 | 0.781 | 0.786 | 0.006 | 0.004 |

**Figure 3: Calibration curve of Etravirine**



**Standrad curve of Etravirine using 0.01 M HCl with SLS**

0.9

0.8

0.7

0.6

0.5

0.4

0.3

0.2

0.1

0

y = 0.7836x

R² = 0.995

0 0.2 0.4 0.6 0.8 1 1.2

**Concentration (µg/ml)**

**Absorbance**

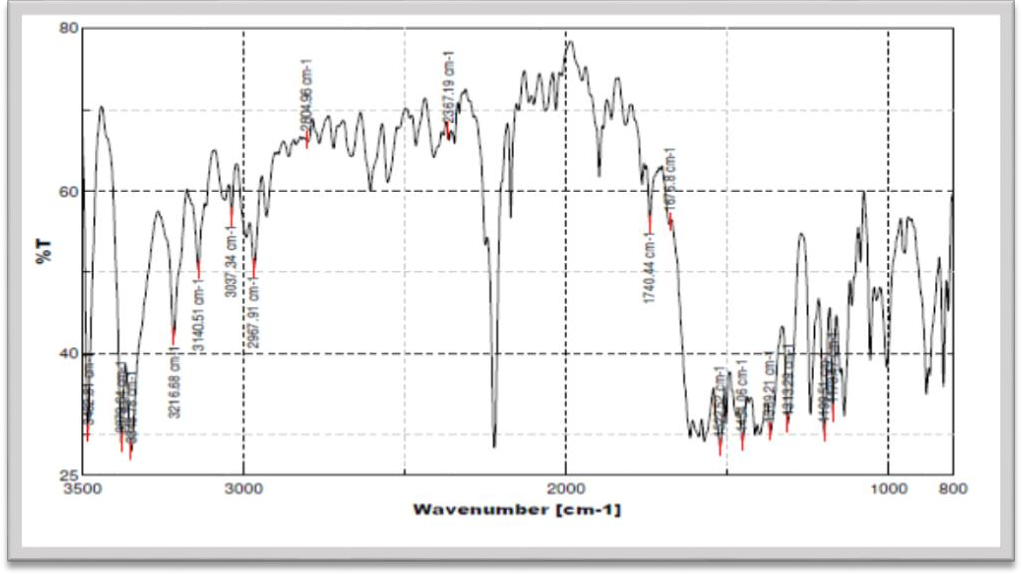
* 1. **COMPATIBILITY STUDIES FOR DRUG AND THE EXCIPIENTS FT-IR (38)**

The interaction of the drug was performed by the FT-IR method and spectra of Etravirine as shown in fig. 4, which was related to average functional group frequencies of Etravirine is shown in Table 9. There is no interaction between the drug and the excipients as shown in fig.5.

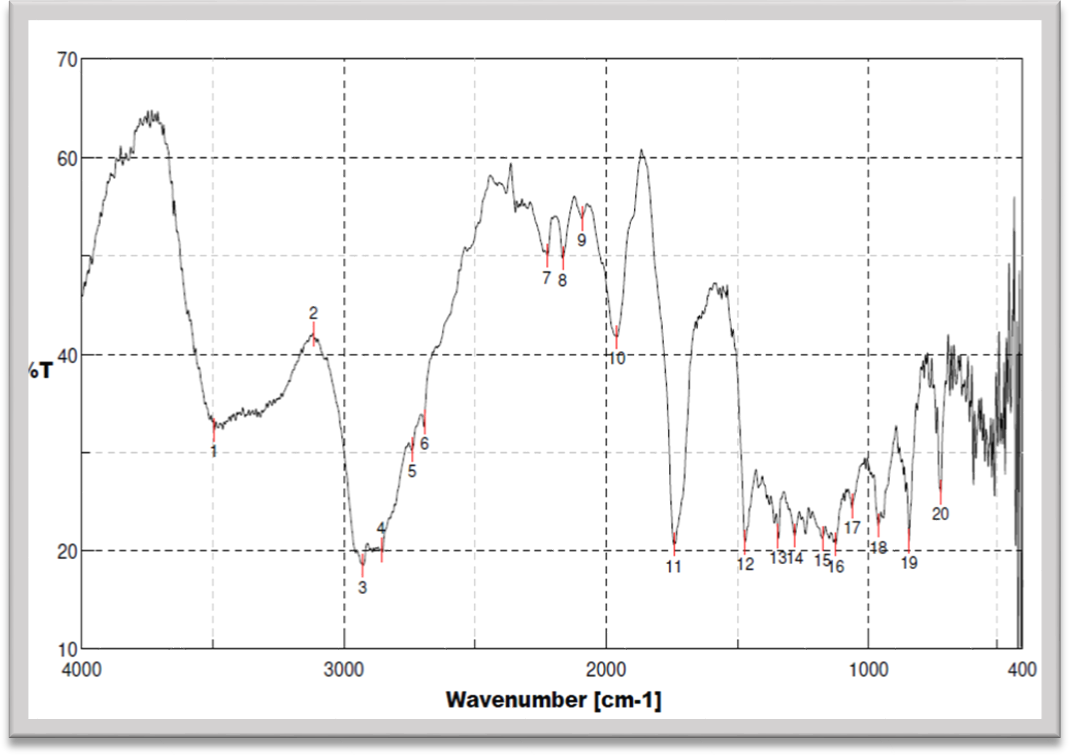
### Table 9: Interpretation of FT-IR spectra

|  |  |  |
| --- | --- | --- |
| **Functional groups** | **Required frequencies** | **Observed frequencies** |
| Aromatic 1° amine stretches | 3410.26 | 3216.68 |
| Aryl C𝑁 stretching | 2367.86 | 2367.19 |
| Aromatic C-H stretching | 2978.86 | 2967.91 |
| 1° & 3° amine stretching | 1365.65 | 1369.21 |
| Ether C-O-C stretching | 1188.19 | 1199.51 |

**Figure 4: FT IR Spectra of Etravirine**



**Figure 5: FT IR Spectra for optimized formulation ET 3**



* 1. **Saturation solubility of Etravirine in different lipids**

Four different solid lipids, namely Compritol 888 ATO, Gelucire 50/13, Glycerol monostearate, and stearic acid has been used to determine the saturation solubility of Etravirine as shown in table 10.

Gelucire 50/13 and Compritol 888 ATO were selected for the formulation of SLN. Gelucire 50/13 (stearoylmacrogol glycerides) was selected for the formulation of SLN because it is an amphiphilic lipid with a high HLB value of 13 and it enhances the solubility and also used as a stabilizer of poorly water-soluble drugs. Compritol 888 ATO (Glycerol Behenate) which is amphiphilic material with melting point of (~70°C) is used for the preparation of SLN. Due to its complex

disposition and less arrangement in the crystal lattice, hence leaving, more space to drug to be settled (19,28).

### Table 10: Saturation Solubility of Etravirine in different lipids

|  |  |  |
| --- | --- | --- |
| **Lipids** | **Melting point** | **Solubility in mg/ml** |
| Gelucire 50/13 | 43° C | 100 mg |
| Compritol 888 ATO | 70 ° C | 50 mg |
| Stearic acid | 69.3 ° C | Not more than 10 mg |
| Glyceryl monostearate | 57.65 ° C | Not more than 10mg |
| COM: GEL | 70° C | 100mg |

* 1. **Characterization of SLNs**

Percentage yield, drug content (%), and % encapsulation of formulations F1 to F8 were determined and is presented in Table 11. The high % encapsulation, drug content, and % yield as shown in table 11. This may be due to high lipid ratio and the surfactant concentration that enhances the solubility of drugs and so loading of them into SLN. Higher HLB values will enhance the encapsulation efficiency depending on the reduction of interfacial tension and enhance the solubilization of poorly water soluble drugs (34).

### Table 11: percentage yield, Drug content and Entrapment Efficiency of formulations ET1 - ET8

|  |  |  |  |
| --- | --- | --- | --- |
| **Formulation** | **% yield** | **Drug content (%)** | **% Encapsulation** |
| ET 1 | 63 | 62.01± 0.98 | 58.4 ±1.20 |
| ET 2 | 69 | 59.33±1.10 | 55.6 ±1.15 |
| ET 3 | 83 | 90.13±1.73 | 87.1 ±1.57 |
| ET 4 | 75 | 90.78±0.52 | 89.3 ±1.29 |
| ET 5 | 76 | 70.65±1.15 | 65.5 ±1.59 |
| ET 6 | 70 | 85.02±1.57 | 81.8 ±1.16 |
| ET 7 | 70 | 75.35±1.64 | 70 ±1.41 |
| ET 8 | 73 | 75.21±1.20 | 70 ±1.75 |

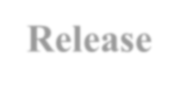
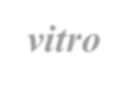
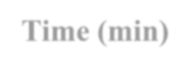
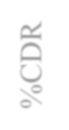
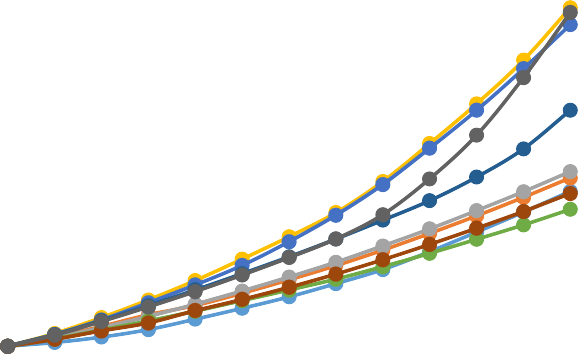
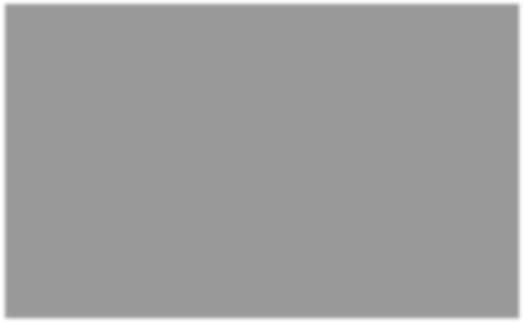
* 1. **Dissolution studies**

Formulations were evaluated for *In-vitro* release. The results are listed in Table 12. Results show the significant differences among different formulations because of long fatty acid chains of the lipid, having greater lipophilic property, and a high melting point. It has shown controlled release. The maximum release was shown in the ET 3. The % cumulative drug release plot was shown in fig.6.

### Table 12: Dissolution profile of formulations

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Time (min)** | **Cumulative % drug release** | | | | | | | | |
| **Pure**  **drug** | **ET 1** | **ET 2** | **ET 3** | **ET 4** | **ET 5** | **ET 6** | **ET 7** | **ET 8** |
| 30 | 0.482 | 1.149 | 1.080 | 1.655 | 1.517 | 0.957 | 1.487 | 0.896 | 1.448 |
| 60 | 1.180 | 2.564 | 2.395 | 3.701 | 3.386 | 2.073 | 3.289 | 1.984 | 3.195 |
| 90 | 2.148 | 4.065 | 3.840 | 5.978 | 5.600 | 3.272 | 5.229 | 3.001 | 5.074 |
| 120 | 3.490 | 5.251 | 5.435 | 8.486 | 7.912 | 4.534 | 7.267 | 4.589 | 7.058 |
| 150 | 4.893 | 6.839 | 7.138 | 11.247 | 10.429 | 5.880 | 9.368 | 6.039 | 9.190 |
| 180 | 6.368 | 8.567 | 8.943 | 14.126 | 13.489 | 7.254 | 11.544 | 7.611 | 11.43 |
| 210 | 8.081 | 10.399 | 10.850 | 17.245 | 16.907 | 8.659 | 13.837 | 9.311 | 13.840 |
| 240 | 9.889 | 12.399 | 12.951 | 21.263 | 20.864 | 10.270 | 16.263 | 11.162 | 16.982 |
| 370 | 12.210 | 14.557 | 15.142 | 26.160 | 25.5447 | 11.984 | 18.785 | 13.143 | 21.605 |
| 300 | 14.725 | 16.822 | 17.502 | 31.283 | 30.437 | 13.8 | 21.822 | 15.229 | 27.235 |
| 330 | 17.302 | 19.212 | 19.949 | 36.915 | 35.806 | 15.650 | 25.467 | 17.395 | 34.663 |
| 360 | 19.999 | 21.731 | 22.541 | 43.689 | 41.502 | 17.667 | 30.461 | 19.706 | 43.062 |

**Figure 6: Drug release for all formulations**



***In-vitro* Release**

50

45

40

35

30

25

20

15

10

5

0

pure drug

ET1 ET2 ET 3

ET 4

ET 5

ET 6

ET 7

ET 8

0 100

200

**Time (min)**

300

400

%CDR

* 1. **Optimization data analysis and model validation**

The objective of the study was to formulate solid lipid nanoparticles by a hot homogenization method and to analyze the effects of design variables on response factors. In order to optimize the prepared formulations, dependent variables, and independent variables were selected, as shown in table 3. These three factors may affect the nanoparticle formulation and two levels of each factor were prepared according to a 23full factorial experimental table 5.

Lower and upper design points in coded and un-coded values for the three factors are shown in Table no 3 & 4. The (+)1 and (-)1 indicate the high and low value of the independent variables. The ranges of responses were 63-83%, 55-90%, and 19- 43%. All the observed responses for all formulations prepared by Design-Expert softwareareshownintable13.

### Table 13: 23 Full-Factorial Design and Response factors

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Factors** | | | | **Responses** | | |
| **STD** | **Run** | **Lipid ratio (Gms)** | **Surfactant conc. (%)** | **Sonication time (minutes)** | **Yield (%) Y1** | **Encapsulation (%)**  **Y2** | **% Drug release Y3** |
| 1 | 1 | -1 | -1 | -1 | 63 | 58.4 | 21.73 |
| 5 | 2 | -1 | -1 | +1 | 69 | 55.6 | 22.50 |
| 8 | 3 | +1 | +1 | +1 | 83 | 87.1 | 43.68 |
| 4 | 4 | +1 | +1 | -1 | 75 | 89.3 | 41.50 |
| 7 | 5 | -1 | +1 | +1 | 76 | 65.5 | 17.66 |
| 2 | 6 | +1 | -1 | -1 | 70 | 81.8 | 30.47 |
| 3 | 7 | -1 | +1 | -1 | 70 | 63.9 | 19.70 |
| 6 | 8 | +1 | -1 | +1 | 73 | 79 | 43.05 |

Multiple linear regression analysis for 23was done to estimate the effect of factors on responses by generating polynomial equation:

### Y= β0+ β1A+β2B+β3C+β12AB+β23 BC+β13AC+β123ABC

Where, Y is the response parameter for each factor level; β0 is an intercept. A B and C are the coded levels of independent variables. The polynomial equation was used to determine the coefficients and the mathematical sign. A positive and negative sign implies a synergistic effect and an antagonistic effect.

To identify the best fit model, results were subjected to a number of regression models; Linear, 2FI, and 3FI in the design experts. Based on the P value, R2 value

predicted R2 value and predicted residual sum of Square (PRESS) value, the appropriate model was observed to be a linear model represented by the equation,

### Y= β0+ β1A+β2B+β3C

From this result, it was also understood that the interaction effect on responses is not significant. The coefficients of the linear equations were produced by Design expert.12 for all the responses (Y1, Y2, Y3) and are given below.

### Coded linear equation generated for the responses are shown below,

**Y1= 72.38+2.87A+3.62B+2.87C Y2= 72.58+11.72A+3.87B-0.77C Y3= 30.04+9.64A+0.59B+1.69C**

**The linear equation for the actual codes for the factors on the responses Y1, Y2, Y3 are given as,**

**Y1**=72.38+ 2.87 Effect of lipid ratio+ 3.62 Effect of surfactant concentration+ 2.87 Effect of sonication time

**Y2**= 72.58+11.72Effect of lipid ratio +3.87Effect of surfactant concentration - 0.775Effect of sonication time

**Y3**= 30.04+9.64Effect of lipid ratio +0.598787Effect of surfactant concentration

+1.69Effectofsonicationtime

### Table 14: Coefficient values of the factors for all the responses

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Responses** | **Interce pt** | **A** | **B** | **C** | **p-value** | **significance** |
| % yield | 72.38 | 2.87 | 3.62 | 2.87 | **0.0013** | significant |
| % Encapsulation | 72.58 | 11.72 | 3.87 | 0.77 | **<0.001** | significant |
| % Drug release | 30.04 | 9.64 | 0.59 | 1.69 | **0.0256** | significant |

ANOVA is to define the significance and magnitude effects of the models on responses. **P<**0.0500 indicates model terms are significant. P>0.100 indicate the model terms are not significant. The model F-value implies that the model is significant and it indicates the adequate signal and can be used to direct the design space (21). R2 measures the proportion of variation in the dependent variables with independent variables for a linear regression model (39). Adjusted R2 is the statistic based on the number of the independent variables in the model. The PRESS indicates the selected model. If the probability value <0.05, PredictedR² is an insensible arrangement with difference of <0.2 with Adjusted R²and Adequate Precision i.e. signal to noise ratio <4 the model is known as significant (40).

From thegenerated linear equation and its coefficient values as shown in table 14. It is observed that three independent variables namely A (Lipid ratio), B (surfactant concentration), and C (sonication time) had a positive effect on percentage yield (Y1) and In-vitro drug release (Y3) but, a negative effect on encapsulation efficiency (Y2). From these values and polynomial equations of response, it was

observed that the main effect of independent variables (A) is a positive significant effect on all the response variables Y1, Y2, Y3. This indicates that the lipid ratio has a significant effect on percentage yield, % encapsulation, and % drug release with a coefficient value of 2.87,11.72 and 9.64.

The P values, R2, adjusted R2, predicted R2, precision value, and F values are given in Table 15. ANOVA for Response 1, 2, and 3 (percentage yield, % encapsulation, and % drug release) are shown in Table 16. As observed from Table 16, model 𝐹-values of all three responses 48.69, 242.28, and 9.85indicates that the models are significant. There is only a 0.01% chance that a “model 𝐹 value” for Response 2, 0.13% for Response 1, and 2.56 % for Response 3 could occur due to noise.

### Table 15: Regression analysis

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Reponses** | **R2** | **Adjusted R²** | **Predicted R2** | **Precision** | **SD** | **% CV** | **p** |
| **% Yield**  **(Y1)** | 0.97 | 0.9534 | 0.8934 | 26.00 | 1.27 | 1.76 | 0.0013 |
| **%**  **Encapsulation (Y2)** | 0.99 | 0.9904 | 0.9781 | 26.96 | 1.30 | 1.79 | <0.001 |
| **%**  **Drug release (Y3)** | 0.88 | 0.7914 | 0.5232 | 416.22 | 5.10 | 16.98 | 0.0256 |

SD: Standard deviation, CV: Coefficient of variation, *P*: Probability

### Table 16: ANOVA for the measured factors

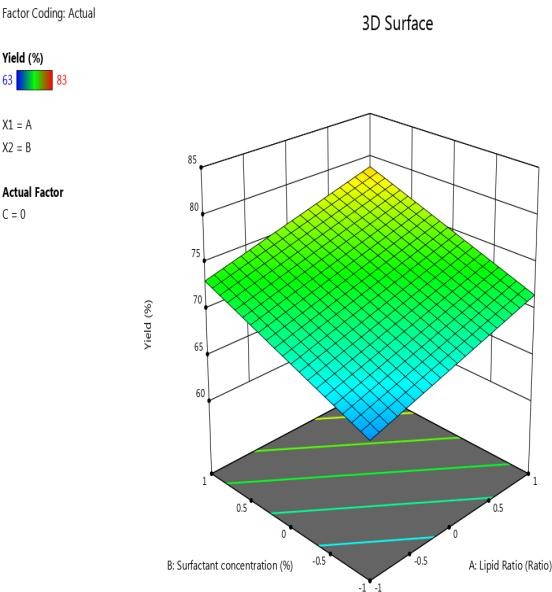
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **df** | **SS** | **MS** | **F** | **Significance** |
| **% Yield** |  |  |  |  |  |
| Model | 3 | 237.38 | 79.13 | 48.69 | Significant |
| Residual | 4 | 6.50 | 1.63 | - |  |
| Total | 7 | 243.83 | - | - |  |
| **% Encapsulation** |  |  |  |  |  |
| Model | 3 | 1224.73 | 408.24 | 242.28 | Significant |
| Residual | 4 | 6.74 | 1.69 | - |  |
| Total | 7 | 1231.74 | - | - |  |
| **% Drug release** |  |  |  |  |  |
| Model | 3 | 768.86 | 256.29 | 9.85 | Significant |
| Residual | 4 | 104.05 | 26.01 | - |  |
| Total | 7 | 872.91 | - | - |  |

df: Degrees of freedom, SS: Sum of square, MS: Mean sum of square, *F*: Fischer’s ratio

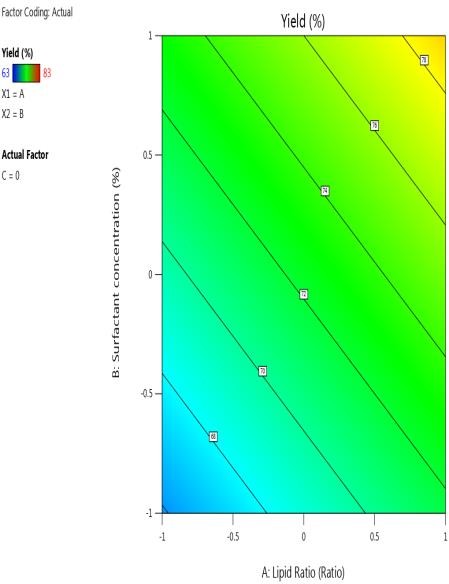
### 6.6 (a) Response surface plot

3D response plots are generated by the software and represented in Figures 7-9 for the responses Percentage yield, % encapsulation, and drug release, respectively.

### Figure 7: (a) 3D and (b) counter plot for percentage yield

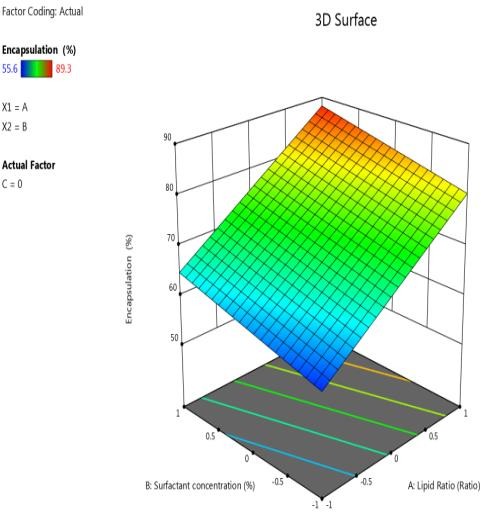


**(a)**

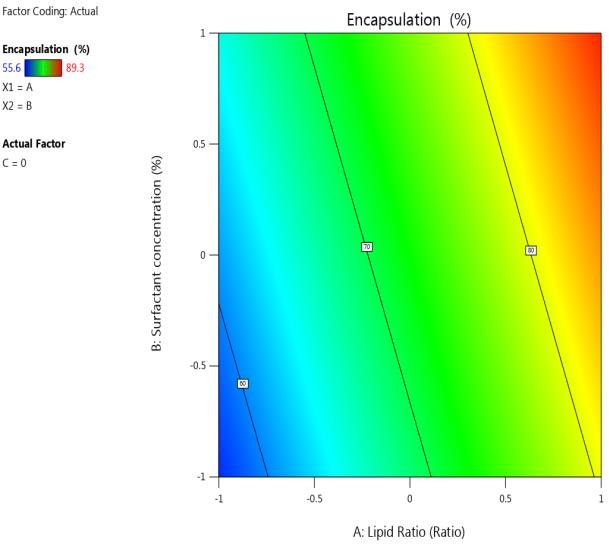


**(b)**

**Figure 8: (a) 3D and (b) counter plot for % encapsulation**

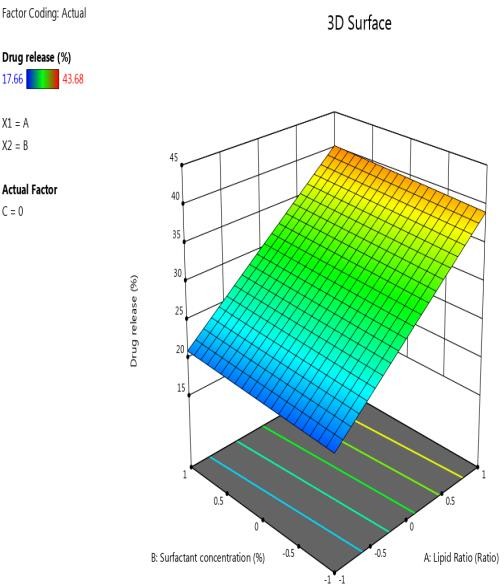


(a)

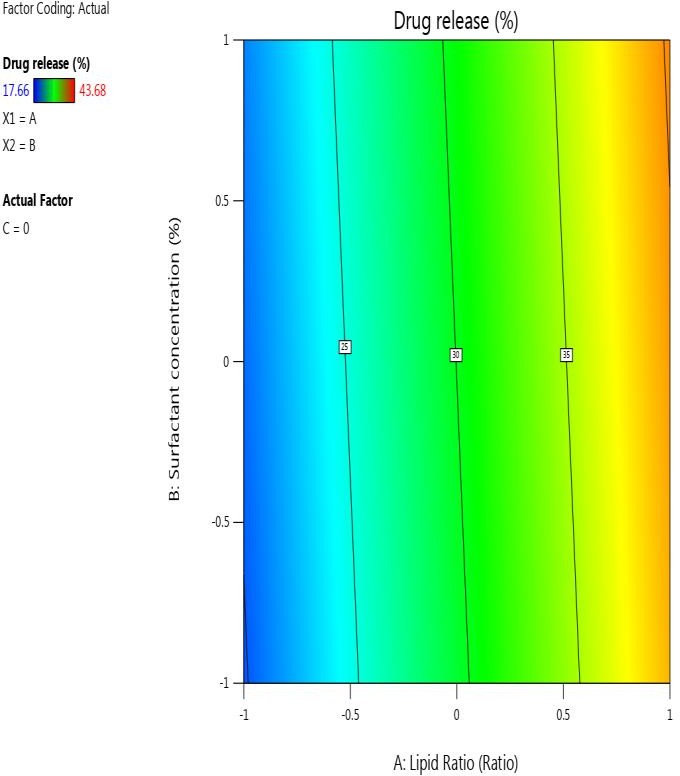


### (b)

**Figure 9: (a) 3D and (b) counter plot for *In-vitro* drug release**



**(a)**



**(b)**

**Figure 7** represents the response surface plot for the effect on the percentage yield. During this study, the volume of the continuous phase and the processing variables such as stirring speed and time remains constant. It is observed that all three independent factors are responsible for the increase in the percentage yield. The % yield is affected by the concentration of lipids and the ratio of COM: GEL mixture. The increase in lipid ratio may increase in % yield (31,37).

**Figure 8** represents the response surface plot for the effect on encapsulation (%). It is observed that an increase in lipid ratio (COM: GEL) and surfactant concentration (poloxamer-188) there is an increase in % encapsulation. This may be due to high lipid concentration and the surfactant concentration that enhances the solubility of drugs and so loading of them into SLN. Also higher HLB value of GELwill enhance the encapsulation efficiency depending on the reduction of interfacial tension and enhances the solubilization of poorly soluble drugs (41-43).

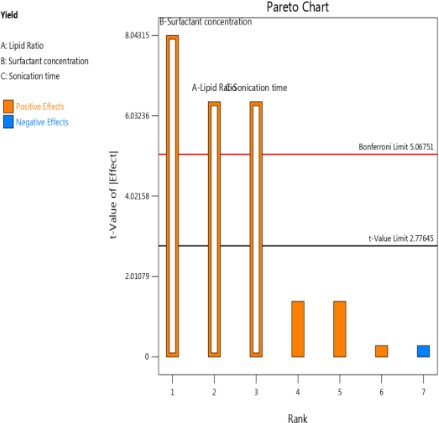
**Figure 9** represents the response surface plot for the effect on the In-vitro release. The lipid type, surfactant concentration, and sonication time are the actual factors for drug release form SLN. An increase in lipid concentration increases the viscosity of the medium and more rigid solidified nanoparticles, it may delay the drug diffusion to the dissolution medium. Particles are stable in varying the surfactant concentration by making a stearic barrier on the surface of the particle and protect form its accumulation. An increase in sonication time decreases the drug release, it may due to its interaction between particles, more aggregation, and increasing the size as well as decreasing its surface area (44,45). The results were

observed that the drug release is mainly affected with the lipid ratio and surfactant in aqueous phase. The drug is lipophilic in nature it diffuses the solid lipid core and shows the sustained release. Change in the lipid ratio, increase in drug release. It is because with the use of different concentrations of lipids used with different chemical natures the results show a significant effect on in-vitro release (21,36).

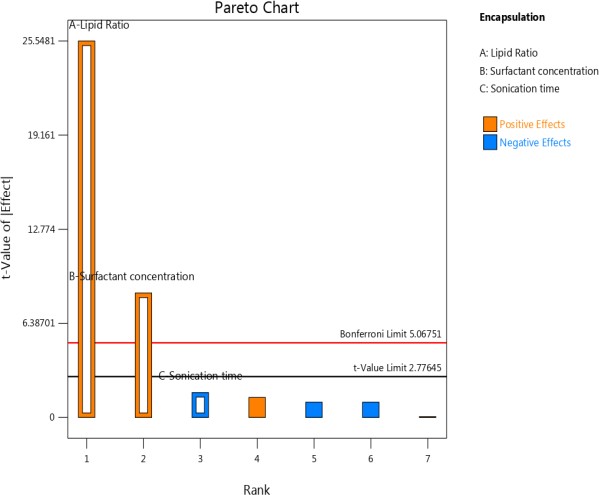
### (b) Pareto chart for all effects

‘t’ value of the effect is analyzed by two limit lines that are Bonferroni limit line and ‘t’ limit line. Coefficient with ‘t’ value between the Bonferroni line and ‘t’ limit line indicates that factor is significant and above the Bonferroni line states that positively significant whereas ‘t’ value is below the ‘t’ limit line it is a statistically insignificant coefficient and not discussed in the analysis (30).

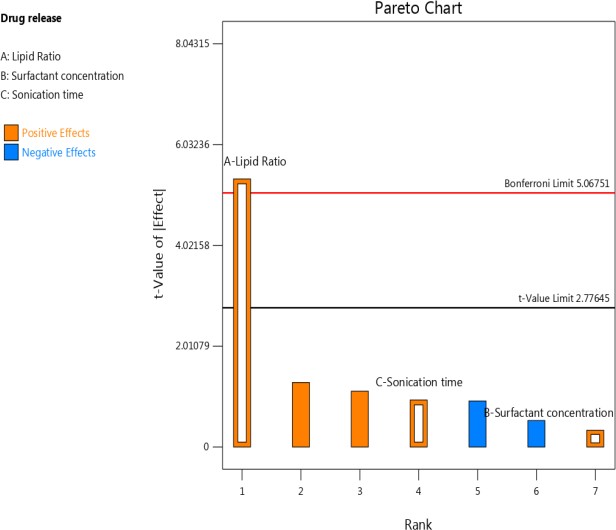
### Figure 10: Pareto chart for % Yield



**Figure 11: Pareto chart for % Encapsulation**



**Figure 12: Pareto chart for % Drug release**



**(c) Optimization and validation**

For generating optimized formulation numerical and graphical optimization was used as a method. Constraints were fixed for both numerical and graphical optimization, by stating the range for independent and dependent variables as shown in table 17.

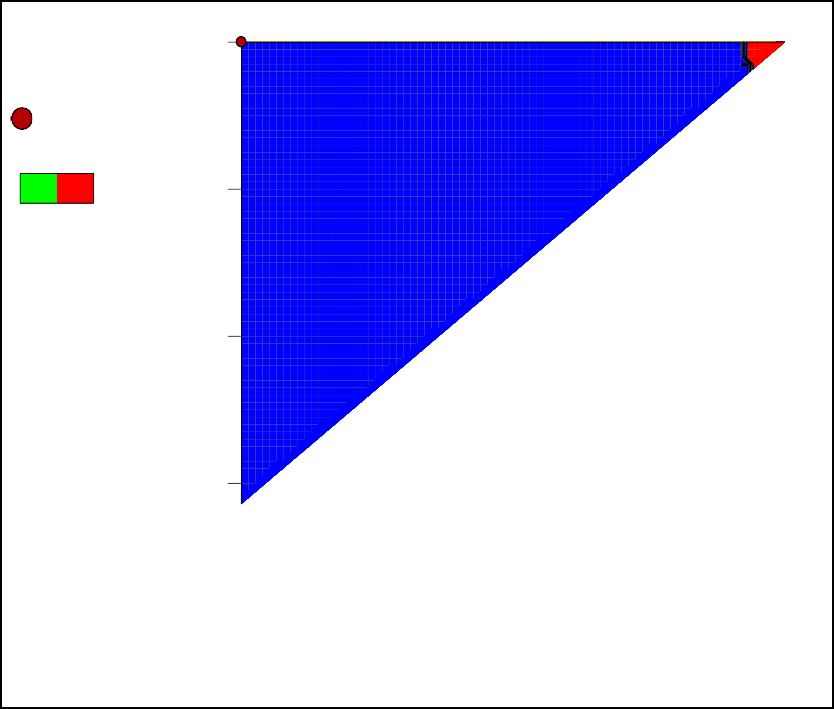
### Table 17: Constraints set for optimization

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Goal** | **Lower Limit** | **Upper Limit** |
| A:Lipid Ratio | Range | -1 | 1 |
| B:Surfactant concentration | Range | -1 | 1 |
| C:Sonication time | Range | -1 | 1 |
| % Yield | Range | 80 | 83 |
| % Encapsulation | Range | 80 | 89 |
| % Drug release | Range | 40 | 43.68 |

Counterplot for the desirability (fig 13) and responses Y1, Y2, and Y3 (figure 14-16), and an overlay plot for responses was generated to identify the design space and to know optimized formulation as shown in fig 17.

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**Figure 13: Counterplot for the desirability**



Factor Coding: Actual

Desirability

1

**Desirability**

Design Points

0

X1 = A X2 = B

1

0.5

**Actual Factor**

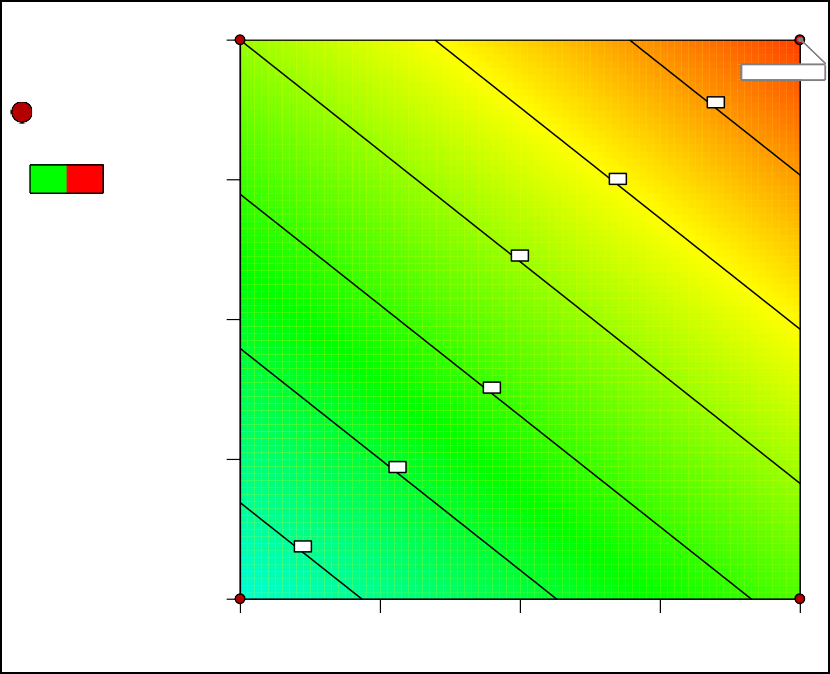
C = 1

0

-0.5

B: Surfactant concentration (%)

**Figure 14: Counterplot for %Yield (Y1)**



Factor Coding: Actual

Yield (%)

1

**Yield (%)**

Design Points

Prediction 81.75

80

63

X1 = A X2 = B

83

0.5

78

76

**Actual Factor**

C = 1

0

74

-0.5

72

70

-1

-1

-0.5

0

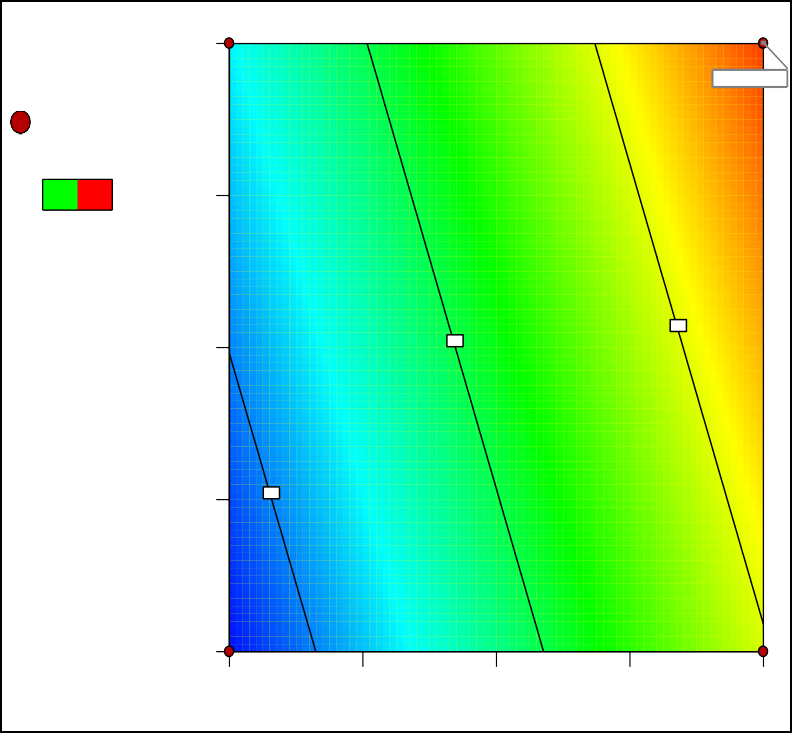
0.5

1

A: Lipid Ratio (Ratio)

B: Surfactant concentration (%)

**Figure 15: Counterplot for %Encapsulation (Y2)**



Factor Coding: Actual

Encapsulation (%)

1

**Encapsulation (%)**

Design Points

Prediction 87.4

55.6

X1 = A X2 = B

89.3

0.5

**Actual Factor**

C = 1

80

0

70

-0.5

60

-1

-1

-0.5

0

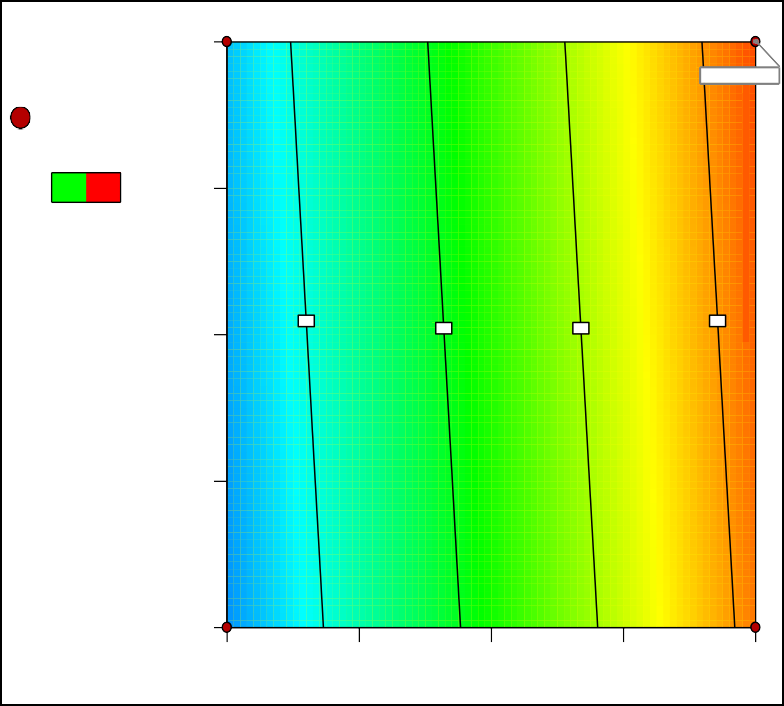
0.5

1

A: Lipid Ratio (Ratio)

B: Surfactant concentration (%)

**Figure 16: Counterplot for % Drug release (Y3)**



Factor Coding: Actual

Drug release (%)

1

**Drug release (%)**

Design Points

Prediction 41.96

17.66

X1 = A X2 = B

43.68 0.5

**Actual Factor**

C = 1

25

0

30

35

40

-0.5

-1

-1

-0.5

0

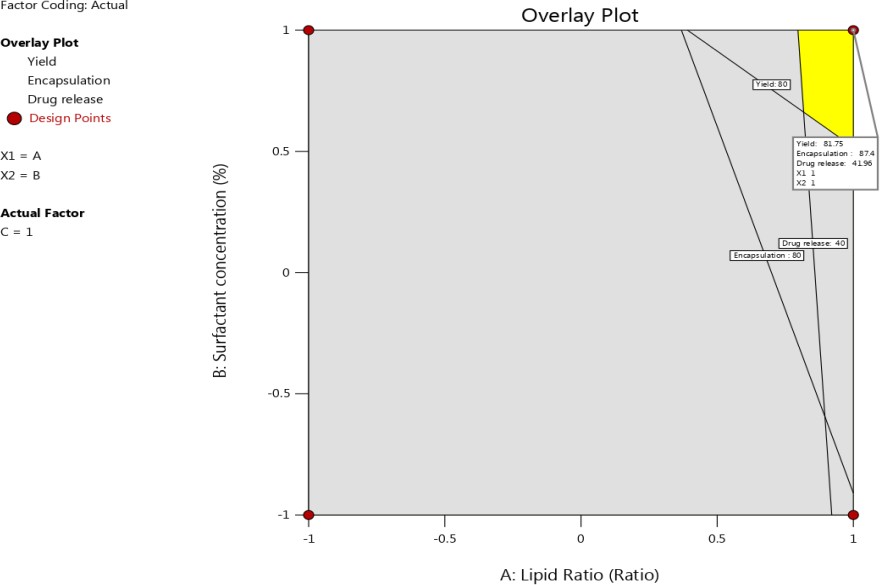
0.5

1

A: Lipid Ratio (Ratio)

B: Surfactant concentration (%)

**Figure 17: Graphical Optimization Involving Design Space**



Form the set criteria (table 13), ET 3 with % yield 87 %, encapsulation 87.1%, and % drug release 43.68% was selected as an optimum formulation with a desirable value of 1. Observed values and predicted values of ET 3 is given in table 18 and could identify that experimental values of the ET 3 were in close relation with predicted values of formulation ET 3.

To validate the model-new set of ET 3 was prepared in triplicates and evaluated for all the response. Predicted mean and average experimental values of ET 3 were subjected to conformation study. The predicted experimental values and average data of formulation 3 prepared in triplicates as shown in table 19.

The average data of the experimentation is within the confirmation view then the model is set. From table 19, it is confirmed that the optimized response value of ET 3 is within low and high prediction intervals showing the statistical similarity of the optimized ET 3 with predicted values. Hence, confirms the validity of the generated linear model.

### Table 18: Point Prediction (predicted and observed value)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Run 3 Response** | **Predicted Mean** | **Predicted Median** | **Observed** | **Std Dev** | **SE**  **Mean** | **95%**  **CI low for Mean** | **95% CI**  **high for Mean** |
| Yield | 81.75 | 81.75 | 83 | 1.27 | 0.90 | 79.24 | 84.25 |
| Encapsulation | 87.4 | 87.4 | 87.1 | 1.29 | 0.91 | 84.85 | 89.94 |
| Drug release | 41.96 | 41.96 | 43.68 | 5.10 | 3.60 | 31.94 | 51.97 |

**Table 19: Confirmation Table**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Run 3 Response** | **Predicted Mean** | **Predicted Median** | **Observed** | **Std Dev** | **n** | **SE**  **Pred** | **95% PI**  **low** | **Avg. data of**  **ET3** | **95% PI**  **high** |
| Yield | 81.75 | 81.75 | 83 | 1.27 | 3.00 | 1.16 | 78.51 | 81.49 | 84.98 |
| Encapsulatio n | 87.4 | 87.4 | 87.1 | 1.29 | 3.0 | 1.18 | 84.11 | 84.39 | 90.69 |
| Drug release | 41.96 | 41.96 | 43.68 | 5.10 | 3.0 | 4.65 | 29.03 | 42.26 | 54.88 |

From the design space, the obtained formulation ET 3 drug release was compared with a pure drug as shown in table 20.

### Table 20: *In-vitro* release for optimized formulation ET 3 and pure drug

|  |  |  |
| --- | --- | --- |
| **Formulation (ET 3)** | **Pure drug** | **% Drug release** |
| **%CDR** | 19.99 % | 43.68% |

**Figure 18: *In-vitro* release for optimized formulation ET 3 and pure drug**



***In-vitro* Release**

50

45

40

35

30

25

20

15

10

5

0

pure drug

ET 3

0 100

200

**Time (min)**

300

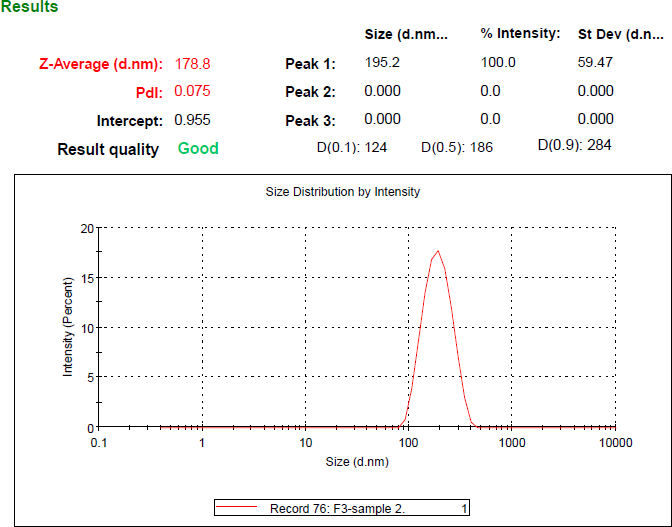
400

**%CDR**

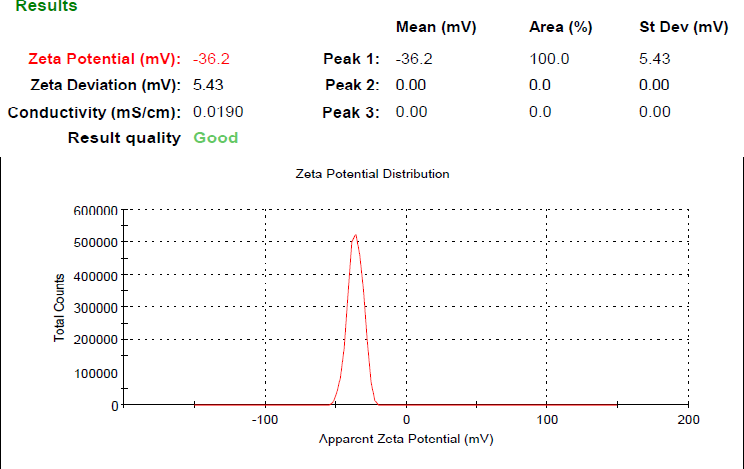
* 1. **Particle size, PDI and zeta potential**

Particle size, PDI, and zeta potential of the solid lipid nanoparticles for the optimized formulation was determined by using Nano Zetasizer.

### Figure 19: Size distribution of ETR nanoparticles



**Figure 20: Size Zeta potential of ETR nanoparticles**



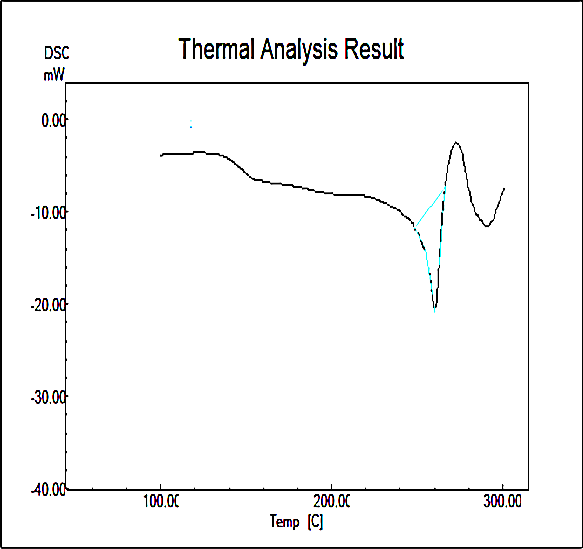
Particles with submicron sizes, specifically smaller than 400nm, may increase the bioavailability of drugs due to decreased particle size (29,46). In the present study size of the particle for optimum ET was found to be 178 nm as shown in fig.19. This result shows that though a high amount of lipid COM is used, GEL acts as a stabilizer to obtain a nanometer range, and also being of a non-ionic hydrophilic surfactant like Poloxamer 188 leads towards lesser particles (41). PDI is a sign of measurement of the particle size distribution. PDI value shows the quality of dispersion generally ranges from 0 to 1. PDI values ≤ 0.1 indicate the highest quality of dispersion (47). PDI was found to be 0.075 as shown in fig.19 indicating a homogenous distribution of SLNs. Increase in concentration surfactant, decrease in PDI value. During homogenization it facilitates the particle partition and decreases surface area with increase in surfactant concentration (15). Positive or negative zeta potential value signifies more repellant forces and repulsion among the particles with like electrical charges and avoids aggregations of particles and therefore, shows a simple distribution (48). Zeta potential was obtained at -36.2 MV as shown in fig. 20, yields a formulation with good physical stability (37).

### Differential scanning calorimetry

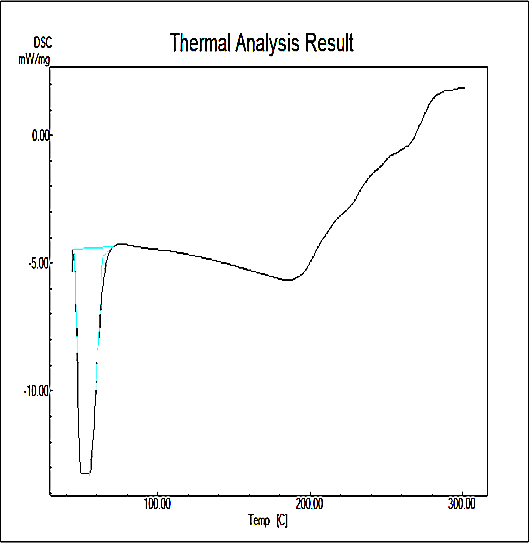
It is used to detect the possible interactions among the components. DSC spectra of drugs, lipids, physical mixture, and optimized preparation of SLN are shown in fig: 21-26. The pure components have single endotherms corresponding to their melting points as shown in fig (21-23). The melting point of Etravirine, GEL, and COM is 260.27°C, 54.87°C, 75.50°Crespectively. In DSC curve of the physical

mixture (fig 25), endotherm of Etravirine was studied at 280°C with the corresponding enthalpy of -116.20 J/g, COM has shown a melting endotherm at 75.01°C and heat of -301.25 J/g, GEL has shown a melting point at 50.93°C and heat of -944.09 J/g. This confirmed that there was no interaction between the ingredients. As shown in fig (26), the absence of endothermic peak within the melting point of Etravirine in SLN preparation neglects the solubility in the lipid matrix, leads in the conversion of the native crystalline state to an amorphous state of ETR (16). Such conversion of drugs from crystalline to amorphous was observed when GEL acts as a carrier in SLN dispersion (49). The melting point depression and sharp peak could be due to the smaller particles (21).

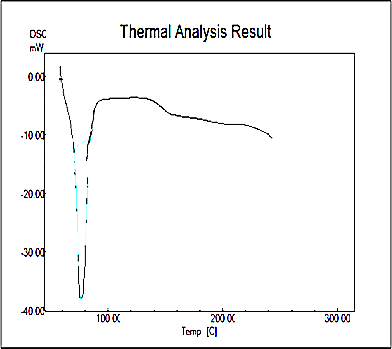
### Figure 21: DSC curve of Etravirine



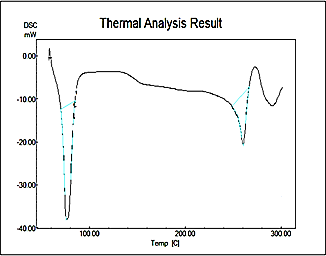
**Figure 22: DSC curve of Gelucire 50/13**

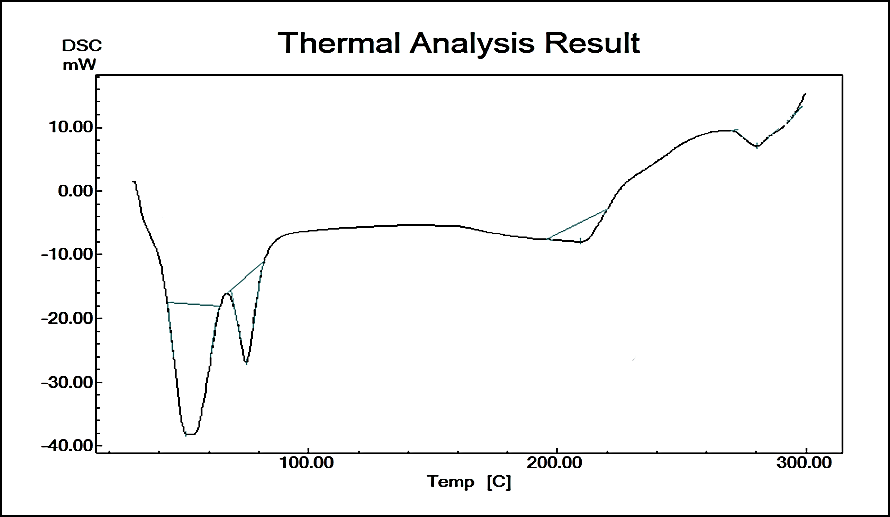


**Figure 23: DSC curve of Compritol 8880 ATO**

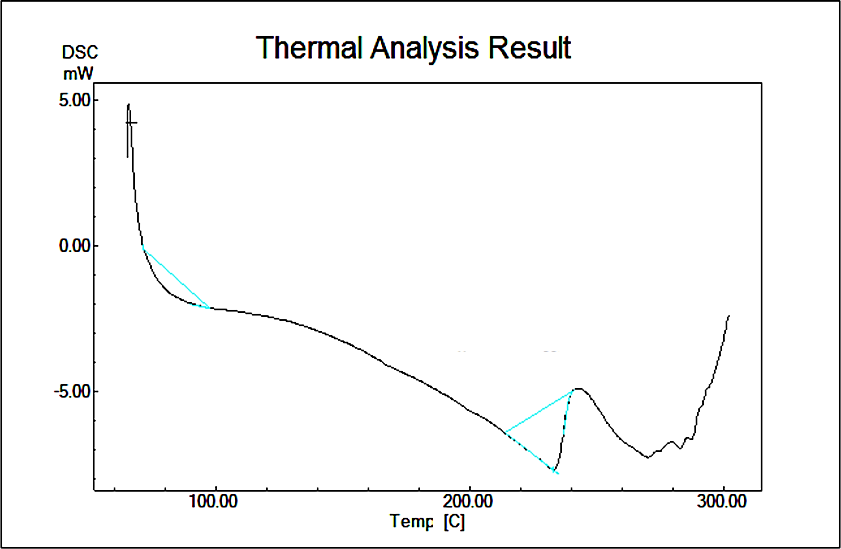


**Figure 24: DSC curve of COM and ETR**



**Figure 25: Physical mixture of COM, GEL, POL, ETR**

**Figure 26: DSC curve of optimized formulation ET 3**



* 1. **Release kinetics**

To study the release kinetics, data obtained from in vitro drug release studies, were plotted in various kinetic models as shown in fig (27-31). zero order, first order, Higuchi’s model, Hixson Crowell model and korsmeyer- peppas model as shown in the table 20. It was found that the *In-vitro* rug release kinetics of solid lipid nanoparticles was best explained by first order kinetics with highest coefficient linearity (r2 = 0.9704) followed by zero order (r2 = 0.9708), Higuchi (r2 =0.9091) respectively shown in table 21. Korsmeyer’s plots indicates a n value of 0.6219, saysthe transport mechanism (50,51) follows by Non-Fickian diffusion as shown in table7.

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**Table 21: Release kinetics for optimized formulation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Models** | **Equation** | **Slope (n)** | **Regression**  **coefficient (r)** |
| Zero order | 𝑄𝑡 = 𝑄𝑜 + 𝐾𝑜𝑡 | 7.870 | 0.9664 |
| First order | 𝐾𝑡  𝑙𝑜𝑔𝑄𝑡 = 𝑙𝑜𝑔𝑄𝑜𝑡 + 2.303 | 0.2068 | 0.9704 |
| Higuchi | 1 𝑀𝑡 1  𝑄 = 𝐾𝐻𝑡2 𝑀 = 𝐾𝑡2  𝑜 | 26.587 | 0.9107 |
| Hixson Crowell | KHc.t3√𝑄 − ∛𝑄  𝑜 𝑡 | -0.0932 | 0.9557 |
| Korsmeyer-  peppas | 𝑀𝑡  𝐹 = = 𝐾𝑚𝑡𝑛  𝑀 | 0.6219 | 0.8614 |

**Figure 27: Zero order kinetics**



**Zero order kinetics**

50

45

40

35

30

25

20

15

10

5

0

y = 7.8705x - 7.122 R² = 0.9664

0 2 4 6 8

**Time(hr)**

**% Cumulative release**

**Figure 28: First order kinetics**



**First order kinetics**

1.8

1.6

1.4

1.2

1

0.8

0.6

0.4

0.2

0

y = 0.2068x + 0.4613 R² = 0.9704

0 2 4 6 8

**Time (hr)**

**log cum % Drug release**

**Figure 29: Higuchi release**



**Higuchi kinetics**

50

45

40

35

30

25

20

15

10

5

0

-5 0

y = 26.587x - 27.573 R² = 0.9107

0.5

1

1.5

2

2.5

3

**SQRT Time**

**%Cumulative drug release**

**Figure 30: Hixson Crowell release**



**Hixson crowell kinetics**

3

2.5

2

1.5

1

0.5

0

y = -0.0932x + 2.83

R² = 0.9557

0 2 4 6 8

**Time(hr)**

**Cube of initial amount - Cube of Amt released**

**Figure 31: Korsmeyer- peppas release**



**Peppas kinetics**

0

-0.2 0

-0.4

-0.6

-0.8

-1

-1.2

-1.4

-1.6

-1.8

-2

0.2

0.4

0.6

0.8

1

y = 0.6219x - 1.7914

R² = 0.8614

**Log Time**

**Log (Mt/M)**

1. **CONCLUSION**

ETR loaded SLNs were successfully prepared by a hot homogenization method followed by probe sonication employing 23 full factorial design. Using the factorial design, with the selected independent variables, it was observed that a suitable composition for the preparation of SLN was found with a higher % yield, % encapsulation, and % drug release. SLN was characterized for the optimum formulation. Particles are in the Nano range which is monodispersed. The controlled release studies of SLN enhances the dissolution of ETR through nanoparticles as a delivery system. Hence, we will conclude that, it is possible to formulate the SLN using 23 factorial design with the selected independent variables and the controlled release studies enhances the dissolution of ETR through nanoparticles as a delivery system.

# SUMMARY

Etravirine (ETR) is an NNRTI, which has low solubility and low permeability and used in the treatment of HIV. To improve the bioavailability of ETR solid lipid nanoparticles (SLN) were formulated by a hot homogenization method followed by probe sonication. Various lipids were examined for the saturation solubility of ETR in different lipids. Compritol 888 ATO and Gelucire 50/13 as lipid carrier and Poloxamer 188 as surfactants were selected for the preparation of SLN. The independent variables like lipid ratio, surfactant concentration, and sonication time were studied in 23 full factorial design to study the effect of response variables % yield, % encapsulation (%E), and % drug release. From the design expert, a polynomial linear equation was generated. The optimized formulation lipid proportion (1:2), surfactant concentration (2%), and sonication time (10 min) were found. SLN was evaluated with high % yield, drug loading, and encapsulation. To increase the oral bioavailability, In-vitro release profiles were studied. Particle size analyzer, differential scanning calorimetry, and FTIR were characterized for optimized formulation ET 3. For the suitable preparation, particles of average size were found to be 178.9 nm, it states that it may improve the oral bioavailability with smaller size particles less than 400 nm. PDI with <0.1 has the highest quality dispersion and the results found to be 0.07. Zeta potential was found to be -36.2Mv, it states the dispersion is stable. DSC studies no interaction between the drugs and excipients. The kinetics of the optimum formulation best fitted is first order kinetics, and the drug mechanism is Non-Fickian transport. In this research work, it can conclude that the ratio of COM: GEL has a higher solubility in drug and enhances the bioavailability of poorly soluble drugs.

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