FORMULATIONS, IN-VITRO CHARATERIZATION AND COMBATING THE THERAPEUTIC POTENTIAL OF ANTI-CANCER DRUGS IBRUTINIB AND GEFTINIB THROUGH LIPID BASED NANO DRUG DELIVERY SYSTEM

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ABSTRACT

Cancer is the second largest disease globally, although conventional chemotherapy has been successful to some extent, the main drawbacks of chemotherapy are its poor bioavailability, high-dose requirements, adverse side effects and low therapeutic indices etc. The main aim in the development of drug delivery vehicles is to improve the bioavailability while reducing adverse side effects. Most of anticancer drugs discovered in the last two decades belongs to biopharmaceutical classification system II, where solubility is the major drawback for the drugs and it decreases the systemic availability of the drug. The main aim of the work is to enhance the solubility of the drugs and improve the bioavailability, hence the dose can be reduced. The model anticancer drugs selected for the study are Gefitinib, Ibrutinib and Pazopanib, were formulated into Lipid based drug delivery systems to enhance solubility and bioavailability of drugs. Ibrutinib is an anticancer drug which binds irreversibly to Bruton's tyrosine kinase (BTK) receptor that binds to a cysteine residue and inhibits BTK active site. Gefitinib is an anticancer drug which inhibits the intracellular phosphorylation of numerous tyrosine kinase associated with transmembrane cell surface receptors, including tyrosine kinase associated with the epidermal growth factor receptor (EGFR-TK).

Screening of excipients was done by determining the saturation solubility studies in various oils, surfactants and co-surfactants by using developed and validated HPLC method. Capryol 90 was selected as an oil phase, Kolliphor EL as surfactant and Transcutol HP as co-surfactant for Ibrutinib, Peceol as oil phase, Labrasol ALF as surfactant and Transcutol P as co-surfactant for Gefitinib.

The best L-SNEDDS formulations were converted to S-SNEDDS by non-solvent adsorption technique by the use of porous carrier neuselin.

INTRODUCTION

1. Cancer is a disease, where a portion of the body's cells develop abnormally and spread to different parts of the body. Cancerous growth can begin in anyplace in the human body, which is pool of cells. Regularly, human cells develop and increase (through a cycle called cell division) to shape new cells as the body needs them and simultaneously when the cells grow old or become damaged and new cells take their place.

Some of the time this precise interaction separates, and abnormal or damaged cells develop and duplicate when they shouldn't. Malignant growths spread into, or attack, close by tissues and can move to distant places in the body to form new cancers which is called as metastasis. Benign tumours don't spread into, or attack, nearby tissues.

Cancer is a main cause of death in numerous nations all throughout the planet. Although, the efficacy of current standard therapies for various of malignant growths is suboptimal.

Firstly, most cancer therapies need lacks specificity, implying that these therapies influence both disease cells and the normal cells3,4.

Second, numerous anticancer drugs are highly toxic, and subsequently, limit their utilization in therapy.

Third, various cytotoxic chemotherapeutics are hydrophobic, which restricts their utility in cancer treatment.

Finally, numerous chemotherapeutic drugs show short half-lives that reduce their efficacy. As a result of these inadequacies, numerous current medicines lead to adverse effects, resistance, patient inconvenience & noncompliance.

The search for new dosage formulations for cancer treatment to achieve enhanced bioavailability, efficacy safety constitutes the main goal of anticancer drug research.

To fight against the cancer, in the last decade of years, scientists & researchers have put great effort in evaluating the potential of drug delivery systems for this purpose. The most promising part of the research is to improve the pharmacokinetic profile of existing anticancer drugs (such as bioavailability, targeting, distribution, drug carriers) by modifications of their delivery routes (new drug formulations).

Various novel drug delivery techniques are used to overcome the difficulties faced by the anticancer drugs.

Cancer is a chronic condition, where therapy lasts for longer time. Hence, the patient needs to take the medication with high compliance and convenience which can be achieved by oral drug delivery.

1.2. Oral route of drug delivery.

Oral drug delivery offers most convenient and self-administered by non-invasive way with a better patient compliance, this is the most common method employed and it accounts 80% of commercially available dosage forms. These dosage forms are economical and available in wide range of designs with least sterility maintenance.

The key factors of oral bioavailability are solubility of drugs in GI fluids and intestinal permeability7,8. The most (nearly 70%) of the newly discovered drug compounds show poor aqueous solubility. Therefore, the need for solubility enhancement for these compounds is the key parameter to enhance the bioavailability the drug. Various approaches to enhance the aqueous solubility of hydrophobic drug compounds have been investigated.

1.3. Lipid-based framework for delivery

Lipid based drug delivery systems (LBDDS) are an umbrella which constitute several types of delivery systems. Various LBDDS which delivers the drugs in efficient manner are divided into three categories. They are a) Emulsion type systems b) Vesicular type systems c) Lipid particulate type systems.

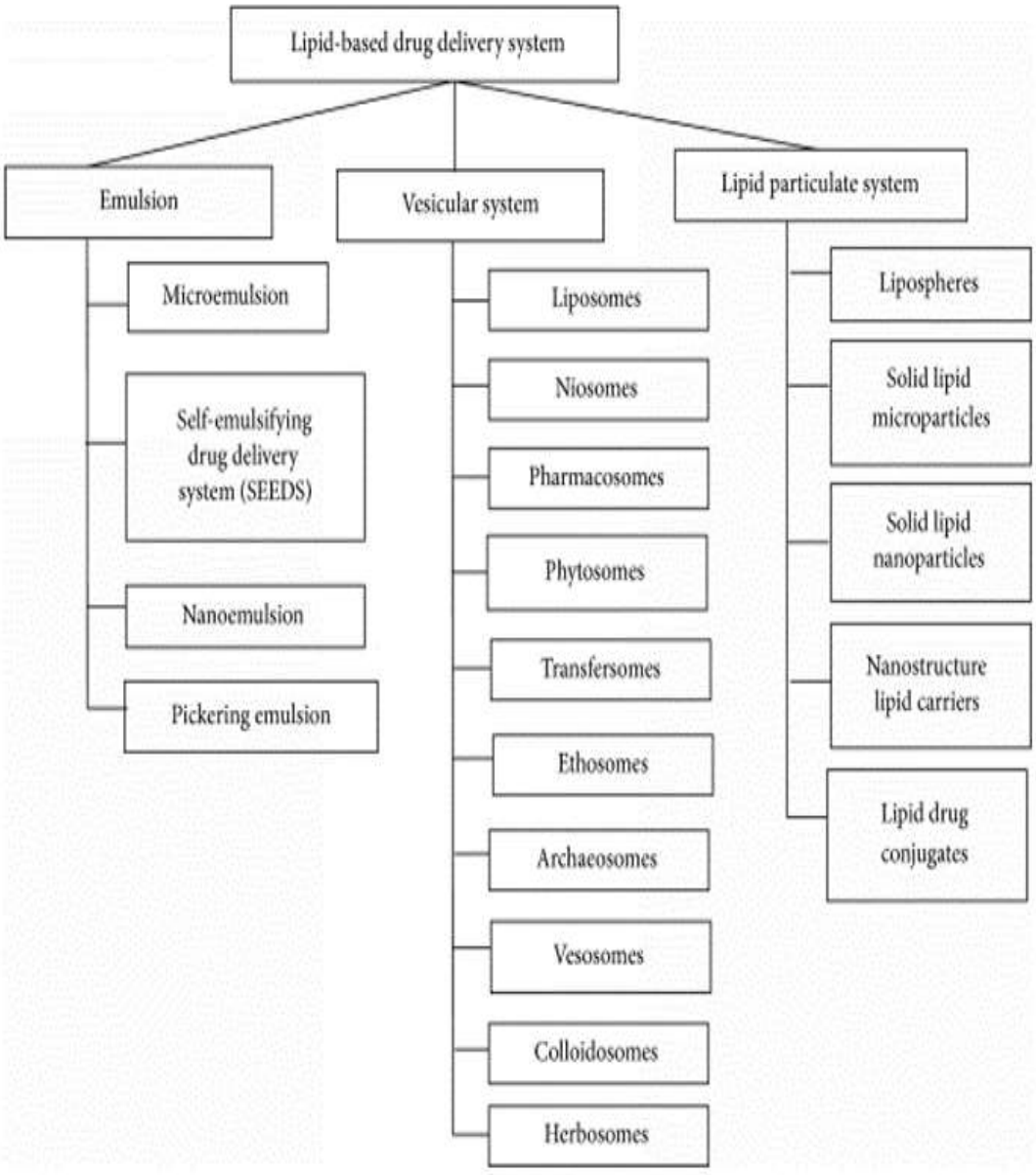

Figure 1: Framework of lipid- based system.

1.4. Self-emulsifying drug delivery systems

Self-emulsifying drug delivery systems (SEDDS) have moved into the limelight of pharmaceutical research worldwide. This advancement is primarily based on several discoveries and improvements of SEDDS have been made in the last decade of years, which resulted in encouraging in-vivo studies confirming the great potential of the formulation approach25. SEDDS emulsifies spontaneously when diluted with mild agitation in aqueous media and forms a transparent liquid.

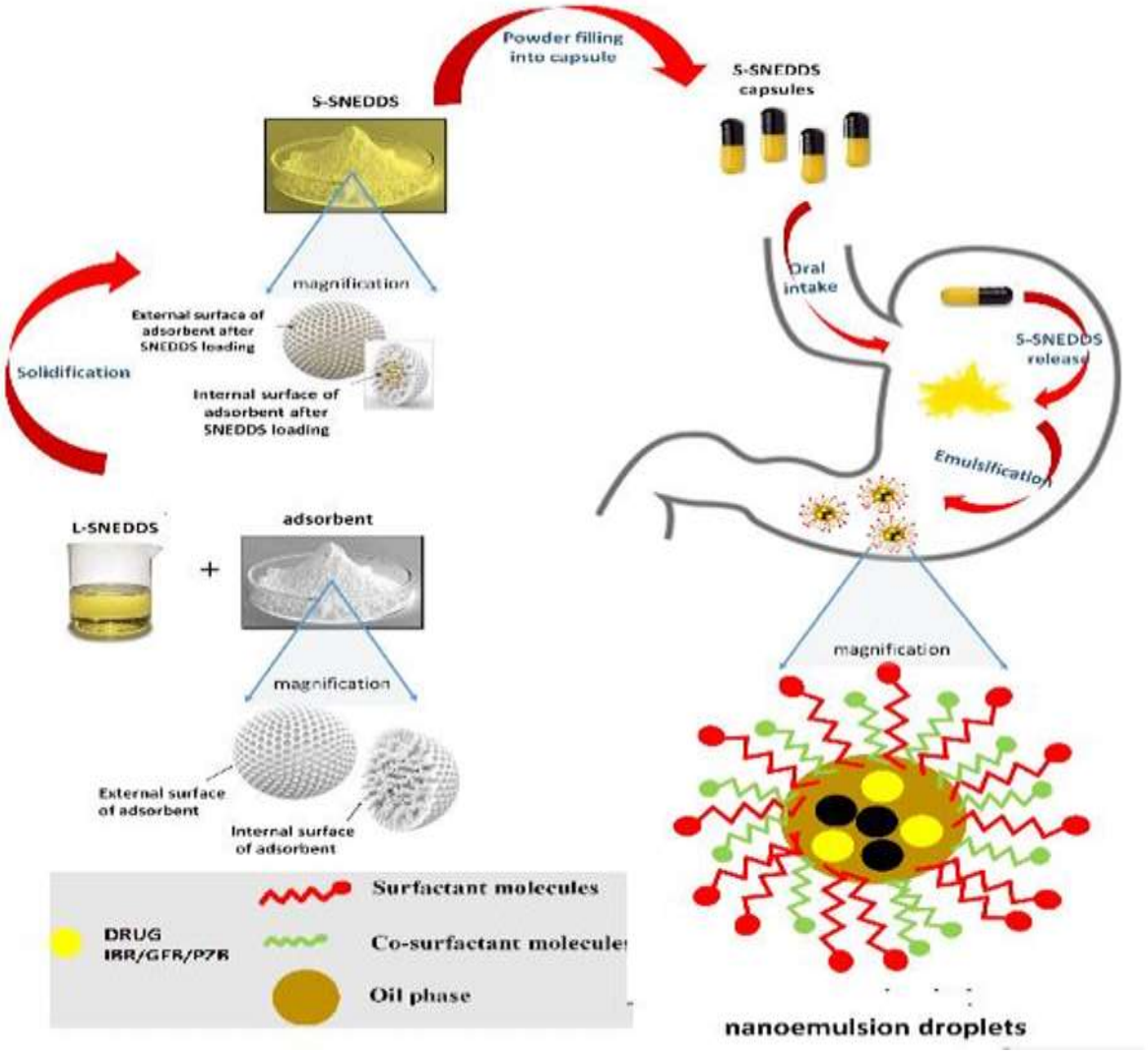

Figure 2: Overview of SEDDS.

1.4.1 Oils

Oils solubilize the hydrophobic compounds and also aids in self-emulsification. Lipids have tendency to elevate the fraction of the drug transported through intestinal lymphatic transport system & thus increasing drug absorption from the GI tract. The nature and structure of oil show greater impact on the emulsification property of oil31,32. Table-1.1 gives the information of various oils used in SMEDDS/SNEDDS.

1.4.2 Surfactants

Surfactants are often used in lipid formulations to improve the drug solubility and self-emulsifying properties of the vehicle, in order to minimize dependence on the digestive factors. Surfactants are important components of SMEDDS / SNEDDS systems as they are responsible for the formation of a stable emulsion when diluted.

A surfactant with an HLB value greater than are used in lipid-based drug delivery systems, they are usually derived from polyethoxylated lipids. Emulsifiers of natural origin are not widely used because of their poor self-emulsifying property.

1.4.3. Co-solvents

Co-solvents, such as ethanol, propylene glycol and low molecular poly ethylene glycols are used to maximize the drug solubilization and also serve to improve the rate of dispersion of lipidic formulations and are often included in SEDDS and SMEDDS36,37.

However, when dispersed in GI fluids, they rapidly get separated out in the aqueous phase and reduce the solvent capacity of the formulation, which leads to precipitation of the drug.

Optimization of SNEDDS formulations by Central composite design Any optimization process is carried out by going through certain phases, firstly Screening; where the identification of a significant factors which are important; & the next Improvement; when it is necessary to identify factors close to the optimum, the last design of the response surface; where the optimal or best product was designed with the Response Surface Method (RSM) by quantifying the relationship between one or more measured responses and the vital input factor.

Choosing a suitable experimental design, which can easily explain many varying solutions, has always been a tedious task. These variables often end with a quadratic surface model. For this type of interpretation, the central composite plane can be an excellent choice.

1.5 Anti-cancer drugs

1.5.1 Ibrutinib

Ibrutinib belongs to the class of acrylamides that is (3 R)-3-[4-amino-3-(4-phenoxyphenyl). pyrazolo [3,4-d] pyrimidin-1-yl] An acryloyl group replaces the nitrogen of piperidine [14]. Ibrutinib is a piperidine-backed acrylamide in which the piperidine's nitrogen has been changed into an acryloyl group. It is used to treat B-cell malignancies because it is a specific and covalent inhibitor of the BTK enzyme.

1.5.2 Mechanism of ibrutinib

BTK is permanently and safely inhibited by ibrutinib. By forming a covalent link with the cysteine residue C481 in the BTK active site, it permanently inhibits the activity of the BTK enzyme. BTK plays a crucial role in the development of several B-cell malignancies, such as MCL, diffuse large B cell lymphoma (DLBCL), follicular lymphoma, and CLL, by acting as a B-cell antigen receptor.

1.5.3 Pharmacology of ibrutinib

The BCR pathway regulates three biological procedures, with propagation, development, then programmed cell death, they are required for both well and malignant B cell survival and activity. Improper BCR signing is critical for the development of B cell malignancies.

1.5.4 Ibrutinib as SMEDDS

Lipid-based preparations (LBFs) have recently attracted widespread interest, which in large amounts dissolve insoluble drugs and disperse to obtain smaller particle sizes to facilitate absorption.

At the same time, the lipophilicity of LBF can improve the absorption significantly. And SMEDDS is the most widely used in the classification of lipid preparations. It is an isotropic mixture composed of lipid, surfactant, and co-surfactant, which belongs to class III B of LBF. After entering the human body, SMEDDS can spontaneously form an O/W emulsion with a particle size of less than 100 nm under the peristalsis of the gastrointestinal tract.
This small particle size can provide a large surface area for drug absorption, which also able to stimulate the production of lipoprotein and chylomicrons and induce lymphatic circulation thus avoiding first-pass metabolism of the liver and ultimately improving drug-release characteristics.

However, SMEDDS is usually supplied in liquid form encapsulated in soft gelatin capsules. And the liquid SMEDDS interacts with the capsule shells, causing leakage. Therefore, it may be prepared into a solid dosage form to solve the above problems.

1.6.1 Gefitinib

Gefitinib, a tyrosine kinase inhibitor, is effectively used in the targeted treatment of malignant conditions. It suppresses the signal transduction cascades leading to cell proliferation in the tumours and is now currently approved in several countries globally as second line and third-line treatment for non-small cell lung cancer.

1.6.2 Mechanism of action

Gefitinib is an inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase that binds to the adenosine triphosphate (ATP)-binding site of the enzyme. EGFR is often shown to be overexpressed in certain human carcinoma cells, such as lung and breast cancer cells. Overexpression leads to enhanced activation of the anti-apoptotic Ras signal transduction cascades, subsequently resulting in increased survival of cancer cells and uncontrolled cell proliferation. Gefitinib is the first selective inhibitor of the EGFR tyrosine kinase which is also referred to as Her1 or ErbB-1. By inhibiting EGFR tyrosine kinase, the downstream signalling cascades are also inhibited, resulting in inhibited malignant cell proliferation.

1.6.3 Pharmacology of Gefitinib

Gefitinib reversibly inhibits the kinase activity of wild-type and certain activating mutations of EGFR, preventing autophosphorylation of tyrosine residues associated with the receptor, thereby inhibiting further downstream signalling and blocking EGFR-dependent proliferation. Gefitinib binding affinity for EGFR exon 19 deletion or exon 21-point mutation L858R mutations is higher than its affinity for the wild-type EGFR.

1.6.4 Ibrutinib as SMEDDS

SNEDDS is an isotropic mixture of oil, surfactant, and co-surfactant which forms oil in water (o/w) nano emulsion with slight agitation. Oil is selected based on their solubility capacity and both surfactant and co-surfactant is selected based on their emulsifying ability. To prevent the precipitation of the drug and to reduce the dosing frequency, suitable precipitation inhibitors can be used (maintains supersaturation state and blocks the formation and growth of the crystals). By introducing precipitation inhibitors into the formulation, the surfactant concentration can be minimized (reduce GI side effects). Hence, Super saturable SNEDDS (S-SNEDDS) is an effective method for the oral delivery of poorly water-soluble drug, in order to improve its bioavailability. Ibrutinib is a selective and covalent inhibitor of the enzyme Bruton's tyrosine kinase (BTK), it is used for treatment of B-cell malignancies.

It has been reported to exhibit pH-dependent solubility as it is slightly soluble at pH 1.2 while practically insoluble at pH 3 to 8, which lead to low bioavailability and impede it’s in vivo antitumor effect after oral administration. The present work described an innovative approach by designing a supersaturated solid-self emulsifying formulation (S-SNEDDS) to improve the solubility and dissolution of a poorly soluble drug, ibrutinib.

1.7.1 Pazopanib

Pazopanib is an oral, multi-targeted, tyrosine kinase inhibitor (TKI) that binds to the vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR) and several other key proteins responsible for angiogenesis, tumour growth and cell survival.

1.7.2 Mechanism of action

Pazopanib is a second-generation multitargeted tyrosine kinase inhibitor against vascular endothelial growth factor receptor-1, -2, and -3, platelet-derived growth factor receptor-alpha, platelet-derived growth factor receptor-beta, and c-kit. These receptor targets are part of the angiogenesis pathway that facilitates the formation of tumour blood vessel for tumour survival and growth.

1.7.3 Pharmacology of Pazopanib

Pazopanib is a **multitargeted tyrosine kinase inhibitor** of the second generation that inhibits the activity of c-kit, platelet-derived growth factor receptor-alpha, platelet-derived growth factor receptor-beta, and vascular endothelial growth factor receptor-1, -2, and -3.

1.7.4 Pazopanib as SMEDDS

The self-micro emulsifying drug delivery systems (SMEDDS) is one of the most prominent techniques for improvising the bioavailability of poorly water-soluble drugs by enhancing their solubility and permeability [15]. SMEDDS has gained interest after the commercial success of Neoral® (cyclosporine A), Norvir® (ritonavir), and Fotovase® (saquinavir) in the market. It is a mixture of drug, oil and surfactant/co-solvent which rapidly emulsifies in the gastrointestinal fluid under gentle agitation of gastrointestinal motility and forms o/w microemulsion resulting in lipophilic drug solubilized in oil system.

The key scientific goal of this study was to develop a pazopanib SMEDDS formulation with improved solubility and faster dissolution rate in comparison to pure drug and marketed formulation.

AIM & OBJECTIVES

AIM

Aim of the research is to formulate Lipid based drug delivery systems (LBDDS) of the selected API drugs (Ibrutinib, Gefitinib & Pazopanib) and to evaluate the prepared formulations for in-vitro studies. The study also focused to depict the lymphatic uptake the SNEDDS formulations.

OBJECTIVES

1. To develop and validate HPLC method for the model drugs.
2. To perform solubility performance of drugs in various excipients. mixtures (oils, surfactants/cosurfactants).
3. To determine Drug – excipient compatibility by using FTIR.
4. To identify Nano emulsion region of blank SEDDS by using pseudo ternary phase diagrams. To optimize the percentage drug content in Oil: Smix combinations by Design of experiments.
5. To prepare and evaluate liquid & solid Self emulsifying drug delivery systems (L-SEDDS) formulate.
6. To perform accelerated stability studies.

MATERIALS

Table 4.1: List of equipments

S.NO	MATERIAL	MANUFACTURER
	HPLC	Waters
	Analytical balance	Sartorious
	Shaking incubator	LabTech
	Mini rotary shaker	Remi
	Cyclomixer	Remi
	Micropipette	Remi
	Cooling Microcentrifuge	Remi
	Water bath	Remi
	Centrifuge	Remi
.	UV spectrophotometer	Schimadzu
.	Dissolution apparatus	Schimadzu
.	FTIR	Bruker (OPUS)
.	Zeta sizer	Malvern zetasizer
.	MilliQ water purifier	Millipore (India) pvt ltd
.	Magnetic stirrer	Remi
.	pH meter	Alpha

Table 4.2: List of materials

S.NO	Material	Category	Manufacturer
	Ibrutinib	API	Natco Pharma ltd.
	Gefitinib	API	Natco Pharma ltd.
	Capryol 90	Oil	SD Fine chem, Mumbai
	Kolliphor EL	Surfactant	SD Fine chem, Mumbai
	TranscutolHP	co-surfactant	SD Fine chem, Mumbai
	TranscutolHPPeceol	oil	SD Fine chem, Mumbai
	LabrasolALF	Surfactant	SD Fine chem, Mumbai
	TranscutolP	co-surfactant	SD Fine chem, Mumbai
	CapmulMCMC8 NF	oil	SD Fine chem, Mumbai
.	Tween 80	Surfactant	SD Fine chem, Mumbai
.	PEG 400	co-surfactant	SD Fine chem, Mumbai
.	Ortho-phosphoric acid	Analytical reagent	Fisher scientific
.	Potassium di hydrogen phosphate	Analytical reagent	Fisher scientific
.	Potassium hydrogen phosphate	Analytical reagent	Fisher scientific

.	Potassium bromide	Analytical reagent	Finar
.	Neusilin	Adsorbent	Fuji chemical industries
.	Cycloheximide	Lymphatic blocker	RPI research product
.	Capsules	HPMC Capsules	Natural capsules Ltd
.	Hydrochloric acid	Analytical reagent	Research lab fine chem industry

5.1 Method development and validation by using HPLC

The Chromatographic system and Conditions for method development trails of the three model drugs, HPLC waters 2998 with a waters 515 pump and a photodiode-array detector was used for chromatographic analysis. The data acquisition was performed by empower software. At room temperature, an isocratic system was used to perform chromatographic operation. The analytical column used for separation was C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column). The injection volume was 10 µl with a run time of 5 min. Prior to use of the buffer, it was filtered through 0.45µm Millipore filter & degassed with the help of bath sonicator.

5.1.1 Method development trails of Ibrutinib

The method development trails of IBR were done with various mobile phases by taking acetonitrile and water in different proportions, but the results were not satisfactory because of tailing of peaks. Hence, the method is switched to acetonitrile and 1% orthophosphoric acid solution with the ratio of 60:40, the peaks obtained were good and acceptable67. To reduce the retention time of peaks the proportion of acetonitrile was increased from 60-70% for obtaining shorter retention time. Mobile phase constituted of acetonitrile & 0.1% orthophosphoric acid in the ratio of 70:30 v/v at a flow rate of 1ml/min and detected at a wavelength 286nm was considered optimized trial with a retention time obtained was 2.5min. The optimized programme for pump A (Acetonitrile) and pump B (0.1% orthophosphoric acid solution) was carried out and the results were good.

5.1.2 Preparation of Solutions for Calibration Curve of Ibrutinib:

Stock solution of Ibrutinib (1000 ppm): 100mg of Ibrutinib was taken into 100mL of volumetric flask and diluted with 20mL of acetonitrile and sonicate for minutes. Then made up the volume to 100mL with acetonitrile.

5.1.3 Working standard solution Ibrutinib (100 ppm): From the stock solution 1ml was taken into 10 mL of volumetric flask and made up the volume to 10mL by acetonitrile as diluent. From the working standard solutions 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 mL was pipetted out and made up the volume to 10mL with acetonitrile to obtain 2,4,6,8,10,12 and 14ppm solutions.

Table 5.1: Optimized chromatographic of Ibrutinib

Parameter	**Optimized condition**
Flow rate	1ml/min
Mobilephase composition	Acetonitrile:0.1% orthophosphoric acid solution (70:30)
Diluent	Acetonitrile
Detector Wavelength	286nm
Column	C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column)
Column temperature	Ambient
Injection volume	10µl
Run time	5min

5.2.1. Method development trails of Gefitinib

Buffer Preparation- Phosphate buffer pH 6.5 (EP):

Phosphate buffer solution pH 6.5. was prepared by dissolving 13.80 g of sodium dihydrogen phosphate monohydrate in 900 ml of distilled water. Adjust the pH (2.3) using a 40 g/l solution of sodium hydroxide. Dilute to 1000 ml with distilled water.

The mobile phase composed of Acetonitrile and 6.5pH Phosphate buffer in a ratio of 70:30 v/v at a flow rate of mobile phase was monitored at 1ml/min and detected at a wavelength 249nm. The injection volume of 20µl with a run time of 10min. Prior to use the buffer was filtered through Millipore 0.45µm filter and degassed on bath sonicator68,69. Different ratios 30:70 to 70:30 of Acetonitrile and pH6.5 phosphate buffer was used as mobile phase and 70:30 of Acetonitrile: pH 6.5 phosphate buffer was selected as an appropriate mobile phase which gave a peak with good retention time and acceptable system suitability parameters was chosen for validation and solubility studies.

5.2.2 Preparation of Solutions for Calibration Curve of Gefitinib

Stock solution of Gefitinib (1000 ppm): 100mg of Gefitinib was taken into 100mL of volumetric flask and diluted with 20mL of acetonitrile and sonicate for minutes. Then made up the volume to 100mL with acetonitrile.

5.2.3 Working standard solution Gefitinib (100 ppm)

From the stock solution 1ml was taken into 10 mL of volumetric flask and made up the volume to 10mL by acetonitrile as diluent. From the working standard solutions 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 mL was pipetted out and made up the volume to 10mL with acetonitrile to obtain 2,4,6,8,10,12 and 14ppm solutions.

Table 5.2: Optimized chromatographic of Gefitinib

Parameter	**Optimized condition**
Flow rate	1ml/min
Mobile phase composition	Acetonitrile: pH 6.5 Phosphate buffer (70:30)
Diluent	Acetonitrile
Detector Wavelength	249nm
Column	C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column)
Column temperature	Ambient
Injection volume	20µl
Run time	10min

5.2 Solubility of the drugs in various Oils, Surfactants and Co-surfactants

The solubility of Ibrutinib/Gefitinib/Pazopanib were studied in various oils, surfactants and cosurfactants. The excess amount of drug was added to 1gm of each excipient in cap vial bottle & cyclo-mixed immediately for 5min on cyclomixer (REMI CM 101) and then the resultant mixtures were equilibrated for 72hours on Shaking incubator (LabTech). The supersaturated solutions were centrifuged at a speed of 3000rpm for 15min to remove the undissolved drug34,81,82. The supernatant was separated and aliquots of supernatant fluid was drawn by using micro pipette and adequately diluted with Acetonitrile. The concentration of drug in each excipient was quantified by validated RP-HPLC method.

5.3. Drug excipient studies by Fourier transform infrared Spectroscopy (FTIR)

Drug-excipients compatibility studies were performed by FT-IR spectrophotometer (Bruker Alpha) with the data acquisition system OPUS software. These studies predict the incompatibility of the drug with various excipients, provides justification for selection of excipients and the plays key process in drug development. The FT-IR spectra of drug and excipients were determined for incompatibilities between them and the spectra of optimized formulation is compared with the pure drug.

5.4 Characterization of L-SNEDDS

5.4.1 Self-emulsification time

The time taken for prepared SNEDDS formulation was monitored visually to form a homogenous mixture upon dilution with water. The SNEDDS (0.1ml) were added to 200ml of distilled water at 37±0.5˚C and gently agitated using magnetic stirrer rotating at constant speed91. The time required for the disappearance of SNEDDS was recorded.

5.4.2 Dispersibility test

The time taken for the formation of nano emulsion was determined by dropwise addition of 1gm of formulation into 250ml of distilled water, 0.1N HCl, pH 6.8 phosphate buffer at 37˚C. The contents were placed on magnetic stirrer at 100rpm. The affinity to form an emulsion was assessed by grades based on the visual appearance and time taken for self-emulsification.

5.4.3 Phase-separation & stability of SNEDDS

Each of the formulation (100µl) was added to a vial containing 5ml of Millipore water, simulated gastric fluid and pH 6.8 phosphate buffer at room temperature and cyclo-mixed for 1min and stored at room temperature and observed for phase separation and precipitation of drug at pre-determined interva94ls for a period of 24 hours

5.5 Size and potential measurements

The optimised blank SNEDDS (Clear transparent/bluish emulsion) from the aqueous titration method/optimised L-SNEDDS formulations by DOE/ reconstituted S-SNEDDS were diluted with milli pore water and droplet size, poly dispersibility and zeta potential were analysed by Dynamic laser light scattering (DLS) spectroscopy by using a Zetasizer Nano ZS 90 version 7.10 (Malvern Zetasizer).

The size analysis was performed at 25˚C placing disposable cuvette and zeta potential was performed by using an electrophoretic cell with an angle of detection of 90˚. The average droplet size affects the in -vivo performance of SEDDS and the least mean droplet size provides greater interfacial area for drug absorption and ensures kinetic stability of the resulting emulsion.

Small value of polydispersity index gives good uniformity of droplet size distribution. High values of zeta potential confirm the electrical stability of emulsion droplets and absence of aggregation.

5.6 Invitro dissolution/drug release studies

In-vitro dissolution studies of pure drug and SNEDDS were assessed using the USP type -I apparatus. The pure drug and SNEDDS containing IBR were added into HPMC capsules “Size 00”. The beaker was filled with 900 ml of simulated gastric fluid (pH 1.2) at 37±0.5°C with a basket rotating speed of 50 rpm using 8-station dissolution apparatus (DS 8000 Lab India) sample was withdrawn at 5, 10, 20, 30, 45, 60, 90 & 120 min and replaced with fresh dissolution medium to keep the volume constant.

The release of drug from the SNEDDS formulation was compared with the pure drug which is filled capsule containing the same amount of drug101,102,103–105. The concentration of drug in the released sample was determined by the developed and validated HPLC method.

5.7 Thermodynamic stability data

The physical stability of the optimized formulation of SNEDDS is essential for its performance during its storage and usage. Poor physical stability of formulation can lead to phase separation of excipients which may affects therapeutic efficacy.

The physical stability was determined by Centrifugation.

5.7.1 Centrifugation.

The optimized SNEDDS formulations were diluted with 100 times with distilled water. The formulations were centrifuged at 3500rpm for 30min. The formulations should not show any physical separation.

5.8 Droplet size & morphology

Morphology of the emulsion droplets analysed by Transmission electron microscopy (TEM) after dilution of SNEDDS preconcentrate to 1000 folds using 1% solution of phospho-tungstic acid.

Droplets should show a spherical shape without any signs of aggregation or drug precipitation. Samples were properly diluted with water for the analysis of particle size by using TEM. A drop of diluted sample was placed on a 300mesh carbon coated copper grid. The grid was left for 5min to settle down the droplets.

Excess of the liquid was removed by adsorbent paper and grid was air dried and a drop of 1% phospho-tungstic acid was added to the grid, it acts as a negative stain. This was left for 5min to settle down and air dried. Finally, the dried grid was visualized under TEM at an operating voltage of 80 kV.

5.9 Formulation of Solid-SNEDDS

Preparation of Ibrutinib/Gefitinib/ SNEDDS

The ibrutinib SNEDDS were prepared by mixing oil phase (capryol 90. TranscutolHP. TranscutolP), surfactant (Cremophor EL. TranscutolHPPeceol. LabrasolALF), and co surfactant (transcutol HP CapmulMCMC8 NF. Tween 80) and warming it at 40 °C, then 120mg of **Ibrutinib Gefitinib/Pazopanib** was added to the mixture and vortexed to facilitate the uniform dispersion of **Ibrutinib /Gefitinib**. The mixture was then allowed to equilibrate at RT. By changing the concentrations of oil phase, surfactant, co-surfactant mixed with 50 mg/g of drug, a total of 5 such experiments were carried out.

The prepared **Ibrutinib /Gefitinib** loaded SNEDDS were filled into size 00 HPMC capsule shells.

The optimized liquid SNEDDS formulation was converted to powder form by using an adsorbent carrier. Through the literature survey, neuselin was chosen as adsorbent for the study, because of its good adsorbent property and flowability. The adsorbent (Neuselin) and L-SNEDDS are taken in the different ratios. The adsorption technique used was solvent free adsorption technique which was simple and stable. The adsorbent and the L-SNEDDS were blended for 5 minutes by placing in closed container for uniform mixing. The powder mixture is evaluated and filled into the 00 capsules.

Table5.3 : Formulation table

S. No	Ibrutinib (120 mg) F1, F2, F3, F4, F5			Gefitinib (120 mg) F1, F2, F3, F4, F5		
	Capryol 90	Kolliphor EL	Transcutol HP	Transcutol HPPeceol	Labrasol ALF	TranscutolP
f	20	15	10	20	15	10
	30	20	15	30	20	15
	40	25	20	40	25	20
	50	30	25	50	30	25
	60	35	30	60	35	30

5.11 Characterization of S-SNEDDS

Determination of Flow properties Powder properties are an important aspect in the scale-up production of dosage forms (solid) as they affect consistency, reproducibility and homogeneity of dosage. Flowability of Powders is influenced by several physical, mechanical & environmental factors. Hence, various parameters like repose angle, bulk density, tapped density, consolidation index & Hausner's ratio must be evaluated to determine the flow properties of powders of S-SEDDS in order to control the nature of the tests. The repose angle is to measure the internal adhesion particle force.

5.12 Globule size of the reconstituted Solid-SNEDDS

The S-SNEDDS were reconstituted with water and analysed droplet size, PDI & zeta potential.

5.13 Invitro dissolution/release studies

The optimized S-SNEDDS and pure drug were filled into a hard gelatine capsule size "00" separately and put into the baskets of dissolution apparatus (DS 8000 Lab India) and immersed into 900ml of water, pH 1.2 at 37±0.5°C with 50 rpm. The samples of 5ml were withdrawn at regular time intervals (5, 10, 20, 30, 45, 60, 90 and 120min) and filtered using a 0.45µm filter and to maintain sink conditions equal volume of dissolution medium was replaced with fresh buffer. The drug content of the samples was analysed using HPLC waters system.

CHAPTER 6

RESULT AND DISCUSSION

6.1 Scan spectrum of Ibrutinib

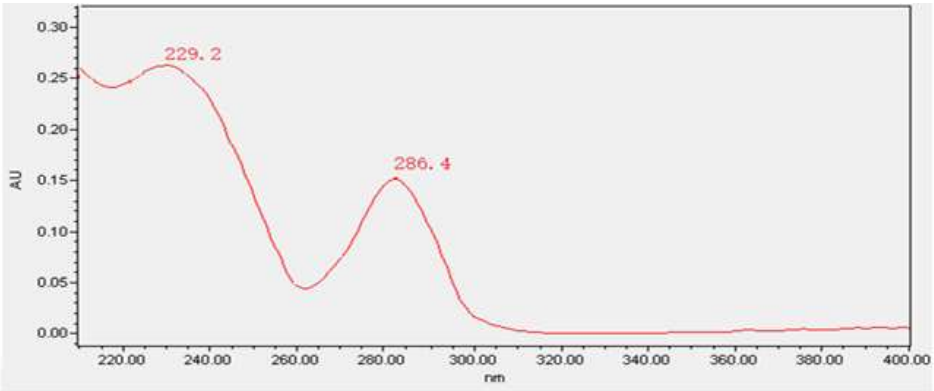

Figure 6.1: Scan spectrum of Ibrutinib

The scan spectrum shown the highest peak area at 229.2nm & 286.4nm.

The mobile phase of optimized trial was Acetonitrile: 0.1% Ortho phosphoric acid 70:30 ratio with a flow rate of 1ml/min and the retention time was 2.5.

The blank and chromatogram of ibrutinib are shown below.

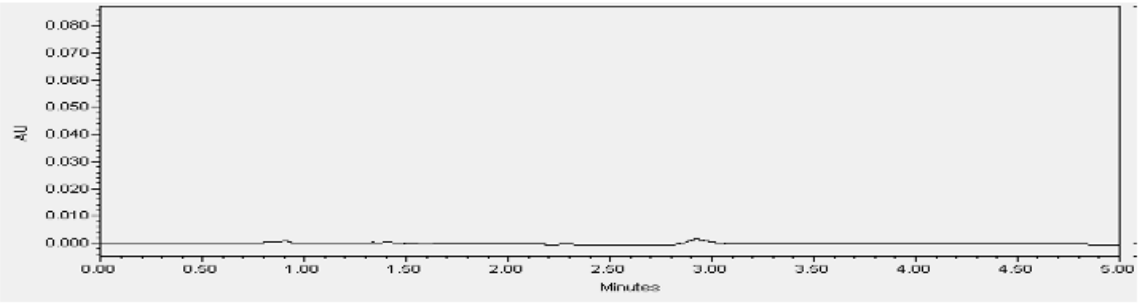

Figure 6.2: Blank chromatogram

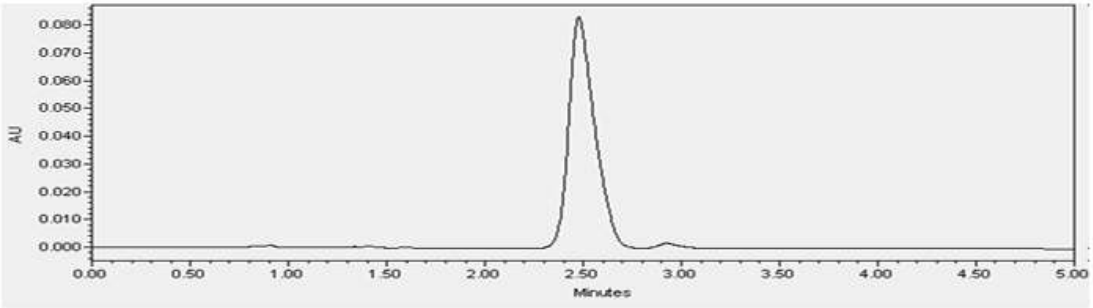

Figure 6.3: Chromatogram of Ibrutinib

6.2 Calibration curve of Ibrutinib

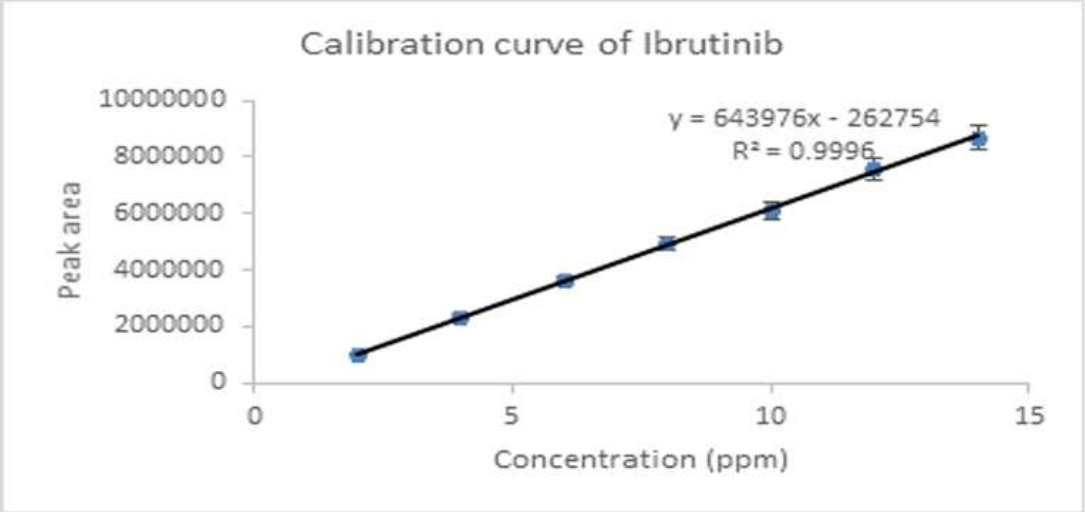

Figure-6.4: Calibration curve of Ibrutinib

Standard calibration curve of IBR was constructed by different concentrations ranging from 0.2 to 4ppm, for which the peak area readings were determined (Figure-7.4). The standard calibration curve was linear over the concentration range studied with a correlation coefficient (R2) 0.9992. The corresponding regression equation was found to be Y = 378526X+28453.

6.3 Solubility studies of Ibrutinib

From the solubility studies results, the maximal solubilizing potential for the drug, among various excipients.

Ibrutinib shown the greatest solubility in the oil Capryol 90 (60.75 mg/ml), Kolliphor EL (39.5 mg/ml) & Transcutol HP (65.77 mg/ml) at 25˚C. The solubility was aimed for determining suitable oil and surfactants for the IBR to formulate LBDDS.

Table 6.1: Solubility of IBR in Various excipients

S.NO	EXCIPIENTS	SOLUBILITY of IBR (mg/ml)
	Capryol 90	62.75
	Kolliphor EL	43.53
	Transcutol HP	67.77

6.4 Drug-excipient compatibility by Fourier infrared spectroscopy for Ibrutinib and excipients

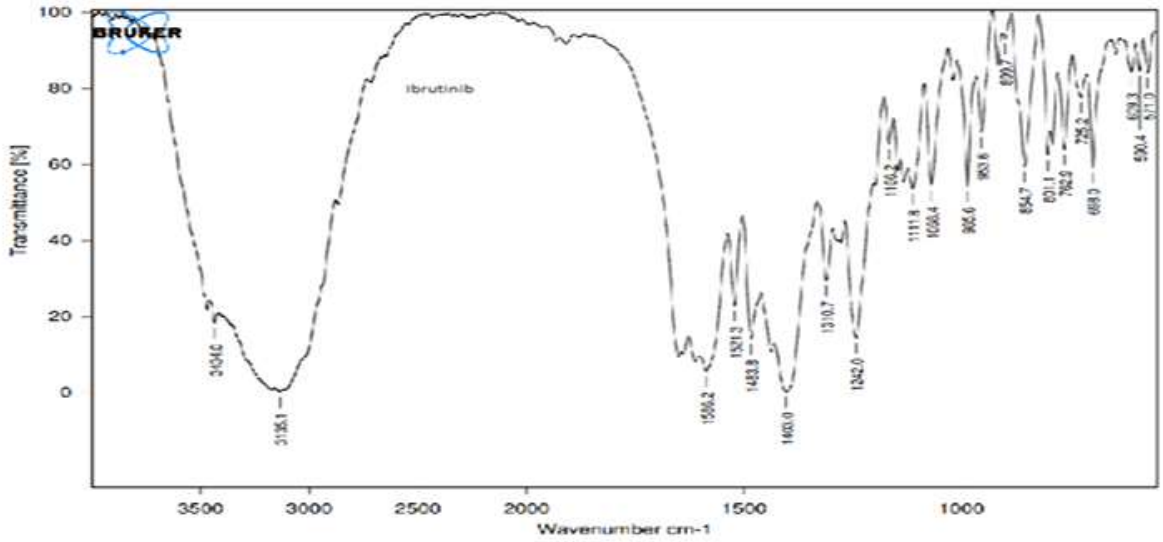

Figure6.5: Spectrum IR of Ibrutinib

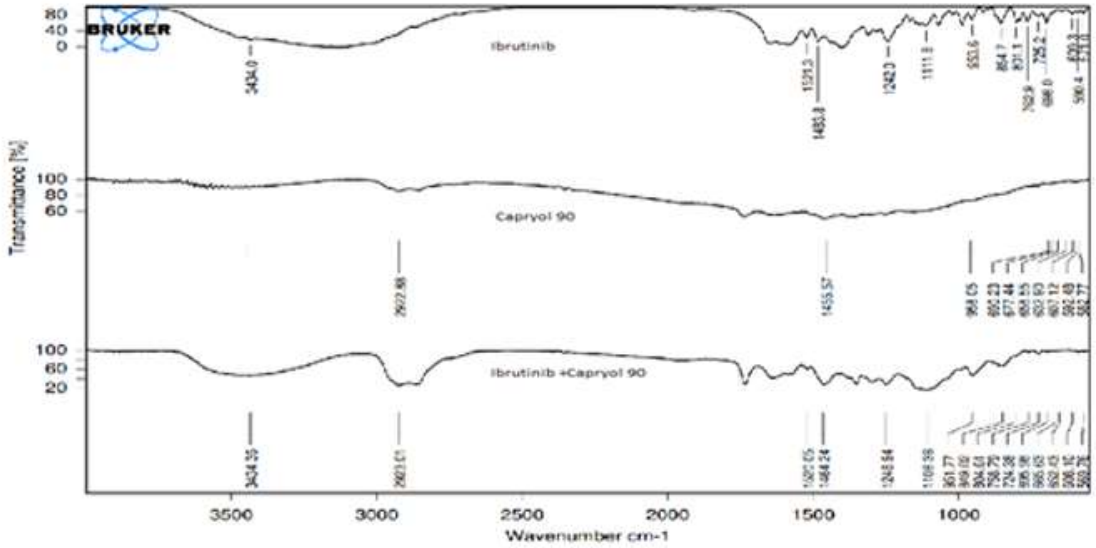

Figure-6.6: Spectrum IR of IBR+Capryol 90

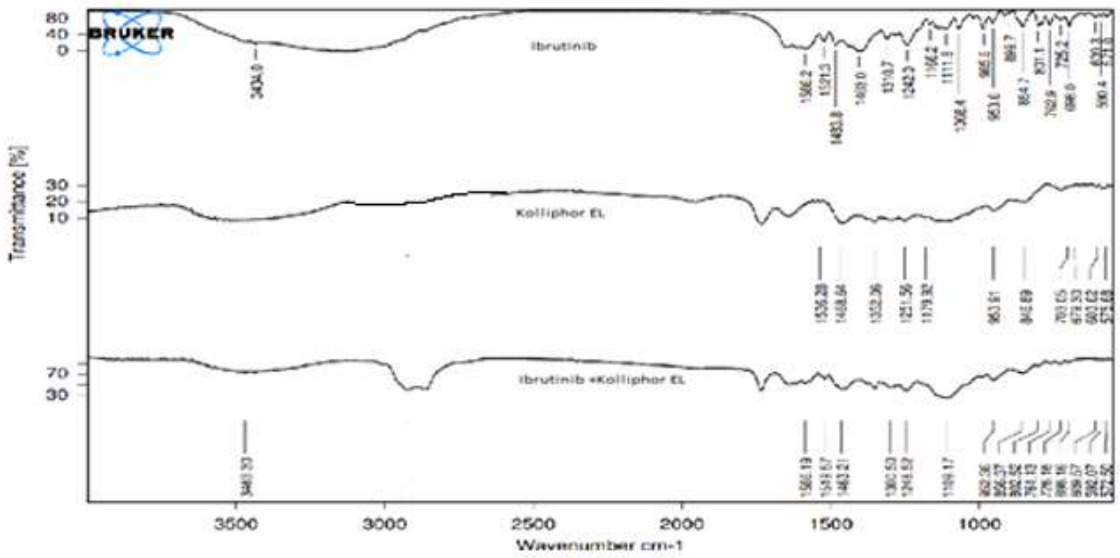

Figure 6.7: Spectrum IR of Kolliphor EL

The drug and excipients are compatible with each other which is determined by FTIR spectrometry.

The FTIR spectra of the Ibrutinib showed noticeable peaks at 3434.0 cm-1 indicates N-H bond, aromatic C-H peak found at 3135.1 cm-1, 1403 cm-1 indicates C=C stretching vibrations, strong peak at which is assigned to be C=N stretching frequencies at 1556 cm-1 and bonding frequencies of HCN and HCH between 1483.8 and 600 cm-1.

6.5 Characterization of L-SNEDDS of Ibrutinib

Table-6.2: Self-Emulsification time (sec)

S.NO	Formulation	Emulsification time(sec)	Emulsion remark
1.	F3	18.23 sec	Good

Table-6.3: Dispersibility test

S.NO	Formulation	Distilled water	0.1N HCl	Phosphate buffer (pH 6.8)
1.	F3	GRADE 1	GRADE 1	GRADE 1

Table-6.4: Phase separation & stability of emulsion

S.no	Formulation	Phase separation	Precipitation
1.	F3	No	No

Table 6.5: Droplet size, zeta potential & PDI

S.no	Formulation	Droplet size (nm)	Zeta potential(mv)	PDI
1.	F3	49.83	-25.67	0.19

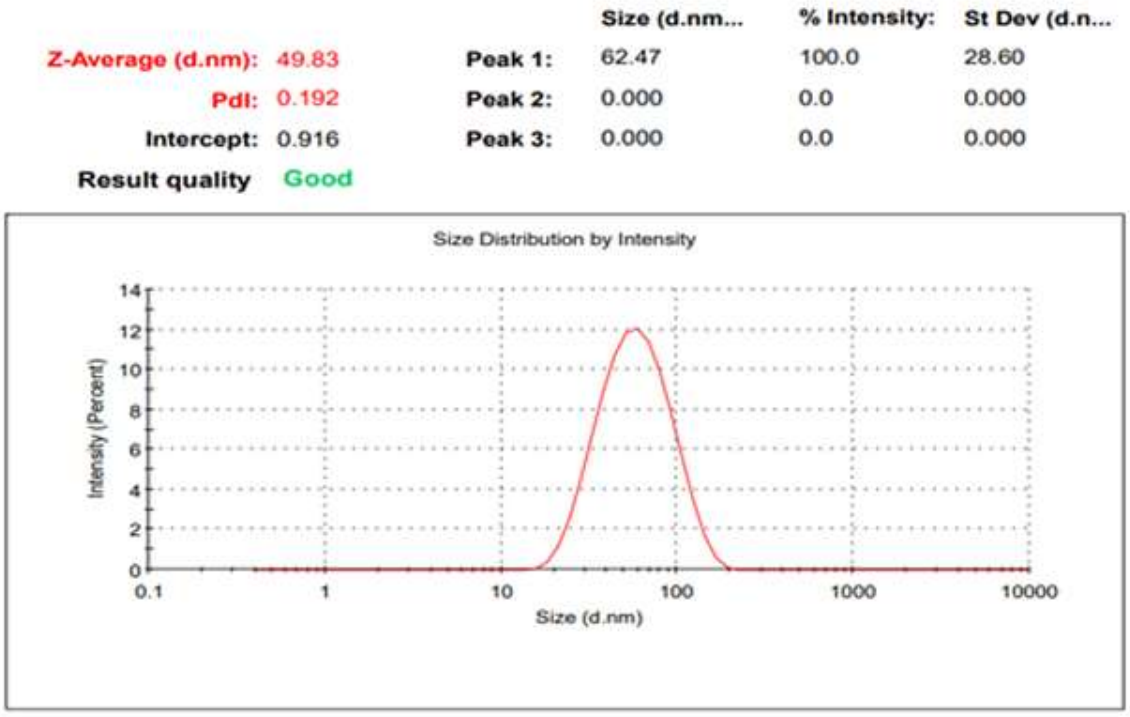

Figure 6.8: Droplet size of F3

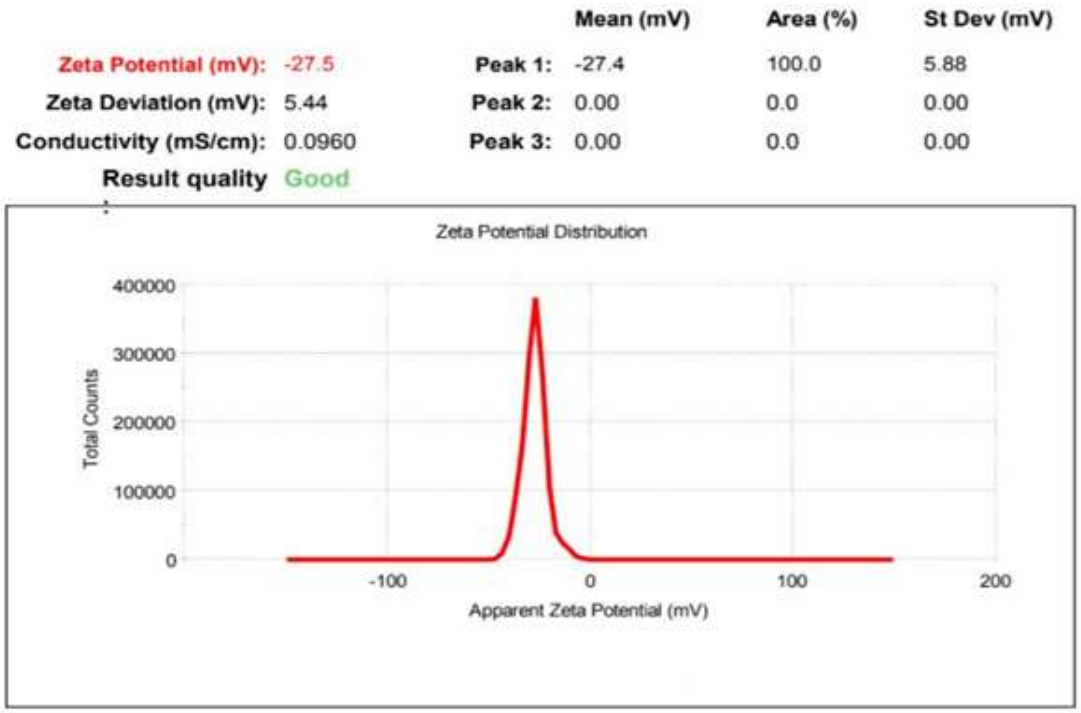

Figure 6.9: Zeta potential of F3.

Table 6.6: In-Vitro release studies of IBR liquid SNEDDS formulations F3

S.no	Time	% Cumulative Drug release of F3
	0	**0**
	5	**12.65±3.863**
	10	**35.64±2.786**
	15	**90.42±4.823**
	30	**95.55±3.576**
	45	**96.05±1.432**
	60	**97.15±2.345**
	90	**98.15±2.675**

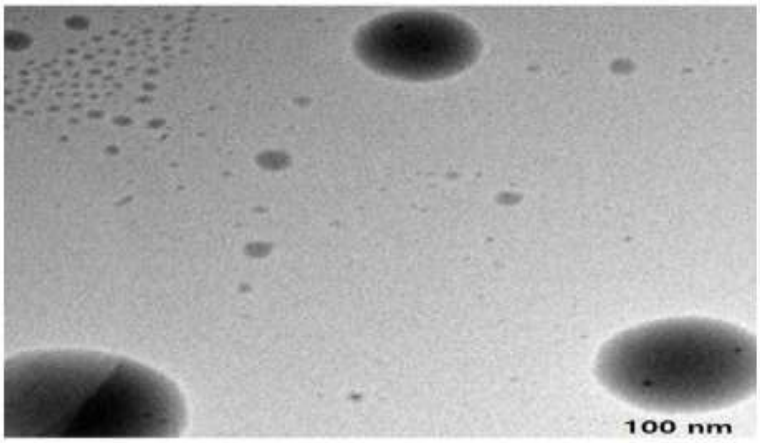

Figure 6.10: TEM image of formulation F3.

6.6 Preparation and Characterization of Solid SNEDDS of Ibrutinib

The Ibrutinib-L-SNEDDS were transformed into free-flowing powder by pouring the Ibrutinib--L-SNEDDS onto the porous carrier. From the literature survey of many solid self-emulsifying drug delivery systems, it was found that Neuselin US2 was selected as an adsorbent phase/ carrier due to its high oil adsorption property and good flowability106-108. Neuselin was taken in the concentration of 0.2% & 0.25%. The neuselin: I-L-SNEDDS ratio 0.25:1 was found to have good flowability.

Table 6.7: Micromeritic properties of L-S-SNEDDS

S.no	Neuselin: L SNEDDS Ratio	Angle of repose, θ	Bulk Density (gm/ml)	Tapped Density	Carr's index	Hausner's ratio (gm/ml)
1.	0.2:1	29.8±1.21	0.362±0.031	0.431±0.0 24	16.01±0.132	1.191±0. 004
2.	0.25:1	24.2±0.89	0.308±0.084	0.349±0.0 6	0.349±0.0 6	1.13±0.0 012

Table 6.8: Invitro drug studies of Ibrutinib SNEDDS

S.no	Time	% Cumulative Drug release of F3(Ibrutinib SNEDDS)
	0	**0**
	5	**10.65±2.458**
	10	**36.64±2.158**
	15	**87.42±4.845**
	30	**90.55±8.265**
	45	**94.05±1.248**
	60	**96.15±2.258**
	90	**97.15±2.158**

From the in-vitro-dissolution studies of liquid SNEDDS of IBR formulations shown F3 was found to release 98.15±2.675% of drug at 90 min which was higher among the L-SNEDDS. The F3(Ibrutinib SNEDDS) was found to release 97.15±2.158 taken as optimized L SNEDDS formulation. From the in-vitro-dissolution, it was found that both the liquid SNEDDS and solid SNEDDS formulations are releasing the drug at similar rates.

6.7 Drug release kinetics of I-S—SNEDDS

Table 6.9: Drug release kinetics I-S-SNEDDS

Time (Min)	%CDR	Log % CDR	SQRT	Log T	$Wo^{1/3}$- $Wt^{1/3}$	% DR	Log % DR
0	0	0	0	0	0	100	2.00000
5	11.55	1.0626	2.2361	0.6990	2.2604	88.45	1.94670
10	29.55	1.4706	3.1623	1.0000	3.0916	70.45	1.84788
15	85.62	1.9326	3.8730	1.1761	4.4075	14.38	1.15776
30	96.01	1.9823	5.4772	1.4771	4.5790	3.99	0.60097
45	98.94	1.9954	6.7082	1.6532	4.6251	1.06	0.02531
60	98.65	1.9941	7.7460	1.7782	4.6206	1.35	0.13033
90	98.02	1.9913	9.4868	1.9542	4.6107	1.98	0.29667

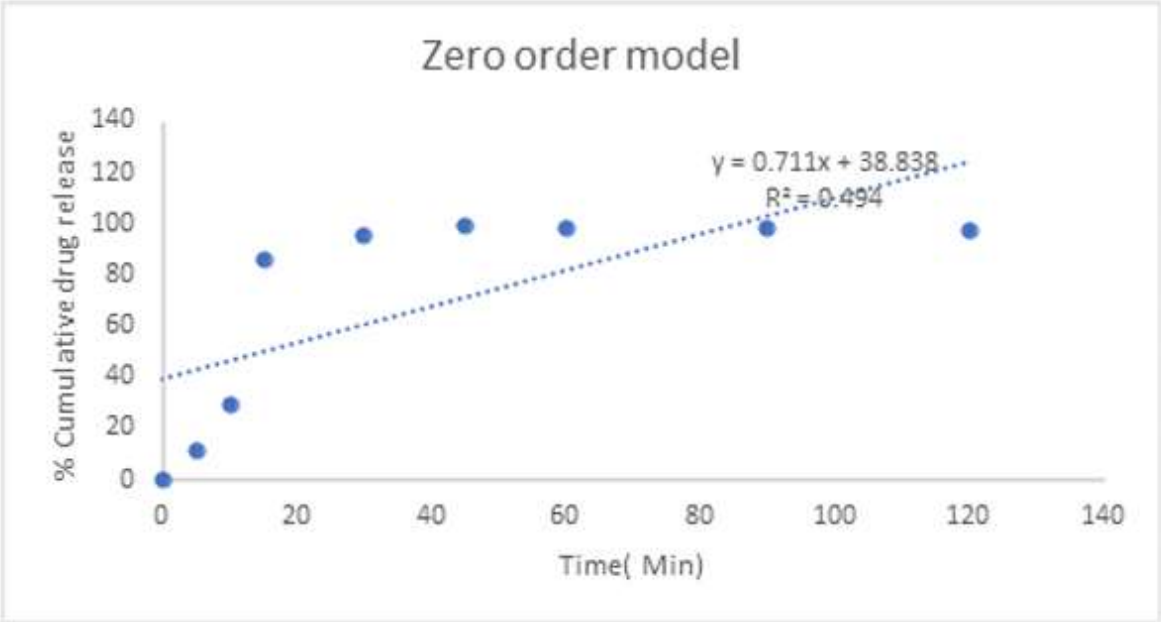

Figure 6.11: Zero order drug release kinetics(F3)

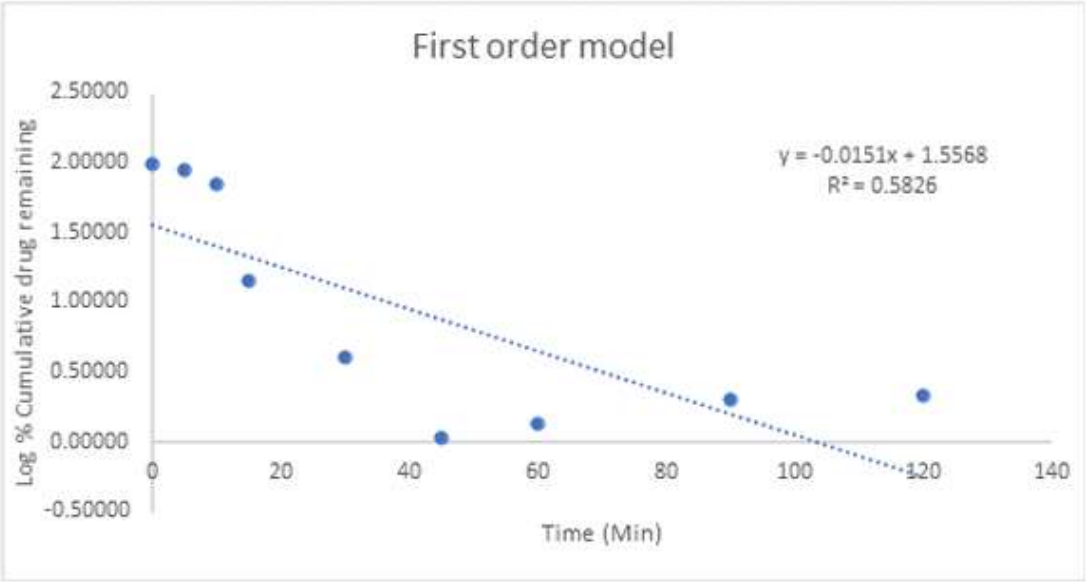

Figure 6.12: First order drug release kinetics(F3)

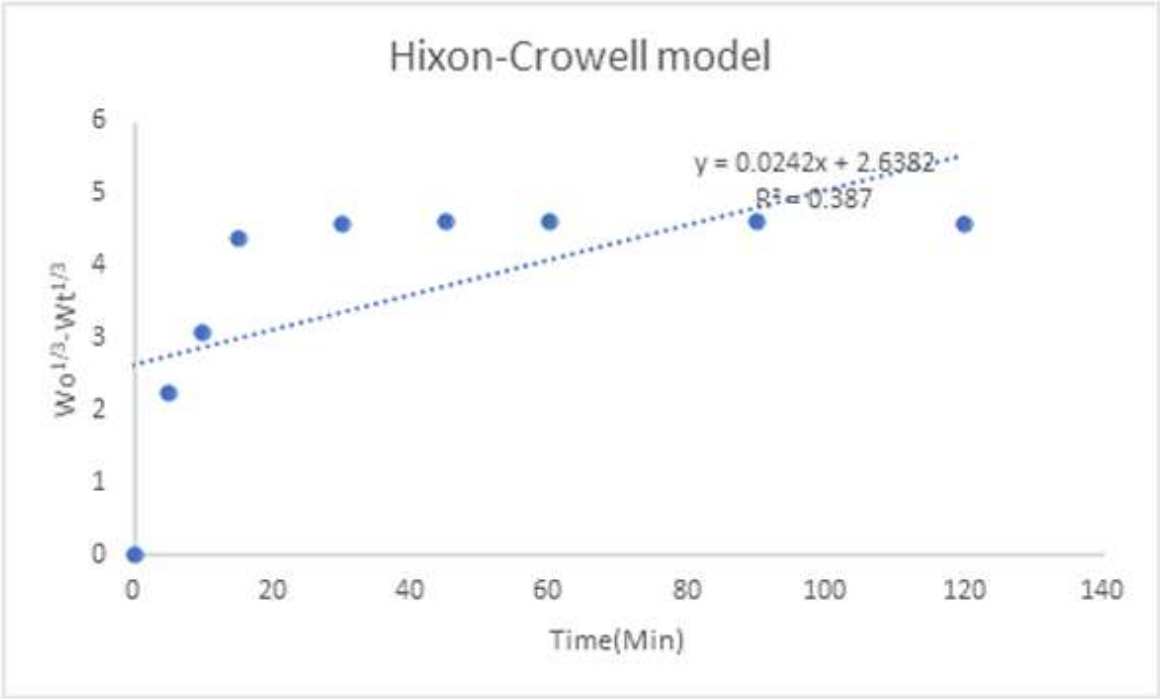

Figure 6.13: Hixon & crowell drug release kinetics

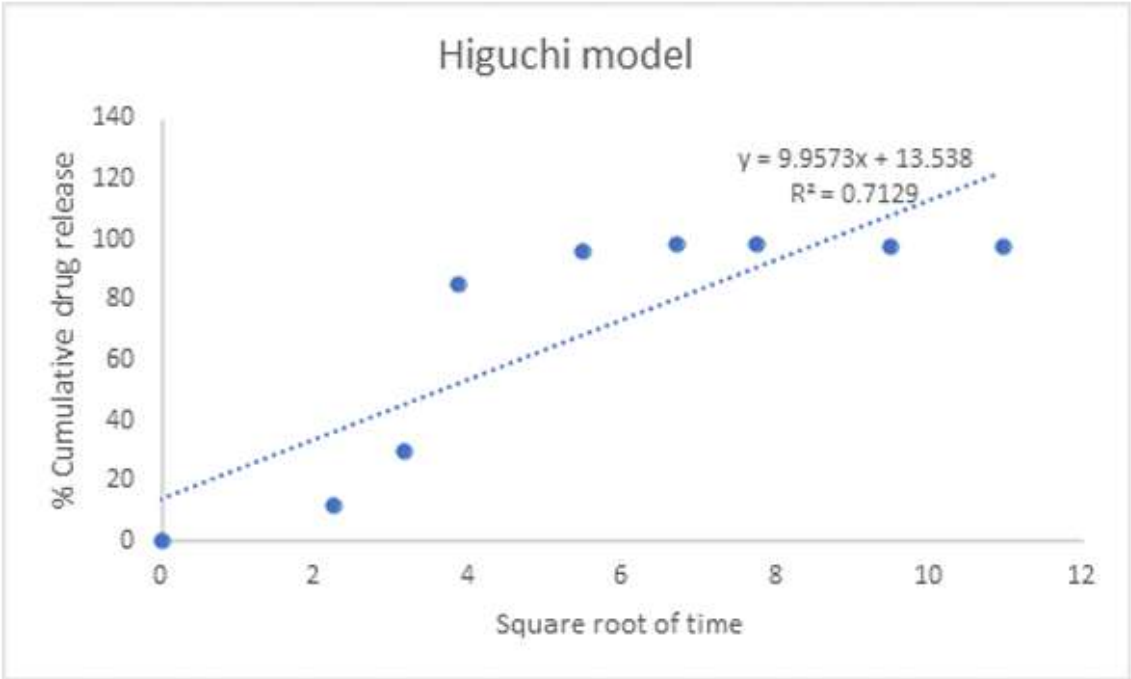

Figure 6.14: Higuchi drug release kinetics

From the various kinetic modelling shown in table 6.9- and figures-6.11-6.14, it was found that none of the model R2 was near to 1. SNEDDS are not dependent on model release kinetics.

6.8 Scan spectrum of Gefitinib

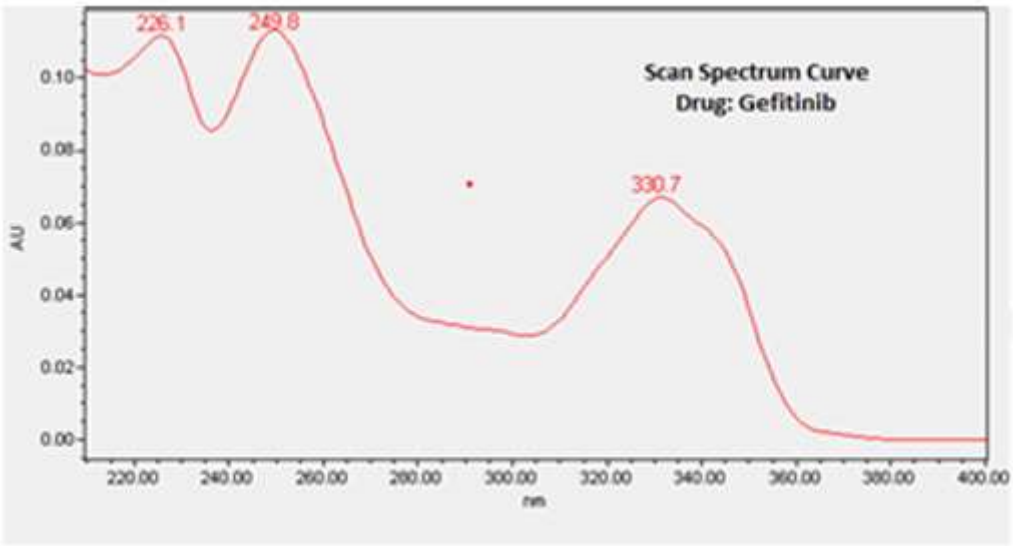

Figure 6.15: Scan spectrum of Gefitinib

The scan spectrum shown the highest peak area at 226.1, 249.8 and 330.7nm.

The mobile phase of optimized trial was Acetonitrile and 6.5pH Phosphate buffer in a ratio of 70:30 ratio with a flow rate of 1ml/min and the retention time was 4.78min.

The blank and chromatogram of Gefitinib are shown below.

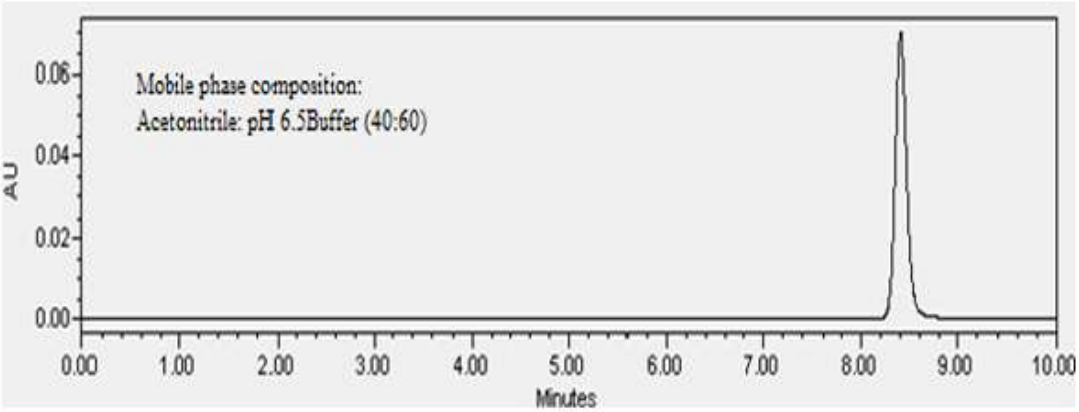

Figure 6.16: Blank chromatogram

6.9 Calibration curve of Gefitinib

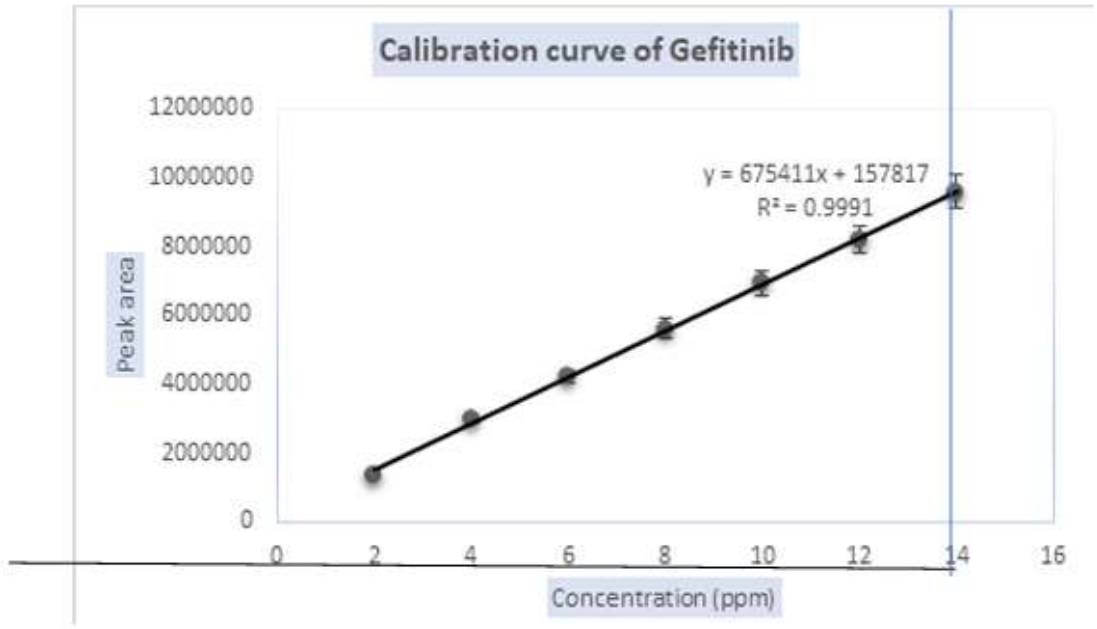

Figure-6.17: Calibration curve of Gefitinib

Standard curve of GFB was constructed by different concentrations ranging from 2 to 16 ppm, for which the peak area readings were determined. The standard curve was linear over the concentration range studied with a correlation coefficient (r2) 0.9991. The corresponding regression equation was found to be Y = 675411X – 157817.

6.10 Solubility studies of Gefitinib

From the solubility studies results, the maximal solubilizing potential for the drug, among various excipients.

Ibrutinib shown the greatest solubility in the oil TranscutolHPPeceol, Labrasol ALF, TranscutolP at 25˚C. The solubility was aimed for determining suitable oil and surfactants for the Gefitinib to formulate LBDDS.

Table 6.10: Solubility of IBR in Various excipients

S.NO	EXCIPIENTS	SOLUBILITY of IBR (mg/ml)
	TranscutolHPPeceol	42.75
	Labrasol ALF	33.53
	TranscutolP	77.77

6.11 Drug-excipient compatibility by Fourier infrared spectroscopy for Ibrutinib and excipients

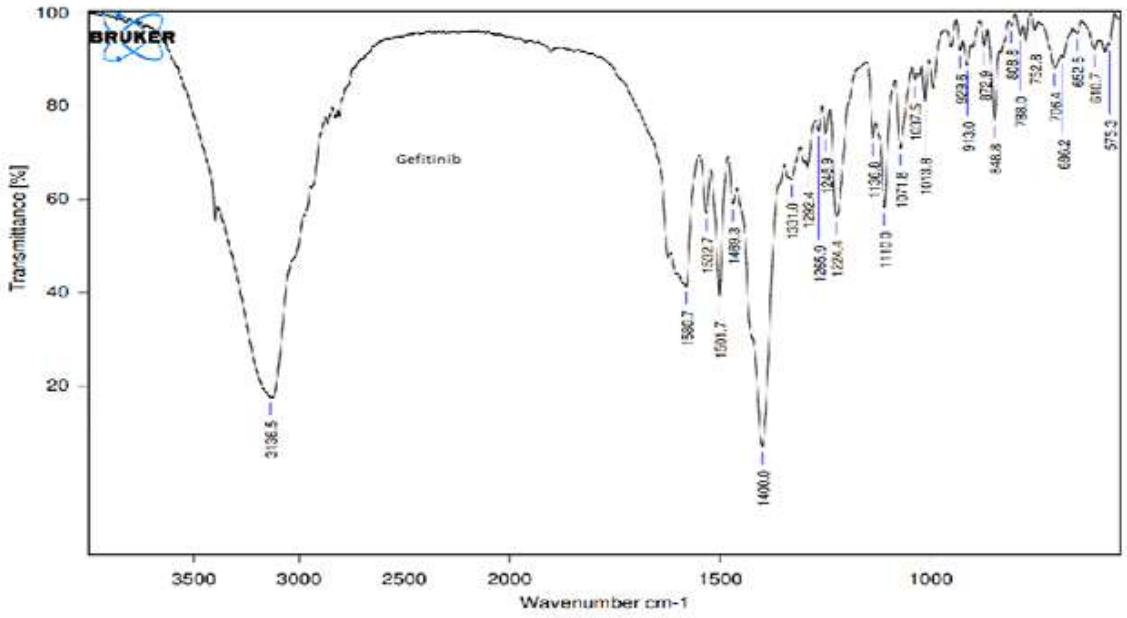

Figure 6.18: Spectrum IR of Gefitinib

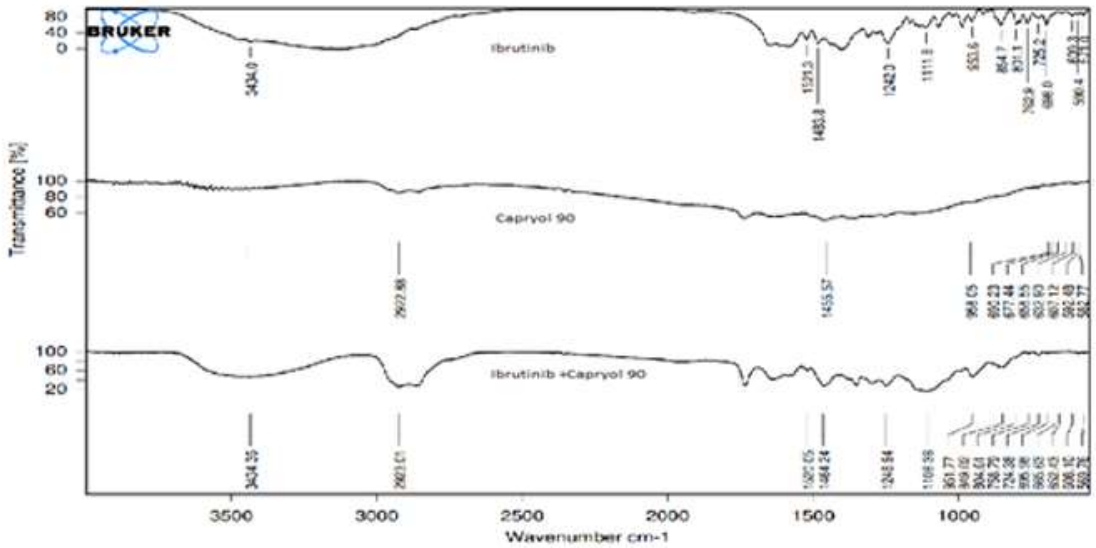

Figure-6.19: Spectrum IR of Gefitinib+ Peceol

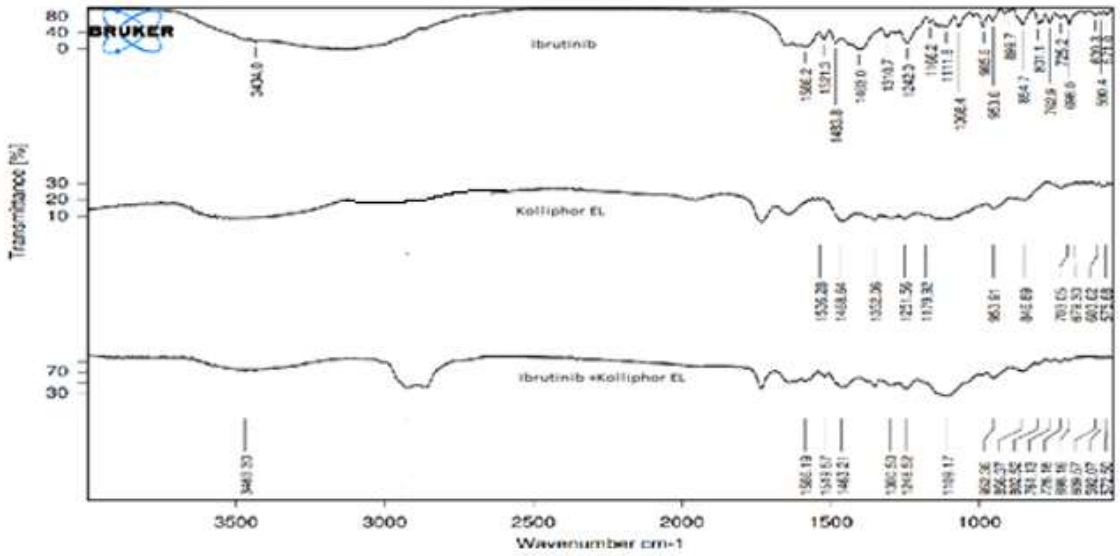

Figure 6.20: Spectrum IR of Gefitinib + Labrasol ALF

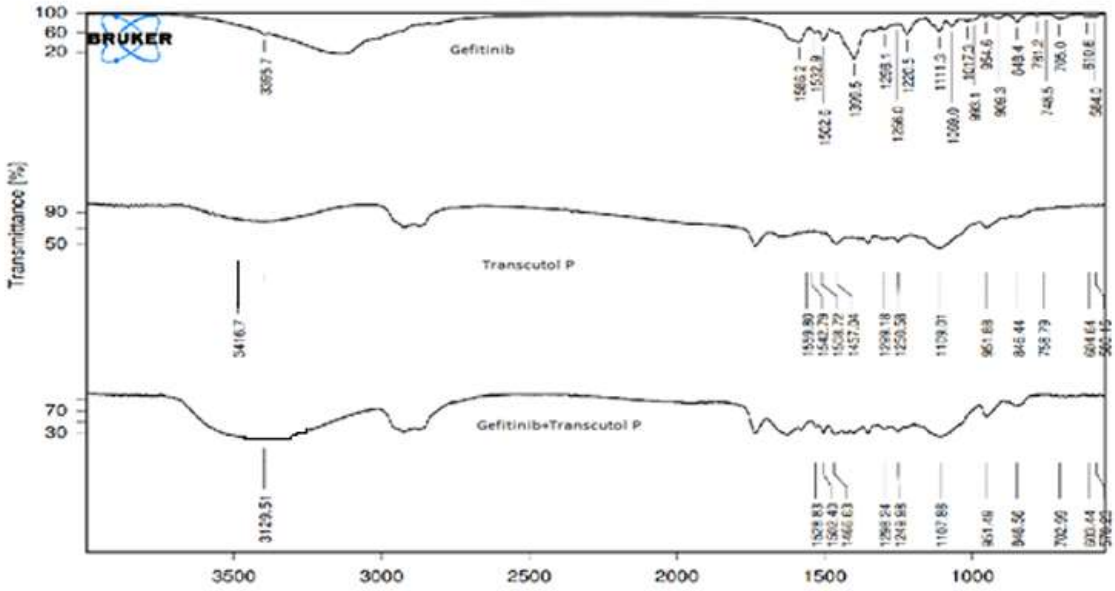

Figure 6.21: Spectrum IR of Gefitinib + Transcutol HP

The drug and excipients are compatible with each other which is determined by FTIR spectrometry.

The spectrum shown various peaks, N-H peak appeared at 3471.05 cm-1, A strong peak of stretching frequency of C=N at 1532 cm-1 and bending frequencies of HCN & HCH have their peaks between 1439.3 cm-1 & 606 cm-1.

6.12 Characterization of L-SNEDDS of Geftinib

Table-6.11: Self-Emulsification time (sec)

S.NO	Formulation	Emulsification time(sec)	Emulsion remark
1.	F4	56.23 sec	Good

Table-6.12: Dispersibility test

S.NO	Formulation	Distilled water	0.1N HCl	Phosphate buffer (pH 6.8)
1.	F4	GRADE 1	GRADE 1	GRADE 1

Table-6.13: Phase separation & stability of emulsion

S.no	Formulation	Phase separation	Precipitation
1.	F4	No	No

Table 6.14: Droplet size, zeta potential & PDI

S.no	Formulation	Droplet size (nm)	Zeta potential(mv)	PDI
1.	F4	26.98	-28.7	0.324

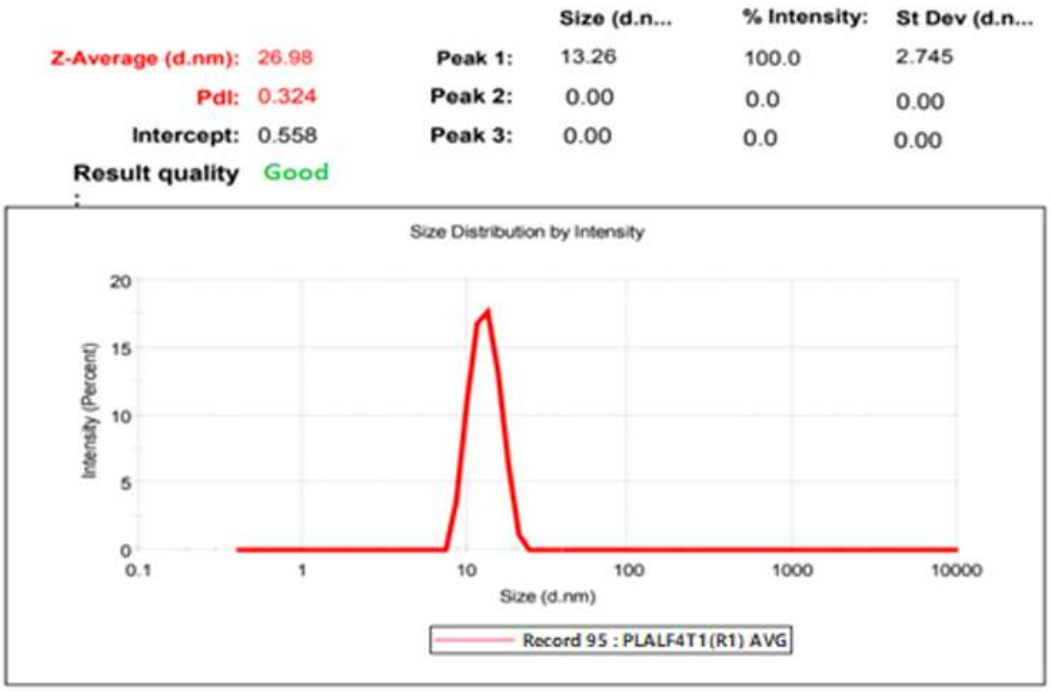

Figure 6.22: Droplet size of F4

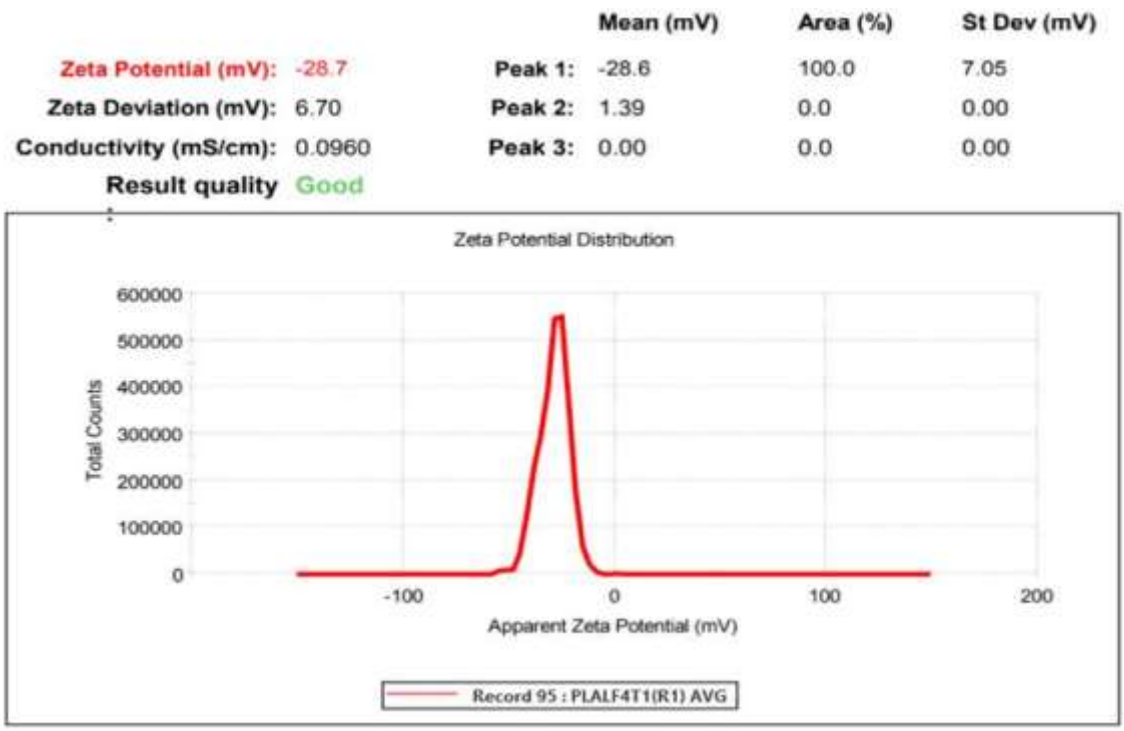

Figure 6.23: Zeta potential of F4.

Table 6.15: In-Vitro release studies of Gefitinib liquid SNEDDS formulations F4

S.no	Time	% Cumulative Drug release of F4
1.	0	0
2.	5	10.65±3.158
3.	10	30.16±2.157
4.	15	80.24±4.124
5.	30	84.45±3.547
6.	45	92.07±1.157
7.	60	95.05±2.452
8.	90	96.75±2.154

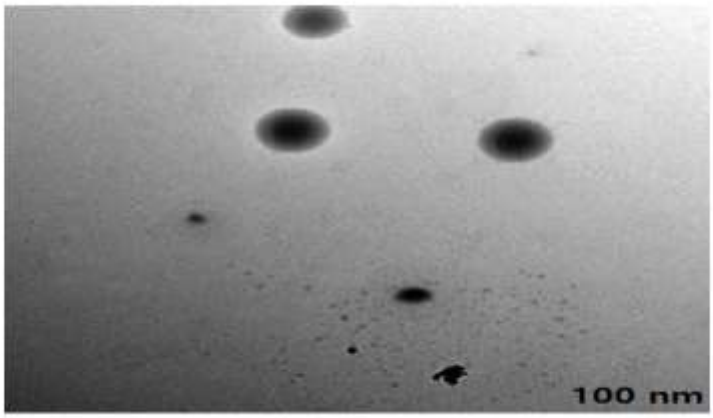

Figure 6.24: TEM image of formulation F4.

6.13 Preparation and Characterization of Solid SNEDDS of Gefitinib

The Gefitinib -L-SNEDDS were transformed into free-flowing powder by pouring the Gefitini -L-SNEDDS onto the porous carrier. From the literature survey of many solid self-emulsifying drug delivery systems, it was found that Neuselin US2 was selected as an adsorbent phase/ carrier due to its high oil adsorption property and good flowability106-108. Neuselin was taken in the concentration of 0.2% & 0.25%.

Table 6.16: Micromeritic properties of Geftinib-S-SNEDDS

S.no	Neuselin: L SNEDDS Ratio	Angle of repose, θ	Bulk Density (gm/ml)	Tapped Density	Carr's index	Hausner's ratio (gm/ml)
1.	0.2:1	27.8±1.53	0.153±0.048	0.425±0.049	19.01±0.482	1.79±0. 001
2.	0.25:1	26.2±0.26	0.142±0.078 9	0.354±0.048	0.429±0.056	1.48+±0.025

Table 6.17: Invitro drug studies of Gefitinib SNEDDS

S.no	Time	% Cumulative Drug release of F4 (Gefitinib SNEDDS)
1.	**0**	**0**
2.	**5**	**11.45±2.159**
3.	**10**	**30.44±2.354**
4.	**15**	**65.12±4.459**
5.	**30**	**85.05±8.182**
6.	**45**	**90.04±1.197**
7.	**60**	**94.05±2.546**
8.	**90**	**96.05±2.481**

From the in-vitro-dissolution studies of liquid SNEDDS of Geftinib formulations shown F4 was found to release **96.75±2.154**% of drug at 90 min which was higher among the L-SNEDDS. The F4(Geftinib SNEDDS) was found to release **96.05±2.481** taken as optimized L SNEDDS formulation. From the in-vitro-dissolution, it was found that both the liquid SNEDDS and solid SNEDDS formulations are releasing the drug at similar rates.

6.14 Drug release kinetics of Geftinib SNEDDS

Table 6.18: Drug release kinetics Geftinib SNEDDS

Time (Min)	%CDR	Log % CDR	SQRT	Log T	$Wo^{1/3}$- $Wt^{1/3}$	% DR	Log % DR
0	0	0	0	0	0	100	2
5	16.35	1.2135	2.2361	0.6990	2.5381	83.65	1.92247
10	28.62	1.4567	3.1623	1.0000	3.0588	71.38	1.85358
15	80.26	1.9045	3.8730	1.1761	4.3135	19.74	1.29535
30	95.44	1.9797	5.4772	1.4771	4.5699	4.56	0.65896
45	99.23	1.9966	6.7082	1.6532	4.6296	0.77	0.11351
60	98.95	1.9954	7.7460	1.7782	4.6253	1.05	0.02119
90	98.41	1.9930	9.4868	1.9542	4.6169	1.59	0.2014

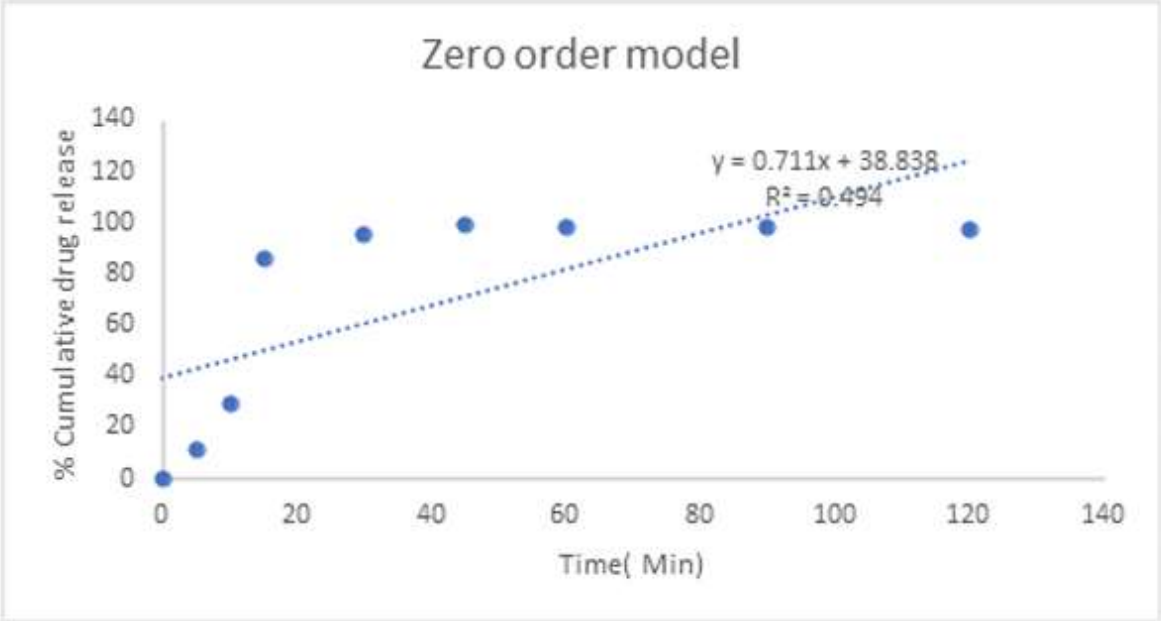

Figure 6.25: Zero order drug release kinetics(F4)

Figure 6.26: First order drug release kinetics(F4)

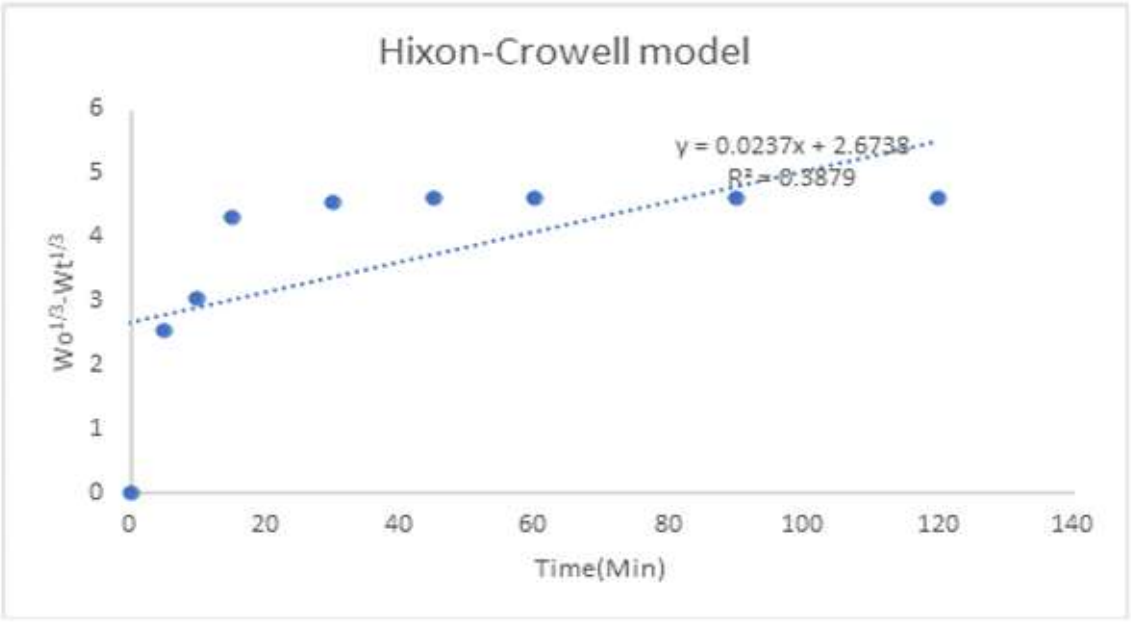

Figure 6.27: Hixon & crowell drug release kinetics

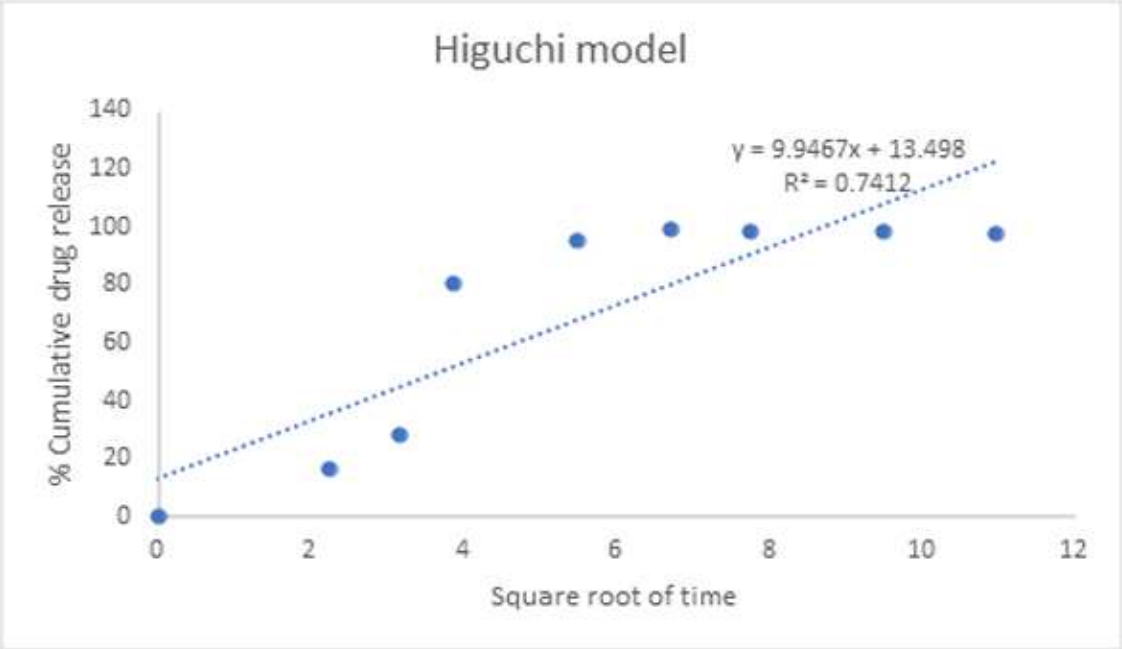

Figure 6.28: Higuchi drug release kinetics

From the various kinetic modelling shown in table 6.9- and figures-6.11-6.14, it was found that none of the model R2 was near to 1. SNEDDS are not dependent on model release kinetics.

CHAPTER 7

SUMMARY AND CONCLUSION

Ibrutinib and Gefitinib, these two model drugs belong to BCS class-II which are with poor solubility in Aqueous Media.

The present work mainly emphasized on the enhancement of solubility of Ibrutinib and Gefitinib by developing them into LBDDS as Self Emulsifying drug delivery systems. The developed and validated method is used to carry solubility studies of different excipients (oils, surfactants and cosurfactants).

The solubility was determined in lipidic excipients by HPLC analysis. Capryol 90(Oil), Kolliphor EL (Surfactant) and TranscutolHP(co-surfactant) were Selected for Ibrutinib. Peceol (Oil), Labrasol ALF(Surfactant) and TranscutolP(co-surfactant) were Selected for Gefitinib.

Using the optimized self-emulsifying mixture, drug loaded Liquid SEDDS were evaluated for their self-micro emulsification tendency, Phase separation & stability, droplet size analysis, poly dispersibility index, zeta potential, TEM analysis, drug release studies etc. are characterized.

In vitro drug release studies showed above above 95% drug release in 45-90 minutes for all the formulations (F3 & F4). The in-vitro release profiles of formulations were good. They showed a significant increased rate of dissolution, when compared with the API.

The optimized L-SNEDDS were converted to S SNEDDS by adsorption technique by using neuselin as carrier.

The SNEDDS will be the promising formulations for the model drugs selected.

CHAPTER 8

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