Design and Optimization by Response Surface Methodology and Lymphatic Uptake Study of Lipid-Based Drug Delivery Systems of Ibrutinib.

K. Anie Vijetha[1], M. Sunitha Reddy[2]

1. Assistant Professor (C), Centre for Pharmaceutical sciences, IST, JNTUH, Kukatpally, Hyderabad, Telangana-500085, INDIA.

2. Associate Professor, Centre for Pharmaceutical sciences, IST, JNTUH, Kukatpally, Hyderabad, Telangana-500085, INDIA.

Corresponding Author*

K. AnieVijetha

Assistant professor (C), Centre for Pharmaceutical sciences, IST,

JNTUH, Kukatpally, Hyderabad, Telangana-500085, INDIA.

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. Most of anticancer drugs discovered in the last two decades belong to biopharmaceutical classification system-I, where solubility is the major drawback for the drugs and it decreases the systemic availability of the drug. Hence, it is prepared as lipid-based drug delivery systems with an aim to enhance its dissolution rate. The objective of the research is to develop Self emulsifying drug delivery systems (SEDDS) of Ibrutinib (IBR). IBR is an anticancer drug, which binds irreversibly to Bruton's tyrosine kinase (BTK) receptor to the cysteine residue and inhibits BTK active site. Screening of excipients was done by determining the saturation solubility studies and Capryol 90 was selected as an oil phase, kolliphor EL as surfactant and transcutol HP as co-surfactant due to their higher solubilization effect. The best composition of oil, surfactant and co-surfactant was obtained by aqueous titration method and further optimized by design of experiments (DOE) by using Central composite model (CCD). In-vivo bioavailability studies were conducted for the pure drug suspension, L-SNEDDS (Liquid-self-nano emulsifying drug delivery systems) and S-SNEDDS (Solid-self-nano emulsifying drug delivery systems) and pharmacokinetics parameters were estimated. The lymphatic uptake of SNEDDS is studied. The in-vivo oral bioavailability studies of optimized SNEDDS

formulation showed 3.57 folds higher than the pure drug Ibrutinib. The I-L-SNEDDS and I-S-SNEDDS were passed Level-A with correlation coefficient of 0.934.

Key Words: Ibrutinib, Self-emulsifying drug delivery systems, capryol 90, kolliphor EL, Transcutol HP, Cancer etc.

1. Introduction

Ibrutinib(IBR) is a tiny molecule that acts as an irreversible potent blocker of Brutons amino acid Tyrosine kinase (BTK)[1]. It is selected as a targeted covalent drug and it presents a really promising activity in B-lymphocyte malignancies. IBR was approved for the treatment of chronic lymphocytic leukaemia and also indicated for the treatment of patients with waldenstrom'sMacroglobulinemia, Breast cancer, Glioblastoma, skin cancer[1,2,3,4]. Upon oral administration, Ibrutinib inhibits BTK activity and preventing each B-cell activation and B-cell-mediated signalling. This ends up in an inhibition of the expansion of malignant B cells that overexpress BTK and blocks the abnormal protein that signals cancer cells multiplication and finally stops dispersion of cancer[5].

IBR belongs to Biopharmaceutics Classification Systems (BCS) **class-II** compound with low solubility(0.003mg/ml) and high permeability with log p value of 3.97, pKa value of 3.79, 3.9% oral bioavailability and is metabolized fastly by CYP3A[6].

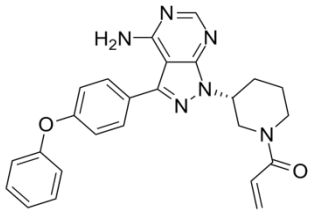


Fig 1: Molecular structure of Ibrutinib

The lipophilic drug compounds have low solubility problems which interprets suboptimal patient outcomes which leads to poor bioavailability. The conventional dosage forms are not enough to solve the issues. Bioavailability of these drugs can be enhanced by Lipid based drug delivery system (LBDDS), solid dispersions, Micronization and nanonization techniques. LBDDS are the best technologies to boost the solubility and bioavailability of poorly soluble drugs. Solubility of these compounds can be enhanced by formulating it into any one of the LBDDS technologies such as Self emulsifying drug delivery systems (SEDDS), liposomes, Solid lipid nanoparticles (SLNs) etc[7,8,9]. SEDDS are gaining global acceptance to enhance the bioavailability potential of lipophilic compounds by enhancing their dissolution and bioavailability[10]. IBR is developed into SEDDS which is an isotropic mixture of oils, surfactants and cosurfactants, that undergo emulsification by slight agitation in presence of aqueous phase (gastric fluid) to make fine emulsion. SEDDS with

globule size range between 20-200nm are known as self-nano emulsifying drug delivery systems (SNEDDS)[11–13].

The potential advantages of SNEDDS are ability to present the drug in solubilized form inside the GIT, provide greater chemical and enzymatic stability by inhibiting p-glycoprotein mediated drug efflux present in the gut enterocytes, overcoming enterohepatic circulation and enhancing lymphatic transport[14–16].

Design of experiments (DOE) helps in understanding the relationship between formulation factors and its quality. CCD is simple, less complex structure, fewer experimental runs, ease of design interpretation and most acceptable approach for optimization of SNEDDS[17–20].

Thus, the overall aim of this study enhancement of bioavailability of IBR and to optimise the Ibrutinib SEDDS to maintain nano-sized globules. The prepared liquid SNEDDS are converted to Solid-free flowing powder. The Solid Self emulsifying drug delivery systems (S-SNEDDS) formulations were studied for in-vitro and in-vivo characterizations and stability studies were conducted for evaluating the safety and stability of the formulation.

2. Materials and methods

2.1. Materials

Ibrutinib, a gift sample from NACTO Pharma Ltd. Hyderabad., Crodamol PC-LQ, Peceol, Capryol 90, Masine CC, Labrafac PG, Labrafac CC, Labrafil M 1944 CS, Labrasol ALF, Transcutol-HP, Transcutol-P, Miglyol 812, Simulsol 1272, Lauroglycol FCC, Lauroglycol 90, Gelucire-44/14, Gelucire50/13, Gelucire-48/16, Lipoid Phosal 53 were supplied from Gattefosse, France. Capmul MCM C8 EP, Capmul MCM NF, Captex 200, Captex 355 are donated by Abitec Corporation, USA. Kolliphor HS 15, Kolliphor RH 40 and Kolliphor EL are gift samples from BASF. The water was obtained from Milli-Q-Water purification system, Millipore. Acetonitrile was HPLC Grade Merck and other chemicals were of analytical grade procured from research lab fine chem.

2.2. Methods

2.2.1. Analytical methodology of IBR

Analytical method is developed for IBR by using HPLC Waters system (Waters with 515 HPLC Pump and SunFire C18 5µm column) and the optimized mobile phase used is acetonitrile and 0.1% orthophosphoric acid solution in the ratio of 70:30, the retention time obtained was 2.5min. The linearity was found to be 0.2 ppm to 4 ppm[21,22].

2.2.2. Saturation Solubility studies of IBR in various oils, surfactants and cosurfactants

The solubility of IBR was determined by dissolving an excess amount of the drug into 1gm of various oils, surfactants and cosurfactants. The excipients were screened for determining the equilibrium solubility of IBR. The samples in the vials were vortexed for 2min using cyclomixer (REMI CM 101) and kept in shaking incubator with constant shaking at 25˚C for 48h. Addition of drug is continued in unsaturated excipients and cyclo-mixed and kept in shaking incubator (LabTech). After saturation of the drug in the particular excipient, the equilibrated samples were centrifuged at 3000rpm for 10min. The supernatant solution was determined for the concentration of IBR using validated method by HPLC[23–25].

2.2.3. Drug-excipient compatibility studies by FTIR spectroscopy

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 are determined for incompatibilities and the spectra of optimized formulation is compared with the pure drug[26–28].

2.2.4. Construction of pseudo-ternary phase diagram

Based on the solubility studies,capryol 90 selected as an oil phase, kolliphor EL as surfactant and transcutol HP as co-surfactant. To determine the composition of oil, surfactant and cosurfactant for a best emulsion and to identify self-nano emulsifying region, a pseudo ternary phase diagram was constructed using aqueous titration method at ambient temperature. The surfactant/co-surfactant (Smix) were also optimised/ by using pseudo ternary phase diagrams. surfactant/co-surfactant (Smix) were mixed in different volume ratios (1:1, 2:1, 3:1 and 4:1). Oil and surfactant/co-surfactant (Smix) were mixed thoroughly in different volume ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) and titrated with water and intermittently vortexed for 2mins during titration and allowed to equilibrate. The change was observed visually from transparent to turbid and marked on the three-component system. The composition of oil, Smix and water were analysed and pseudo-ternary plot was constructed using Triplot generator. The components used for construction of pseudo-ternary phase diagram are capryol 90 as oil, kolliphor EL as surfactant, transcutol HP as co-surfactant and double distilled water as aqueous phase.

The samples which produced transparent and slightly bluish colour were considered as nanoemulsions[29–34].

2.2.5. Optimization of SNEDDS by using Central composite design

The self-nanoemulsifying region was identified by pseudo-ternary phase diagrams, for the desired component ratios of SNEDDS. For the Preparation of SNEDDS, Capryol 90 (X_1), Kolliphor EL (X_2) and Transcutol HP (X_3) were chosen and finally selected as three factors for further optimization of the formulation. To design the experiment, a central composite design (CCD) was used to optimise SNEDDS using DesignExpert® Version 13 (Stas-ease) where, the three independent variables X1 (Oil), X2 (surfactant), X3 (co-surfactant) were varied inthe minimum and maximum range, selected from the nanoemulsion region (Pseudo ternary phase diagrams). Based on the experimental formulations 20 runs were formulated by mixing various proportions of oil, surfactant and cosurfactant as per the CCD proportions (Table-). IBR (40 mg) was accurately weighed and added to weighed amount of oil into a glass vial, the mixture was cyclomixed for a few minutes (Cyclomixer, Remi CM 101), then the mixture was sonicated inan ultrasonic bathwith occasional cyclo-mixing until the IBR was dissolved to maximum extent and the formulations were centrifuged for the sedimentation of undissolved drug and supernatant was evaluated for the drug content. Each formulation was rated for response Y (dependent variable), i.e. active ingredient content. The optimization goal was to maximize % Drug content(Y_1) and self-emulsification(Y_2)(> 99%)[35–37].

2.2.6. Characterization of SNEDDS

2.2.6.1. 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) was added to 200ml of distilled water at 37±0.5˚C and gently agitated using magnetic stirrer rotating at constant speed. The time required for the disappearance of SNEDDS was recorded[38,39].

2.2.6.2. 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[40,41].

Grade I: Rapidly forming (In 1 min) Nano emulsion having a Clear (or) Bluish appearance.

Grade II: Rapidly forming, slightly less Clear emulsion, having a Bluish white appearance.

Grade III: Fine Milky emulsion is formed within 2min.

Grade IV: Dull, greyish white emulsion with a slight oily appearance that is slow to emulsify (More than 2 min).

2.2.6.3. Phase separation and stability study of emulsions

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 intervals for a period of 24 hours[40,42,43].

2.2.6.4. Robustness on dilution

The optimized SNEDDS formulations were subjected to various dilutions from 1:50 to 1:500 with water, 0.1N HCl, phosphate buffer pH 7.5 to access the effect on dilution of the SNEDDS formulation and these systems were stored at ambient temperature for 24 hours then visually observed for any signs of phase separation[38,39,43,44].

2.2.6.5. Droplet size and zeta-potential measurements

The optimised blank SNEDDS (Clear transparent/bluish emulsion) from the aqueous titration method and optimised formulations was 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 is performed at 25˚C placing disposable cuvette and zeta potential is performed by using an electrophoretic cell with an angle of detection of 90˚ measurement. 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[39,40,43–45].

2.2.6.6. In vitro drug release studies

In-vitro dissolution studies of IBR and IBR 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 IBR from the SNEDDS formulation was compared with the pure drug IBR which is filled capsule containing the same amount of drug[43,46,47]. The concentration of IBR in the released sample was determined by the developed HPLC method.

2.2.6.7. Thermodynamic stability studies

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 is determined by Centrifugation and Freeze thaw cycles[44,47].

2.2.6.7.1. **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.

2.2.6.7.2. **Freeze Thaw cycle:** In this study the stability of nano-emulsion was determined by exposing the formulations between -20˚C and +25 ˚C for each temperature cycles and this was repeated for 5 times for each formulation up to 48hours. The samples were observed for phase separation (or) precipitation.

2.2.6.8. 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 is left for 5min to settle down and air dried. Finally, the dried grid was visualized under TEM at an operating voltage of 80 kV[48–50].

2.2.7. Formulation of S-SNEDDS

The prepared L-SNEDDS are converted to S-SNEDDS by adsorption on the carrier (Neuselin). The adsorption process involves addition of the L-SNEDDS formulation onto

the carrier by mixing in a blender and dried until a free-flowing powder is obtained. The resultant powder is filled into the capsules. etc[39,40,44].

2.2.8. In-vivo bioavailability studies of SNEDDS and determination of drug in rat plasma by HPLC in presence and absence of Lymphatic blocker (Cyclohexidine)

The bioavailability studies are carried out in wistar breed healthy male rats (200±20g) were secured from the animal house. Institutional animal ethical committee with Reg. No. 1662/PO/Re/S/12/CPCSEA approved the animal study. The work was permitted by the animal ethical facilities guidelines from the ethical committee of animal care centre, Mallareddy institute of pharmaceutical sciences, Maisammaguda, Hyderabad with clearance no. 001/MRIPS/CPSCEA-IAEC/Hyd/2020. The animals were maintained in animal cages and 6 animals were kept per cage with a 12hours light & dark cycles at a temp of 25±2˚C. The rats were acclimatized to laboratory conditions for one week before the experimental work. To avoid food-drug interactions the animals were kept fasted for a period of 12 hours prior to the experiment and had continuous availability to water throughout the experimental period. The animals were given with general diet after 1 hour of rat dosing[51,52,53]. The total number of animals taken for the study are 24 and divided into 4 groups. Group-1 was administered orally aqueous suspension of drug (control group), L-SNEDDS was given to Group-2 & S-SNEDDS was given as Group-3. As both L-SNEDDS and S-SNEDDS were showing nearly same rate and extent of drug release in the body, L-SNEDDS were preferred for studying lymphatic uptake. Group 4 rats were used to evaluate the lymphatic system uptake of L-SNEDDS by the enterocytic cells of intestine, in vivo evaluations were performed by Cycloheximide (CXI), which is lymphatic uptake inhibitor. CXI inhibits the production of chylomicrons by the enterocytic cells, which stops the lymphatic delivery of the lipoidal particles without interfering passive and active absorption pathways. Lymphatic uptake of L-SNEDDS was studied by administering (10mg/kg of body weight) of cyclohexidine (Lymphatic blocker) solution through IV route through the tail vein. The L-SNEDDS was administered to Group-2 rats and for the Group-4 rats, L-SNEDDS was administered orally 10 min after the injecting the cyclohexidine solution.

The formulations were administered to wistar rats by feeding needle through oral gavage. By chloroform anaesthesia, blood samples were withdrawn (0.5 mL) by the retro-orbital blood collecting route at different time scheduled 15, 30, 60, 120, 240, 360, 480, 600, 720 & 1440min by into K2 EDTA tubes. The aliquots were centrifuged at 10,000rpm for 20min at a temp of 4°C. The blood samples (100µL) processed & 1mL acetonitrile

was added to samples to precipitate the plasma proteins. The samples were centrifuged by using cooling centrifuge at 10,000 rpm at 4°C/10 min, the supernatant fluid was injected into the HPLC and the concentrations of drug estimated by using the developed and validated HPLC waters system consisting of photo-diode array detector. Empower software of waters was used to process the data for the determination of drug content by developed chromatographic method. The bioavailability of selected SNEDDS formulation was compared with the pure drug (Active pharmaceutical ingredient) and difference in bioavailability of SNEDDS in presence and absence of Cycloheximide.

2.2.9. Pharmacokinetic Parameters and Statistical Analysis

Non compartmental pharmacokinetic analysis was opted to study the PK behaviour of drugs. PK Solver 2.0 (Add-in software of windows microsoft Excel) which was chosen to calculate the various PK parameters for the study[46,54]. The Oral bioavailability of the optimized SNEDDS to pure drug is calculated as follows % Percentage relative bioavailability = $AUC_{SNEDDS}/AUC_{Pure\ drug}$. Statistical data analysis was performed using Student's *t*-test with $p < 0.05$ as the minimal level of significance.

2.2.10. Invitro-invivo correlation studies

Invitro-invivo correlation was developed by Phoenix Winnolin, fraction of drug dissolved in invitro dissolution studies vs fraction of drug absorbed in the bioavailability studies conducted on male wistar rats. The IVIVC model was validated by predicting the in-vivo data from the in-vitro data (deconvolution) as internal validation and in-vitro data from the in-vivo data. The percentage prediction error (%PE) was within ±15% which provides confidence that the IVIVC model is validated and could be used for further predictions[55,56].

2.2.11. Accelerated stability studies

Stability studies for the prepared SNEDDS are carried out as per ICH guidelines for a period of 3 months. The Ibrutinib SNEDDS were stored at 40±2˚C and 75±5% RH. The samples were withdrawn at pre-determined time intervals and the samples were analysed for the drug content, droplet size, zeta potential and in-vitro drug release studies[57–59].

3. Results and Discussion

3.1. Analytical methodology

IBR was estimated by HPLC Waters system. The λmax of IBR was found to be 286nm shown in Fig-2. The concentration range of 0.2 to 4 ppm was found to be linear with R^2 value 0.9992 shown in Fig-3.

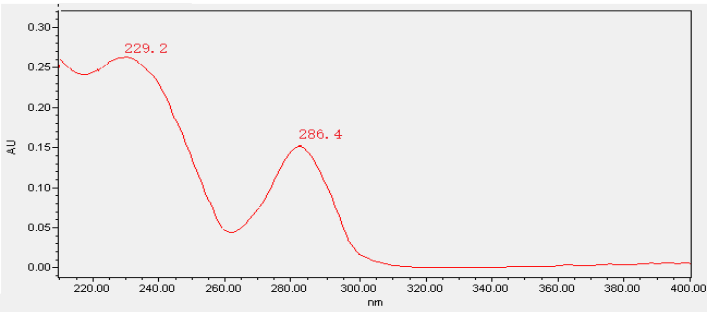


Fig-2: HPLC Scan spectrum of IBR

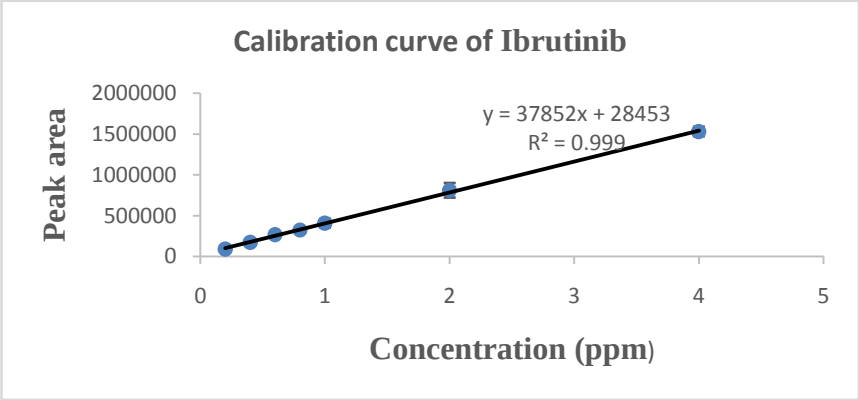


Fig-3: Calibration curve of IBR by HPLC

3.2. Saturation Solubility studies of IBR in various oils, surfactants and cosurfactants

Solubility studies were performed for identifying a suitable oil, surfactant and cosurfactant for development of IBR SNEDDS. The suitable excipient having maximal solubilizing potential for the drug is selected to achieve optimum drug loading. Among various excipients, Ibrutinib showed the highest solubility in capryol 90 (60.75 mg/ml), kolliphorEL(39.5 mg/ml) and transcutol HP (65.7 mg/ml) at 25˚C shown in Fig-4,5 & 6. The solubility studies aimed for identifying suitable oily phase and surfactants for the Ibrutinib to formulate Lipid based drug delivery systems.

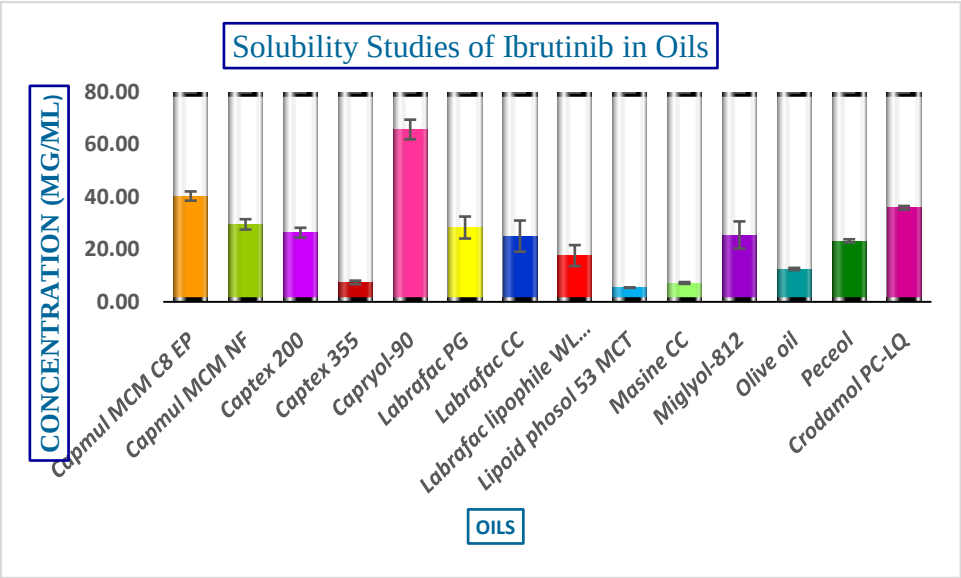


Fig-4: Solubility of IBR in various Oils

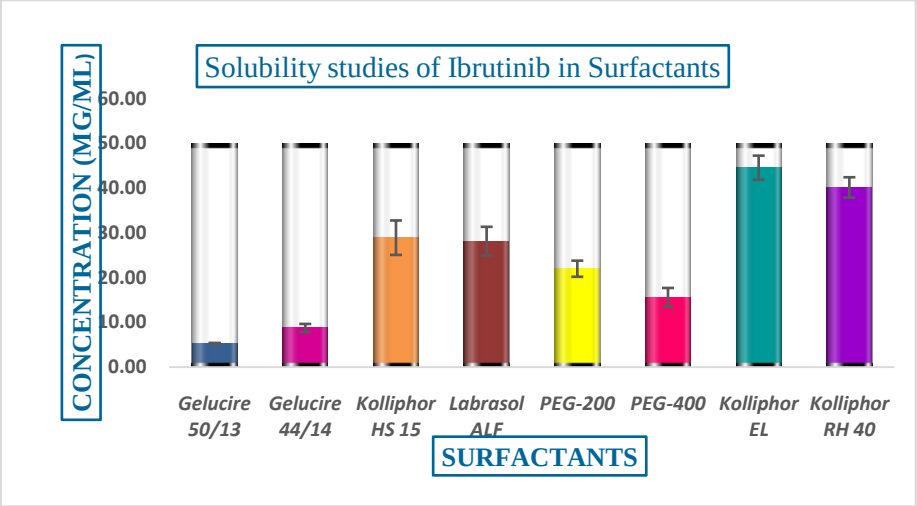


Fig-5: Solubility of IBR in various Surfactants

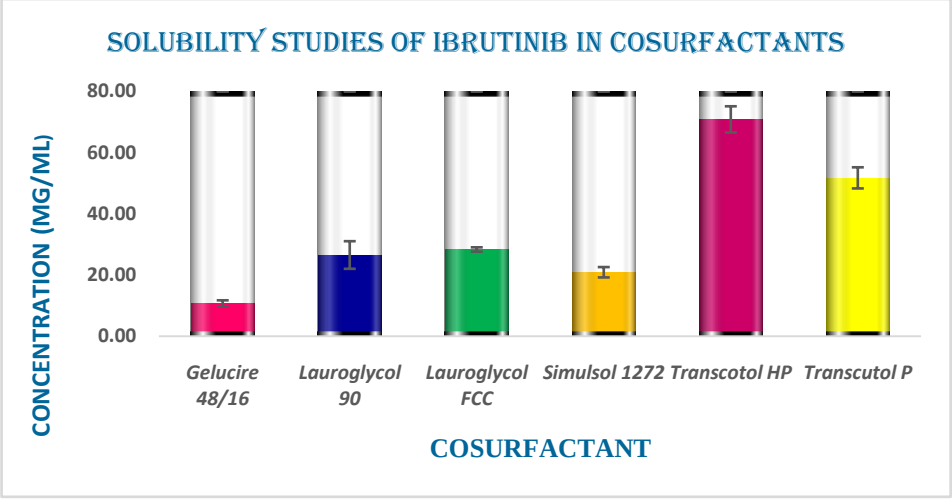


Fig-6: Solubility of IBR in various Co-surfactants

3.3. Drug-excipient compatibility studies by FTIR spectroscopy

The drug and excipients were compatible with each other which is determined by FTIR spectrometry and shown in figures 7.8 to 7.14. 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}. These peaks can be considered as characteristic peaks of IBR and were not affected and prominently observed in IR spectra of IBR and IBR S-SNEDDS shown in the Fig-7 & 8.

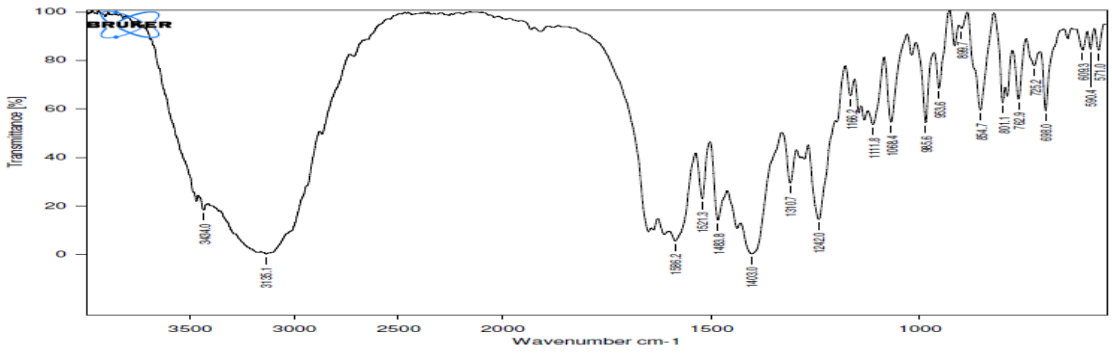


Fig-7: FTIR Spectra of IBR

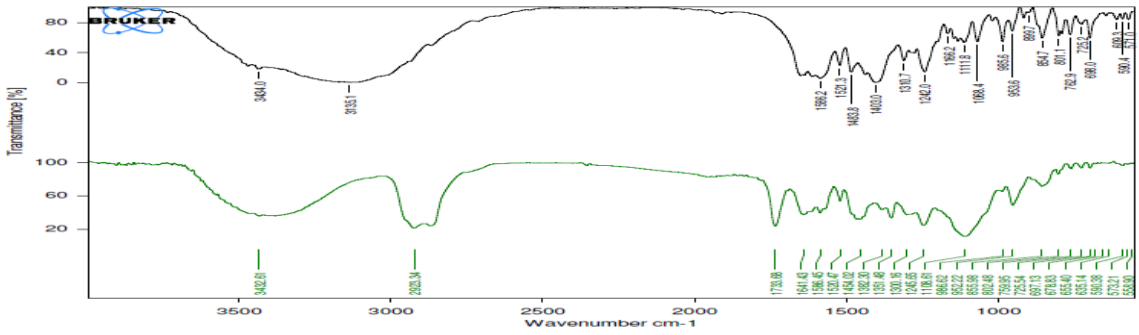


Fig-8: FTIR Spectra of IBR-S-SNEDDS

3.4. Construction of pseudo-ternary phase diagram

The study of the ternary phase behaviour is helpful to choose the appropriate excipient concentration, thatis,the ratioof oil and the optimum ratio of Smix to prepare an emulsion with good stability. Phase diagram of Smix 3:1 was shown the figure-9. In the present study, capryol 90 is taken as oil phase, kolliphor EL as surfactant and transcutol HP as cosurfactant. In order to let know the self-emulsifying regions and also for the

optimization of the percentages of different liquid SNEDDS components, the ternary phase diagrams were developed in absence of IBR. Based on the data obtained from solubility studies, Capryol 90 used as oily phase, Kolliphor EL used as surfactant &Transcutol HP was used as a co-surfactant to construct ternary phase diagrams. The Smix ratio of 3:1 & 4:1 produced nano emulsion areas at 4:6 to 1:9 of Oil:Smix ratios for 3:1 Smix i.e., CKEL3T1(4:6) to CKEL3T1(1:9) and 3:7 to 1:9 Oil:Smix ratios for 4:1 Smix i.e., CKELT4T1(3:7) to CKELT4T1(1:9). CKEL3T1 were selected for further studies because of larger emulsification areas in pseudo-ternary phase diagrams shown in Fig-9.

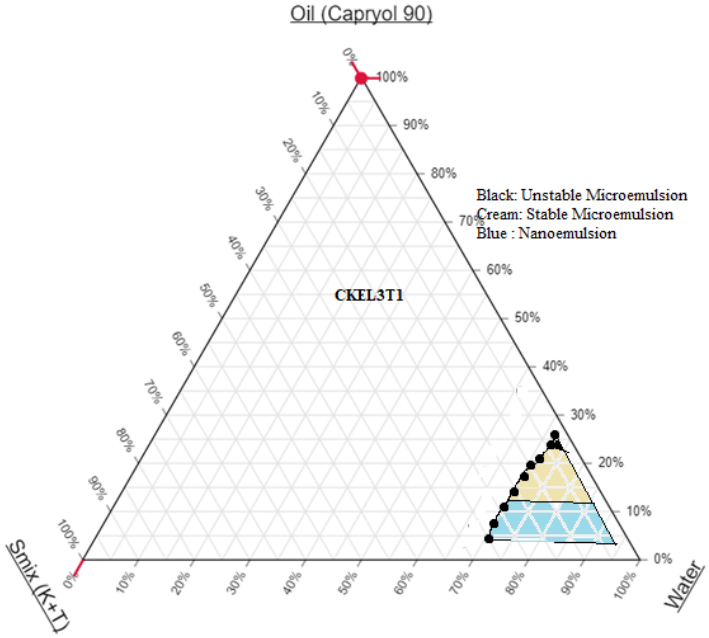


Fig-9: Pseudo-ternary phase diagram of CKEL3T1

3.5. Optimization of SNEDDS by using Central composite design

The optimized blank SNEDDS ratios from the pseudo-ternary phase diagrams were selected, the lowest and highest concentrations of oil, surfactant and cosurfactant are calculated and the data added to Design expert software (version 13) using response surface graphs of central composite design. The design produced 20 runs and to the optimized compositions of the three factors (oil, surfactant and cosurfactant) obtained in various runs, IBR (25 mg) added to 20 runs which is mentioned as R1 to R20. The response surface graphs were shown in Fig-10, 11 & 12 The response i.e. percentage maximizeY_1 (> 99%) and minimize self-emulsification time(Y_2) for the 20 runs is analysed, where the percentage drug content for runs R2, R11 & R14 was found to have ≥99 % drug content with good self-emulsification time. The least and highest values of

the best composition of oil &Smix obtained from the aqueous titration method (Pseudo-ternary phase diagrams) was given in DOE by using Design expert software version 13, designed by opting response surface methodology-central composite design (CCD), where block 1 is chosen and 20 runs are generated by software for the 3 factors given oil, surfactant & cosurfactant and the responses analysed were percentage drug content and self-emulsification time. The responses for run 2, 11 & 12 (i.e. CKELT31(R2), CKELT31(R11) &CKELT31(R14)) were found to be best with 99.02, 99.64 & 99.81 % and self-emulsification time was 18, 20 & 17 seconds for both the runs which was depicted in the Table-1. CKELT31(R2), CKELT31(R11) &CKELT31(R14) were evaluated for dispersibility test, robustness to dilution, thermodynamic stability studies, particle size, poly-dispersiblity index, zeta potential, phase separation and stability test & invitro dissolution studies.

Table-1: The low & high values of excipients by design of experiments (DOE)

	Name	Units	Low	High	-alpha	+alpha
A	I-Oil (Capryol 90)	Mg	50	203	2.15715	255.157
B	I-Surfactant (Kolliphor EL)	Mg	223.5	339	184.126	378.374
C	I-Cosurfactant (Transcutol HP)	Mg	74.5	113	61.3755	126.125

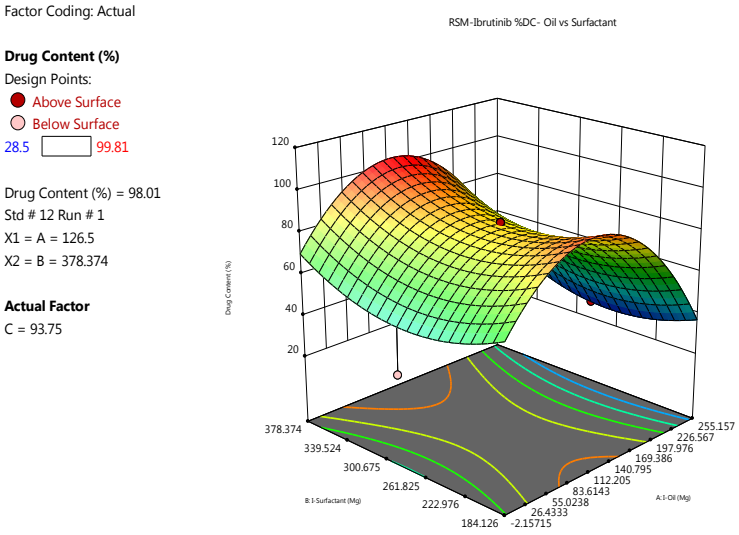


Fig-10: RSM of IBR % drug content- Oil vs surfactant

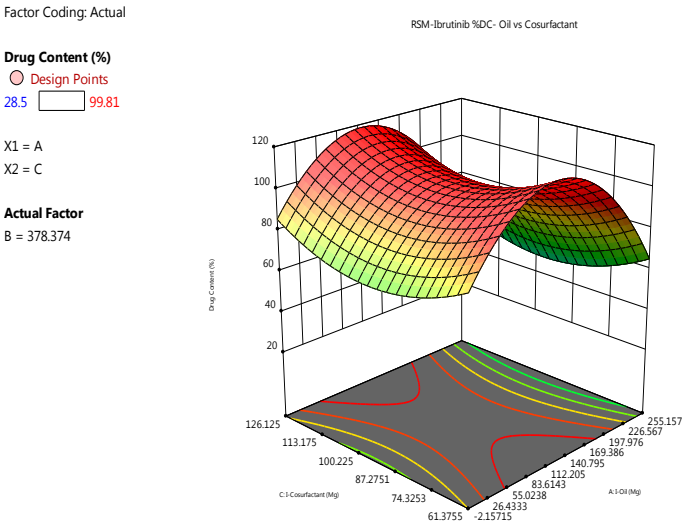


Fig-11: RSM of IBR % drug content- Oil vs cosurfactant

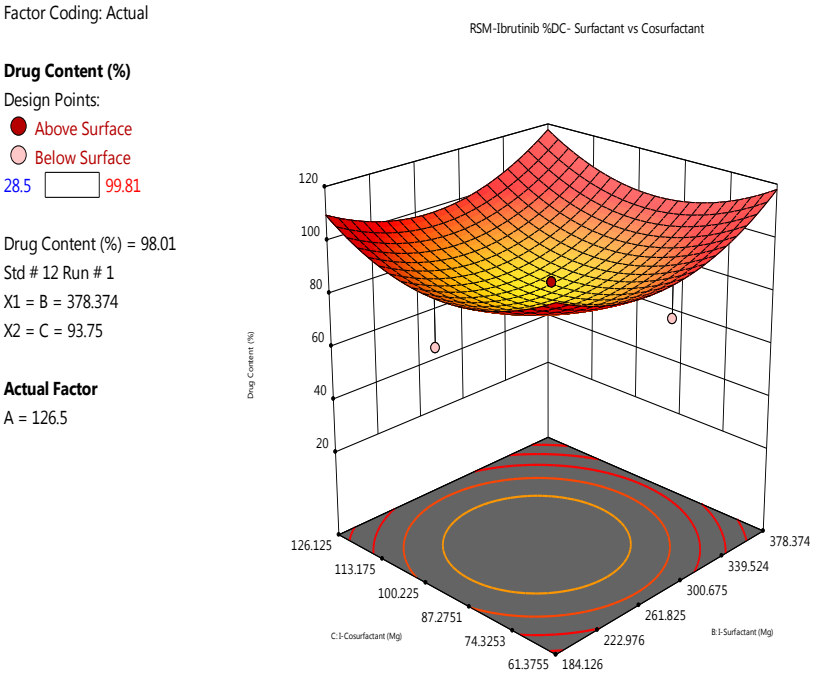


Fig-12: RSM of IBR -%DC- surfactant vs co-surfactant

3.6. Characterization of L-SNEDDS

The dispersibility test of optimized SNEDDS i.e CKEL3T1(R2, R11 & R14) have shown Grade 1 type of dispersion in water, 0.1N HCl and phosphate buffer pH 6.8. It was observed that all the three formulations(runs) showed neither precipitation nor phase separation of the drug in the formulation after 2,4,6,8,12,24 hrs of the study, representing that all formulations are stable emulsions. To check the robustness to dilution the formulations were diluted with excess of water, 0.1 N HCl and phosphate buffer (pH 6.8) and was stored for 24 hours. No precipitation or phase separation was found which

indicate that all the formulations were stable on dilution. Thermodynamic stability studies were conducted for the formulations by centrifuging at 3,500rpm for 30min&Freeze thaw cycle is conducted at -20^{o}C and +25^{o}C, where the formulations have passed the studies.

Self-emulsification is primarily assessed visually. The emulsificationrate is an important indicatorin evaluating the emulsification efficiency, thatis, SNEDDS must disperse completely and rapidly when diluted withwaterwith gentle agitation. Emulsification time studies haveshown that all three runs emulsified in the 18 to 30 second range.

The evaluation tests were performed for the best formulations obtained from the DOE (CKEL3T1(R2), CKEL3T1(11) & CKEL3T1(R14)). The evaluations performed were Self-emulsification time, dispersibility test, robustness to dilution, phase separation and stability test & thermodynamic stability testing and droplet size analysis and zeta potential were performed and all were found to be within the limits. Particle size was least for CKEL3T1(R14) with a good PDI and Zeta potentialand invitro drug release studies showed 99.72±2.432 % drug was released within 45min. TEM analysis was performed for CKEL3T1 (R14) and the droplets were found to be less than 100nm shown in the Fig-13.

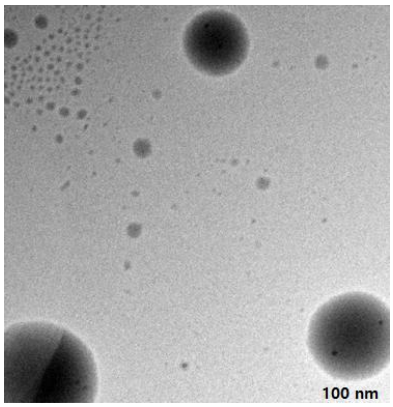


Fig-13: TEM image of CKEL3T1(R14)

3.7. Preparation and Characterization of Solid SNEDDS of Ibrutinib

From the evaluations performed on the I-L-SNEDDS formulations, CKEL3T1 (R14) was selected as an optimized formulation. CKEL3T1(R14) has passed all the evaluations with a globule size of 18.98nm, the poly-dispersibility index of 0.391 & zeta potential -29.6mV, shown in fig-14 & 15 and invitro dissolution studies of optimized I-L-SNEDDS (CKEL3T1(R14)) was found to release 99.72±2.432% at 45min. The I-L-SNEDDS were transformed into free-flowing powder by pouring the I-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 flowability. Neuselin was taken in the concentration of 0.2% & 0.25%.

The flow properties were good for 0.25:1 ratio of Neuselin: L-SNEDDS with good values for angle of repose ($24.2^{O}\pm0.89^{)}$, carr's index (10.9) and hausner's ratio (1.13). The S-SNEDDS were prepared with 0.25:1 ratio of Neuselin: L-SNEDDS. From the in-vitro-dissolution studies of liquid SNEDDS of IBR formulations shown CKEL3T1(R14) was found to release 99.72% of drug at 45min which was higher among the L-SNEDDS. The CKEL3T1(R14) was 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.

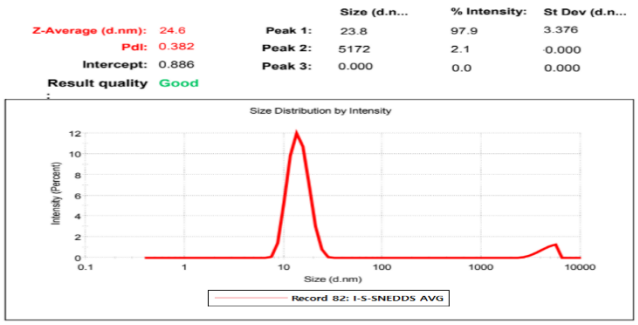


Fig-14: Droplet size of I-S-SNEDDS

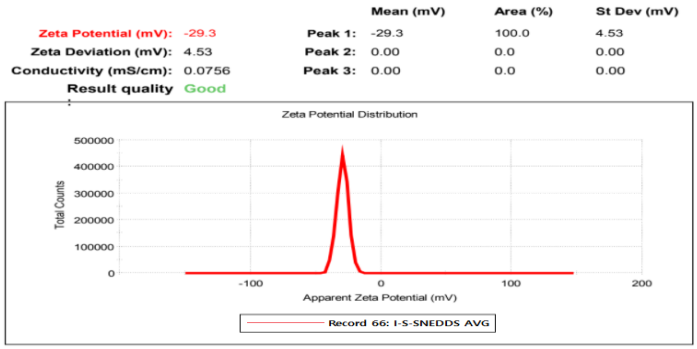


Fig-15: Zeta potential of I-S-SNEDDS

3.8. In-vivo bioavailability study of Ibrutinib and Ibrutinib formulations

From the in-vivo studies the optimized I-L-SNEDDS shown greater rise in plasma drug concentration compared with the pure drug IBR, and pharmacokinetic parameters are depicted in Table-2. The in-vivo pharmacokinetic studies in male wistar rats shown enhanced values of peak concentration of drug (Cmax) and area under curve (AUC) for I-

L-SNEDDS were 3440ng/ml & 19651.98 ng*hr/ml and I-S-SNEDDS 3325ng/ml & 18721.88ng*hr/ml compared to pure drug Ibrutinib which was 360.13 ng/ml & 3357.99 ng*hr/ml respectively. The Oral bioavailability of I-S-SNEDDS showed an enhanced bioavailability of 3.57 times greater than that of the pure drug Ibrutinib.

When cyclohexidine (lymphatic blocker) is given orally before administering I-L-SNEDDS formulation, to evaluate the lymphatic uptake of SNEDDS, there was a great decrease in plasma drug concentration which indicates lymphatic uptake increasing the bioavailability of IBR SNEDDS shown in fig-16 pharmacokinetic parameters are depicted in Table-3. The bioavailability of Group-2 (I-L-SNEDDS)rats were found to be higher than the Group-4 rats (CXI + I-L-SNEDDS administered rats = I-L-SNEDDS-C). The Cmax and AUC of I-L-SNEDDS were 3440ng/ml & 19651.98 ng*hr/ml and I-L-SNEDDS-C were 2432 ng/ml & 14521.75 ng*hr/ml. It clearly indicates that the lymphatic uptake is enhanced the bioavailability of drug.

Table-2: Pharmacokinetic parameters of Ibrutinib & Formulations

Parameter	IBR	I-L-SNEDDS	I-S-SNEDDS
Cmax (ng/mL)	360.13	3440.00	3325.00
Tmax(hrs)	4.00	1.00	1.00
Clast(ng/mL)	35.00	72.50	96.00
Tlast(hrs)	24.00	24.00	24.00
AUCt(ng*hr/mL)	3357.99	19651.98	18721.88
AUMC(ng*hr2/mL)	23996.93	118090.48	117907.31
MRTt(hrs)	7.15	6.01	6.30
T1/2(hrs)	8.32	4.12	4.75
AUCi(ng*hr/mL)	3778.07	20082.59	19379.10
AUMCi(ng*hr2/mL)	39120.62	130982.77	138180.07
MRTi(hrs)	10.35	6.52	7.13
CL(mL/min)	13.23	2.49	2.58
Vd (lts)	158.84	14.79	17.66

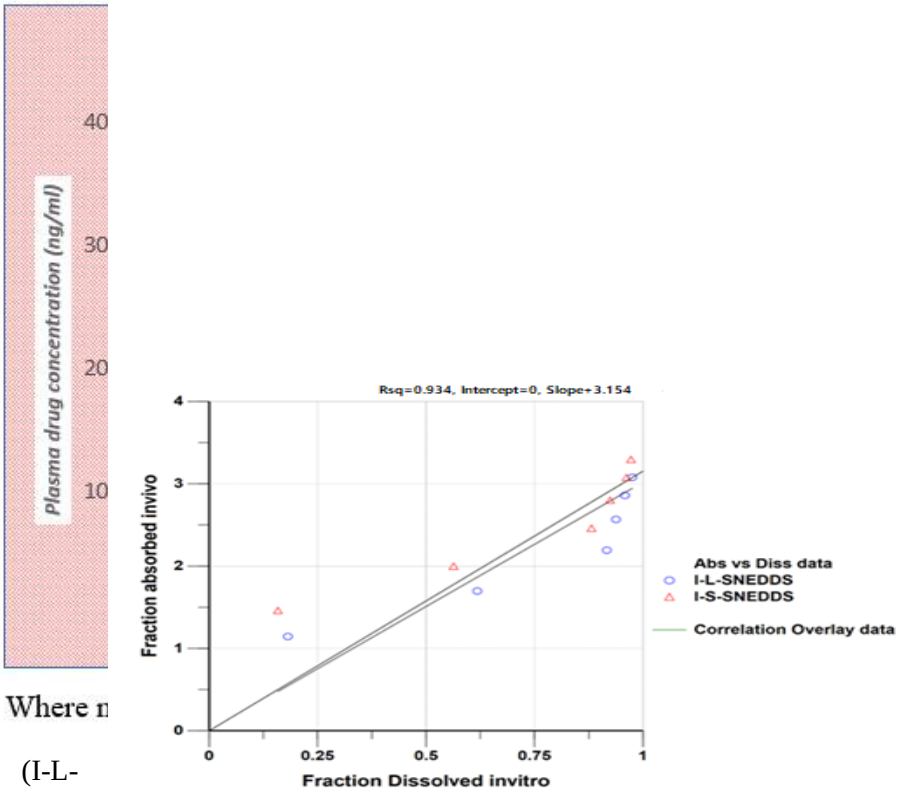
IBR=Ibrutinib, I-L-SNEDDS=Ibrutinib Liquid SNEDDS, I-S-SNEDDS= Ibrutinib solid SNEDDS

Table-3: Pharmacokinetic parameters to study Lymphatic uptake

Parameter	I-L-SNEDDS	I-L-SNEDDS-C
Cmax (ng/mL)	3440.00	2432.00

Tmax(hrs)	1.00	1.00
Clast(ng/mL)	72.50	65.00
Tlast(hrs)	24.00	24.00
AUCt(ng*hr/mL)	19651.98	14521.75
AUMC(ng*hr2/mL)	118090.48	84534.81
MRTt(hrs)	6.01	5.82
Lz	0.17	0.15
AUCi(ng*hr/mL)	20082.59	14960.76
AUMCi(ng*hr2/mL)	130982.77	98036.30
MRTi(hrs)	6.52	6.55
CL(mL/min)	2.49	3.34
Vd (lts)	14.79	22.57

I-L-SNEDDS=Ibrutinib Liquid SNEDDS, I-L-SNEDDS C= Ibrutinib Liquid SNEDDS with Cyclohexidine(Lymphatic blocker)



Where n

(I-L-SNEDDS-Ibrutinib Liquid SNEDDS, I-L-SNEDDS-C-I-L-SNEDDS With Cycloheximide)

Fig-16:Study of invivo lymphatic uptake of I-L-SNEDDS

3.9. Invitroinvivo correlation

The I-L-SNEDDS and I-S-SNEDDS were passed Level-A (point to point correlation) with correlation coefficient of 0.93.

3.10. Accelerated stability studies of I-S-SNEDDS

The conditions maintained during accelerated stability testing were at higher temperature (40 ± 2˚C) and relative humidity (75 ± 5%). The optimized formulation I-S-SNEDDS was physically stable with no apparent changes in the physical appearance. The three samplings were done during the stability testing period i.e. 0, 3 and 6 months and globule size is slightly increased during the period. The globule size, PDI and ZP of the I-S-SNEDDS were shown in the Table-4. It is clear that the I-S-SNEDDS formulations prepared by using Capryol 90, Kolliphor EL and Transcutol HP were stable during the accelerated stability testing.

Evaluation Parameter	0-Month	3-Month	6-Month
Globule Size (nm)	36.22	36.38	40.76
Poly dispersibility index	-26.1	-24.2	-22.8
Zeta potential (mV)	0.164	0.398	0.129
% Drug content	99.54	99.47	99.35

Table-4: Accelerated stability studies of I-S-SNEDDS

4. Conclusion

The oral bioavailability of self-nano-emulsified formulation CKEL3T1(R14) showed an enhanced bioavailability of 3.57 times greater than that of the pure drug suspension Ibrutinib. The SNEDDS will be the promising formulation for the Ibrutinib.

Acknowledgements

Immense support from my guide Dr. M. Sunitha Reddy, Associate professor, Centre for pharmaceutical sciences, Institute of science and technology, Jawaharlal Nehru university of technological sciences is heartfully acknowledged., The authors are thankful to ABITEC Corporation (Cleveland, USA), Gattefossé (Saint-Priest, France) I also thank Mallareddy institute of pharmaceutical sciences for providing animal studies facilities is kindly acknowledged.

Ethics declaration

All experimental procedures of the animal studies were conducted in accordance with the CPCSEA guidelines for the care and use of laboratory animals. As well as the animal facilities guidelines approved from the ethical committee of experimental animal care centre, Mallareddy institute of pharmaceutical sciences, JNTUH (Clearance No. 1662/PO/Re/S/12/CPCSEA/Hyd/2020).

References

1. Wang, J. *et al.* Ibrutinib, a Bruton's tyrosine kinase inhibitor, exhibits antitumoral activity and induces autophagy in glioblastoma. *J. Exp. Clin. Cancer Res.* (2017) doi:10.1186/s13046-017-0549-6.

2. Prabaharan, C. B. *et al.* Ibrutinib as a potential therapeutic option for HER2 overexpressing breast cancer – the role of STAT3 and p21. *Invest. New Drugs* (2020) doi:10.1007/s10637-019-00837.

3. Capitani, N. *et al.* LMW-PTP targeting potentiates the effects of drugs used in chronic lymphocytic leukemia therapy. *Cancer Cell Int.* (2019) doi:10.1186/s12935-019-0786-1.

6. Swapna, M. S. R. and G. No Title. *Int. J. Pharm. Biol. Sci.***9**, 174–189 (2019).

7. Rajalakshmi, G., Dhanapal, C. K. & Sundhararajan, R. An Insight to Nanostructured Lipid Carrier System. *J. Drug Deliv. Ther.* (2020) doi:10.22270/jddt.v10i6-s.4589.

8. Akhlaquer Rahman, M., Harwansh, R., Aamir Mirza, M., Hussain, S. & Hussain, A. Oral Lipid Based Drug Delivery System (LBDDS): Formulation, Characterization and Application: A Review. *Curr. Drug*

Deliv. (2011) doi:10.2174/156720111795767906.

9. Zhang, Z., Gao, F., Jiang, S., Ma, L. & Li, Y. Nano-based Drug Delivery System Enhances the Oral Absorption of Lipophilic Drugs with Extensive Presystemic Metabolism. *Curr. Drug Metab.* (2012) doi:10.2174/138920012802850100.

10. Savale, S. K. a Review - Self Nanoemulsifying Drug Delivery System (Snedds). *Int. J. Res. Pharm. Nano Sci.* (2015).

11. Buya, A. B., Beloqui, A., Memvanga, P. B. & Préat, V. Self-nano-emulsifying drug-delivery systems: From the development to the current applications and challenges in oral drug delivery. *Pharmaceutics* (2020) doi:10.3390/pharmaceutics12121194.

12. Singh, D. Self-nano emulsifying drug delivery system: A versatile carrier for lipophilic drugs. *Pharm. Nanotechnol.* (2021) doi:10.2174/2211738509666210422124023.

13. Čerpnjak, K., Zvonar, A., Gašperlin, M. & Vrečer, F. Lipid-based systems as a promising approach for enhancing the bioavailability of poorly water-soluble drugs. *Acta Pharm.* (2013) doi:10.2478/acph-2013-0040.

14. Bannow, J., Yorulmaz, Y., Löbmann, K., Müllertz, A. & Rades, T. Improving the drug load and in vitro performance of supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS) using polymeric precipitation inhibitors. *Int. J. Pharm.* (2020) doi:10.1016/j.ijpharm.2019.118960.

15. Morakul, B. Self-nanoemulsifying drug delivery systems (SNEDDS): An advancement technology for oral drug delivery. *Pharm. Sci. Asia* (2020) doi:10.29090/psa.2020.03.019.0121.

16. Kuruvila, F. S., Mathew, F. & Kuppuswamy, S. Solid Self Nanoemulsifying drug delivery system (Snedds) Devolopment , Applications and Future Perspective : A Review. *Indo Am. J. Pharm. Sci.* (2017).

17. Singh, B., Khurana, L., Bandyopadhyay, S., Kapil, R. & Katare, O. O. P. Development of optimized self-nano-emulsifying drug delivery systems (SNEDDS) of carvedilol with enhanced bioavailability potential. *Drug Deliv.* (2011) doi:10.3109/10717544.2011.604686.

18. Zhao, Z., Cui, X., Ma, X. & Wang, Z. Preparation, characterization, and evaluation of antioxidant activity and bioavailability of a self-nanoemulsifying drug delivery system (SNEDDS) for buckwheat flavonoids. *Acta Biochim. Biophys. Sin. (Shanghai).* (2021) doi:10.1093/abbs/gmaa124.

19. Jain, A., Hurkat, P. & Jain, S. K. Development of liposomes using formulation by design: Basics to recent advances. *Chemistry and Physics of Lipids* (2019) doi:10.1016/j.chemphyslip.2019.03.017.

20. Aziz, D. E., Abdelbary, A. A. & Elassasy, A. I. Implementing Central Composite Design for Developing Transdermal Diacerein-Loaded Niosomes: Ex vivo Permeation and In vivo Deposition. *Curr. Drug Deliv.* (2018) doi:10.2174/1567201815666180619105419.

21. Sabir, A. M., Moloy, M. & Bhasin, P. S. HPLC METHOD DEVELOPMENT AND VALIDATION: A REVIEW. *Int. Res. J. Pharm.* (2016) doi:10.7897/2230-8407.04407.

22. N. V. R. and R. D. Santhoshillendula, Dhandempally Priyanka, V. S. A New Simple Method Development and Validation of Ibrutinib In Bulk and Pharmaceutical Dosage Form By RP-HPLC. *Int. J. Pharm. Biol. Sci.***9**, 36–46 (2018).

23. Nasr, A., Gardouh, A. & Ghorab, M. Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartan medoxomil: Design, formulation, pharmacokinetic and bioavailability evaluation. *Pharmaceutics* (2016) doi:10.3390/pharmaceutics8030020.

24. Kazi, M. *et al.* Enhancing oral bioavailability of apigenin using a bioactive self-nanoemulsifying drug delivery system (Bio-SNEDDS): In vitro, in vivo and stability evaluations. *Pharmaceutics* (2020)

doi:10.3390/pharmaceutics12080749.

25. Mohsin, K. *et al.* Development of self-nanoemulsifying drug delivery systems for the enhancement of solubility and oral bioavailability of fenofibrate, A poorly water-soluble drug. *Int. J. Nanomedicine* (2016) doi:10.2147/IJN.S104187.

26. Dole, M. N., Patel, P. A., Sawant, S. D. & Shedpure, P. S. Advance applications of fourier transform infrared spectroscopy. *Int. J. Pharm. Sci. Rev. Res.* (2011).

27. Upadhyayula, S. S. N., Sureshkumar, R., Janani, S. K. & Karthika, C. Lipid-based nano formulation approach to target brain for the management of tuberculosis through intranasal delivery: Formulation development and evaluation. *Indian J. Pharm. Educ. Res.* (2020) doi:10.5530/ijper.54.2s.75.

28. Rojek, B. & Wesolowski, M. FTIR and TG analyses coupled with factor analysis in a compatibility study of acetazolamide with excipients. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* (2019) doi:10.1016/j.saa.2018.10.020.

29. Parmar, N., Singla, N., Amin, S. & Kohli, K. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. *Colloids Surfaces B Biointerfaces* (2011) doi:10.1016/j.colsurfb.2011.04.016.

30. Nasr, A., Gardouh, A., Ghonaim, H., Abdelghany, E. & Ghorab, M. Effect of oils, surfactants and cosurfactants on phase behavior and physicochemical properties of self-nanoemulsifying drug delivery system (Snedds) for irbesartan and olmesartan. *Int. J. Appl. Pharm.* (2016).

31. Beg, S., Swain, S., Singh, H. P., Patra, C. N. & Rao, M. B. Development, optimization, and characterization of solid self-nanoemulsifying drug delivery systems of valsartan using porous carriers. *AAPS PharmSciTech* (2012) doi:10.1208/s12249-012-9865-5.

32. Patel, J., Kevin, G., Patel, A., Raval, M. & Sheth, N. Design and development of a self-nanoemulsifying drug delivery system for telmisartan

for oral drug delivery. *Int. J. Pharm. Investig.* (2011) doi:10.4103/2230-973x.82431.

33. Beg, S. *et al.* Development of solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential. *Colloids Surfaces B Biointerfaces* (2013) doi:10.1016/j.colsurfb.2012.06.031.

34. Beg, S. *et al.* Solid self-nanoemulsifying systems of olmesartan medoxomil: Formulation development, micromeritic characterization, in vitro and in vivo evaluation. *Powder Technol.* (2016) doi:10.1016/j.powtec.2016.02.023.

35. Dash, R. N., Mohammed, H., Humaira, T. & Ramesh, D. Design, optimization and evaluation of glipizide solid self-nanoemulsifying drug delivery for enhanced solubility and dissolution. *Saudi Pharm. J.* (2015) doi:10.1016/j.jsps.2015.01.024.

36. Xue, X., Cao, M., Ren, L., Qian, Y. & Chen, G. Preparation and Optimization of Rivaroxaban by Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Enhanced Oral Bioavailability and No Food Effect. *AAPS PharmSciTech* (2018) doi:10.1208/s12249-018-0991-6.

37. Dash, R. N., Mohammed, H. & Humaira, T. Design, optimization, and evaluation of ezetimibe solid supersaturatable self-nanoemulsifying drug delivery for enhanced solubility and dissolution. *J. Pharm. Investig.* (2016) doi:10.1007/s40005-015-0225-9.

38. Raghuveer, P., Vanithakamal, J., Madhuri, D. & Rani, A. P. Design development and evaluation of self nanoemulsifying drug delivery system of simvastatin. *Res. J. Pharm. Technol.* (2018) doi:10.5958/0974-360X.2018.00221.4.

39. Reddy, S. M., Sunitha Reddy, M., Srikanth Reddy, N. & Muralidhar Reddy, O. Formulation and evaluation of novel lipid based solid self nano emulsifying drug delivery systeme of repaglinide. *Int. J. Pharm. Pharm. Sci.* (2014).

40. Pan Gao,1 Zhujun Jiang,2 Qiao Luo,1 Chengqiao Mu,2 Mengsuo Cui,2 and Xinggang Yang2,3.Preparation and Evaluation of Self-emulsifying Drug Delivery System (SEDDS) of Cepharanthine. AAPS PharmSciTech (2021) 22: 245.

41. Aparna, K. & Meenakshi, B. A Self Emulsifying Drug Delivery System (SEDDS) for Nisoldipine: Characterization, Dissolution and Conversion into Solid Sedds. *J. Pharm. Res.* (2012) doi:10.18579/jpcrkc/2012/11/4/79371.

42. Cardona, M. I., Nguyen Le, N. M., Zaichik, S., Aragón, D. M. & Bernkop-Schnürch, A. Development and in vitro characterization of an oral self-emulsifying delivery system (SEDDS) for rutin fatty ester with high mucus permeating properties. *Int. J. Pharm.* (2019) doi:10.1016/j.ijpharm.2019.03.036.

43. Reddy, M. S., Goud, P. S. & Apte, S. S. Solubility Enhancement of Candesartan Cilexetil By Self Emulsifying Drug Delivery Systems. *Ijpsr* (2012).

44. Sunitha Reddy, M. & Sowjanya, N. Formulation and in-vitro characterization of solid self nanoemulsifying drug delivery system (S-SNEDDS) of Simvastatin. *J. Pharm. Sci. Res.* (2015).

45. Reddy, M. S., Sowmya, S. & Fazal, S. M. Formulation and in-Vitro Characterization of Self Microemulsifying Drug Delivery Systems of Rivaroxaban. *Int. J. Pharm. Sci. Res.* (2017).

46. Balakumar, K., Raghavan, C. V., selvan, N. T., prasad, R. H. & Abdu, S. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. *Colloids Surfaces B Biointerfaces* (2013) doi:10.1016/j.colsurfb.2013.08.025.

47. Reddy, M. S., Reddy, N. S. & Reddy, S. M. Solubility enhancement of poorly water soluble drug efavirenz by solid self emulsifying drug delivery systems. *Int. J. Pharma Res. Rev.* (2014).

48. Khan, A. W., Kotta, S., Ansari, S. H., Sharma, R. K. & Ali, J. Self-

nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: Design, characterization, in vitro and in vivo evaluation. *Drug Deliv.* (2015) doi:10.3109/10717544.2013.878003.

49. Kazi, M. *et al.* Development and optimization of sitagliptin and dapagliflozin loaded oral self-nanoemulsifying formulation against type 2 diabetes mellitus. *Drug Deliv.* (2021) doi:10.1080/10717544.2020.1859001.

50. Batool, A. *et al.* Formulation and evaluation of hyaluronic acid-based mucoadhesive self nanoemulsifying drug delivery system (SNEDDS) of tamoxifen for targeting breast cancer. *Int. J. Biol. Macromol.* (2020) doi:10.1016/j.ijbiomac.2020.02.275.

51. Vishal N Kushare & Saravanan S. Invitro and invivo evaluation of anticonvulsant herbal drug loaded self nano emulsifying drug delivery system. *Int. J. Pharm. Res. Life Sci.* (2018) doi:10.26452/ijprls.v6i2.1279.

52. Menzel, C. *et al.* In vivo evaluation of an oral self-emulsifying drug delivery system (SEDDS) for exenatide. *J. Control. Release* (2018) doi:10.1016/j.jconrel.2018.03.018.

53. Shao, B. *et al.* Enhanced oral bioavailability of piperine by self-emulsifying drug delivery systems: In vitro, in vivo and in situ intestinal permeability studies. *Drug Deliv.* (2015) doi:10.3109/10717544.2014.898109.

54. Zhang, Y., Huo, M., Zhou, J. & Xie, S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput. Methods Programs Biomed.* (2010) doi:10.1016/j.cmpb.2010.01.007.

55. Kazi, M., Al amri, R., Alanazi, F. K. & Hussain, M. D. In vitro Methods for In vitro-In vivo Correlation (IVIVC) for Poorly Water Soluble Drugs: Lipid Based Formulation Perspective. *Curr. Drug Deliv.* (2018) doi:10.2174/1567201815666180116090910.

56. Nguyen, M. A. *et al.* A survey on IVIVC/IVIVR development in the

pharmaceutical industry – Past experience and current perspectives. *European Journal of Pharmaceutical Sciences* (2017) doi:10.1016/j.ejps.2017.02.029.

57. Magari, R. T., Murphy, K. P. & Fernandez, T. Accelerated stability model for predicting shelf-life. *J. Clin. Lab. Anal.* (2002) doi:10.1002/jcla.10043.

58. Parmar, K., Patel, J. & Sheth, N. Self nano-emulsifying drug delivery system for Embelin: Design, characterization and in-vitro studies. *Asian J. Pharm. Sci.* (2015) doi:10.1016/j.ajps.2015.04.006.

59. Alhasani, K. F., Kazi, M., Ibrahim, M. A., Shahba, A. A. & Alanazi, F. K. Self-nanoemulsifying ramipril tablets: A novel delivery system for the enhancement of drug dissolution and stability. *Int. J. Nanomedicine* (2019) doi:10.2147/IJN.S203311.