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DESIGN AND PHARMACOKINETIC EVALUATION OF IBRUTINIB BY SELF-NANO-EMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT: The objective of present research work is design and *in-vivo* evaluation of ibrutinib self-nanoemulsifying drug delivery system (SNEDDS) for the enhancement of oral bioavailability. Box-Behnken design was used for the study and the results analysed using response surface methodology. Ibrutinib SNEDDS were prepared with various oils, surfactants and co-surfactants and tested particle size, PDI, zeta potential, refractive index, drug release and TEM studies. The optimized ibrutinib SNEDDS has the composition of capryol 90, cremophore EL and transcutol HP. Based on the particle size and entrapment efficiency and *in-vitro* dissolution studies; F2 is identified as optimized formulation with 85% drug released, whereas less than 1% was released from pure drug at end time of 30 min. *In-vivo* bioavailability data reported in male wistar rats indicate higher concentration of drug in plasma implying enhanced systemic absorption of ibrutinib from SNEDDS formulation. The C_{max} and AUC_{0-inf} values of optimized SNEDDS was considerably higher (p<0.05) than pure drug formulation. The oral bioavailability of optimized SNEDDS increased around 12 folds when compared with pure drug. Ibrutinib SNEDDS was successfully prepared, and results indicate that oral bioavailability of ibrutinib optimized SNEDDS was significantly improved when compared with pure drug. Therefore, the results suggest that the optimized formulation has a great potential for clinical application in the effective management of lymphocytic leukaemia.

INTRODUCTION: Ibrutinib is recently approved drug for treating chronic lymphocytic leukaemia. However, ibrutinib exhibits low oral bioavailability and widespread first-pass effects. Due to poor solubility and hepatic first pass effects, it is commercially available in capsular dosage form with very high doses.

In the last few years, nanoemulsions and SNEDDs have been found to be effective approaches for the targeting of several anticancer drugs like 5-fluorouracil, 5-FU conjugates, methotrexate, paclitaxel and isoniazid analogue to melanoma cells for enhanced therapeutic effects of these drugs. All the materials used in the preparation of ibrutinib SNEDDS are nontoxic and fall under generally regarded as safe category of excipients [1, 2].

Recently, the response surface methodology (RSM), by proper experimental designs, has become widely used for formulation optimization.

RSM is generally applied to experimental situations where several independent variables influence a response variable. The Box–Behnken design (BBD) is RSMs for understanding the effects of independent and dependent factors [3-6]. In the present study ibrutinib SNEDDS was prepared and performed *in-vivo* bioavailability studies on wistar rats.

MATERIALS AND METHODS:
Materials: Ibrutinib was kindly gifted by Caplin Point Laboratories Ltd, Bangalore, Labrasol, Cremophor EL, Transcutol HP obtained from MSN labs, Hyderabad, acetonitrile (ACN) was obtained from Sigma–Aldrich (St. Louis, MO). Dialysis tubing was purchased from Hi-Media Laboratories (Mumbai, India) and other chemicals were from analytical grade.

Formulation of Ibrutinib SNEDDS: Solubility Studies indicate the drug was significantly more soluble in capryol 90 hence chosen as oil phase for formulating the SNEDDS system [7, 8]. Evaluation of sixteen surfactants was performed by screening their ability of oil phase emulsification [9]. Eight co surfactants [Capmul MCM C8, lauroglycol 90, PEG 400, PG, EG, plurol oleique CC497, triacetin, transcutol P] were tested for emulsification capability with oil phase [10].

Construction of Ternary Phase Diagrams: Based on solubility study data and the emulsification tendency, capryol 90 (oil), cremophor EL (surfactant) and transcutol HP (co-surfactant) were chosen respectively. Ternary diagrams of the three components prepared to identify the emulsifying region with reported method [11].

Optimization Experiments: Ibrutinib loaded SNEDDS optimization was conducted using DoE. Response surface methodology (RSM) is a statistical method for various processes using specially designed experimental programs to achieve desired response. Experimental design with RSM consists of a group of empirical techniques devoted to the evaluation of relations existing between a cluster of controlled experimental factors and the measured responses according to one or more criteria. Identification and evaluation of the most significant parameters and their interaction during the experimental process can be done by

statistical evaluation and experimental design thereby minimizing the number of runs.

Experimental Design:
Box–Behnken Experiment Design: A three-factor, three-level Box-Behnken Design employed to explore and optimize the interaction and quadratic effects of various ingredients in the formulation on the performance of the SNEDDS [12]. The variables that were chosen as dependent and independent are specified in **Table 1**. **Table 2** shows 17 randomized experimental run for the selected factors, including 5 replicates at mid point (asterisk-marked) generated from a three factor, three-level BBD and their corresponding responses. The BBD matrix was generated using Design Expert® software (Version8.0, Stat-Ease Inc., Silicon Valley, CA, USA), and the data obtained were analysed by the same software. Design Expert software was utilized for fitting all the responses into second-order quadratic model [13, 14].

Preparation of Ibrutinib SNEDDS: The ibrutinib SNEDDS were prepared by mixing oil phase (capryol 90), surfactant (cremophor EL), and co surfactant (transcutol HP) and warming it at 40 °C, then 140mg of ibrutinib was added to the mixture and vortexed to facilitate the uniform dispersion of ibrutinib. 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 17 such experiments was carried out using experimental design. The prepared ibrutinib loaded SNEDDS were filled into size 0 HPMC capsule shells.

Characterization of SNEDDS: Droplet size and polydispersity index of seventeen varying SNEDDS formulations analysis by Auto measure software (Malvern Instruments) using reported method [15].

Zeta meter system was utilized for measurement of zeta potential of diluted SNEDDS. Surface morphology measured using electron microscopy studies (JEOL JEM 2100 F, USA) [16]. Refractive index (RI) was measured using Abbes type Refractometer (Precision Testing Instruments Laboratory, Germany) at room temperature; mean values were recorded after performing the experiment in triplicate.

TABLE 1: DEPENDENT (FACTORS) AND INDEPENDENT (RESPONSES) VARIABLES

Factors		Level			
		Unit	Low(-1)	Middle (0)	High (+1)
A	Amount of capryol 90	mg	10	20	30
B	Amount of cremophor EL	mg	30	50	70
C	Amount of Transcutol HP	mg	10	20	30
Responses			Target		
Y1	Droplet size	nm	Minimize		
Y2	PDI		Minimize		
Y3	Drug release after 15 min	%	Maximize		

PDI, polydispersity index

TABLE 2: BOX BEHNKEN DESIGN WITH OBSERVED RESPONSES

Run	Amount of capryol 90 (mg)	Amount of cremophor EL (mg)	Amount of transcutol HP (mg)	Droplet size (nm)	PDI	Drug release after 15 min (%)
1	20	50	20	185.4	0.262	80.44
2	20	30	30	236.62	0.312	60.9
3	30	50	10	175.99	0.232	81.92
4	20	50	20	184.2	0.222	83.78
5	20	50	20	184.4	0.208	82.38
6	10	30	20	186.5	0.246	55.59
7	10	70	20	163.62	0.218	61.32
8	20	30	10	197.2	0.24	65.61
9	20	50	20	182.3	0.234	81.62
10	30	70	20	167.26	0.248	71.57
11	10	50	10	157.07	0.212	63.61
12	10	50	30	164.35	0.256	79.22
13	20	50	20	183	0.228	82.96
14	20	70	10	188.6	0.206	72.4
15	20	70	30	167.7	0.21	77.48
16	30	50	30	183.9	0.22	70.46
17	30	30	20	221.85	0.28	54.28

Percentage Drug Content: Drug content was estimated by dissolving each formulation in 10ml methanol, 10 min vortexing the same followed by filtration of contents with a membrane filter (00.45 mm) and then analysing the filtrate for amount of drug at 260 nm against blank by a spectrophotometer.

***In-vitro* Release Studies of Ibrutinib SNEDDS:** *In-vitro* release studies on developed ibrutinib SNEDDS and IBR suspension (ibrutinib suspension was prepared in sodium carboxymethyl cellulose) were performed using dialysis membrane. The drug release investigation conducted in USP XXIV dissolution apparatus containing pH 6.8 phosphate buffer (900ml) as dissolution medium. The speed of apparatus set to 50 rpm, temperature maintained at 37 ± 0.5 °C. About 1 ml of each ibrutinib SNEDDS and ibrutinib suspension was filled into ready to- use dialysis bag and tied with dissolution apparatus. At predefined intervals 3ml of sample was withdrawn with simultaneous replacement with fresh dissolution media. The ibrutinib content in each dissolution sample was quantified spectrophotometrically at the wavelength of 260 nm as reported in literature.

Pharmacokinetic Study of Ibrutinib:
Animal Preparation: Healthy wistar rats were (weighing 150-180g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (temperature 25 ºC, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100% fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water *ad libitum.* The protocol of animal study was approved by the institutional animal ethics committee no: ARTI/CPCSEA/2017/22/A.

Study Design: The male wistar rats were categorized into two groups that were offered with food post four hours of dosing. Group I administered orally with pure ibrutinib suspension in 0.5% methocel and group II with administered prepared ibrutinib SNEDDS diluted in 0.5% methocel at a dose of 10mg/kg as per animal body weight .500 µL sample of blood collected from the femoral artery of the animals at varying time intervals of 0, 0.50, 1, 1.50, 2, 2.50, 3, 4, 5, 6, 8, 12, 16, 20, 24 h post administration and mixed with heparin to check clotting. The plasma isolated by centrifugation 5000 rpm and stored at −20 °C [17, 18, 19].

Pharmacokinetic Analysis: Various pharmaco-kinetic parameters like C_{max}, T_{max}, AUC and $t_{1/2}$ were evaluated [20].

RESULTS AND DISCUSSION:
Construction of Ternary Phase Diagram: Three component system chosen for the ibrutinib loaded

SNEDDS preparation was capryol 90 - cremophor EL40 - transcutol P **Fig. 1**. The selection was done based on preliminary trials performed. Self-nanoemulsifying region that was termed efficient was shown as grey region of the diagram. Each component ranges on the basis of diagram selected as follows: 10% ≤ Capryol90 ≤ 30%, 45% ≤ Cremophor El ≤ 75%, 10% ≤ Transcutol HP ≤ 30%.
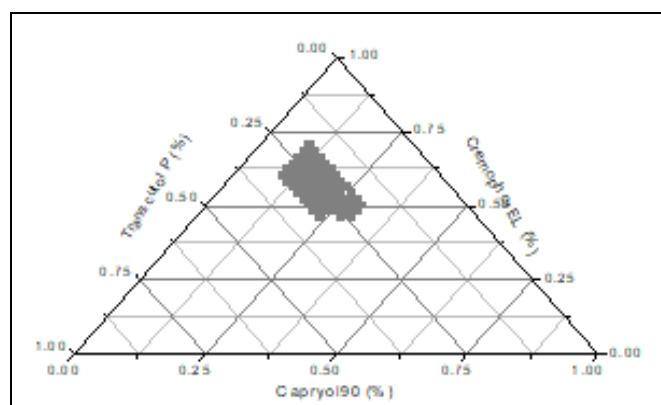

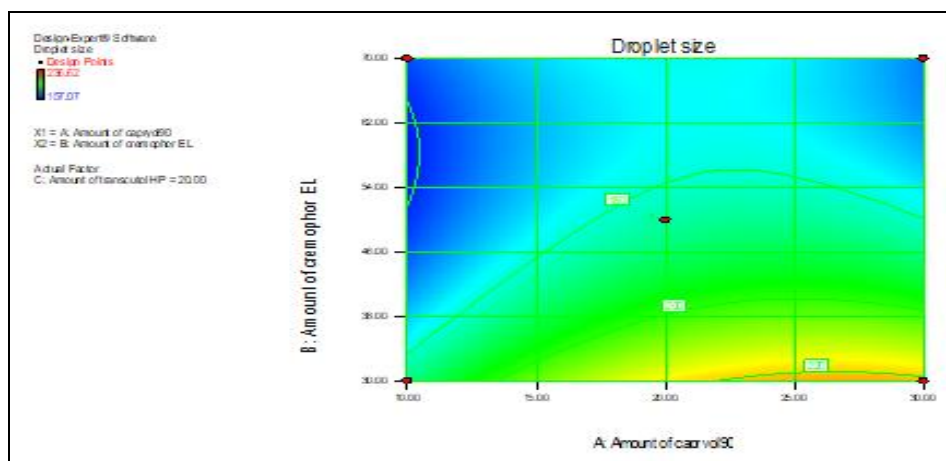**FIG. 1: IBRUTINIB SNEDDS TERNARY PHASE DIAGRAM**

FIG. 2A: CONTOUR PLOT DISPLAYING THE INFLUENCE OF AMOUNT OF CAPRYOL90 AND AMOUNT OF CREMOPHOR EL ON DROPLET SIZE FIXED LEVEL OF C
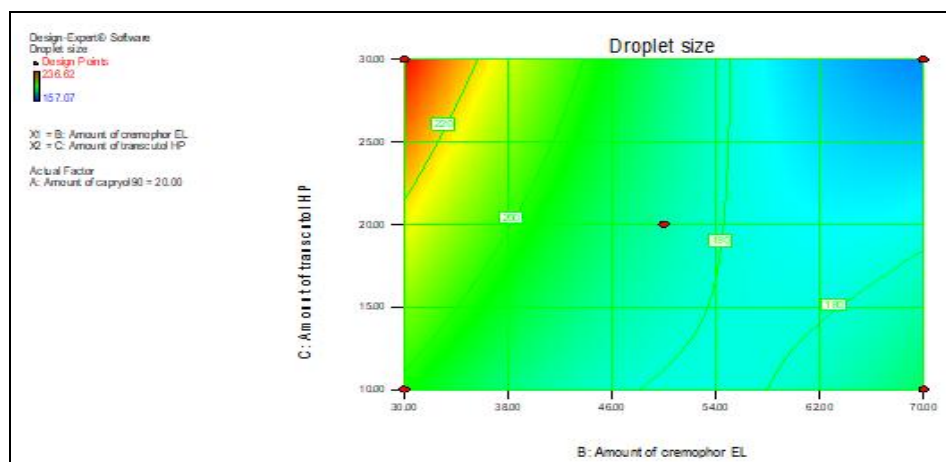

FIG. 2B: CONTOUR PLOT DISPLAYING THE INFLUENCE OF THE AMOUNT OF CREMOPHOR EL AND AMOUNT OF TRANSCUTOL HP ON DROPLET SIZE FIXED LEVEL OF A

Statistical Analysis of the Designed Experiment: Box–Behnken design formed the basis for carrying out series of experiments. **Table 2** specifies the independent variable along with their corresponding responses. The droplet size range (Y1) for all runs observed between 157.07 – 236.62 nm.

Similarly, the range for PDI (Y2) was 0.206 – 0.312 and cumulative drug release in percent (Y3) was within 54.28-83.78%. A second quadratic model was used for data fitting whose adequacy was justified by Design- Expert software provided multiple correlation coefficient, lack of fit and ANOVA tests.

Response Surface Analysis: Stat-Ease Design Expert ® software V8.0 was utilized for analysing

data, to get regression equation, regression coefficient and analysis of variance (ANOVA). The particle size of nanoparticles that was between 157.07-236.62 nm. Significant influence of amount of capryol 90, cremophor EL amount and amount of transcutol HP on droplet size are represented in **Fig. 2A** and **2B**.

The polydispersity index of nanoparticles that ranged from 0.206 – 0.312. At low levels of A, Y2 decreased from 0.256 to 0.212; Y2 decreased from 0.28 to 0.22 at high levels. At low levels of B, Y2 decreased from 0.312 to 0.24, at high levels Y2 decreased from 0.248 to 0.206. At low levels of C, Y2 decreased from 0.24 to 0.206while at high Y2 decreased from 0.312 to 0.21 **Fig. 3**.
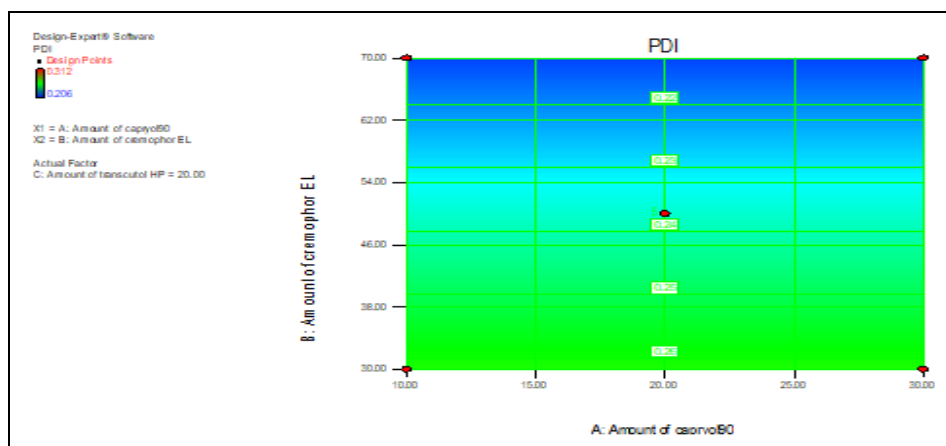

FIG. 3: CONTOUR PLOTS DISPLAYING THE INFLUENCE OF AMOUNT OF CAPRYOL90 AND AMOUNT OF CREMOPHOR EL ON POLYDISPERSITY INDEX AT FIXED LEVEL OF C

The percent drug dissolution from nano formulations in 15 min that ranged from 54.28 - 83.78%. Y3 increased from 55.59 to 79.22% and from 54.28 to 81.92% at lower and higher levels of A. At low levels of B, Y3 increases from 54.28 to

65.61% while at high levels it increases from 61.32 to 77.48%. At lower C levels, Y3increased from 63.61 to 81.92% while at higher levels it increased from 60.9 to 79.22%.
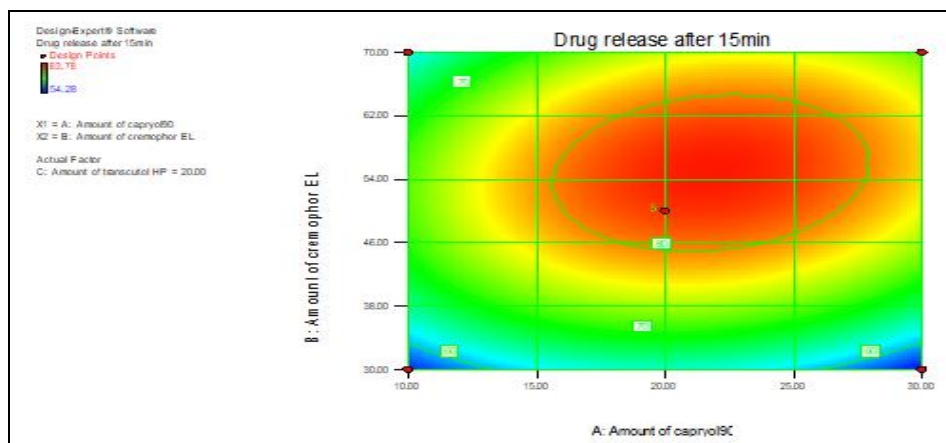

FIG. 4A: CONTOUR PLOT SHOWING THE INFLUENCE OF AMOUNT OF CAPRYOL 90 AND AMOUNT OF CREMOPHOR EL ON PERCENT DRUG RELEASE AT FIXED LEVEL OF C

The respective contour plots indicating influence of capryol 90, cremophor EL and transcutol HP on % drug release are as shown in **Fig. 4A, B** & **C**. Y3 increased from 55.59 to 79.22% and from 54.28 to 81.92% at lower and higher levels of A. At low

levels of B, Y3 increased from 54.28 to 65.61%. Similarly, at high levels of B, Y3 increased from 61.32 to 77.48%. At low levels of C, Y3 increased from 63.61 to 81.92%. Similarly, at high levels of C, Y3 increased from 60.9 to 79.22%.
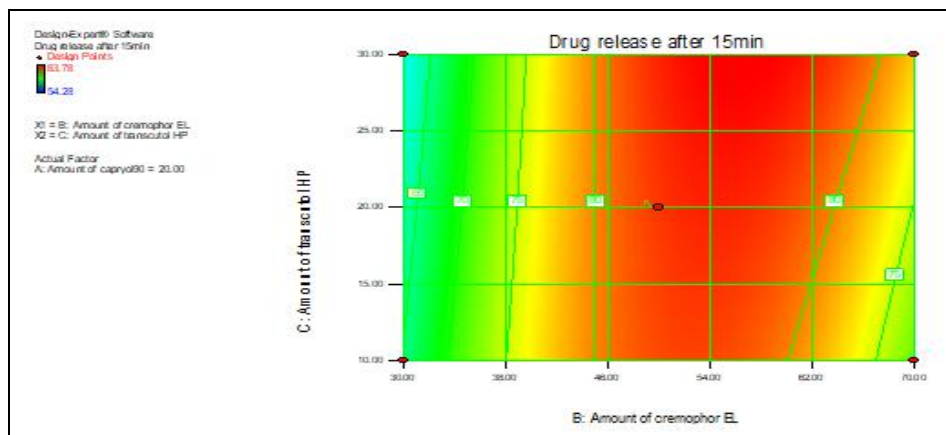

FIG. 4B: CONTOUR PLOT SHOWING THE INFLUENCE OF THE AMOUNT OF CREMOPHOR EL AND AMOUNT OF TRANSCUTOL HP ON PERCENT DRUG RELEASE AT FIXED LEVEL OF A
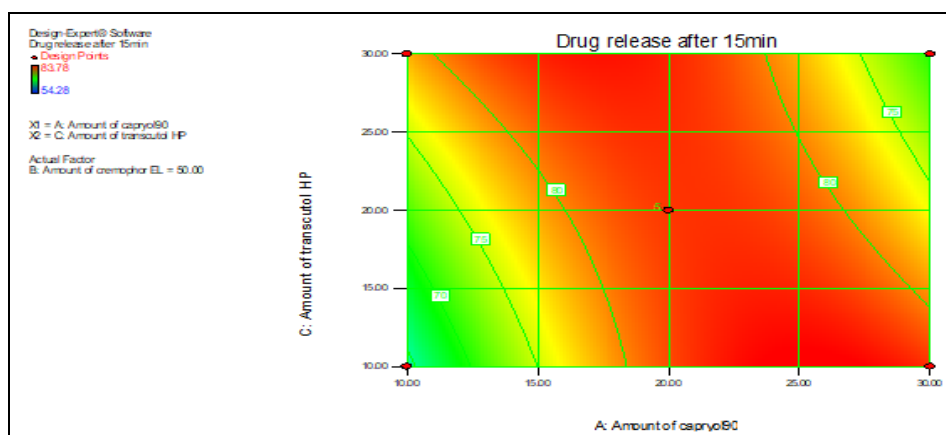

FIG. 4C: CONTOUR PLOT SHOWING THE INFLUENCE OF AMOUNT OF CAPRYOL90 AND AMOUNT OF TRANSCUTOL HP ON PERCENT DRUG RELEASE AT FIXED LEVEL OF B

Optimization by Desirability Function: Simultaneous optimization of three responses was undertaken with desirability function. Respective transformation of responses: size of droplet (Y1), polydispersity index (Y2), and cumulative amount of percent drug release in 15min (Y3) into the desirability scale was done.

Characterization of SNEDDS: Enhancement of drug absorption by oral delivery can be achieved by decreasing the size of the particle to nanorange. The drug loaded nanoparticles were sonicated prior to size and morphology determination. All the three formulations were well dispersed in aqueous media with no aggregates. Particle size distribution and ZP of all formulations was analysed for all the formulated batches. The particle size of

nanoparticles loaded with drug ranged from 167.9 nm to 181.7 nm The PDI values ranges from 0.208 to 0.228, indicating the narrow range of size distribution. Inclusion of ibrutinib in SLN's lipid matrix was indicated by the negative surface charge of prepared formulations that is a key regulating factor for particle stability. This zeta potential ranged between -17.6 mV to -21.4mV for all prepared formulations.

Refractive Index: Isotropic nature of nano-emulsion produced from SNEDDS is characterized by determination of refractive index. Even after conversion to nanoemulsion, prepared three batches were isotropic which was confirmed by determining refractive index that was in range of 1.35-1.45.

Transmission Electron Microscopy: Transmission electron microscopy (TEM) was carried out to determine structure and morphology of optimized batches. Micelles formation in spheres in size range 150-200nm was revealed by TEM images. Analysis of globule size was in accordance with these results.

Percentage Drug Content: Almost 100% drug content was observed on analysis with low standard deviations suggestible of drug's uniform dispersion in the prepared formulation.

***In-vitro* Dissolution Testing of Ibrutinib SNEDDS: Fig. 5** depicts plain ibrutinib and ibrutinib SNEDDS formulation's dissolution profile in virtual intestinal fluid (SIF, pH 6.8) media. To understand the dissolution mechanism of ibrutinib SNEDDS formulations, the drug release profiles of formulation 2 (F2) were analyzed. Dissolution test results were subjected to further analysis by % dissolution efficiency (%DE), mean dissolution time (MDT), relative dissolution (RD), difference factor (f1) and similarity factor (f2). The dissolution parameters such as percent dissolution efficiency at different time points ($\%DE_{30min}$, $\%DE_{60min}$, $\%DE_{6h}$, $\%DE_{12h}$ and $\%DE_{24h}$), relative dissolution rate (RD_{60min}), and mean dissolution time at 180 min (MDT_{180min}) are calculated. More than 85% of drug was dissolved from F2 after 30 min. However, the original ibrutinib powder showed only approximately 0.1% dissolved after the same time period. This result suggested that the SNEDDS formulation significantly enhanced the dissolution of ibrutinib.
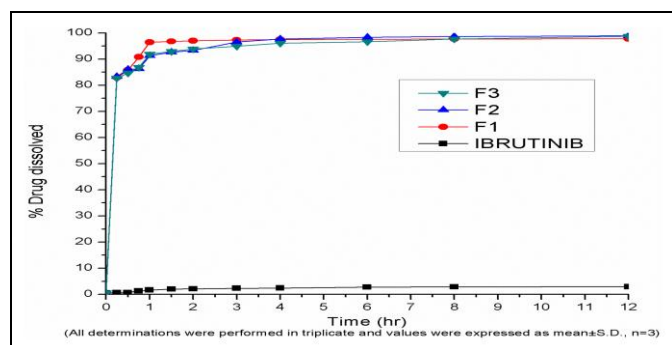

FIG. 5: DISSOLUTION PROFILE OF PURE IBRUTINIB AND IBRUTINIB SNEDDS FORMULATIONS

Pharmacokinetic Analysis: The retention time of ibrutinib and vortioxetine acid (Internal Standard) were found to be 4.6min and 9.7min respectively. The linearity of developed HPLC method checked

by injecting seven samples of varying concentration into the HPLC. The average peak areas *vs.* concentrations was plotted for linearity evaluated using calibration curve to calculate correlation coefficient, slope and intercept. The curve indicated the correlation coefficient (r^2) value of 1.00 that lies within the accepted range the slope (m) of 4646.

Pharmacokinetic Parameters Comparison: Fig. 6 depicts the plasma concentration *vs.* time plot in male wistar rats after administration of first oral dosage of ibrutinib SNEDDS formulation as compared to ibrutinib pure suspension. At any points, ibrutinib concentrations in rat plasma administrated with SNEDDS formulation was comparatively higher than those administrated with pure drug pharmacokinetic parameters of ibrutinib **Table 3**. The C_{max} of the SNEDDS 67.23±4.54 ng/ml was significant (p<0.05) than pure drug suspension 5.8±0.42 ng/ml. T_{max} of SNEDDS and pure drug suspension were 4.00±0.03 h and 6.00±0.01 h, respectively. $AUC_{0-\infty}$ infinity for SNEDDS formulation was higher (399.1±5.14 ng.h/ml) than the pure drug 32.3±6.22 ng.h/ml.
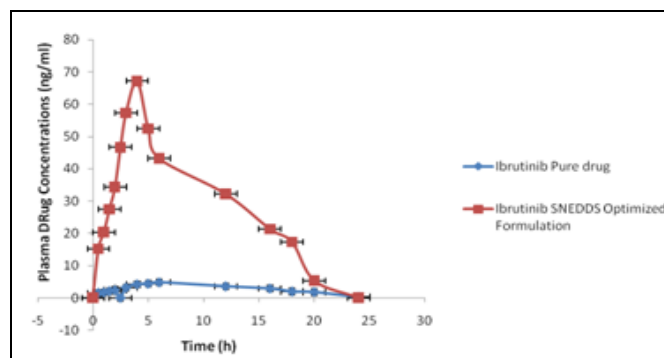

FIG. 6: PLASMA CONCENTRATION PROFILES OF IBRUTINIB SNEDDS AND PURE DRUG

TABLE 3: PHARMACOKINETIC STUDY DATA

Parameters	Ibrutinib Pure drug	Ibrutinib –SNEDDS Optimized Formulation
C_{max} (ng/ml)	5.8±0.42	67.23±4.54
AUC_{0-t} (ng.h/ml)	28.2±3.62	374.4±4.61
AUC_{0-inf} (ng.h/ml)	32.3±6.22	399.1±5.14
T_{max}(h)	6.00±0.01	4.00±0.03
$t_{1/2}$ (h)	10.52±0.02	8.02±0.02

The AUC_{0-t} of the SNEDDS formulation was significantly higher (p<0.05) as compared to pure drug suspension formulation. Higher drug concentrations in plasma are indicative of better

absorption of ibrutinib from SNEDDS formulation as compared to the pure drug suspension formulation.

CONCLUSION: In the present study, the optimized ibrutinib SNEDDS has the composition of capryol 90, cremophore EL and transcutol HP. Based on the particle size and entrapment efficiency and *in-vitro* dissolution studies; F2 is identified as optimized formulation with 85% drug released, whereas less than 1% was released from pure drug at end time of 30 min. *In-vivo* bioavailability data reported in animals indicate higher concentration of drug in plasma implying enhanced systemic absorption of ibrutinib from SNEDDS formulation. The C_{max} and AUC_{0-inf} values of optimized SNEDDS was considerably higher ($p<0.05$) than pure drug formulation. The oral bioavailability of optimized SNEDDS increased around 12 folds when compared with pure drug. Therefore, from our results, the study suggests that the ibrutinib loaded formulation has a great potential for clinical application in the effective management of lymphocytic leukaemia.

CONFLICTS OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflicts of interest.

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