ORIGINAL ARTICLE

Development and Optimization of Self-nanoemulsifying Drug Delivery System of Ibrutinib

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Abstract

Objective: The present research demonstrates the formulation of novel self-nanoemulsifying drug delivery system (SNEDDS) for potential delivery of ibrutinib by oral route. **Materials and Methods:** Ibrutinib SNEDDS formulation optimized using three-factor, three-level Box–Behnken design and the responses of dependent and independent variables fitted to the second-order quadratic equations and statistical validation calculated by analysis of variance. Various response surface graphs and contour plots were constructed to understand the effects of different factor level combinations on the responses. All optimized SNEDDS formulations were characterized for particle size, zeta potential (ZP), refractive index, % transmission, % drug content, Fourier transform infrared, transmission electron microscopy, and drug release study. **Results:** The formulations prepared using Capryol 90, Cremophor RH40, and Transcutol P (40%) indicated close comparison of the predicted values and experimental values whose droplet size, polydispersity index (PDI), and cumulative drug release varied between 157.07–236.62 nm, 0.206–0.312, and 54.28–83.78%, respectively. Based on the physicochemical parameters and *in vitro* dissolution studies, F2 is identified as optimized formulation with droplet size of 167.9 nm, PDI of 0.228 and −21.2 ± 1.62 mV ZP, maximum % transmission, and 100% drug loading with 85% drug released at end time of 30 min. **Conclusion:** A conclusion can thus be drawn from the present study stating the requirement for potential of ibrutinib delivery as SNEDDS in the effective management of lymphocytic leukemia. Stability test for a period of 3 months revealed the formulation to be stable for the specified time.

Key words: Box–Behnken design, ibrutinib, lymphocytic leukemia, self-nanoemulsifying drug delivery system, zeta potential

INTRODUCTION

Ibrutinib is recently approved drug for the treatment of chronic lymphocytic leukemia. However, ibrutinib exhibits low bioavailability oral bioavailability and widespread first-pass effects that lead to commercially available capsular dosage form with very high doses. In the past few years, nanoemulsions and self-nanoemulsifying drug delivery systems (SNEDDSs) have been found to be effective approaches for the targeting of several anticancer drugs such as 5-fluorouracil (5-FU), 5-FU conjugates, methotrexate, paclitaxel, and isoniazid analog to melanoma cells for enhanced therapeutic effects of these drugs. Therefore, in the current work, various ibrutinib SNEDDSs were formulated by aqueous phase titration method for enhanced treatment of mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL). All the materials used in the preparation of ibrutinib SNEDDS are non-toxic 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

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(BBD) is RSMs for understanding the effects of independent and dependent factors.[3-6]

MATERIALS AND METHODS

Materials

Ibrutinib was kindly gifted by Caplin Point Laboratories Ltd., Bangalore; Labrasol, Cremophor EL, Transcutol HP, and chromatographic acetonitrile were obtained from Sigma-Aldrich (St. Louis, MO). Dialysis tubing was purchased from HiMedia Laboratories (Mumbai, India).

Instrumentation

UV-visible spectrophotometer (Jasco, Tokyo, Japan, Model V-570) was used for UV analysis. IR spectroscopy performed on Fourier transform infrared (FTIR) spectrophotometer (Shimadzu FTIR 8400S, Japan). Powder X-ray diffraction analysis carried out on Bruker D8 Advance diffractometer. A PerkinElmer DSC/7 differential scanning calorimeter was used for thermal analysis. The morphology of finely ground particles was observed under scanning electron microscopy (JOEL SEM, Model 6400F, Japan) and Vortexer (GeNei, Bangalore, India).

Formulation of ibrutinib-loaded SNEDDS

Selection of oil, surfactant, and cosurfactant

Oil phase selection was based on solubility of ibrutinib in the oils. Higher the solubility of the drug, higher will be the drug loading potential.[7] Solubility studies indicate that the drug was significantly more soluble in Capryol 90 hence chosen as oil phase for formulating the SNEDDS system.[8]

Evaluation of 16 non-ionic surfactants was performed by screening their ability of oil phase emulsification. A 300 mg of various surfactants mixed with Capryol 90, vortexed for 1 min. A 100 mg of resultant isotopic system was diluted with distilled water. Emulsion resultant from the above process was subjected evaluation for phase separation and visual transparency after a storage period of 24 h at a temperature of 25°C.[9]

Eight cosurfactants (Capmul MCMC8, Lauroglycol 90, polyethylene glycol [PEG] 400, PG, EG, Plurol Oleique CC497, triacetin, and Transcutol P) were tested for emulsification capability with oil phase.

One hundred milligrams of cosurfactant, 200 mg surfactant, and 300 mg selected oil phase were mixed together and evaluated for surfactant screening.[10]

Construction of ternary phase diagrams

On the basis of solubility studies and the emulsification tendency, Capryol 90 (oil), Cremophor EL (surfactant), and Transcutol HP (cosurfactant) were chosen, respectively. Ternary diagrams of the three components prepared to identify the emulsifying region. Mixtures with varying compositions of oil (25% w/w–70% w/w), surfactant (25% w/w–75% w/w), and cosurfactant (25% w/w–75% w/w) were prepared, vortexed, and diluted with distilled water and the ease of emulsification was analyzed visually. Either clear or slight bluish-colored dispersions were considered in the region of nanoemulsion of the diagram.[11] Ternary phase diagrams are used to determine the ambient self-emulsifying region, that is, the feasibility of nanoemulsion formation at extreme values of the excipients. Therefore, the extreme and middle levels of the independent variables, consisting of the oil, surfactant, and cosurfactant, were selected for further study.

Optimization experiments

Ibrutinib-loaded SNEDDS optimization was conducted using DoE. RSM is a statistical method for various processes using specially designed experimental programs to achieve the 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 BBD employed to explore and optimize the interaction and quadratic effects of various ingredients in the formulation on the performance of the liquid SNEDDS.[12] The variables that were chosen as dependent and independent are specified in Table 1.

Table 2 shows 17 randomized experimental runs for the selected independent variables, including five replicates at the center point (asterisk marked) generated from a three-factor, three-level BBD and their corresponding responses.

The BBD matrix was generated using Design-Expert® software (Version 8.0, Stat-Ease Inc., Silicon Valley, CA, USA), and the data obtained were analyzed by the same software. Design-Expert software was utilized for fitting all the responses into the second-order quadratic model.[13,14] The second-order quadratic equation is approximated from the mathematical model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_2 X_3 + \beta_6 X_1 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 \quad (1)$$

Preparation of ibrutinib-loaded SNEDDS

The ibrutinib-loaded SNEDDSs were prepared by mixing oil phase (Capryol 90), surfactant (Cremophor EL), and cosurfactant (Transcutol HP) and warming it at 40°C, then 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, surfactant and cosurfactant along with 50 mg/g of drug, a total of 17 such experiments were carried out using experimental design. The prepared ibrutinib-loaded SNEDDSs were filled into size 0 HPMC capsule shells.

Table 1: Dependent and independent variables from Box–Behnken design

Independent variables			Levels		
Variable	**Name**	**Units**	**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
Dependent variable			**Goal**		
Y1	Droplet size	nm	Minimize		
Y2	PDI		Minimize		
Y3	Drug release post 15 min	%	Maximize		

PDI: Polydispersity index

Characterization of SNEDDS

Developed ibrutinib SNEDDSs were physicochemical evaluated for droplet diameter, polydispersity index (PDI), zeta potential (ZP), refractive index (RI), percentage of transmittance (% T), and surface morphology.

Droplet size and PDI

Droplet size and PDI of 17 varying liquid SNEDDS formulations (500 mg) containing 5% ibrutinib were prepared followed by dilution of 100 µg of each formulation to 100 ml with simultaneous stirring at 37°C. The formulations sonicated for minimum period and the droplet size analyzed by Zetasizer Nano ZS90 at wavelength of 635 nm. The PDI and Z-average diameter, also referred to as the harmonic intensity-weighted average hydrodynamic diameter of the emulsion, was derived from cumulated analysis by Automeasure software (Malvern Instruments).

ZP

Zeta meter system was utilized for the measurement of ZP of diluted SNEDDS. Dilution of SNEDDS with distilled water at a ratio of 1:2500 (v/v) was carried out with proper mixing with magnetic stirrer. Zeta potential of the resulting microemulsion was determined using a Zetasizer. The analysis performed using purified water with 0.9% w/v NaCl. This was followed by documentation of mean values of z for the three independent samples.

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

PDI: Polydispersity index

RI

RI was measured using Abbe type refractometer (Precision Testing Instruments Laboratory, Germany) at room temperature, mean values were recorded after performing the experiment in triplicate.

Percentage transmittance

Dilution of optimized formulations to 1000 times was done that was then allowed to stand for 2 h followed by evaluation at 650 nm by UV spectrophotometer for determining percentage transmission.

Surface morphology

Transmission electron microscopy (TEM) studies (JEOL JEM 2100 F, USA) were carried out for optimized formulation by diluting the same with distilled water to 1000 times and then plunging on a 2% uranyl acetate solution stained carbon grid.

FTIR spectroscopy

FTIR spectrophotometer (Shimadzu FTIR 8400S, Japan) was used to record the FTIR spectra of pure drug and drug-loaded SNEDDS in 4000–400 cm^{-1} range.

Percentage drug content

Drug content was estimated by dissolving each formulation in 10 ml methanol, 10 min vortexing the same followed by filtration of contents with a membrane filter (00.45 mm) and then analyzing the filtrate for the 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 release studies were carried out in USP XXIV dissolution apparatus containing pH 6.8 phosphate buffer (900 ml) as dissolution medium. The speed of apparatus sets 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, 3 ml 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.

RESULTS

Selection of oil (solubility studies)

The solubility data of ibrutinib in different components at 37°C are listed in Table 3. Different oils having varying saturation degrees were used. Among different oils, the highest solubility of ibrutinib was recorded in Capryol 90 (57.34 mg/ml), followed by Capmul PG8 (42.78 mg/g), Paceol (36.89 mg/g), Captex 355 (36.12 mg/g), IPA (29.79 mg/g), and Miglyol 812 (24.38 mg/g). The aqueous solubility of IBR was recorded as 0.12 mg/ml. Based on these results, Capryol 90 was selected as oil phase. The selection of surfactant and cosurfactant was subsequently done based on their ability to emulsify the oil.

Selection of surfactant (emulsification study)

In this study, 16 different non-ionic surfactants were tested for emulsification potential. Evaluation of these characteristics was done from % T and ease of emulsification. The number of inversions and percentage transparency of different surfactants is shown in Figure 1. The quantity of oil that underwent emulsification by different surfactants is shown in Figure 2. Cremophor EL (PEG-35-castor oil) has the ability to enhance permeability and uptake of drugs that are susceptible to p-glycoprotein-mediated efflux and is well tolerated for oral administration. Cremophor EL was chosen as surfactant of choice for the preparation of ibrutinib-loaded SNEDDS.

Table 3: Solubility of ibrutinib in various oils and other solvents

S. No	Oil/solvent	Solubility (mg/ml)
1	Capmul MCM	23.21
2	Captex 355	36.12
3	Capmul PG8	42.78
4	Capryol 90	57.34
5	Imwitor 742	14.03
6	IPM	13.12
7	Labrafil M2	16.34
8	Labrafac CC	17.78
9	Labrafac Lipophile WL 1349	4.28
10	Maisine 35-1	2.42
11	Miglyol 812	24.38
12	Paceol	36.89
13	Sefsol 218	23.56
14	Olive oil	4.18
15	Oleic acid	1.98
16	Castor oil	7.78
17	IPA	29.79
18	1-butanol	9.69
19	2-butanol	3.12
20	0.1 N HCl	23.67
21	pH 4.5 acetate buffer	19.35
22	pH 6.8 phosphate buffer	8.12
23	pH 7.4 phosphate buffer	2.45
24	Water	0.12

Selection of cosurfactant (emulsification study)

The selection of cosurfactant was carried out by mixing eight cosurfactants with previously optimized surfactant (Cremophor EL) at a fixed (1:1) surfactant:cosurfactant ratio. Oil phases were better emulsified with hydrophilic cosurfactants (Transcutol P, PEG 400, PG, Capmul MCMC8, and EG) than with lipophilic cosurfactants (Lauroglycol 90, triacetin, and Plurol Oleique CC 497). The number of inversions and percentage transparency of different cosurfactants is shown in Figure 3. Transcutol P was selected as cosurfactant of choice for the SNEDDS preparation due to its % transmission which was highest in addition to its ease of emulsification.

Construction of ternary phase diagram

Three-component systems chosen for the ibrutinib-loaded SNEDDS preparation were Capryol 90, Cremophor EL40, and Transcutol P [Figure 4]. The selection was done based on preliminary trials performed. Self-nanoemulsifying region that was termed efficient was shown as gray region of the diagram. Each component ranges on the basis of diagram selected as follows: 10% ≤ Capryol 90 ≤ 30%, 45% ≤ Cremophor El ≤ 75%, 10% ≤ Transcutol *P* ≤ 30%.

Statistical analysis of the designed experiment

BBD 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 and 236.62 nm. Similarly, the range for PDI (Y2) was 0.206–0.312 and cumulative drug release in percentage (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 analysis of variance (ANOVA) tests.

Response surface analysis

Stat-Ease Design-Expert® software V8.0 was utilized for analyzing data, to get regression equation, regression

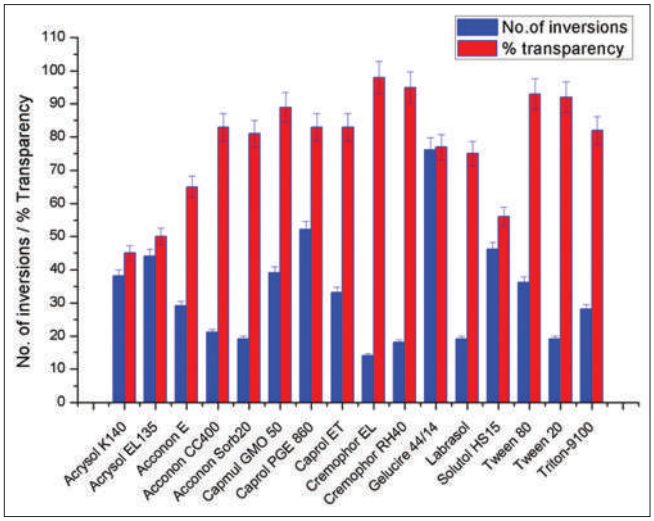

Figure 1: Emulsification study of surfactant Capryol 90

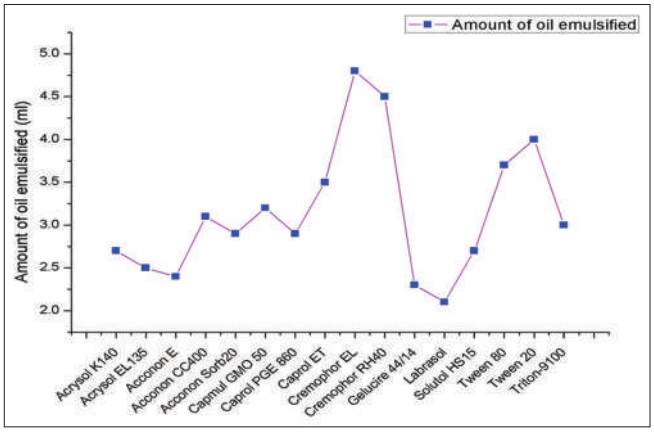

Figure 2: Emulsification study of Capryol 90 amount of oil emulsified

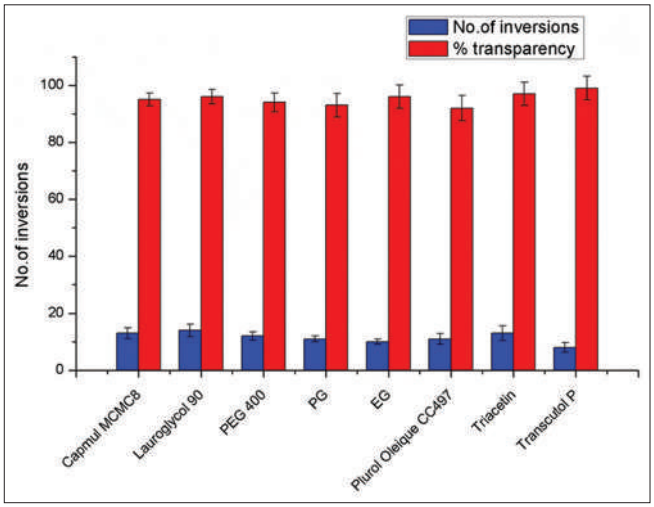

Figure 3: Emulsification study of Capryol 90 for selection of cosurfactant

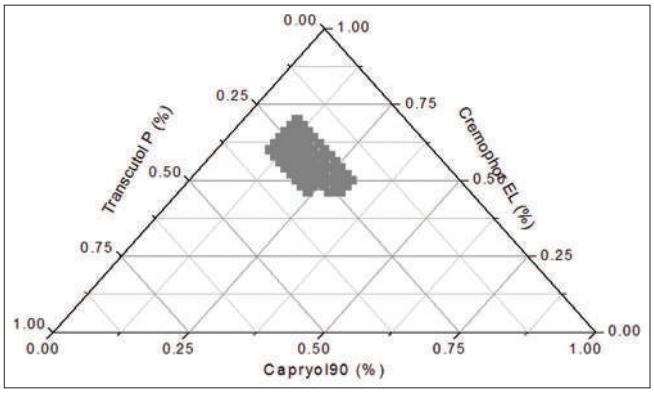

Figure 4: Ibrutinib-loaded self-nanoemulsifying drug delivery system ternary phase diagram

coefficient, and ANOVA. Table 4 shows the mathematical relationships for specified variables generated using multiple linear regression analysis.

A key regulator for SNEDDS assessment is droplet size since the reduction in droplet size gives access to a greater interfacial surface area for absorption of drug in addition to a faster rate of release. Table 5 specifies particle size of nanoparticles that were between 157.07 and 236.62. Significant influence of the amount of Capryol 90, Cremophor EL amount, and amount of Transcutol HP on droplet size came into light by the generated quadratic model. A reasonable good correlation was found between theoretical (predicted) values and the observed values [Figures 5, 6a and b, 7a and b].

This shows the PDI of nanoparticles that ranged from 0.206 to 0.312. The quadratic model generated revealed that the amount of Cremophor EL and amount of Transcutol HP have a significant influence on the PDI. A good agreement was observed between theoretical (predicted) values and the observed values. Significance of generated mathematical model for PDI (Y2) was depicted by its F = 6.68A good correlation coefficient of 0.8109 which was observed for factorial equation generated for PDI. Independent variables of main and interactive effects on PDI were elucidated by the use of contour, 3D response plots, and perturbation. Figures 8 and 9 is the perturbation plot depicting the effects of B and C on PDI (Y2) that clearly indicates major effect of B on Y2 followed by C that has a moderate effect on Y2. 3D response surface plots and corresponding contour plots indicated the dependent and independent variables relationship. A and B interaction on PDI at constant level of C is indicated in Figure 9a. The respective contour plots are shown in Figure 9b. At low levels of A, Y2 decreased from 0.256 to 0.212; Y2 decreased from 0.28 to 0.22 at high levels of A. At low levels of B, Y2 decreased from 0.312 to 0.24. Similarly, at high levels of B, Y2 decreased from 0.248 to 0.206. At low levels of C, Y2 decreased from 0.24 to 0.206. Similarly, at high levels of C, Y2 decreased from 0.312 to 0.21.

Table 5 shows percentage drug release from nanoformulations in 15 min that ranged from 54.28 to 83.78%. The quadratic model generated revealed that the amount of Capryol 90, amount of Cremophor EL, and amount of Transcutol HP have a significant influence on the droplet size. Significance of the model significance of the mathematical model generated for percentage drug release in 15 min (Y3) can be depicted

Table 4: Regression equations for the responses

Response	Regression equation
Y1	183.69+9.68 A–19.37 B+4.21 C–7.93 AB–15.08 BC–13.15 A^2+14.05 B^2
Y2	0.24+6.0 A–0.025 B+0.014 C
Y3	82.23+2.31 A+5.80 B+0.56 C+2.89 AB–6.77 AC+2.45 BC–8.42 A^2–13.13 B^2

Table 5: Optimized values obtained by the application of constraints on Y1, Y2, and Y3

Independent variable	Nominal values	Predicted			Batch	Observed			
		Droplet size (Y1) (nm)	Polydispersity index (Y2)	Percent drug release in 15 min (Y3)		Droplet size (Y1) (nm)	Polydispersity index (Y2)	Percentage drug release in 15 min (Y3)	ZP (mV)
Amount of Capryol 90 (A)	30 mg	173.379	0.2120	82.305	1	172	0.208	82.88	–17.6±1.28
Amount of Cremophor EL (B)	59.6 mg				2	167.9	0.228	83.12	–21.2±1.62
Amount of Transcutol HP (C)	10 mg				3	181.7	0.216	82.20	–21.4±0.98

ZP: Zeta potential

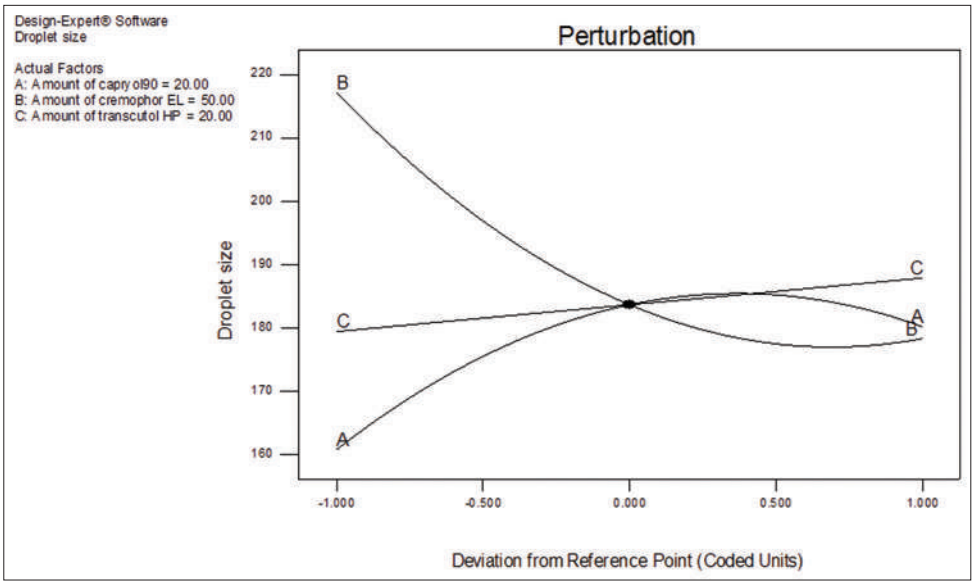

Figure 5: Influence of A, B, and C on droplet size

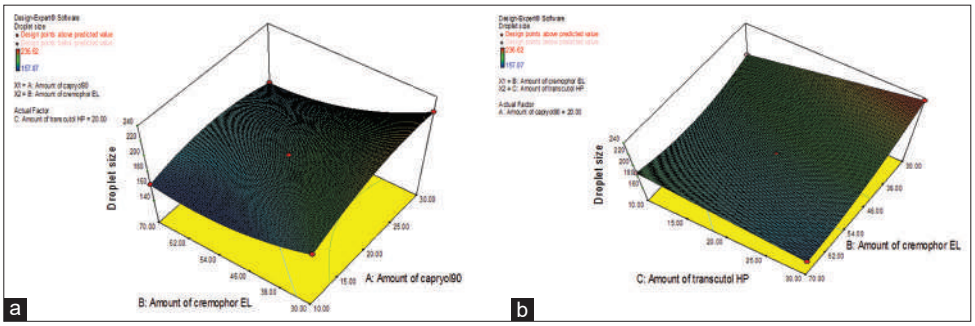

Figure 6: (a) Response surface plot indicating influence of amount of Capryol 90 and amount of Cremophor EL on droplet size at constant C. (b) Response surface plot indicating the effect of Cremophor EL and amount of Transcutol HP on droplet size at constant level of A

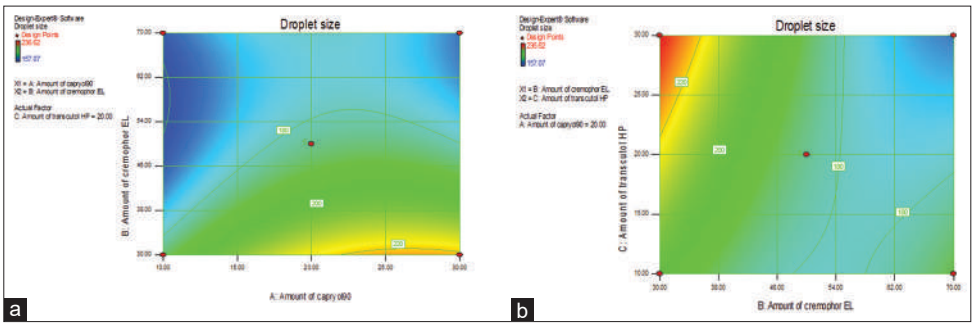

Figure 7: (a) Contour plot displaying the influence of the amount of Capryol 90 and amount of Cremophor EL on droplet size fixed level of C. (b) Contour plot displaying the influence of the amount of Cremophor EL and amount of Transcutol HP on droplet size fixed level of A

by its F = 195.28. Chance of occurrence of "Lack of Fit F-value" this large due to noise was only 87.56%. Significant effect of B in comparison to that of A and C is indicated by equation results. A good correlation coefficient of 0.9949 was observed for the factorial equation for percentage drug release. Perturbation, contour, and 3D response plots further elucidated main and interactive effects influence on particle size by variables that were independent. Figure 10 is the perturbation plot depicting the main effects of A, B, and C on size of the particle (Y3) which gives a clear indication of foremost and major influence of B on Y1 that is followed by A and C having a moderate influence on Y3. Percentage drug release was negatively influenced by higher values of A and B. 3D response surface plots and corresponding contour plots depicted the dependent and independent variables relationship. The interaction between A and B on percentage

release of drug at a fixed level of C is shown in Figure 11a. The interaction between B and C on droplet size at A level fixed is illustrated in Figure 11b. The effect on droplet size by interaction of A and C at level B fixed is depicted in Figure 11c. The respective contour plots are shown in Figure 12a-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%. Surfactant concentration had a major hand in enhancement of cumulative drug release from the formulation that may be accredited instant dispersion of formulation in medium on capsule shell dissolution that resulted in faster self-emulsification of the same. Spontaneity in the formation of oil-water interface is observed due to the requirement of very low free energy which, in turn, increases penetration of water into oil droplet leading to interface disruption; hence, a decrease in droplet size and enhancement in release rate is observed. An observation of increase in drug release on addition of cosurfactant was also made which could be due to cosurfactant penetration into surfactant monolayer which further increases self-emulsification performance of SNEDDS.

Optimization by desirability function

Simultaneous optimization of three responses was undertaken with desirability function. Respective transformation of responses: Size of droplet (Y1), PDI (Y2), and cumulative amount of percentage drug release in 15 min (Y3) into the desirability scale were done. Table 5 depicts these results. Model validity was affirmed by fine agreement existing between predicted and observed values and also indicated BBD success in combination with desirability function in optimization and evaluation of SNEDDS.

Characterization of SNEDDS

Developed ibrutinib SNEDDSs were physically, chemically evaluated in terms of droplet diameter, PDI, ZP, RI, percentage of transmittance (% T), and surface morphology.

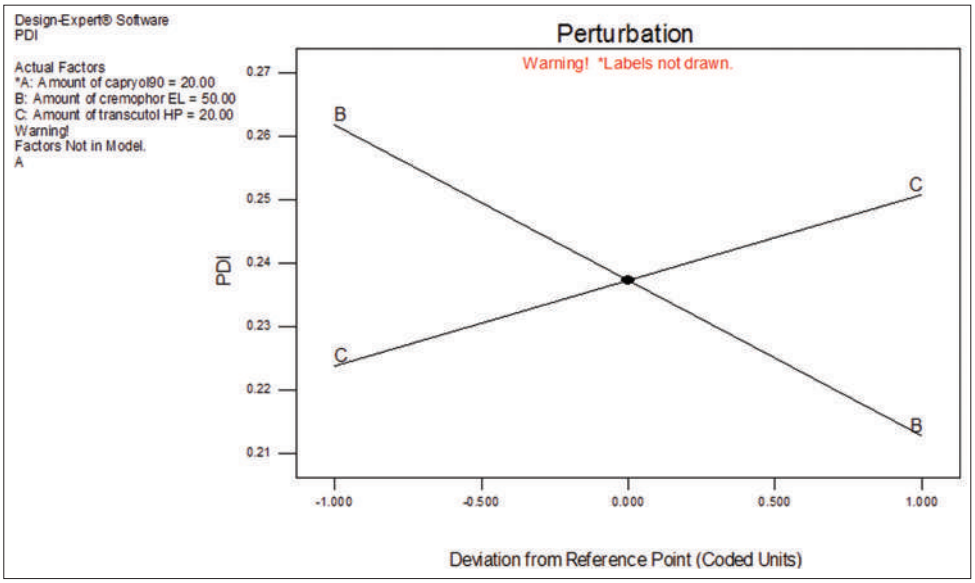

Figure 8: Perturbation plot displaying the effect of B and C on polydispersity index

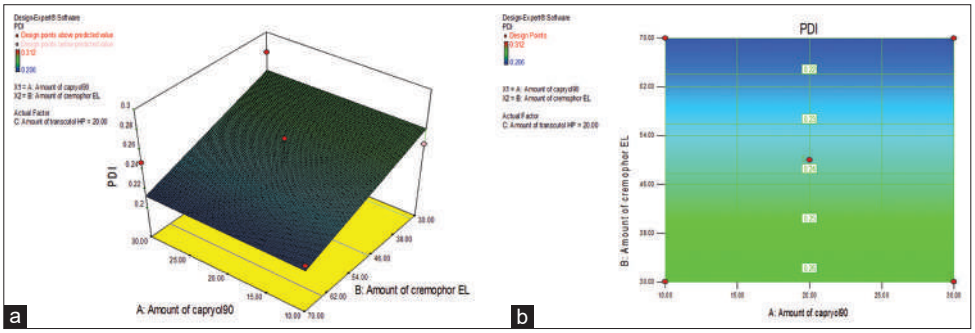

Figure 9: (a) Response surface plot showing the influence of the amount of Capryol 90 and amount of Cremophor EL on polydispersity index at fixed level of C. (b) Contour plots displaying the influence of the amount of Capryol 90 and amount of Cremophor EL on polydispersity index at fixed level of C

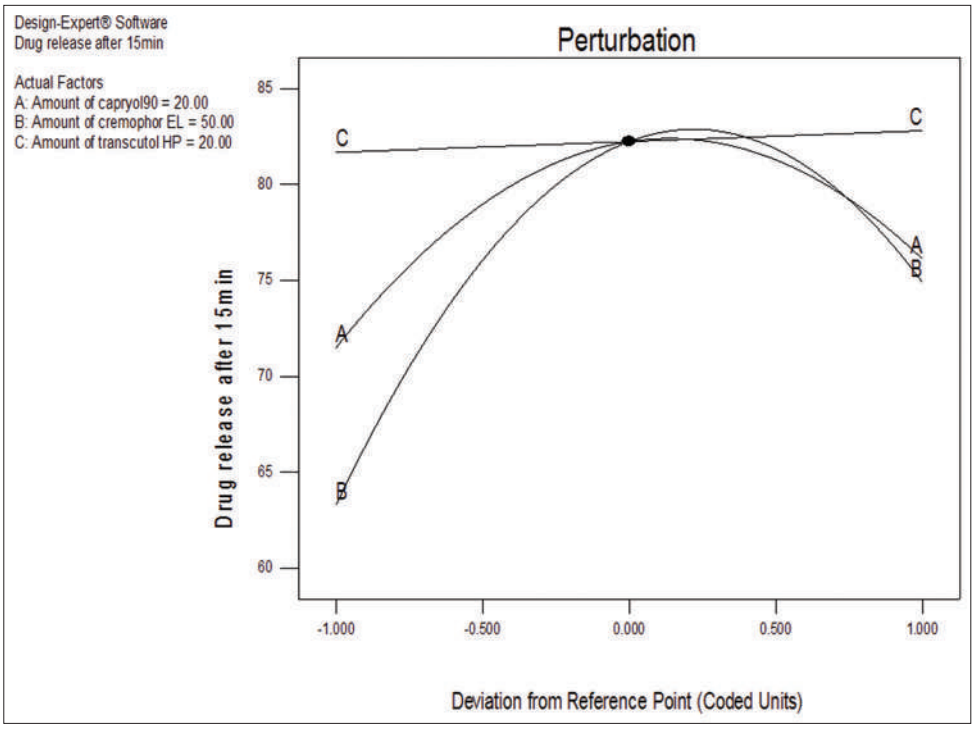

Figure 10: Plot indicating the effect of A, B, and C on percentage drug release (Y3)

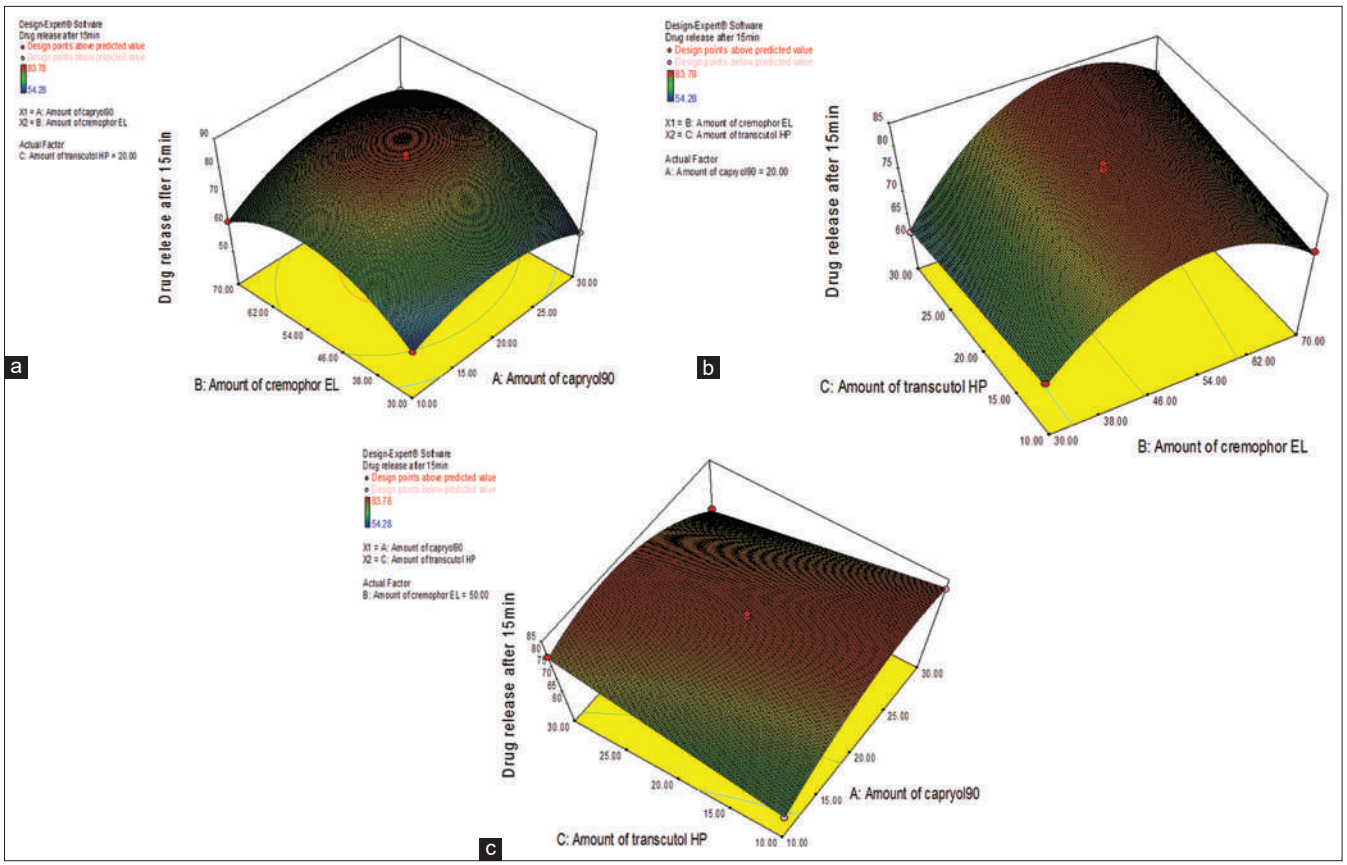

Figure 11: (a) Response surface plot showing the influence of amount of Capryol 90 and amount of Cremophor EL on percentage drug release at fixed level of C. (b) Response surface plot showing the influence of the amount of Cremophor EL and amount of Transcutol HP on percent drug release at fixed level of A. (c) Response surface plot showing the influence of the amount of Capryol 90 and amount of Transcutol HP on percentage drug release at fixed level of B

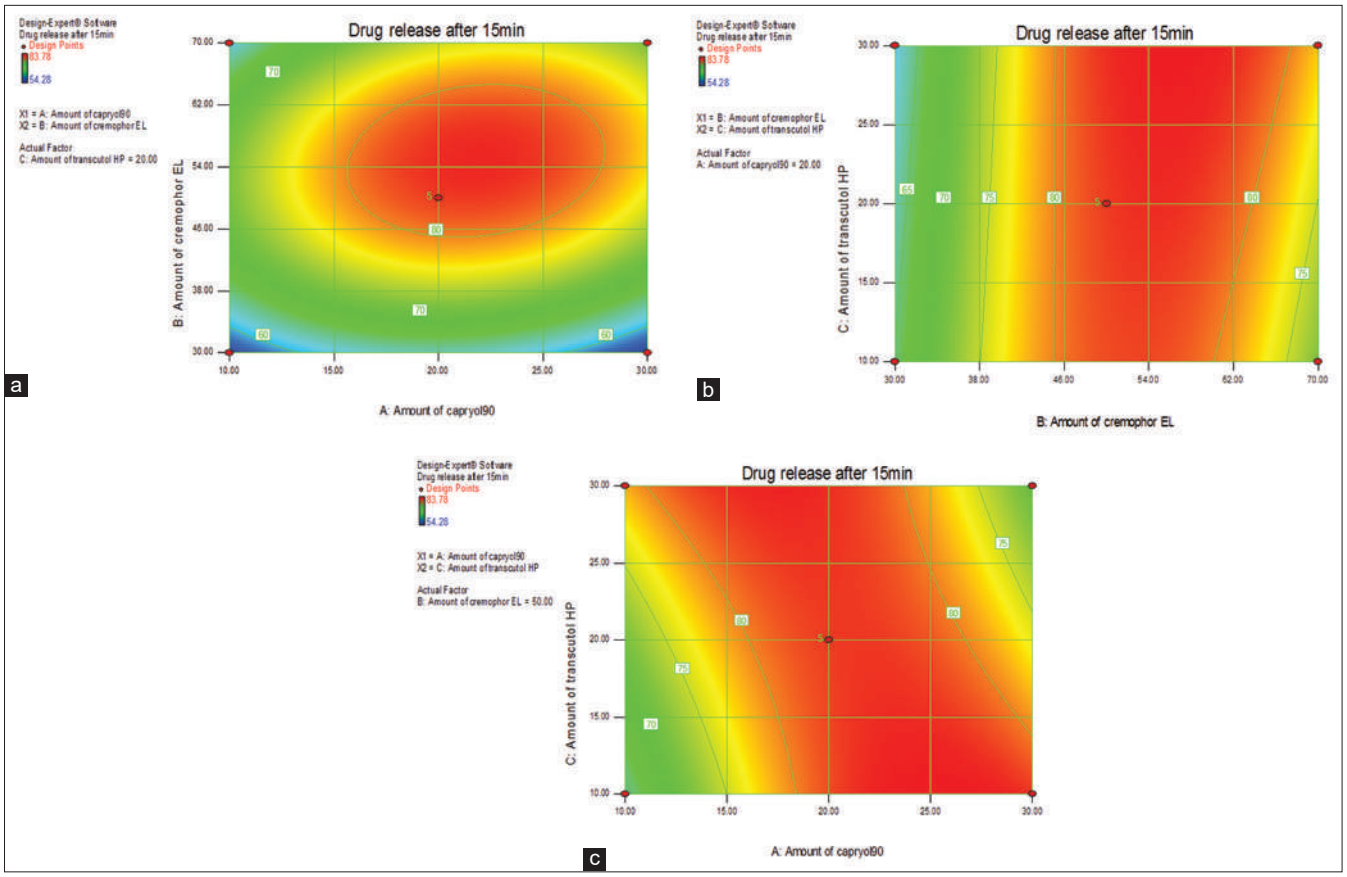

Figure 12: (a) Contour plot showing the influence of amount of Capryol 90 and amount of Cremophor EL on percentage drug release at fixed level of C. (b) Contour plot showing the influence of the amount of Cremophor EL and amount of Transcutol HP on percentage drug release at fixed level of A. (c) Contour plot showing the influence of amount of Capryol 90 and amount of Transcutol HP on percentage drug release at fixed level of B

Droplet size, PDI, and ZP

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 before 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 were analyzed for all the formulated batches. Figures 13 and 14 denote the optimized formulation's particle size and ZP, respectively. The particle size of nanoparticles loaded with drug ranged from 167.9 nm to 181.7 nm [Table 5]. The PDI values range from 0.208 to 0.228, indicating the narrow range of size distribution. Inclusion of ibrutinib in SNEDDS was indicated by the negative surface charge of prepared formulations that are a key regulating factor for particle stability. This zeta potential ranged between −17.6 mV and −21.4 mV for all prepared formulations.

RI

Isotropic nature of nanoemulsion produced from SNEDDS is characterized by the determination of RI. Even after conversion to nanoemulsion, prepared three batches were isotropic which was confirmed by determining RI that was in the range of 1.35–1.45.

Percentage transmittance

Transmittance study is another study for characterization of isotropic nature of SNEDDS, the results of which indicated transmittance of 100% of all batches.

TEM

TEM was carried out to determine the structure and morphology of optimized batches. Micelles formation in spheres in the size range of 150–200 nm was revealed by TEM images [Figure 15]. Analysis of globule size was in accordance with these results.

Characterization of ibrutinib-loaded SNEDDS

FTIR spectroscopy

FTIR spectra of pure drug showed peaks characteristic of the functional groups present in drug's chemical structure which

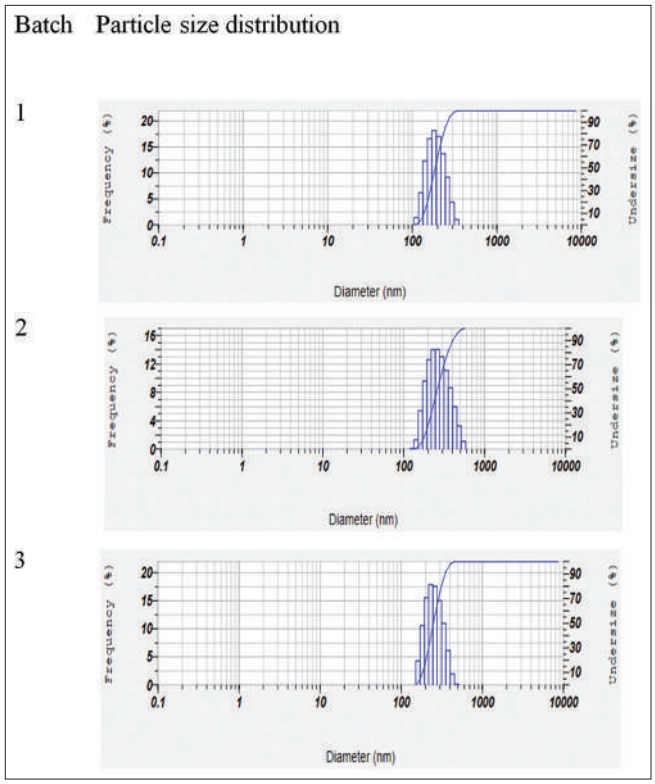

Figure 13: Particle size distribution of ibrutinib self-nanoemulsifying drug delivery system

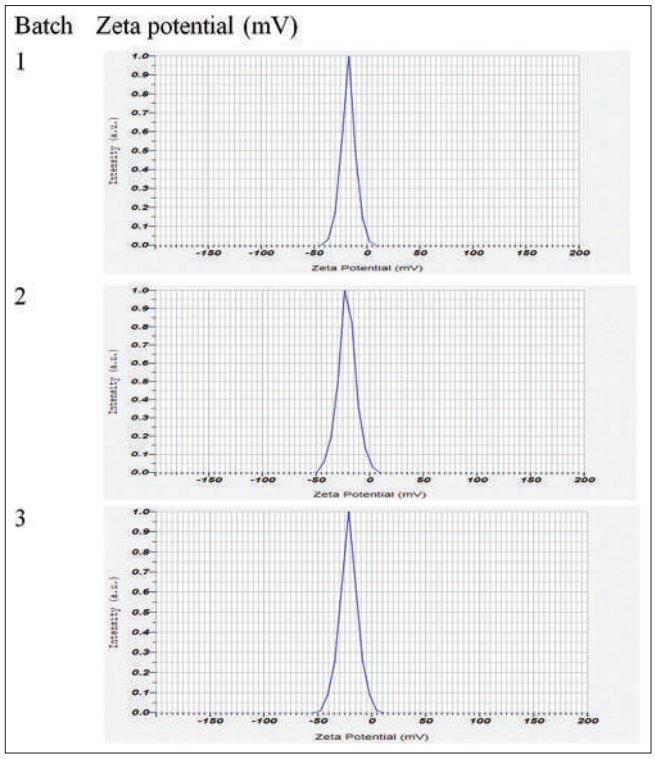

Figure 14: Zeta potential of ibrutinib self-nanoemulsifying drug delivery system

were also seen in the spectra of SNEDDS loaded with the drug, but were broadened that might be due to drug-excipient hydrogen boding [Figure 16].

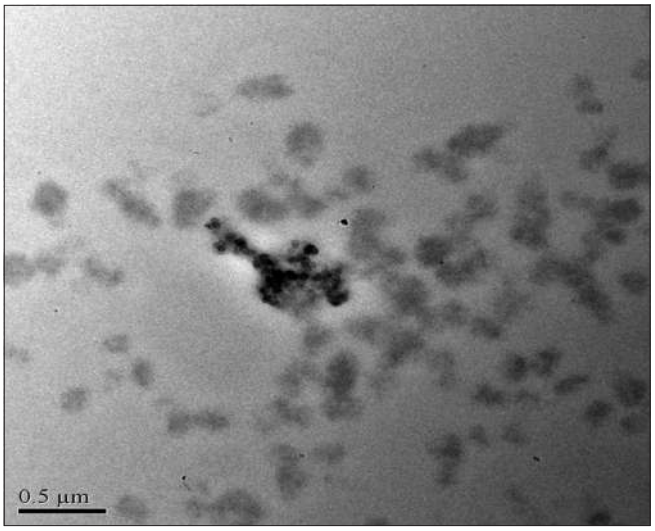

Figure 15: Transmission electron microscopy photomicrographs for the optimized batches of ibrutinib-loaded self-nanoemulsifying drug delivery system

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

Dissolution testing plays an important role in the pharmaceutical industry for drug formulation development, quality control testing for batch manufacturing consistency and specification setting, and establishment of *in vitro* and *in vivo* relationships between drug release from dosage form and drug absorption. Figure 17 depicts plain ibrutinib and ibrutinib SNEDDS formulation's dissolution profile in simulated intestinal fluid (SIF, pH 6.8) media. To understand the release mechanisms of ibrutinib SNEDDS formulations, the drug release profiles of formulation 2 (F2) were analyzed. Dissolution test results were subjected to further analysis by percentage 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 MDT at 180 min (MDT_{180min}) are calculated from Figure 18. 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.

The results indicate that the value of $\%DE_{60min}$ was enhanced from 1.1 for plain ibrutinib to 96.45 for SNEDDS formulation. Similar to $\%DE_{60min}$ values, the value of RD_{60min} was higher for the SNEDDS formulation. Not even 2% release from original ibrutinib was observed within 3 h. The SNEDDS formulation

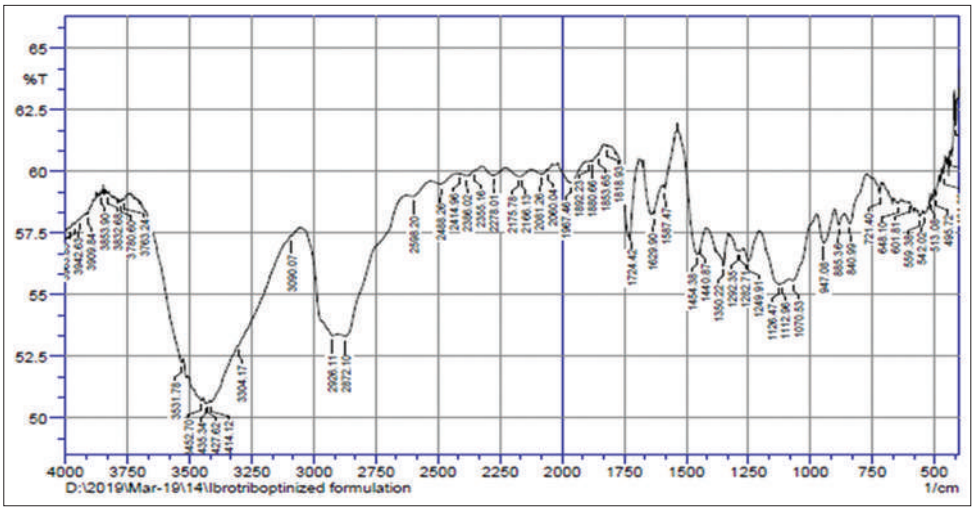

Figure 16: Fourier transform infrared spectra of ibrutinib-loaded self-nanoemulsifying drug delivery system formulation

reached 82% dissolution in 15 min. MDT is an indication of the rate of drug release from a dosage form. About 180 min of MDT for SNEDDS formulation was observed that was much lower than MDT of plain ibrutinib powder suggestible of greater dissolution rate in comparison to ibrutinib powder. Further, comparison between ibrutinib dissolution profiles was made by studying similarity factor (f2) and difference factor (f1). The dissolution profiles of SNEDDS formation and plain ibrutinib powder were not similar as f1 values for ibrutinib nanoparticles were higher than 15. All these studies are a clear indication of enhancement of *in vitro* dissolution profile with lowered MDT by SNEDDS formulation in comparison to pure ibrutinib.

Kinetic analysis of ibrutinib release data

Drug release mechanism and order were found by fitting the *in vitro* release data of optimized formulation (F2) in different kinetic equations such as first-order, zero-order, Korsmeyer–Peppas, and Higuchi plots. Korsmeyer–Peppas model was found to be more appropriate in determining the release mechanism due to its correlation coefficient (R^2 = 0.94572) with a non-Fickian diffusion indicated by the value of release component or n equal to 0.836 [Figures 17 and 19].

Stability study

Stability tests indicate changes in drug substance or product's quality with time brought about by varied environmental factors such as light, temperature, and humidity. Insignificant difference in particle size and entrapment efficiency ($P < 0.05$) was observed for optimized formulation stored at room temperature and refrigerated conditions, as indicated in Table 6.

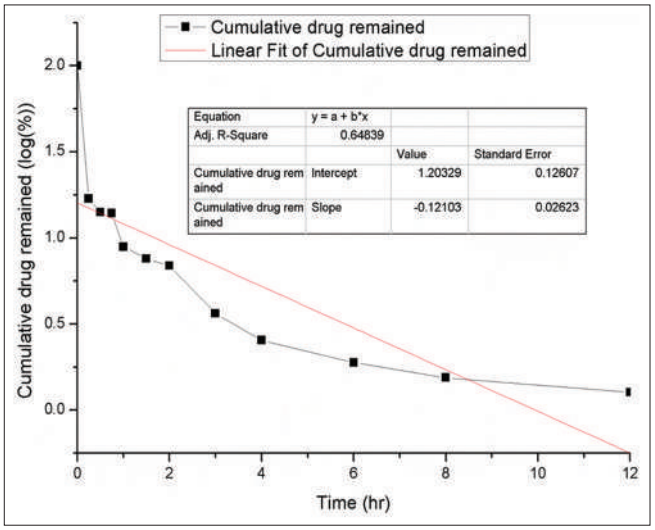

Figure 17: Plot of first-order release kinetics

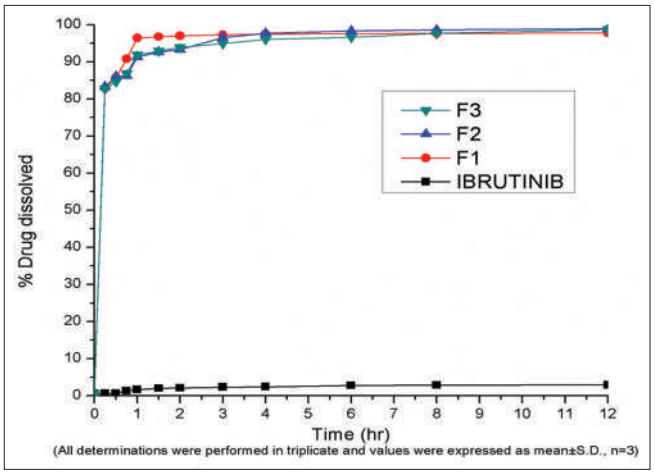

Figure 18: Dissolution profile of pure ibrutinib and ibrutinib self-nanoemulsifying drug delivery system formulations

DISCUSSION

Based on preliminary solubility studies and ternary phase diagram, the component systems chosen for the ibrutinib-loaded SNEDDS preparation were Capryol 90, Cremophor EL40, and Transcutol P. A three-factor, three-level BBD combined with a desirability function chosen to optimize

Table 6: Stability study data of ibrutinib SNEDDS formulation for 90 days

Temperature (°C)	Particle size (nm)		Release data (% CDR)			
	0 day	90 days	0 day		90 days	
			2 h	4 h	2 h	4 h
4±1	167.9±2.12	169.12±3.26	93.11±0.56	97.45±1.45	91.56±1.34	94.12±2.15
25±2	167.9±2.12	168.27±1.86	93.11±0.56	94.6±0.92	90.12±1.16	93.82±1.13

n=3 (*P*<0.05). SNEDDS: Self-nanoemulsifying drug delivery system, CDR: Charging data record

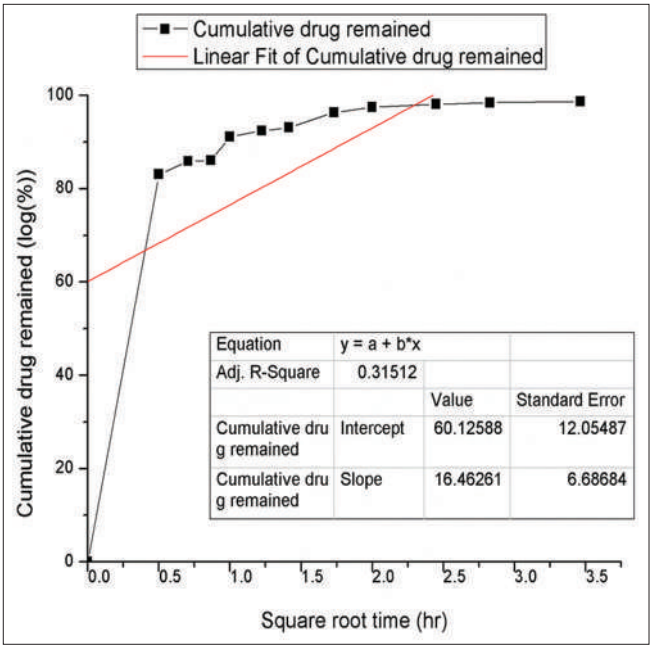

Figure 19: Plot of Korsmeyer–Peppas release kinetics

the formulation parameters. The independent factors were the amount of Capryol 90, amount of Cremophor EL, and amount of Transcutol HP, while dependent variables include droplet size, PDI, and drug release post 15 min. The responses were fitted to a second-order quadratic model and statistical validation of the fitted models was carried out by ANOVA. Various response surface graphs and contour plots were constructed to understand the effects of different factor level combinations on the responses.

The formulations using 30 mg Capryol 90, 59.6 mg Cremophor EL40, and 10 mg Transcutol P were prepared, and comparison of the predicted values and experimental values was found to be in close agreement. The droplet size, PDI, and cumulative drug release varied between 157.07 and 236.62 nm, 0.206 and 0.312, and 54.28 and 83.78%, respectively. Formulation F2 with minimum droplet size (167.9 nm), minimum PDI (0.228), and maximum drug release was chosen as optimized formulation and further characterized for physicochemical parameters.

The results indicated a desirable ZP value of −21.2 mV, maximum % transmission, and 100% drug loading with 85% drug released at end time of 30 min. The droplet size analyses revealed <200 nm as size of the droplet, while FTIR studies revealed no interaction among the drug and polymers. The reaction kinetics indicated that drug release followed the first-order release mechanism and is found stable for 3 months.

CONCLUSION

In the present study, the optimal SNEDDS formulation showing significant improvement in dissolution profile of SNEDDS loaded with ibrutinib when compared with pure ibrutinib was successfully developed. The SNEDDS readily released the lipid phase to form a fine oil-in-water nanoemulsion, with a narrow distribution size. The *in vitro* dissolution test showed that the SNEDDS had a faster *in vitro* release rate than the pure ibrutinib with the first-order release mechanism. The RSM using the BBD could be a suitable approach for understanding formulation variables and for efficiently optimizing the formulation.

Thus, the prepared SNEDDS for the delivery of ibrutinib would be a promising dosage form for the treatment of MCL and CLL.

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