Application of Box–Behnken design and desirability function in the development and optimization of self-nanoemulsifying drug delivery system for enhanced dissolution of ezetimibe

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Abstract

Background: This study is focused on developing and optimizing a self-nanoemulsifying drug delivery system (SNEDDS) of BCS class II drug (ezetimibe) through Box–Behnken design (BBD) and desirability function for enhanced dissolution. Pseudoternary phase diagrams were created by taking oil (Pecol), surfactant (Tween80), and co-surfactant (Transcutol-P) and the concentration ranges were identified for generating BBD. The composition of ezetimibe-SNEDDS was optimized through various response variables viz. globule size ($Y_1$), %transmittance ($Y_2$), self-emulsification time ($Y_3$), dissolution after 5 min and 40 min ($Y_4$, $Y_5$). Optimized formulation was characterized for various physicochemical properties.

Results: Pseudoternary phase diagram having maximum nano-emulsification area was selected to formulate SNEDDS. Derived polynomial equation and model graphs were exercised to investigate the impact of formulation variables on the responses. Significant effect of formulation composition on the responses was observed ($p < 0.05$). The formulation with least oil (10%) and high surfactant (60%) exhibited low globule size ($24.4 \pm 2.07$ nm), low emulsification time (55 s) but high %transmittance (101.2%) and drug release (49.21% after 5 min; 95.27% after 40 min). Based on the desirability function, the optimized formulation was selected and reformulated. The optimized formulation (FF1) was found to be uniform, stable, and showed similar observed and predicted responses.

Conclusion: The potential of SNEDDS in improving the dissolution profile of weakly soluble drug and the applicability of BBD with desirability function in optimizing a SNEDD formulation has made it possible to identify the impact of various independent variables on optimization of the formulation for better responses.

Keywords: SNEDDS, BCS, Box–Behnken design, Ezetimibe, Desirability function, Optimization
Background
Administering drugs by means of oral route is considered ideal for a variety of drugs and hence, 80% of the available dosage forms are being administered orally. Moreover, oral administration of drug is also preferred for various kinds of dosage forms (controlled release, sustained release, fast release dosage forms, etc.) with a far different preparation methodology. However, it is the physicochemical property of the drug which needs to be explored and assessed for selecting an ideal route of administration. For example, very low aqueous solubility of the majority of drugs (68% of oral drugs have poor solubility, < 100 µg/mL) lead to inadequate dissolution in the gastric milieu after oral administration which eventually show low bioavailability and thus suboptimal efficacy [1, 2].

These drugs are classified as class II drugs by the Biopharmaceutical Classification System, drugs with poor aqueous solubility and high permeability [3]. If the oral administration route is constrained for giving drugs having irrational property then the solution lies in developing a tailored dosage forms. Therefore, a lot of emphasis has been given by the scientists and researchers in recent years, on improving the solubility of such scantily water-soluble drugs either by using formulation strategies like crystal engineering [4], solid dispersion [5], cyclodextrin complexation [6], micronization, and nanosization [7], or chemical strategies such as salt formation [8], prodrugs [9], and lipidic systems [10] including SNEDDS which is considered to be one of the prospective nanotechnology-based drug delivery approach used to optimize the dissolution time and hence bioavailability of low soluble drugs.

SNEDDS are lipid-based formulations and are basically the blend of oils from the natural or synthetic origin and surfactants, or it can be a mixture of hydrophilic solvents and cosolvents/surfactants used for improving the solubility of lipophilic drugs (Log P > 3) [14]. On oral ingestion, the preconcentrate of SNEDDS transform into oil-in-water type emulsion having fine globules of size ranges to micron (microemulsion) or nano (nanoemulsion). The transformation of SNEDDS preconcentrate into fine globules takes place due to mild agitation (in vitro) or digestive motility (in vivo) in the presence of simulated fluid or Gastric fluid (in vivo) and thus it is considered to be an important phenomenon for self-emulsification [11].

Formation of stable formulation that can be filled into capsules, high entrapment efficiency for drug, spontaneous formation of emulsion which aid in bypassing the dissolution step and preventing degradation of the drug in gastric media are some common advantages linked with SNEDDS. In addition, the spontaneous emulsification of SNEDDS preconcentrate results in the formation of micro/nanosized globule which will provide a large surface area for absorption thereby, enhancing the rate and magnitude of absorption that will ultimately result in improved bioavailability [12]. On oral administration, lipase enzymes (lingual and pancreatic) act on the lipid/oily phase of the SNEDDS and form emulsified glycerides and fatty acids. Thereafter, intestinal mixed micelles are formed from glycerides and fatty acids in the presence of bile acids. These mixed micelles then passed through intestinal cell (enterocytes) and form chylomicrons. These formed chylomicrons, containing drug, facilitate lymphatic transport thus bypassing the liver (first-pass effect) and thereby enhance the bioavailability of lipophilic drugs [13].

Selection of excipients for the formulation of potent, stable SNEDDS is very critical and thus requires a comprehensive knowledge of the oil phase, surfactants, and co-surfactants used to prepare SNEDDS. Physicochemical properties of lipid or oil phase (e.g., polarity, viscosity and molecular volume) considerably preside the spontaneity of the nano-emulsification, globule size, solubility of drug, and biological fate of the nanoemulsions and the entrapped drug [14]. The surfactant which possesses high-solubilization capacity for the drug and have the ability to lower the interfacial tension at the water and oil interface should be selected for the formulation of SNEDDS.

The high HLB (HLB > 12) and thus surfactant’s hydrophilicity is a prerequisite for the instantaneous development of oil droplets in water and/or fast distribution of the formulation in the gastric milieu or aqueous environment, ensuring an excellent self-emulsifying/dispersing performance [15]. However, it is difficult for a single surfactant to produce transient negative interfacial tension which necessitates the inclusion of a co-surfactant [15].

Co-surfactant helps in building a fluid interfacial film which affects the interfacial bending stress by reducing it and thus cater an ample flexibility to the interfacial film for obtaining different curvatures obligatory to form nanoemulsions over a broad array of composition [12, 16]. Hence, the scalability of SNEDDS is dependent on the optimized selection of its components.

In the present investigation, Box–Behnken design (BBD) along with desirability function was used to design and optimize SNEDDS formulation containing a BCS class II model drug, ezetimibe (EZT, water solubility = 0.00846 mg/mL, log P = 4.5) [17], to enhance its dissolution profile. Further, the effect of SNEDDS components (oil; Peceol, surfactant; Tween 80, and co-surfactant; Transcutol P) on various response variables like SNEDDS globule size, their % transmittance, Self emulsification time, Dissolution (% drug release) after 5 min and 40 min was also assessed.

Methods
Materials
Ezetimibe was obtained from Jubilant Generics Limited as a kind gift sample. Peceol, Maisine 35-1, Labrafac Lipophile...
WL-1349, Labrafil PG, Capryol PGMC, Labrafil M2125, Lauroglycol 90, Labrasol ALF, and Transcutol P were received as gift samples by Gattefossé (Saint-Priest Cedex, France). Tween 80 (Polyoxyethylene 20 sorbitan monoleate) was supplied by Sigma–Aldrich Chemical Company, St. Louis, USA. All other chemicals used in this study were of analytical grade.

**Methods**

**Solubility studies (screening of excipients)**
The selection of excipients (oil, surfactant, and co-surfactants) was based on their ability to dissolve a maximal drug. Various oils (Peceol, Maisine 35-1, Labrafil Lipophile WL-1349 and Labrafil PG, surfactants (Tween 80 and Labrasol ALF), and co-surfactants (Capryol PGMC, Labrafil M2125, Lauroglycol 90, and Transcutol P) were screened on solubility basis using the shake-flask method. An excess quantity of EZT was added to each 2 mL of tested excipients in a test tube and these mixtures were thoroughly mixed using vortex shaker while maintaining the temperature at 37 °C. Each of the systems was then centrifuged using high-speed centrifuge at 10,000 rpm for 10 min. The supernatant portion of each system was separated and its drug content was estimated after suitable dilution with methanol at 233 nm using UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) against blank (methanol). The study was conducted in triplicate and their mean values were recorded [18, 19].

**Construction of pseudoternary phase diagram**
For the identification of the optimal concentration of oil, surfactant, and co-surfactant for formulating SNEDDS and also to specify the existence of maximum self-emulsifying region, the pseudoternary phase diagram for all mixtures were constructed without incorporating drug using CHEMIX School 3_60 version. Based on the results acquired from preliminary solubilization study, components like oil, surfactant, and co-surfactant were utilized as apex of ternary phase diagram. The procedure reported by Craig et al. (1995) was used in this study [20]. Two batches were prepared, first comprised of Peceol as oil, Labrasol as surfactant, and Transcutol P as co-surfactant while the second batch contains Peceol as oil, Tween 80 as surfactant, and Transcutol P as co-surfactant. Surfactant:co-surfactant ratio (Smix) was prepared by mixing selected surfactant and co-surfactant in different volume ratios (1:1, 1:2, 2:1, 3:1, and 3:1.5). Each phase diagram was constructed by mixing oil and exact Smix in nine ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1; %w/w). 0.3 mL of the mixture of oil, surfactant, and co-surfactant (ternary mixture) was gently stirred on a mechanical stirrer with 300 mL of distilled water in a glass beaker while maintaining the temperature at 37°C. The propensity of the system to emulsify spontaneously and the spread of emulsion droplets were investigated. The emulsions were subjected to stand for 2 h and their transmittance was assessed at 638.2 nm by a double beam UV-Vis spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) using water as blank [20, 21].

**Box–Behnken experimental design**
A three-factor, three-level BBD was produced by software of experimental design (Design Expert 11). Among the various techniques of response surface methodology (RSM), Box–Behnken design (BBD) is a suitable approach for ascertaining the effects of formulation ingredients/variables (independent factors) and their associated effect on the response variables (dependent factors). BBD is a three-factor, three-level statistical screening approach which was applied in our study to evaluate main as well as interaction effects of the formulation variables (oil, surfactant, and co-surfactant concentrations or amounts) on measured responses of prepared SNEDDS and applicability of desirability function to optimize the formulation. BBD requires 15 experimental runs with 3 central points to determine the experimental error and the precision of the design [22]. The non-linear quadratic model equation produced by the BBD is of the form (Eq. 1):

$$
Y = \alpha_0 + \alpha_1X_1 + \alpha_2X_2 + \alpha_3X_3 + \alpha_4X_1X_2 + \alpha_5X_2X_3 + \alpha_6X_1X_3 + \alpha_7X_1^2 + \alpha_8X_2^2 + \alpha_9X_3^2
$$  

where, Y is the dependent or measured response of the dependent variables associated with each factor-level combination; $\alpha_0$ is the intercept; $\alpha_1$–$\alpha_9$ are the regression coefficients; while the independent variables used in the study is denoted by $X_1$, $X_2$, and $X_3$, which were listed in Table 1.

The amounts of oil, surfactant, and co-surfactant in each of the 15 formulations were given in Table 2.

**Preparation of ezetimibe-loaded SNEDDS**
After studying pseudoternary phase diagram, self-emulsifying region was identified and the concentrations of surfactant (20–60%), co-surfactant (10–30%), and oil (10–70%) were selected to prepare the drug-loaded formulations. Fifteen formulations were formed incorporating ezetimibe at a concentration of 10 mg/g. First, the drug was dissolved in the co-surfactant, followed by the addition of an appropriate amount of surfactant. After proper mixing, the oil component was then added into the homogenized mixture. All the components were mixed gently using vortex shaker and were then warmed at 40 °C on a magnetic stirrer until a clear homogenized mixture was obtained. The preconcentrate remained clear at room temperature and was kept for further study.
**Table 1 Variables in the Box–Behnken design**

| S.no. | Independent variables | Dependent variables | Goal for dependent variables |
|-------|-----------------------|---------------------|------------------------------|
| 1.    | Amount of oil added ($X_1$) | Globule size ($Y_1$) | Minimize                    |
| 2.    | Amount of surfactant added ($X_2$) | Percentage transmittance ($Y_2$) | Maximize                    |
| 3.    | Amount of co-surfactant added ($X_3$) | Self-emulsification time ($Y_3$) | Minimize                    |
| 4.    | –                      | Dissolution after 5 minutes ($Y_4$) | Maximize                    |
| 5.    | –                      | Dissolution after 40 minutes ($Y_5$) | Maximize                    |

**Evaluation of SNEDDS**

**Droplet size and zeta potential determination**

The average droplet size, zeta potential, and polydispersity index (PDI) of the prepared 15 formulations (F1–F15) containing the drug were determined using Malvern® Zetasizer Version 7.12 (Malvern® Instruments Limited, Worcestershire, UK). Prior to the measurement, sample from each formulation was diluted with distilled water in the ratio of 1:100 and agitated gently to ensure proper distribution of the formulation in aqueous media. All the measurements were taken in triplicate.

**Table 2 Design layout of Box–Behnken design batches for prepared SNEDDS**

| Experimental run | Coded factor levels | Factor 1 ($X_1$) | Factor 2 ($X_2$) | Factor 3 ($X_3$) |
|------------------|---------------------|------------------|------------------|------------------|
| 1                | 0 0 0               | 0 0 0            |                  |                  |
| 2                | 0 1 0               | 0 1 0            | −1 −1 0          |                  |
| 3                | 1 0 −1              | 0 0 0            | −1 −1 0          |                  |
| 4                | 0 0 0               | 0 0 0            | −1 −1 0          |                  |
| 5                | 1 0 −1              | 0 0 0            | −1 −1 0          |                  |
| 6                | 1 0 −1              | 0 0 0            | −1 −1 0          |                  |
| 7                | 1 0 −1              | 0 0 0            | −1 −1 0          |                  |
| 8                | −1 1 0              | 0 0 0            | −1 −1 0          |                  |
| 9                | 1 1 0               | 0 0 0            | −1 −1 0          |                  |
| 10               | −1 0 0              | 0 0 0            | −1 −1 0          |                  |
| 11               | 0 0 0               | 0 0 0            | −1 −1 0          |                  |
| 12               | −1 0 0              | 0 0 0            | −1 −1 0          |                  |
| 13               | 0 1 1               | 0 0 0            | −1 −1 0          |                  |
| 14               | 0 −1 1              | 0 0 0            | −1 −1 0          |                  |
| 15               | −1 −1 0             | 0 0 0            | −1 −1 0          |                  |

**Dissolution study**

Drug-release studies for the 15 SNEDDS formulations were done using USP XXIII dissolution apparatus I (basket type) with phosphate buffer (pH 6.8, 900 mL) as the medium at 37 ± 0.5 °C. The rotation speed of the basket was adjusted to 50 rpm. Then, 0.5 g of the EZT-loaded SNEDDS formulations (equivalent to 5 mg of EZT) was placed in hard gelatin capsules, size “0.” At predetermined time intervals, an aliquot (3 mL) of the sample was collected, filtered, and analyzed for EZT contents by double beam UV-Vis spectrophotometer measured at 233 nm. An equivalent volume (3 mL) of fresh dissolution medium was immediately added to keep the sink condition. Moreover, drug release profile from a suspension of pure EZT (in carboxymethyl cellulose) was also observed and compared with that of prepared EZT-SNEDDS formulations. The dissolution studies were performed in triplicate.

**Optimization using desirability function**

In the study, all the five responses were simultaneously optimized using a numerical optimization technique (desirability function approach) given by Derringer and Suich.
In this approach, a specific goal was assigned to each response (Table 1). A partial desirability function is associated with an individual response, where value 0 is assigned to an undesired/unacceptable response while for an acceptable response, the value lies between 0 and 1. The value between 0 and 1 indicates the closeness of the response to its target value (i.e., minimum to most desirable). Therefore, the desirability function helps in ascertaining the most favorable and appropriate point in the design space that accomplishes the set goals for dependent variables (response). In our study, Design Expert 11 was utilized to conclude the maximum desirability value after assigning desired goals to the responses.

**Evaluation of optimized formulation**

**Effect of composition of optimized formulation on response variables**

On the basis of desirability function, optimized formulation (FF1) was selected and prepared. The prepared optimized formulation was assessed for the parameters/response variables (globule size, percentage transmittance, self-emulsification time, dissolution after 5 min and 40 min), in accordance with the above-mentioned procedure. The observed responses for the optimized formulation were compared with that of predicted values. The other parameters like robustness to dilution, cloud point measurement, and stability studies were also conducted for FF1.

**Robustness to dilution**

Different dissolution media (distilled water, 0.1M HCl buffer (pH 1.2) and phosphate buffer (pH 6.8)) were used to dilute the optimized formulation to 10, 100, and 1000 times. The diluted samples were checked for any occurrence of instability (phase separation or precipitation) after storing for 24 h.

**Cloud point measurement**

The cloud point measurement was done to assess the stability of EZT-loaded SNEDDS at physiological temperature as in the case of in vivo. The cloud point value of optimized SNEDDS formulations was determined. The formulation was diluted 100 times with distilled water and placed on a water bath with a gradual increase in temperature (approximately 2 °C/min, range 25 to 80 °C). The cloud point was recorded as the point of temperature at which an abrupt appearance of the cloudiness was observed visually [24].

**Transmission electron microscopy**

The morphological characteristic of the optimized formulation (FF1) of EZT was evaluated by transmission electron microscopy (TEM) (Hitachi H-7500). Prior to the measurement, the sample of the optimized formulation was diluted with distilled water (1:100) and agitated gently to assure proper dispersion of the formulation. The reconstitution of the formulation was followed by negative staining using a phosphotungstic acid solution.

**Stability studies of optimized SNEDDS**

The thermodynamic stability and phase integrity of the optimized formulation was assessed under variable conditions of temperature and centrifugal force [25]. The effect of temperature and centrifugal force was studied by subjecting the optimized formulation to the following test [26]:

**Centrifugal test**

In this study, centrifugation (5000 rpm for 30 min) of the optimized formulation was done after diluting with distilled water (1:25). The nanoemulsion was then checked for any sign of instability like phase separation, creaming, etc. [27].

**Heating-cooling cycles**

The preconcentrate of optimized SNEDDS was subjected to three cycles of heating and cooling by storing the formulation between 4 °C and 45 °C for not less than 48 h at each temperature. Then, the formulation was initially assessed for drug precipitation and phase separation followed by its dilution with distilled water. The obtained nanoemulsion was then checked for instability [28].

**Freeze-thaw cycle**

This involved three freeze-thaw cycles in which formulation was stored between −20 °C and +25 °C for not less than 48 h at each temperature. Then, centrifugation of the formulation was done for 5 min at 3000 and then the formulation was visually inspected for phase separation and drug precipitation. Similarly, as discussed above, formulation was then diluted with distilled water and examined for some instability [28].

**Results**

**Solubility studies**

The excipients used in the preparation of SNEDDS should be able to solubilize maximum drug as well as they should exhibit a larger self-emulsification region in the pseudo ternary phase diagram. The excipients were chosen on the basis of solubilizing capacity, their safety, and compatibility with the incorporated drug. The equilibrium solubility of EZT in different excipients is represented in Fig. 1.

In our study, four oils were examined to use as lipid phase in the preparation of SNEDDS. It was evident from the study that EZT showed the highest solubility in Peceol (11.75 ± 1.63 mg/mL) and Maisine 35-1 (12.25 ± 1.78 mg/mL) and the least was observed in Labrafac PG and Labrafac lipophile WL-1349.

In our study, two non-ionic surfactants (Labrasol ALF and Tween 80) were used. Both surfactants showed reasonable solubilizing potential for EZT and also possess high HLB value (Labrasol ALF-12 and Tween 80-15). Hence, both the surfactants were selected for delineating a stable nanoemulsion region.
Co-surfactants are the substance which helps the surfactants in solubilization of drug and hence the one which shows maximum solubility of EZT was chosen. Among the co-surfactants, Transcutol P was selected as it showed maximum solubility of EZT (> 393.85 ± 6.74 mg/mL).

**Pseudoternary phase diagram**

On the basis of preliminary trials on the drug solubility in various vehicles, Peceol (oil), Labrasol and Tween 80 (surfactant), and Transcutol P (co-surfactant) were selected for the development of pseudoternary phase diagrams of the three systems (with different surfactant and cosurfactant ratio). Two batches were prepared, batch A comprised of Peceol as oil, Labrasol as surfactant, and Transcutol P as co-surfactant, while batch B contains Tween 80 as surfactant instead of Labrasol ALF (while other two components were same as batch A). The phase diagram gives the relationship between the concentration range of components and their phase behavior in the formation of nanoemulsion.

Based on the result of percentage transmission and visual appearance first batch (batch A) containing Peceol, Labrasol ALF, and Transcutol P was rejected as the percentage transmittance was low and appeared turbid, (Supplementary Tables 1 and 2), which revealed the formation of coarse emulsion instead of nanoemulsion.

The pseudoternary phase diagrams for batch B containing Peceol, Tween 80 and Transcutol P, were shown in
Fig. 2. Figure 2a–e represents five systems (I–V) having different Smix ratios. The shaded area in Fig. 2 represents the nanoemulsifying regions. From Fig. 2e, it was clearly observed that system V displayed a broader nanoemulsification region as compared to other four systems. The Smix concentration of system V which is 3:1.5 w/w, produced nanoemulsion which can emulsify up to 60% w/w of an oily composition. However, systems I, II, and III with Smix concentration of 1:1, 1:2, and 2:1, respectively, produced clear emulsion and found to emulsify only 20% (for system I) and 30% (for systems II and III) of the oil phase. Moreover, it was noticed that surfactant concentration below 40% w/w resulted in the turbid emulsion for systems I and III, while system V produced transparent stable nanoemulsion with a good percentage of transmittance at even 20% w/w of surfactant.

The system IV containing Smix at a concentration of 3:1 was able to emulsify 40% of oil, but the low level of co-surfactant in this system renders the system inefficient to emulsifying more amount of oil.

On the basis of range of nanoemulsion formation in the phase diagram of system V (Fig. 2e), the constraints of independent variables (10% ≤ Oil (X1) ≤ 70%, 20% ≤ Surfactant (X2) ≤ 60%, 10% ≤ Co-surfactant (X3) ≤ 30%) were selected for further optimization.

Experimental design
In the present investigation, BBD was selected and applied to analyze the consequence of three independent variables (components of SNEDDS) on dependent variables. The constraints of the dependent and independent factors are depicted in Tables 1 and 2. As per the BBD, 15 formulations were formulated and assessed for their response variables, i.e., globule size (Y1), percentage transmittance (Y2), self-emulsification time (Y3), dissolution after 5 min (Y4), and dissolution after 40 min (Y5).

All data were obtained using Design Expert 11. Each response was separately fitted to a full quadratic equation; significance of model was assessed by ANOVA, lack of fit test, and multiple correlation coefficient (R2) test. For the model to be fitted well in the quadratic equation, the model p value should be less than 0.05 (significant). The variation of data around the fitted value is analyzed by lack of fit test and it should be insignificant (p value > 0.05) relative to the pure error. The amount of variation around the mean is expressed by R2 value (multiple correlation coefficient test). The value of R2 should be close to 1 [29].

Evaluation of SNEDDS
Globule size, zeta potential, and effect of formulation composition on globule size (Y1)
Emulsion globular size is considered to be an important factor because it governs the rate and extent of drug release and absorption [30]. The globule size, PDI, and zeta potential of the formulations were measured using Malvern Zeta sizer and the results obtained are depicted in Table 3.
The globule size of EZT-SNEDDS was found to range between 24.4 (Fig. 3) and 102.01 nm. It was clear from the results that there was a remarkable diminution in globule size with an increased concentration of surfactant and decreased concentration of oil.

The PDI is a dimensionless quantity which measures the width of the size distribution and its value lies between 0 and 1. Values close to 0 indicate a monodisperse system while greater values indicate a heterogeneous system with broader size distribution. All the 15 formulations showed PDI below 0.3, indicating a good globular system with a monodisperse size distribution and decreased concentration of oil.

Based on the BBD, the independent factor combinations of Peceol (X1), Tween 80 (X2), and Transcutol P (X3) resulted in different response variables for globule size (Y1). The mathematical relationship in the form of a polynomial equation for the measured response (globule size), Y1, is mentioned below (Eq. 2):

\[
Y_1 \text{ (Globule size)} = 47.86 + 1.040X_1 - 0.020X_2 - 0.694X_3 \\
- 0.00126X_1X_2 + 0.00701X_2X_3 \\
- 0.00092X_1X_3 + 0.00259X_1^2 \\
+ 0.00439X_2^2 - 0.0176X_3^2
\]  
(2)

The above equation reflect the quantitative effect of independent variables (X1−X3) and their interactions (coefficient with more than one-factor term, X1X2, X1X3, X2X3) on the response Y1. The p value (< 0.05) of the coefficient indicated their significant effect on Y1 (Table 4). The positive sign of coefficient signifies collegial effect while the negative sign signifies the opposing effect of independent variables on response. The larger coefficient value of the factor indicates its substantial effect on the response. All responses were fitted well to the quadratic model. The efficiency of the model was verified by ANOVA and multiple correlation test (R^2). The result of ANOVA and multiple correlation test (R^2) of the quadratic model is shown in Table 4. The p value and R^2 value was found to be < 0.05 and 0.9965, respectively. The observed p value (< 0.05) and R^2 value (close to 1) confirmed that independent variables had a significant effect in predicting the response (Y1). In addition, the multicollinearity of the independent factors was assessed by variance inflation factor (VIF) and it was found that there was no multi-collinearity amid the independent variables (X1−X3) in the quadratic model, as the value VIF is less than 10 (VIFs greater than 1 indicate multi-collinearity. As a general rule, VIFs less than 10 are tolerable).

The response surface plot, contour plots, and predicted vs actual plot for Y1 between X1 and X2 at middle levels of X3 are depicted in Figs. 4a, 5a, and 6a, respectively.

### Percentage transmittance and effect of formulation composition on percentage transmittance (Y2)

Percentage transmittance was studied to ascertain that the nanoemulsion formed was transparent and clear. Clear solution/dispersions give higher transmittance while the cloudier/turbid solutions/dispersion give lower transmittance.

| Run | Formula-tion code | Globule size (nm) | PDI | Zeta potential (mV) | Transmittance (%Y2) after 5 min | Self-emulsification time (sec) | % drug release after 5 min | % drug release after 40 min |
|-----|--------------------|-------------------|-----|---------------------|-------------------------------|----------------------------|--------------------------|--------------------------|
| 1   | F1                 | 55.3 ± 3.08       | 0.275 | 25.5 ± 6.46  | 100.1 ± 3.46 | 90 ± 1.33 | 38.17 ± 2.64 | 70.54 ± 3.54 |
| 2   | F2                 | 45.7 ± 2.89       | 0.287 | 23.9 ± 5.81  | 108.2 ± 2.94 | 82 ± 2.54 | 40.27 ± 2.98 | 75.62 ± 3.02 |
| 3   | F3                 | 102.1 ± 8.88      | 0.444 | 17 ± 4.73   | 93.8 ± 5.61 | 182 ± 4.67 | 32.22 ± 2.11 | 58.21 ± 2.14 |
| 4   | F4                 | 76.8 ± 3.65       | 0.298 | 22.9 ± 4.81  | 97.2 ± 4.32 | 145 ± 5.12 | 36.19 ± 2.14 | 66.72 ± 2.11 |
| 5   | F5                 | 55.8 ± 2.14       | 0.299 | 26.2 ± 3.35  | 100.3 ± 2.54 | 94 ± 3.31 | 39.85 ± 2.13 | 71.29 ± 2.56 |
| 6   | F6                 | 89.9 ± 3.57       | 0.302 | 18.3 ± 3.37  | 98.2 ± 2.15 | 170 ± 4.98 | 33.29 ± 1.97 | 60.16 ± 2.81 |
| 7   | F7                 | 81.83 ± 2.68      | 0.271 | 23.9 ± 6.38  | 98.6 ± 3.68 | 125 ± 4.41 | 34.09 ± 2.56 | 63.45 ± 2.49 |
| 8   | F8                 | 24.4 ± 2.07       | 0.247 | 26.4 ± 6.52  | 101.2 ± 1.97 | 55 ± 3.54 | 49.21 ± 2.99 | 95.27 ± 2.38 |
| 9   | F9                 | 76.2 ± 3.02       | 0.315 | 24 ± 5.15    | 97.4 ± 2.41 | 118 ± 2.63 | 34.62 ± 3.03 | 65.81 ± 2.62 |
| 10  | F10                | 31.8 ± 2.15       | 0.276 | 26.3 ± 2.48  | 99.3 ± 3.15 | 67 ± 1.14 | 43.18 ± 2.91 | 84.21 ± 2.51 |
| 11  | F11                | 56 ± 3.27         | 0.322 | 26.1 ± 3.64  | 100.2 ± 4.81 | 97 ± 1.89 | 39.13 ± 2.10 | 70.85 ± 1.45 |
| 12  | F12                | 32.2 ± 2.08       | 0.259 | 16.6 ± 3.52  | 98.1 ± 3.12 | 68 ± 2.14 | 45.69 ± 2.13 | 91.23 ± 3.05 |
| 13  | F13                | 38.8 ± 2.45       | 0.268 | 27.1 ± 5.88  | 97.9 ± 3.65 | 85 ± 3.05 | 41.56 ± 1.95 | 78.26 ± 2.33 |
| 14  | F14                | 63.91 ± 3.91      | 0.308 | 11.2 ± 3.52  | 97.6 ± 2.88 | 105 ± 2.54 | 37.02 ± 3.13 | 68.61 ± 2.84 |
| 15  | F15                | 38.4 ± 2.31       | 0.302 | 21.7 ± 5.08  | 98.7 ± 2.69 | 70 ± 2.96 | 42.17 ± 2.52 | 82.24 ± 2.08 |
transmittance, as the latter will scatter more of incident radiations resulting in lower transmittance. The result of percentage transmittance lies between 80.4 and 102.8%. It was observed that percentage transmittance increases with a decrease in oil content and an increase in the content of Smix, respectively.

The full quadratic polynomial equation for the measured response (percentage transmittance), \( Y_2 \) is given below (Eq. 3):

\[
Y_2 \text{% transmittance} = 94.71 - 0.1679 X_1 + 0.1749 X_2 + 0.054 X_3 + 0.000042 X_1 X_2 - 0.00162 X_1 X_3 - 0.000160 X_2^2 + 0.00136 X_3^2
\]

The above polynomial equation represents the quantitative effect of predictor variables (independent variables) on the measured response (\( Y_2 \)). The result of regression analysis for \( Y_2 \) depicted negative sign for \( X_1 \) (oil) and positive sign for \( X_2 \) (surfactant) and \( X_3 \) (cosurfactant). This suggested that with a decrease in the amount of oil (Peceol) and an increase in surfactant (Tween 80) and co-surfactant (Transcutol P) concentration, the % transmittance increases. ANOVA analysis of the model equation generated suggested that the independent variables had significantly affected (\( p < 0.05 \)) in predicting the response (\( Y_2 \)). F8 exhibited a higher percentage of transmittance (102.8%). The result of the response plot, contour plot, and predicted vs actual plot are illustrated in Figs. 4b, 5b, and 6b, respectively. The increase in percentage transmittance with a decrease in oil content could be due to the presence of a large amount of emulsifier for the emulsification process [17].

Self-emulsification time and effect formulation composition on self-emulsification time (\( Y_3 \))

The result of self-emulsification time study is presented in Table 3. It was observed that the emulsification time vary from 55 to 182 s.

The full quadratic polynomial equation for the measured response (self-emulsification time), \( Y_3 \) is given below (Eq. 4):

\[
Y_3 \text{ Self-Emulsification Time} = 134.7 + 2.275 X_1 - 2.204 X_2 - 3.996 X_3 - 0.02042 X_1 X_2 + 0.05375 X_2 X_3 - 0.03833 X_1 X_3 - 0.00880 X_1^2 - 0.01167 X_2^2 + 0.0592 X_3^2
\]

The above polynomial equation represents the quantitative effect of predictor variables (independent variables) on the measured response (\( Y_3 \)). Since the \( R^2 \) value of 0.9970 is in close agreement with the adjusted \( R^2 \) of 0.9915 (as the difference is less than 0.2), the above equation showed a good fit to the response variable (\( Y_3 \)). The result of regression analysis for \( Y_3 \) showed a positive sign for \( X_1 \) (oil) and a negative sign for \( X_2 \) (surfactant)
and $X_3$ (cosurfactant). This suggested that with an increase in the amount of oil (Peceol) and a decrease in surfactant (Tween 80) and co-surfactant (Transcutol P) concentration, the self-emulsification time increases. ANOVA analysis of the model suggested that the independent variables had significantly affected ($p < 0.05$) in predicting the response ($Y_3$) and the coefficient terms with $p$ value less than 0.05 had a significant effect on the prediction efficacy of the model. The result of the response plot, contour plot, and predicted vs actual plot are illustrated in Figs. 4c, 5c, and 6c, respectively.

**Dissolution studies and effect of formulation composition on dissolution after 5 min ($Y_4$) and after 40 min ($Y_5$)**

In the present investigation, in vitro drug release study was done using USP dissolution apparatus I. Each formulation (containing 5 mg of drug) was filled in a hard gelatin capsule (size 0). Percent drug release in phosphate buffer (pH 6.8) was observed at different time intervals. The result of the study was illustrated in Fig. 7. It was observed that F8 showed the highest cumulative percent drug release (49.21% after 5 min and 95.27% after 40 min of the study) while the lowest drug release was observed with the formulations containing a high percentage of oil (F3, F6, F7, F9). Despite this, all the formulations exhibited enhanced dissolution profile, as the highest amount of drug from the formulations was released within 1 h. While drug release from EZT suspension (sodium carboxymethyl cellulose) was only 5.23% and 38.95% after 5 min and 1 h of the study. This indicates the feasibility of SNEDDS in improving the dissolution of poorly soluble drugs (EZT).

| Coefficient | Response | $Y_1$ (nm) | $Y_2$ (%) | $Y_3$ (s) | $Y_4$ (%) | $Y_5$ (%) |
|-------------|----------|------------|-----------|-----------|-----------|-----------|
| $a_0$       |          | 47.86      | 94.71     | 134.7     | 36.63     | 81.40     |
| $a_1$       | +0.1040  | –0.1679    | +2.275    | –0.1218   | –0.5950   |           |
| $a_2$       | –0.020   | +0.1749    | –2.204    | +0.1585   | +0.114    |           |
| $a_3$       | –0.604   | +0.054     | –3.996    | +0.258    | +0.115    |           |
| $a_4$       | 0.027    | 0.143      | 0.000     | 0.052     | 0.004     |           |
| $a_5$       | 0.512    | 0.942      | 0.001     | 0.028     | 0.047     |           |
| $a_6$       | +0.00701 | –0.00162   | +0.05375  | +0.00057  | +0.00094  |           |
| $a_7$       | 0.248    | 0.364      | 0.002     | 0.773     | 0.732     |           |
| $a_8$       | –0.00092 | –0.00008   | –0.03833  | –0.00142  | –0.0031   |           |
| $a_9$       | 0.808    | 0.942      | 0.001     | 0.308     | 0.131     |           |
| $a_{10}$    | +0.00259 | –0.001349  | –0.00880  | +0.000449 | +0.003861 |           |
| $a_{11}$    | 0.091    | 0.016      | 0.007     | 0.351     | 0.001     |           |
| $a_{12}$    | +0.00439 | –0.000160  | 0.01167   | +0.000253 | +0.00254  |           |
| $a_{13}$    | 0.176    | 0.857      | 0.050     | 0.807     | 0.118     |           |
| $a_{14}$    | –0.0176  | +0.00136   | +0.0592   | –0.00391  | +0.00395  |           |
| $a_{15}$    | 0.176    | 0.705      | 0.023     | 0.365     | 0.496     |           |

* $p < 0.05$ indicate significant terms

| Model statistics | $R^2$ | Adjusted $R^2$ | Model $F$ value | Model $p$ value | Lack of fit $F$ value | Lack of fit $p$ value |
|------------------|-------|----------------|----------------|-----------------|----------------------|----------------------|
|                  | 0.9965| 0.9965         | 0.9970         | 0.9911          | 0.9968               |
|                  | 0.9903| 0.9903         | 0.9915         | 0.9751          | 0.9910               |
|                  | 160.05| 160.00         | 183.37         | 61.86           | 172.90               |
|                  | < 0.0001| < 0.0001    | < 0.0001       | 0.0001          | < 0.0001             |
|                  | 17.17 | 1.11          | 0.9797         | 0.6681          | 11.88                |
|                  | 0.0555| 0.5056        | 0.5410         | 0.6459          | 0.0786               |
The full quadratic polynomial equation for the measure response dissolution after 5 min ($Y_4$) is given below (Eq. 5):

$$Y_4 \text{ (Dissolution after 5 min)} = 36.63 - 0.1218X_1 + 0.1585X_2 + 0.258X_3 - 0.001933X_1X_2 + 0.00057X_2X_3 + 0.000253X_3^2 - 0.00391X_3^2 \quad (5)$$

The above equation represents the effect of factors on response $Y_4$. It showed a goodness of fit to response variable as the $R^2$ of 0.9911 was in reasonable agreement with the adjusted $R^2$ of 0.97511 (difference is less than 0.2). Adequate precision ratio of 26.0612 depicts an adequate signal, confirming the utility of this model to navigate the design space. The result of regression analysis for $Y_4$ showed negative sign for oil and positive sign for surfactant and co-surfactant. This suggested antagonistic effect of oil (Peceol) content on response while surfactant (Tween 80) and co-surfactant (Transcutol P) showed synergistic effect on response. ANOVA analysis of the model suggested significant effect ($p < 0.05$) of independent factors ($X_1, X_2, X_3$) in predicting the response.

The model graphs (response plot, contour plot and predicted vs actual plot) are shown in Figs. 4d, 5d, and 6d.

The full quadratic polynomial equation for the measured response (dissolution after 40 min) $Y_5$ is given below (Eq. 6):

$$Y_5 \text{ (Dissolution after 40 min)} = 81.40 - 0.5950X_1 + 0.114X_2 + 0.115X_3 - 0.002263X_1X_2 + 0.00094X_2X_3 - 0.00311X_1X_3 + 0.003861X_1^2 + 0.00254X_2^2 + 0.00395X_3^2 \quad (6)$$

The above equation showed a goodness of fit to the response variable as the $R^2$ of 0.9968 was in reasonable agreement with the adjusted $R^2$ of 0.9910 (difference is less than 0.2). The result of the regression analysis for $Y_5$ shows a negative sign for oil and a positive sign for surfactant and co-surfactant. This suggested a antagonistic effect of oil content on response while surfactant and co-surfactant showed synergistic effect on response. The result of ANOVA analysis suggested a significant effect ($p < 0.05$) of
independent factors in predicting the response. The results were analogous with the above-discussed response ($Y_4$).

The response surface plot and contour plots between factors ($X_1$ and $X_2$) and their effect on response ($Y_5$) are shown in Figs. 4e and 5e, respectively. The predicted vs actual plot showing the relation between actual and predicted response is shown in Fig. 6e. The 3D surface plot obtained was somewhat curvilinear.

Identification and evaluation of optimum formulation using desirability function

The desirability function approach was applied in the present study using Design Expert 11. The constraints were set for all the responses. The independent variables (factors) were set in range as depicted in Table 1. Among the responses, $Y_1$ and $Y_3$ were set to be minimized while $Y_2$, $Y_4$, and $Y_5$ were set to be maximized. Equal weight (1) and importance (+++) were given to all responses (weight and importance are the constraints of the software used where 3 pluses (+++) is a default setting which indicates equal importance of all responses). In the desirability function approach, individual desirability function is calculated which is required for combining all the responses in one measurement. This will help in forecasting the optimum levels for the independent factors [32]. Best formulation (FF1) with the best desirability function, fulfilling maximum requirement of response variables was selected. The selected optimized formulation contains $X_1 = 10\%$ w/w, $X_2 = 60\%$ w/w, $X_3 = 27.12\%$ w/w, and the overall desirability was found to be 0.714. The predicted value of the optimized formulation (FF1) for the response $Y_1$, $Y_2$, $Y_3$, $Y_4$, and $Y_5$ was 24.72 nm, 102.64\%, 67.99 s, 49.39\% and 97.12\%, respectively. To confirm and validate the optimization, optimized formulation was prepared in triplicate. All the responses were evaluated for each formulation as observed values. The comparison of the observed and predicted value is shown in Table 5. Figure 8 shows the counterplots for the desirability function between $X_1$ and $X_2$ ($X_3$ at an actual concentration of 27.12\% w/w). The percent biased range between $+1.82$ and $-6.04\%$.

Robustness to dilution

Optimized formulation (FF1) was tested for robustness in different dilution media (distilled water, 0.1M HCl buffer (pH 1.2), and phosphate buffer (pH 6.8)) with variable dilution fold (10, 100, and 1000 times) to resemble the
in vivo condition and to assure the formation of uniform emulsion. With the gradual increase in dilution and change in dilution media, there was no drug precipitation observed, even after 24 h of the study.

Cloud point measurement
In our study, the optimized formulation exhibited a cloud point at 63.8 °C.

Transmission electron microscopy
Figure 9 depicted the morphology of optimized formulation (FF1) which was examined using transmission electron microscope.

Stability studies of optimized SNEDDS
The optimized SNEDDS (FF1) preconcentrate and its resulting emulsion after aqueous dilution was assessed for thermodynamic stability. The result of the study showed no sign of instability (like precipitation, phase separation, creaming, etc.), neither by the preconcentrate nor by its emulsion.

Discussion
The high solubility of EZT in Peceol and Maisine was possibly ascribed to their complex composition (monoglyceride content-32-52% and diglyceride content (30–50%), as they belong to the class of long-chain triglycerides (LCT) containing mixed glycerides (mono and di-glycerides and free fatty acids). It is well reported in the literature that presence of mixed glycerides in formulation enhances the solvent capacity of lipid formulation. Moreover, their similarities with the lipid digestion product make them advantageous to use in the lipid formulations [33]. The high solubility of dug in the lipid phase is important for self-emulsification, as in SNEDDS drug should be present in dissolved form, providing a high concentration gradient which will serve as a driving force for the permeation of drug through GI tract [34]. Peceol was selected as oil phase for further investigations because of its high solubilization capacity for EZT. However, due to the high viscosity and gritty consistency of Maisine 35-1, it was not selected as the oil phase for the formulation development.

Selection of surfactant is important in the lipid system as they are mainly responsible for the stabilization of the emulsion. Non-ionic surfactants are safer, less irritant, and
exhibit greater stability of emulsion over a broad region of pH and ionic strength than ionic surfactants. Further, they expedite the adsorption of a drug by producing reversible alteration in the permeability of intestinal mucosa [12, 35]. Therefore, two non-ionic surfactants (Labrasol ALF and Tween 80) were selected for the preparation of SNEEDS.

Cosurfactants are important component of SNEDDS as they help in forming and stabilizing micelles with the surfactant in the aqueous phase. In addition, they increase the drug solubility in micelles as well as prevent precipitation of drug [36]. Transcutol P was selected as it solubilizes maximum amount of drug.

Two batches (batches A and B) of formulations were prepared; among the batches, batch A was rejected as it resulted in the formation of a coarse emulsion. Moreover, the poor affinity of Labrasol ALF to the oil led to decreased adsorption (surface assimilation) of surfactant on to the oil globules [37], resulting in the formation of coarse emulsion and eventually phase separation.

In the phase diagram, the shaded area depicted nano-emulsification region delineating best chances or probability to form nanoemulsion while the segment surrounding this area represents biphasic formulation or formulation with poor emulsification property.

System which contains high amount of surfactant (System IV; Smix-3:1) does not show emulsification of high amount of oil. This may be ascribed to the possibility of development of liquid crystal region at high surfactant concentration, as the cosurfactant concentration is not sufficient to minimize this region [38].

The system V was selected to define the boundaries of the nanoemulsion as it showed the wider self nanoemulsification zone. The wider nanoemulsion region for system V could be due to an appropriate concentration of Smix (3:1.5, w/w). A perfect Smix can emulsify greater quantity of oil and thus the molecular structure of both surfactant (linear structure of Tween 80) and cosurfactant (short-chain amphiphilicity of Transcutol P) is responsible for the easy penetration of Transcutol P into the surfactant monolayer and form a proper arrangement with Tween 80. The hydrocarbon region of the interfacial film formed due to oil and surfactant is fluidized by the Transcutol P

**Table 5** Comparison of predicted and observed experimental values of optimized EZT-SNEDDS (FF1)

| Response | Predicted value | Observed value | Percent bias (%) |
|----------|----------------|----------------|------------------|
| Y1 (nm) | 24.72          | 26.31 ± 2.64   | −6.04            |
| Y2 (%)  | 102.64         | 101.4 ± 3.91   | 1.22             |
| Y3 (s)  | 67.99          | 69.26 ± 2.56   | −1.83            |
| Y4 (%)  | 49.39          | 48.74 ± 2.29   | 1.33             |
| Y5 (%)  | 97.12          | 95.38 ± 3.67   | 1.82             |

*Percent bias (%) = (predicted value-observed value)/predicted value × 100*

**Fig. 7** Drug release profile of pure EZT-suspension and different prepared EZT-SNEEDS. (All values are mean ± SD, n = 3)
generating void space between the molecules of surfactant which ultimately results in the reduction of interfacial tension and decreased bending stress at the interface. Thus, a wider zone of nanoemulsion was observed [39].

When a complex process needs to be developed and optimized, conventional experiments require more material, efforts and thus turn out to be a tedious process. Among various experimental designs, response surface methodology (RSM) will help in overcoming these problems. This methodology is better over conventional optimization approaches because it utilizes one variable at a time (OVAT) and provides extensive information with the least number of experimentations. There are many types of response surface designs like central composite design (CCD), Box–Behnken design (BBD), Doehlert design, etc. [40].

The Box–Behnken design (BBD) is a three factor-three level design which is preferable over other because it obligates fewer experimental runs, rotatable, and it does not contain a combination of extremes (highest or lowest levels) points of the cubic region simultaneously; therefore, chances of getting unsatisfactory results are minimized [22, 41]. Therefore, BBD was selected and applied in the present investigation to comprehend the effect of three independent variables (components of SNEDDS) on dependent variables.

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**Fig. 8** Contour plot of optimized formulation as a function of $X_1$ and $X_2$. (FF1) (a) for overall desirability (D); b for globule size ($Y_1$); c for % transmittance ($Y_2$); d for self-emulsification time ($Y_3$); e for dissolution (% drug release) after 5 min ($Y_4$); f for dissolution (% drug release) after 40 min ($Y_5$).

**Fig. 9** TEM micrograph of optimized formulation of EZT.
As per the BBD, 15 formulations were prepared and evaluated for their response variables (globule size, percentage transmittance, self-emulsification time, dissolution after 5 min and dissolution after 40 min). The effect of formulation composition on the response variables was also studied.

There was a clear indication from the results of globule size study that the formulation components impose a great effect on globule size. A remarkable diminution was observed with increasing concentration of surfactant and decreasing concentration of oil.

Zeta potential governs the physical stability of the emulsion. Under the electric field, the potential of a colloid particle exerted at the slipping/shear plane is termed as zeta potential [42]. As reported in various literature zeta potential values of ± 0–10 mV are highly unstable, ± 10–20 mV are relatively stable, ± 20–30 mV are moderately stable, and above ± 30 mV are highly stable system [43]. However, some colloidal systems exhibit low zeta potential but are stable, which may be assigned to the additive effect of van der Waals attractive forces and electrostatic repulsive forces of the electrical double layer (as per DLVO theory) [44]. Some steric interactions are also responsible for the colloid stability [45].

The formulations would not exhibit any coalescence as the negative zeta potential and steric effects, as the oil phase surface and mono-oleate tail of surfactant (Tween 80) exert a hydrophobic interactions which form high-energy barriers between the dispersed globules. In addition, some non-DLVO forces like high content of non-ionic surfactant and hydration of its polar head group which helps in maintaining the intrinsic stability of the system [46, 47]. The negative charge of the free fatty acid and esters present on the oil droplets might be responsible for the observed negative zeta potential value [48].

As it was clear from the results that when the quantity of Tween 80 increased, globule size ($Y_1$) decreased. This might be attributed to the localization of surfactant molecules at the interface, forming a mechanical barrier and providing a compact layer around the droplets, thereby decreasing the interfacial tension and thus prevents coalescence of oil globules [49]. The results obtained were in consonance with the study conducted by Tang et al. reporting a linear/direct relationship between surfactant and the globule size (improved emulsion stability and reduction in globule size with increasing amount of surfactant) [31].

The amount of oil also had a significant effect on $Y_1$. It was observed that system containing 70% w/w oily phase produced nanoemulsion with larger globules. This could be ascribed to the distortion of the interfacial film by the penetration of oil droplets into surfactant chain which in turn changes the surface curvature of the globule leading to an increase in size [50].

The increase in % transmittance with decreased oil content is might be owing to the presence of more quantity of emulsifier for the emulsification process [18]. It is an index for assessing the efficiency of emulsification i.e. ability of SNEDDS to disperse properly and rapidly when exposed to dilution with water under mild agitation. This indicates the time required for the emulsification process and helps in assuring dispersion of drug in dispersed emulsion without further precipitation.

The emulsification time was found to be decreased with increasing concentration of Smix.

It was clear from the model equation as well as from the model graphs that there is a relative increase in emulsification time with rise in oil content. The emulsification process tend to become slow on increasing the oil concentration, as the high oil content led to an increase in viscosity of the system which require large shear forces for dispersion [49, 51]. Moreover, regarding effect of surfactant concentration, it was observed that there was a linear decrease in emulsification time with increase in surfactant concentration. This could be due to the more stabilization of interface with increased surfactant content. In general, the property of surfactant molecules to get adsorbs on the interface resulted into reduction in the interfacial tension. This decrease in interfacial tension depends on concentration of surfactant (Gibb’s isotherm). The adsorption of surfactant on the interface helps in stabilization of emulsion either by steric stabilization or electrostatic stabilization. In the present investigation, there is a formation of o/w emulsion which was reported to be electrostatically stabilized by the repulsive forces that are generated when electrical charged surface approach other at the interface [52]. In addition, Tween 80 is composed of a C18 hydrophobic chain and a double bond which would give rise to a looser film and thus helps in spontaneous formation of emulsion on dilution with aqueous media [53].

Similarly, increased co-surfactant (Transcutol P) amount also decreases the emulsification time. This might be as a consequence of cosurfactant ability to lower the interfacial tension by penetrating the surfactant film, fluidizing the hydrocarbon region of the interfacial film which induces formation of void spaces between the surfactant molecules. This help in emulsification process and thus lowers the emulsification time [54].

Dissolution testing is an essential tool in predicting drug release characteristics and consistency of a formulation/product. In some cases, dissolution testing could help in estimating in vivo dissolution profile of the drug (dissolution limited systems).

The model graphs which showed effect formulation composition on dissolution after 5 min ($Y_2$) indicated a linear decrement in cumulative percent drug release after 5 min with increase in oil content. This was in agreement with
the results of globule size and percentage transmittance. The increased oil content is responsible for increasing the viscosity of the system, which would slow down the emulsification process and thus retard the drug release [55]. The pattern was similar with results of self-emulsification time. Moreover, as discussed in the above section, the globule size of the formulations containing high oil content was large as compared to other, therefore small interfacial film area would be available for drug release.

The increasing surfactant concentration from 20% w/w to 60% w/w, significantly increases the drug dissolution \((p < 0.05)\). This was due to rapid formation of emulsion by the formulation when dispersed in the aqueous medium after dissolution of capsule shell. Due to self-emulsification, the free energy required is low which resulted in spontaneous formation of oil-water interface. Eventually, there is an increase in penetration of oil droplets which disrupt the interface and thereby decrease the globule size. The smaller globule size favors increase in dissolution, as large surface area is available at the interface. As mentioned in the above section, addition of cosurfactant also enhances the emulsification process and thus it also contribute in drug release [28, 56].

Apart from emulsification process, drug’s solubility in different components of SNEDDS may also affect drug dissolution. The highest solubility of EZT was observed in cosurfactant (Transcutol P). Therefore, it might be possible that drug molecules which are solubilized in cosurfactant might dissolve more rapidly in the medium prior to emulsification [57]. This could be the reason of large amount of drug release (49.21%) from F8 within 5 min of the study.

The effect of independent variables on response (dissolution after 40 min \((Y_3)\)) was similar as that observed with response \(Y_3\). Mainly oil and surfactant content had significantly affected the drug release. As mentioned earlier, the reduction in drug release with increasing oil content is might be due to increased viscosity of the system. Although, high surfactant concentration is also associated with high viscosity and liquid crystal formation which could retard the drug release, but in this investigation drug release was found to be increased with increase in surfactant content. This could be due to two reasons: firstly, high surfactant content stabilized the emulsion by forming layer around the interface and decreasing the interfacial tension, thereby increasing the rate of emulsification and eventually drug release [58]. Secondly, the cosurfactant (Transcutol P) provides adequate flexibility to the film at the interface by decreasing the bending stress of the interface. In addition, cosurfactant like alcohol, Transcutol P had been shown to reduce the viscosity and help in destroying liquid crystal phase of the surfactant by distorting its chain. This loses the domain structure of the interface, thus resulting in increased mobility of both water and surfactant [59].

Desirability function is based on the conversion of all responses into a dimensionless value (desirability function). The value of desirability function ranges from 0 to 1. The value 0 is observed when the factors give undesirable results, whereas the value 1 is attributed to optimal response for the factors under study [60]. This approach is used to produce desired robust formulation complying the maximum need of all responses within the given constraints.

After generating the polynomial equation and studying the effects of independent variables on responses, the process was optimized using desirability function. It was clearly observed and elucidated from the results that all the obtained values were in consonance with the predicted values, indicating BBD combined with desirability function as a promising approach for the optimization and evaluation of SNEDDS.

At higher dilution in vivo, there is a greater probability of drug precipitation which might significantly affect the drug absorption and its performance. Therefore, robustness to dilution was done to ensure that uniform emulsion is formed from SNEDDS [61, 62]. The results clearly suggested that the optimized formulation was robust to dilution and will maintain its performance in vivo [63].

Cloud point of an emulsion is a temperature at which the emulsion becomes cloudy, as the dissolved drug no longer remains in solution, followed by its precipitation resulting in cloudiness of emulsion [64].

The cloud point is an important parameter for assessing the stability of emulsion containing non-ionic surfactant such as Tween 80. Chemically, Tween 80 is polyoxyethylene sorbitan monooleate and has a temperature dependent behavior. On increasing the temperature, the HLB value of surfactant changes (shifted towards lipophilicity) since the surfactant starts losing its hydrophilicity which is due to dehydration of the polyoxyethylene moiety of Tween 80 [65, 66]. This results in precipitation of drug and eventually turns the clear emulsion to cloudy one. Therefore, the formulation should show cloud point above 37 °C to circumvent phase separation and phase inversion of emulsion at the physiological temperature of GIT, thereby averting drug precipitation and cloudiness [67]. The result suggested that emulsion might show highest chances of stability at normal body temperature.

The TEM micrographs showed that the nanoemulsion droplets of optimized formulation were uniform, spherical in shape and discrete.

The stability study indicates that the preconcentrate should be able to withstand the temperature variation on storage without undergoing drug precipitation and phase separation. preconcentrate and its resulting emulsion after aqueous dilution The result of the stability study revealed that the optimized formulation preconcentrate and its resulting emulsion after aqueous dilution were found to be stable and does not show instability sign.
Conclusion
The present study demonstrates the applicability of BBD approach of RSM combined with desirability function for the designing and optimization of SNEDDS containing BCS class II drug (EZT), in an attempt to enhance the dissolution profile of the poorly soluble drug. The polynomial equations and model graphs (response surface plot and contour plots) were used for ascertaining the effects of different formulation variables (oil, surfactant and co-surfactant) on the responses (globule size, their % transmittance, self-emulsification time, dissolution after 5 min and 40 min). It was clear from the study that formulation variables poses a significant effect ($p < 0.05$) on the measured responses. High oil content in the formulation, resulted in formation of emulsion with large globules, decreased % transmittance, more self-emulsification time and reduced drug release. The reverse phenomenon was observed on increasing surfactant content, which might be due to the increased stabilization and reduce interfacial tension at the interface. The drug release study showed enhanced dissolution profile of EZT incorporated in SNEDDS, as the drug release was maximum within 1 h of the study.

The polynomial equation and model graphs help in predicting the values of formulation ingredients for the preparation of optimized EZT-SNEDDS (FF1) with desired characteristics. The observed response values of the optimized formulation were found to be in consonance with the predicted values, suggesting effectiveness of the design model. Further, the optimized formulation showed no signs of instability. The result of this study suggested the potential of SNEDDS in improving the dissolution profile of poorly soluble drugs. Furthermore, BBD with desirability function could also be considered as a promising approach for investigating the effect of formulation variables and to optimize their values for the preparation of efficient optimized formulation. Also, the scope of this research is not confined to these studied parameters, but the performance of the system both in fasting state and fed state could also be in the area of further assessment.

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Authors’ contributions
We declare that this work was done by the authors named in this article: PY conceived and designed the study. PY and VR carried out the laboratory work, collected and analyzed the data and drafted the manuscript. AV supervised the work and assisted in the data analysis. All authors have read and approved the final manuscript.

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Supplementary information
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Additional file 1. Supplementary Table 1. Percentage transmittance and visual appearance of various prepared formulation with Smix (Labrasol: Transcutol P) at 1:1 ratio. Supplementary Table 2. Percentage transmittance and visual appearance of various prepared formulation with Smix (Labrasol: Transcutol P) at 2:1 ratio.

Abbreviations
BBD: Box-Behnken design; BCS: Biopharmaceutical Classification System; EZT: Ezetimibe; PDI: Polydispersity index; SNEDDS: Self-nanoemulsifying drug delivery system.
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