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Design of Experiments-Guided Development of Lansoprazole Incorporated SNEDDS: Formulation and Characterization

Astha Singh[1], NikitaYadav[2], Komal Rao[3]

[1,2]School of Pharmaceutical Sciences, Starex University, Gurugram, Haryana, India
[3]School of Pharmaceutical Sciences, MVN University, Palwal, Haryana, India

Abstract

Introduction: One of the main obstacles in drug delivery is the poor aqueous solubility of many therapeutic agents, which limits their bioavailability and clinical effectiveness. Lansoprazole (LNS), a proton pump inhibitor classified under BCS class II, suffers from low solubility and dissolution in gastrointestinal fluids. To address this issue, the present study focused on the development of a Lansoprazole-loaded self-nanoemulsifying drug delivery system (SNEDDS) optimized using a Box–Behnken design (BBD).

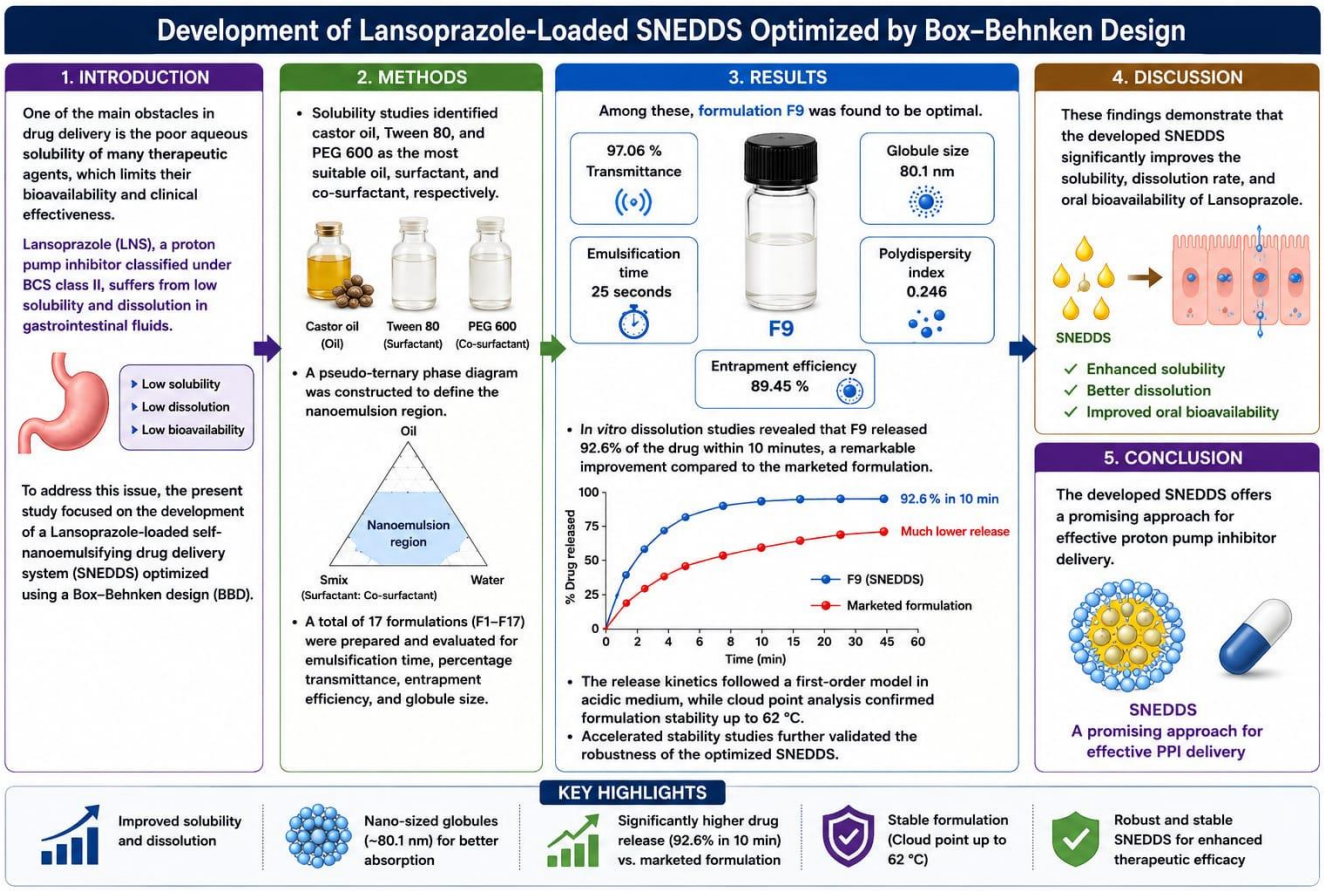
Methods: Solubility studies identified castor oil, Tween 80, and PEG 600 as the most suitable oil, surfactant, and co-surfactant, respectively. A pseudo-ternary phase diagram was constructed to define the nanoemulsion region, and a total of 17 formulations (F1–F17) were prepared and evaluated for emulsification time, percentage transmittance, entrapment efficiency, and globule size.

Results: Among these, formulation F9 was found to be optimal, showing 97.06% transmittance, a rapid emulsification time of 25 seconds, globule size of 80.1 nm with a polydispersity index of 0.246, and an entrapment efficiency of 89.45%. In vitro dissolution studies revealed that F9 released 92.6% of the drug within 10 minutes, a remarkable improvement compared to the marketed formulation. The release kinetics followed a first-order model in acidic medium, while cloud point analysis confirmed formulation stability up to 62 °C. Accelerated stability studies further validated the robustness of the optimized SNEDDS.

Discussion" These findings demonstrate that the developed SNEDDS significantly improves the solubility, dissolution rate, and oral bioavailability of Lansoprazole.

Conclusion: The developed SNEDDS offers a promising approach for effective proton pump inhibitor delivery.

Keywords: Lansoprazole, Optimization, SNEDDS, Box–Behnken statistical design, Enhanced solubility and dissolution, oral bioavailability.



List of Abbreviations

Abbreviation	Full Form
BBD	Box–Behnken Design
BCS	Biopharmaceutics Classification System
EE	Entrapment Efficiency
LNS	Lansoprazole
PDI	Polydispersity Index
PEG	Polyethylene Glycol
PPI	Proton Pump Inhibitor
SNEDDS	Self-Nanoemulsifying Drug Delivery System

Abbreviation	Full Form
Smix	Mixture of surfactant and co-surfactant

1. Introduction

70% of new pharmaceuticals have low water solubility, according to drug detection, which indicates that the number of poorly soluble compounds has grown recently [1]. The factors influencing these oral formulations' in-vivo bioavailability are their poor solubility and rate of dissolution in gastrointestinal fluids. As a result, in vitro dissolution, increasing the rate at which low-soluble medications dissolve, and increasing their bioavailability have been recognized as crucial problems in drug development, posing a significant challenge to pharmaceutical professionals [2]. One of a distinctive molecule's inherent material properties is its solubility in different solvents. The molecules must dissolve in physiological intestinal fluids and be absorbed at the site of absorption after dissolution in order to exhibit the intended pharmacological activity. The primary measure of intestinal fluid solubility and its possible role in bioavailability problems is aqueous solubility [3]. Class II and class IV compounds of the Biopharmaceutics Classification System (BCS) are hydrophobic and have an aqueous solubility of less than 100g/ml. Class II compounds have a high permeability in spite of their weak solubility. BCS class IV compounds are frequently poor medication candidates because to their poor intestinal permeability and low solubility (unless the dosage is very low) [4, 5].

Twelve chemicals were classed as class II, whereas 14 of the 100 chemicals that were introduced between 1995 and 2002 were designated as class I. In addition, class III applied to 35 and class IV to 46. Many medications have been developed since then, but the issue of low permeability and low solubility persists [6]. However, this issue has been researched and addressed utilizing a variety of methods that could even somewhat improve a drug's solubility. The issue of low water solubility has been addressed in a variety of ways, including particle size reduction, cyclodextrin complexations, salt creation, solid dispersions, surfactant application, nanoparticles, and more [7, 8].

1.1. The path to SNEDDS

The development of a self-emulsifying drug delivery system [SNEDDS], which overcame the limiting factors such as bitter taste, inability to transport through hard or soft gelatin capsules, hydrolysis of drug molecules due to oil content, larger aqueous content, and extended-term storage, was prompted by the fact that NE had good stability but lacked several other aspects [9]. Nearly thirty years ago, it was demonstrated that nano/micro emulsions could improve the GI absorption of hydrophobic medications. Additionally, NE must be ingested at a greater rate volume in order to achieve the necessary drug concentration to produce a therapeutic effect. For example, quercetin and carbamazepine are poorly soluble in any pharmaceutically acceptable oils. This may significantly hinder patient adherence [10].
Compared to NE, SNEDDS can offer benefits like:

- It can enhance chemical and/or physical stability.
- The storage profile is significantly longer.
- Because SNEDDS can be readily filled in hard or soft capsules, it increases patient compliance and economic feasibility.

- Taste masking is accomplished [10].

The necessary pharmacological molecule, lipid, surfactant, and hydrophilic co-surfactant are all present in SNEDDS, an isotropic mixture [11, 12]. SNEDDS are either anhydrous nanoemulsion forms or nanoemulsion preconcentrates [13]. The droplet size of the spontaneous nanoemulsion created by SNEDDS self-emulsification is 100 nm or smaller [14]. SNEDDS have shown great promise in resolving limitations related to the oral administration of several medications. Examples of these limitations include minimal solubility in the GI tract, enzymatic degradation, inconsistent dissolution, and unexpected intestinal absorption. SNEDDS's lipid and surfactant components can cooperate to enhance GI tract medication absorption. Additionally, these elements can be easily changed to create SNEDDS, which is useful for both lipophilic and lipophobic medications [15].

In SNEDDS,

- Oil globules are less than 100 nm;
- The dispersion has a translucent appearance;
- The required HLB value is greater than 12;
- Additionally, the characterisation of a pseudo-ternary phase diagram may be required for development.
- Peptic ulcers are digestive system lesions brought on by stomach acid that produce cracks in the mucosa that extend into the submucosa. The stomach or proximal duodenum are the typical sites for peptic ulcers, but they can also develop in the esophagus or Meckel's diverticulum.
- Prior to the discovery of Helicobacter pylori infection and the widespread use of non-steroidal anti-inflammatory drugs (NSAIDs) in the second half of the 20th century, the majority of PUDs were thought to be caused by a hyper secretory acidic environment, along with dietary factors or stress. [16].

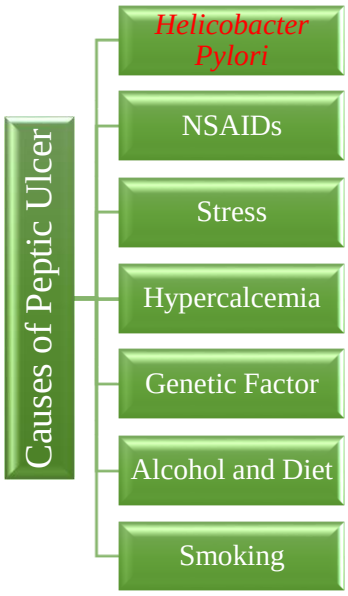


Fig.1 Schematic representation of the major etiological factors contributing to peptic ulcer disease (PUD), including Helicobacter pylori infection, non-steroidal anti-inflammatory drugs (NSAIDs), smoking, alcohol consumption, stress, and excessive gastric acid secretion. **[17]**

2. Materials and Methods:

MATERIALS: Lansoprazole was bought from Dr. Morepen, India. Castor oil was supplied by Arora Pharmaceutical's, New Delhi, India. Methanol was purchased from Rankem, Thane, Maharashtra, India. TWEEN 80 was purchased from Central Drug House, New Delhi, India. PEG 600 was purchased from Central Dug House, New Delhi, India. Rest of the ingredients were used as provided by the laboratory.

METHODS: The formulation of Lansoprazole-loaded SNEDDS consists of three main components.

a) **Screening of oil and Smix**
b) **Construction of Pseudo-Ternary phase diagram**
c) **Formulation Preparation with Design of Experiment**

a) Screening of oil and Smix

The first and most crucial phase while formulating is the screening of the components. Solubility study was performed to understand the capacity of LNS to solubilize in the excipients and to achieve the maximum amount of drug concentration while formulating SNEDDS. The oils used for the study are Oleic acid, castor oil, Coconut oil and Olive oil as they have anti-inflammatory effects while the selection of surfactant (Tween80, Tween60 and Span80) and co- surfactant (PEG600, PEG400, Ethanol and propylene glycol) was dependent on its lipophilicity. The choice of excipient was also based on its oral suitability and lastly availability.

An excess quantity of the drug was added to 2ml of each excipient in a test tube which was then subjected for vortexing for 10min or until a homogenous mixture was formed. The mixture was transferred in a 5ml stoppered vials. To achieve equilibrium solubility, these vials were shaken in a thermostatically controlled water bath at 25°C for 24 hours. After that, the samples were taken out and centrifuged for 10 minutes at 5000 rpm. To eliminate any remaining drug, the supernatants were filtered through filter paper and diluted with methanol. The filtered solutions were suitably diluted by methanol and the concentration of the drug was determined by UV spectrophotometer at 283nmCareful measurements were made, and the solubility was reported as the mean value (mg/mL) [18].

b) Construction of Pseudo-Ternary phase diagram:

From the above screening of the excipient Castor oil, Tween 80 and PEG 600 were selected as Lipid phase, Smix respectively was chosen as the working excipients. Pseudo-ternary phase was employed to find the region of maximum nano-emulsification.

The phase diagram was plotted by observing the formation of emulsions formed by introducing the S_{mix} and oil phase in specified amount of water. 3 different ratios of the S_{mix} were chosen i.e. 1:1, 2:1 and 1:2. The S_{mix} were chosen in increasing concentration of the Surfactant with respect to the co-surfactant. Nine combination of S_{mix} and oil 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 were prepared for analysis. The mixture of oil and S_{mix} was prepared by mixing it on magnetic stirrer for 20min and them vortexing it for 5min, it was then titrated with water and the appearance was observed. Emulsions that were clear, transparent and imparted bluish tinge were considered excellent also the emulsions which were slightly opaque but had the bluish tinge were also considered good. Rest of the emulsions

which were milky, dull, and grayish or had phase separation were not considered in the phase diagram. Once the observation it done the amount of water added was also noted. The recorded data was filled in the pseudo-ternary phase diagram to find the best nanoemulsion range. In order to determine the greatest range of self-emulsification, the ternary phase diagram was drawn without LNS [19].

c) Formulation Preparation with Design of Experiment:

To investigate and plan the experiment and comprehend the primary effects, interaction effects, and quadratic impacts of the excipients on the overall performance of the liquid SNEDDS, a three-factor, two-level Box Behnken Experimental Design [20] was employed. A total of 17 runs with 5 similar composites ere depicted to assess the effect of independent constraints [oil, surfactant and co-surfactant] on the dependent constraints [transmittance and emulsification time]. Ternary phase diagram was used to set the limits for the design. From the plot it was concluded that the Smix which had maximum amount of surfactant showed the best nano-emulsification range and decrease in the concentration of oil caused clear and translucent nanoemulsion, so the limits were set accordingly [21].

The Ranges for each component were established as shown in table 1:

Table 1: Ranges and coded levels of the independent variables investigated using the Box–Behnken experimental design for the optimization of the Lansoprazole-loaded SNEDDS formulation.

S.no.	Independent Variables	Levels Low[-]	High [+]
1.	Oil [% w/w]	6	16.5
2.	Surfactant [% w/w]	32	78
3.	Co-surfactant [%w/w]	8	52

The key response factors utilized to evaluate the quality of SNEDDS were percentage transmittance (Y1) and emulsification time (Y2). The goal of using these particular constraints was as follow:

Responses	Goal
Percentage Transmittance (Y_1)	Maximize
Emulsification time (Y_2)	Minimize

A quadratic polynomial model explained by a nonlinear equation 1 was used to calculate the results for each response:

$$y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_2X_3 + \beta_6X_1X_3 + \beta_7X_1^2 + \beta 8X_2^2 + BX_2^3 \ldots (1)$$

Where,
y= Measured Response
β_0-β_9 = Regression coefficients
X_1, X_2 and X_3= Independent factors [22]

Table 2: Experimental matrix generated by the Box–Behnken design (BBD) showing the coded levels of the independent formulation variables.

S. No.	Independent Factors Oil X_1	Surfactant X_2	Co-surfactant X_3
1.	16.5	55	30
2.	6	55	8
3.	28	78	30
4.	16.5	55	30
5.	28	32	30
6.	16.5	32	8
7.	6	32	30
8.	6	78	30
9.	16.5	78	52
10.	16.5	55	30
11.	16.5	32	52
12.	16.5	55	30
13.	28	55	52
14.	16.5	78	8
15.	28	55	8
16.	6	55	52
17.	16.5	55	30

Simple mixing method was used to formulate LNS-SNEDDS. The amount of each component were taken according to the DOE as shown in table 2. PEG600 and Tween80 were taken in a beaker plus mixed on the magnetic stirrer until homogenous mixture was acquired. To that specified amount of Castor oil was added drop wise, the systems temperature was kept persistent [45^{o}C] at 50 rpm. Once all the mixture were uniformly homogenized, it was transferred into a beaker and vortexed for about 10min or till a clear and translucent solution was formed. The LNS-SNEDDS were kept in tight sealed glass bottle in the refrigerator until next use [23, 24].The Models were validated by using ANOVA, Lack of fit and multiple co-relation (R^2) tests. Response Surface methodology was used to depict the inter-correlation of the variables.

4.4. Thermodynamic stability testing:

The test measures the stability of the SNEDDS in terms of phase separation and precipitation before evaluating the SNEDDS. The following test were executed to check the SNEDDS stability:

4.4.1. Heating and cooling cycle:

This test is used to determine how stable a formulation is at various predetermined temperatures. Six cycles of each temperature were used in this study at two different temperatures (1°C & 50°C), and each temperature was maintained for more than 48 hours. The qualified formulations were chosen for further research [25].

4.4.2. Centrifugation:

The selected formulation stability was determined using this study. To assess the formulation stability in terms of phase separation or precipitation, the chosen formulations were centrifuged for 30min. at 3500rpm. The qualified formulation was send for the last and final test [26].

4.4.3. Freeze-Thaw Cycle:

The temperature range used for this study was -21°C to +25 °C. The formulas were kept for 48hrs at each temperature while being visually monitored. The formulations that passed this test have been chosen for additional research [27].

Characterization of formulated LNS-SNEDDS:

The selected SNEDDS were evaluated on the basis of Emulsification time and Entrapment efficiency.

Emulsification Time:

The rate of emulsification was evaluated using the emulsification time and total time taken to form nano-emulsion.

1ml of the SNEDDS was introduced in USP 2 paddle type dissolution apparatus. The basket contained 300ml of distilled water which was kept at 37°C. The rotating time of the apparatus was kept 100 rpm. Initial time at which the SNEDDS was added in the system was noted as well as the final time at which a homogenous solution formed [18]. The total time taken by a SNEDDS to form nanoemulsion was measured. All the SNEDDS were assessed using the following grading system as shown in table 3:

Table 3: Grading system used for the visual assessment of the self-nanoemulsifying drug delivery system (SNEDDS) based on emulsification efficiency and appearance. **[28]**

S. No.	Grade	Appearance	Time
1.	Grade A	Clear nanoemulsion	>1min
2.	Grade B	Translucent/slightly bluish	>1min
3.	Grade C	Slightly bluish and translucent/unclear	>2min
4.	Grade D	Milky white appearance	>2min
5.	Grade E	greyish emulsion with either no emulsion formation or poor emulsification and big oil globules on the surface	>5min

Optimization of formulation using design expert:
The formulation was optimized to produce SNEDDS that outperformed all other SNEDDS. The optimized ratio was based on the dependent variables' output. Using Design software, the independent variables were adjusted to produce a favorable response. SNEDDS were also optimized on the basis of results of its emulsification time and percentage transmittance evaluation. For the characterization, the formulation that took the least amount of time to form nanoemulsion and had the highest percentage transmittance was chosen.

Characterization of optimized formulation:
By optimizing the SNEDDS we were able to eliminate the one's that did not had good performance. Only one out of 17 formulations was chosen for further characterization.

1. **PDI and globule size:**

In a volumetric flask, 0.1 ml of the formulation was diluted with 100 ml of distilled water to measure the globule size of LNS SNEDDS. After that, the flask was inverted to thoroughly mix the diluted mixture until a homogenous solution was achieved.

After achieving homogeneity, the resultant solution was subjected to globule size analysis using a Zetasizer instrument. The Zetasizer utilizes laser diffraction or dynamic light scattering techniques to measure the size distribution of particles or droplets within a sample [29].

Entrapment efficiency: To find the Entrapment efficiency of the SNEDDS 0.1ml of SNEDDS was diluted carefully with methanol and checked under UV at 283nm. The entrapment effectiveness of the SNEDDS was then calculated using the obtained absorbance [30,31].

$$\text{Entrapment Efficiency\%} = \frac{\text{Concentration of drug in the formulation}}{\text{Initial amount of drug}} \times 100$$

1. **Cloud point determination:** The cloud point is an important component in the evaluation of SNEDDS which are composed of non-ionic surfactants. It is essential to measure the cloud point of the formulation for the development of a stable nanoemulsion. Irreversible phase separation occurs when the temperature upsurges overhead the cloud point**.** 1ml of SNEDDS was transferred into a 100ml beaker, to its 100ml of water was filled and placed on the water bath. The temperature at which a cloudy appearance formed at the bottom the beaker was noted [31].

2. **Comparative drug release study of Marketed formulation and Optimized formulation**:

The selected formulation and a commercial tablet was compared for its release time within 15min. USP II equipment was used for the dissolution investigation. To maintain the volume and sink conditions, 1 ml of samples were removed at regular intervals and replaced with equivalent amounts of new medium. Same test was carried out for both of the dosage form. The aliquots which were obtained from the experiment were subjected for UV analysis and the results were observed [32].

Accelerated Stability Studies:

According to ICH guidelines, the stability was evaluated at 40 ± 2°C/75 ± 5% RH, 30 ± 2°C/70 ± 5% RH, and 25 ± 2°C/65 ± 5% RH. The optimized formulation was stored for 3 months to determine any kind of changes or alteration that can be caused during this period of time. The % drug release and % transmittance was assessed to determine any changes at 0, 1, 2 and 3 months. Sample were also assessed visually to find as physical change like change in colour, phase separation etc. [33].

3. Results and Discussion:

The formulation and design of the SNEDDS were based on the most suitable excipients selected during screening, further pseudo ternary phase diagram was used to find the best nanoemulsion region. The formulation was designed using designed expert.

Screening of oil and Smix:

Solubility is critical for attaining the right concentration of the drug in systemic circulation to elicit the intended pharmacological reaction [34]. Solubility of the drug in different vehicles plays major role in formulation of a stable dosage form. Maximum solubility is required so that the SNEDDS formed do not form precipitation upon oral administration.

SNEDDS comprises three basic excipients, Oil phase, Smix which plays foremost role in forming desired stable formulation with increase aqueous solubility. High solubility of drug in oil phase is essential since it helps in maintaining high Solubilization. The solubility of LNS in oil/ Smix was determined at room temperature.

In this study Tween80, Twee60 and Span80 were evaluated. Tween80 (6.13mg/ml) showed the maximum solubility as compared to Tween60 (6.08mg/ml) and Span80 (1.01mg/ml) as shown in table 4 and figure 2. Tween 80 has HLB value 15 which makes it even more suitable for the formulation since for o/w emulsion the surfactant should have HLB value >10 [15]. Olive oil, Coconut oil and Oleic acid showed lesser solubility, which was not sufficient for formulation. From the solubility data it was observed that LNS showed maximum solubility in Castor oil (17mg/ml) as shown in Table 7 followed by Oleic acid (3.56 mg/ml), Coconut oil (1.6mg/ml) and olive oil (0.0371mg/ml). Therefore, Castor oil was selected as the oil phase of the formulation. While in the case of co-surfactant Propylene glycol, Ethanol and Polyethylene glycols were chosen. PEG600 (9.65ml/ml) had the highest solubility as compared to others. Moreover, it can reduce local irritancy caused by surfactant and help in maintain dose variability.

Table 4: Solubility of Lansoprazole (LNS) in different oils, surfactants, and co-surfactants used for the selection of SNEDDS components.

S.No.	Lipid/Surfactant/Co-surfactant	Solubility in mg/ml
1.	Castor oil	17±0.37
2.	Oleic Acid	3.56±0.67
3.	Coconut oil	1.6±0.35
4.	Olive oil	0.0371±0.49
5.	Tween 80	6.13±0.49

6.	Tween 60	6.08±0.52
7.	Span 80	1.31±0.35
8.	PEG 600	9.65±0.41
9.	PEG 400	7.119±0.19
10.	Propylene Glycol	1.09±0.32
11.	Ethanol	0.29±0.56

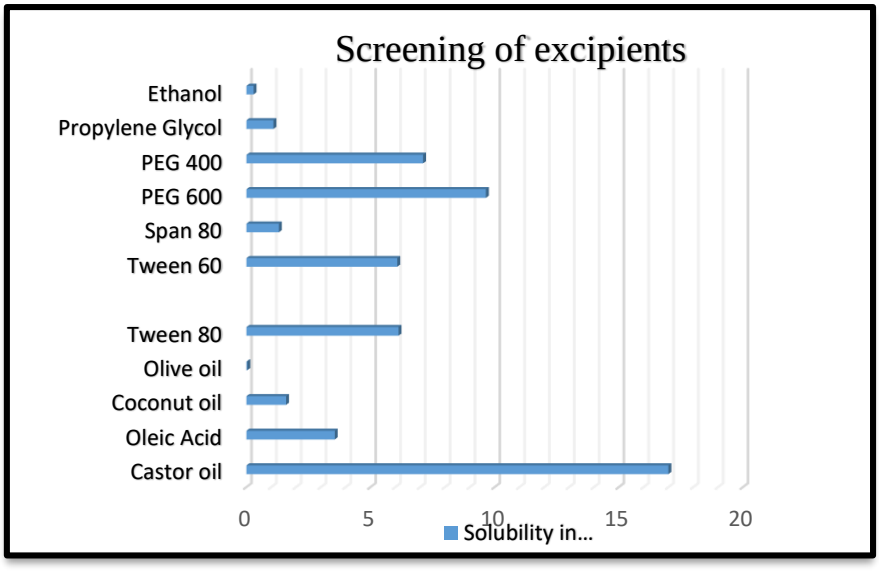


Fig. 2 Graphical representation of the solubility of Lansoprazole (LNS) in different oils, surfactants, and co-surfactants used for the selection of SNEDDS excipients.

Construction of Pseudo-Ternary phase diagram:

Pseudo ternary phase diagram is used to identify the region of maximum emulsification. The interaction of SNEDDS with aq. Phase triggers the formation of nanoemulsion. The primary criteria for the development of SNEDDS lies in the ratio of surfactant to co-surfactant. This is where pseudo ternary plot helps in estimating the amount of Smix as well as oil.

During the preliminary solubility testing it was found the castor oil had the maximum capability of solubilizing LNS. Whereas Tween80 and PEG 600 were used for S_{mix} since they found to have comparably more solubility of LNS as compared to others surfactant and co-surfactant. Among the different ratio of S_{mix} 2:1 ratio of Tween80 and PEG600 was employed, this ratio showed wide nanoemulsion range when compared to 1:1 and 1:2 ratio as shown in figure 3,4,5 and table 5,6,7. A decrease in surface tension and fluidization of the interfacial surfactant layer produced by the S_{mix} could be the reason for the large emulsifying zone. Additionally, it was observed that the development of the formulation is significantly influenced by the composition of PEG600 and Tween80.

Due to high HLB value of 12, PEG600 was chosen as the co-surfactant. Phase separations were noticed in preparations with higher PEG600 ratios. While no phase separation was seen when using a low concentration of PEG600, it was also observed that higher PEG600 concentration samples, had higher

viscosities and were more difficult to mix. Low concentrations of PEG600 and high concentrations of Tween 80 in the formulation combined with an ideal Castor oil concentration to create highly stable SNEDDS [18].

When the concentration of oil phase was kept low, clear and transparent nanoemulsion was observed whereas increase in the concentration of oil phase resulted in the formation of oil globules on the surface as well as phase separation was also found. This may cause due to either of the two reasons, Increase in the amount of oil phase can overwhelm the surfactant which can lead to increase in the interfacial tension or the solubilizing capacity of surfactant can be also affected by increasing the amount of oil phase.

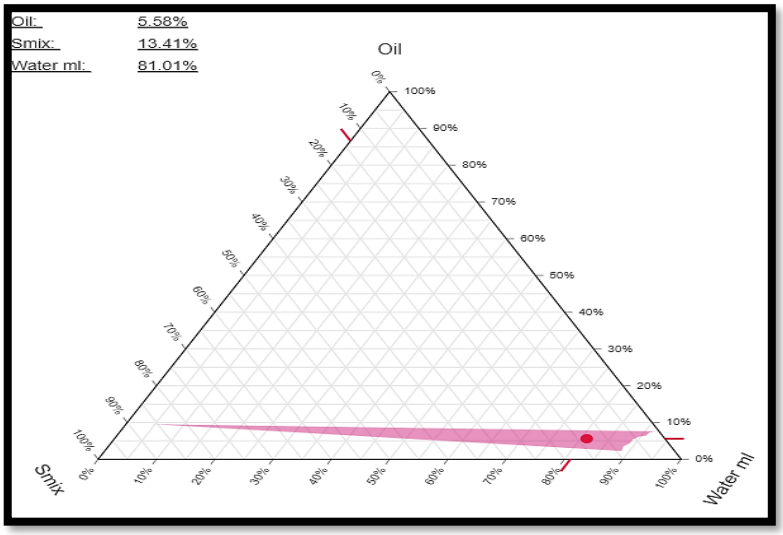


Fig 3: Pseudo-ternary phase diagram illustrating the nanoemulsion region formed using castor oil, Tween 80, and PEG 600 at a Smix (Tween 80:PEG 600) ratio of 1:2.

Table 5: Composition of Smix (Tween 80:PEG 600, 1:2), oil, and water used for the construction of the pseudo-ternary phase diagram.

S. No.	Oil	S_{mix} (1:2)	Water (ml)
1.	9	1	109
2.	8	2	99.6
3.	7	3	98.6
4.	6	4	87.5
5.	5	5	81.36
6.	4	6	80.54
7.	3	7	75.5
8.	2	8	78
9.	1	9	55

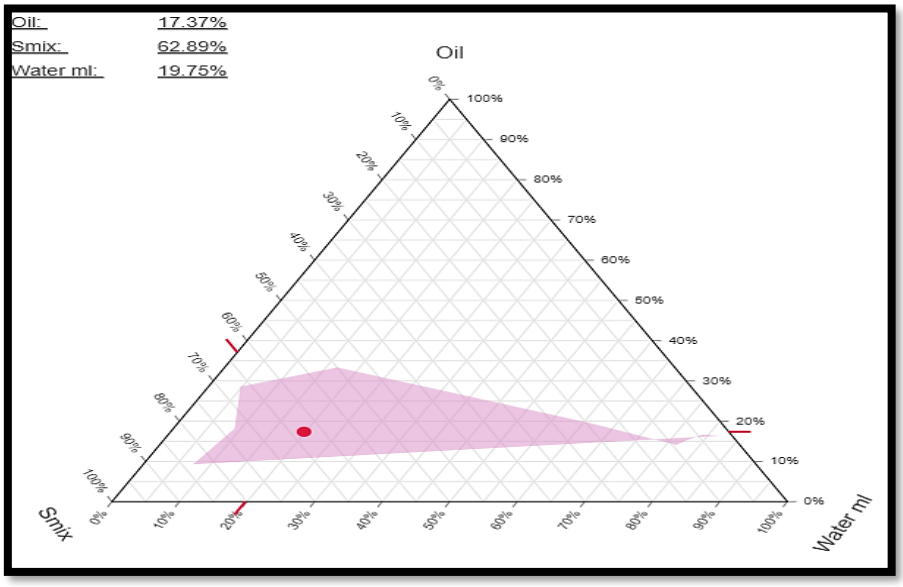


Fig 4: Pseudo-ternary phase diagram illustrating the nanoemulsion region formed using castor oil, Tween 80, and PEG 600 at a Smix (Tween 80:PEG 600) ratio of 2:1.

Table 6: Composition of Smix (Tween 80:PEG 600, 2:1), oil, and water used for the construction of the pseudo-ternary phase diagram.

S. No.	Oil	S_{mix} (2:1)	Water (ml)
1.	9	1	45
2.	8	2	38
3.	7	3	35
4.	6	4	32.5
5.	5	5	15.6
6.	4	6	2
7.	3	7	0.5
8.	2	8	1
9.	1	9	0.8

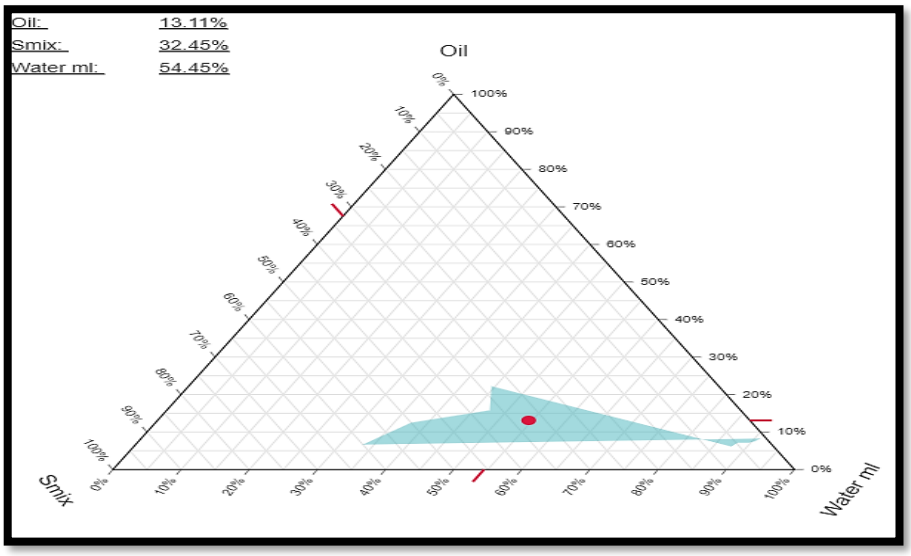


Fig 5: Pseudo-ternary phase diagram illustrating the nanoemulsion region formed using castor oil, Tween 80, and PEG 600 at a Smix (Tween 80:PEG 600) ratio of 1:1.

Table 7: Composition of Smix (Tween 80:PEG 600, 1:1), oil, and water used for the construction of the pseudo-ternary phase diagram.

S. No.	Oil	S_{mix} (1:1)	Water (ml)
1.	9	1	99
2.	8	2	95.7
3.	7	3	88.9
4.	6	4	74
5.	5	5	71.6
6.	4	6	8.1
7.	3	7	9.15
8.	2	8	6
9.	1	9	6.5

Preparation of Formulation using Design of Experiment:

A three level, two factor BBD was used to Design the experiment which contained a total of 17 runs. By using a limited number of experimental runs, BBD is perfect for investigating quadratic response surfaces and creating second order polynomial models, which help with process optimization [35].

The aim of using BBD was to formulate LNS-SNEDDS in order to achieve less emulsification time (Y_2) and maximum percentage transmittance (Y_1). The nanoemulsion regions was identified using pseudo ternary phase diagrams, and the optimum levels for the three excipients were determined for the production of SNEDDS. Tween80 and PEG600 in the S_{mix} ratio of 2:1 was considered for the formulation, as it had a wide area of nanoemulsion zone (Fig. 4). This may be related to the fact that the Smix ratio 2:1 has a

larger concentration of Tween 80 than the other ratios (1:1 and 1:2). Tween 80's high content in the 3:1 ratio may aid in emulsifying oil at the oil-water interface because it is a surfactant with a high HLB value. The ranges for the Independent variables were, for oil it was 6-16.5% w/w, for surfactant it was 32-78% w/w and for co-surfactant it was ranged from 8-52% w/w. The runs for factorial batches were presented in Table 8.

The mixture design proposed 17 formulae, the time of emulsification and percentage transmittance of the SNEDDS was determined (Table 8). The formulated SNEDDS were categorized from F1 to F17 respectively. The percentage transmittance of SNEDDS ranged from 83.73 (F5) to 97.06% (F9), according to the results. Whereas the time of emulsification ranged from 25 (F9) - 147 (F5) sec, signifying that there were some of the SNEDDS that took more than 60sec to emulsify. The software suggested that the best fitted model was Quadratic in both of the responses.

However, all the formulation took less than 180sec to emulsify and most of the formulation had acceptable percentage transmittance (>85) indicating clear homogenized SNEDDS. No such particular pattern was found for the responses (Y_1 and Y_2) in between the formulation. The responses were modelled according to a quadratic model, and multiple correlation coefficient (R^2), ANOVA, and lack-of-fit tests were implemented to assess the model's suitability.

Table 8: Box–Behnken experimental design showing the independent formulation variables and the corresponding dependent responses used for the optimization of the Lansoprazole-loaded SNEDDS.

S. No.	Independent Factors			Percentage Transmittance [%]	Emulsification Time [sec]
	Oil X_1	Surfactant X_2	Co-surfactant X_3		
1.	16.5	55	30	96.14	63
2.	6	55	8	91.8	64
3.	28	78	30	94.65	82
4.	16.5	55	30	96.06	64
5.	28	32	30	83.73	147
6.	16.5	32	8	87.4	106
7.	6	32	30	94.78	61
8.	6	78	30	96.75	47
9.	16.5	78	52	97.06	25
10.	16.5	55	30	94.66	65
11.	16.5	32	52	92.62	37
12.	16.5	55	30	96.04	62
13.	28	55	52	89.7	77
14.	16.5	78	8	94.73	44

15.	28	55	8	87.6	136
16.	6	55	52	97	35
17.	16.5	55	30	96.39	64

In the ANOVA test, the p-values generated by the responses Y_1 and Y_2 were <0.0001 & <0.0001 respectively, thus from the p-values of this model it can concluded that each and every response produced by Y_1 and Y_2 fitted the quadratic model well ($p<0.05$). A further useful statistical parameter for the evaluation of model's improved fitness is the lack of a fit test as shown in table 9. It compares the pure error from the replicated design points with the residual error. All the fitted responses showed a non-significant deficiency of fit test that is highly desirable.

Table 9: Analysis of variance (ANOVA) and lack-of-fit test results for the quadratic model developed for the response variables in the Box–Behnken experimental design.

Responses		F-value	p-value
Y_1	Model	82.63	<0.0001
	Lack of fit	0.5049	0.7864
Y_2	Model	948.59	<0.0001
	Lack of fit	2.37	0.2114

The responses Y1 and Y2 in our investigation had R2 values of 0.990 and 0.9992, respectively, this value signifies the measure of the amount of variation around the mean. Whereas the adequate precision was found to be 29.4505 for Y_1 and 109.9191 for Y_2 indicating an adequate signal as shown in table 10. Adequate precision measures the signal to noise ratio. To be able to travel design space, this signal's ratio must be greater than 4.

Table 10: Summary of the regression analysis of the response variables obtained from the Box–Behnken experimental design for the optimization of the Lansoprazole-loaded SNEDDS.

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	SD	CV (%)
Y_1	0.9907	0.9787	0.9568	29.4505	0.5827	0.6241
Y_2	0.9992	0.9981	0.9911	109.9191	1.44	2.07

Response Surface and Analysis of Contour Plots

Between the responses and the independent factor, two-dimensional contour plots and three-dimensional response surface plots were created. As seen in Figures 6, 7, 8, and 9, this aided in clarifying the connection between the causes and responses. However, there are certain limitation to this plot, one independent

variable must always be fixed because these kinds of plots can only express two independent variables against the response at once [36].

Influence of Formulation Composition Factors (X_1, X_2 and X_3) on Percentage Transmittance [Y_1]

The Percentage Transmittance (%T) of the prepared SNEDDS was found to be in range of 83.73 (F5) to 97.06% (F9) as shown in Table 10. The data was fed and the following equation was generated:

$Y_1 = +95.86 - 3.08A + 3.08B + 1.86C + 2.24AB - 0.7750AC - 0.7225BC - 2.40A^2 - 0.9765B^2 - 1.93C^2$…… Eqn.1

From the polynomial equation Eqn. 1, the overall interaction between the different constraints can be elucidated. It may be inferred that the term 'A' (oil) has a negative impact on the %T. This might be true since increase in the amount of oil can cause surfactant coalescence, surfactant might not be able to reduce the interfacial tension due to overwhelming amount of oil. Formulation F5 is a good example of this phenomenon, since the amount of oil is almost close to that of surfactant and co-surfactant there is a decrease in the %T. However in the case of 'B' (surfactant) and 'C' (co-surfactant), it can be concluded from the eqn. that they have positive impact on the %T. This could be the result of accessibility of A and B at the oil/water interface, which might help to reduce the interfacial tension leading to an increase in the %T as show by formulation F8 and F9. Similar results were seen in the case of atorvastatin calcium SNEDDS.

On the other hand, simultaneous increase in the amount of oil and surfactant can increase the transmittance but its opposite in the case of oil and co-surfactant. In this model all the terms A, B, C, AB, AC, BC, A^2, B^2, and C^2 were considered to be important. The predicted R^2 was 0.9568 and the adjusted R^2 was 0.9787, were found to be in reasonable agreement.

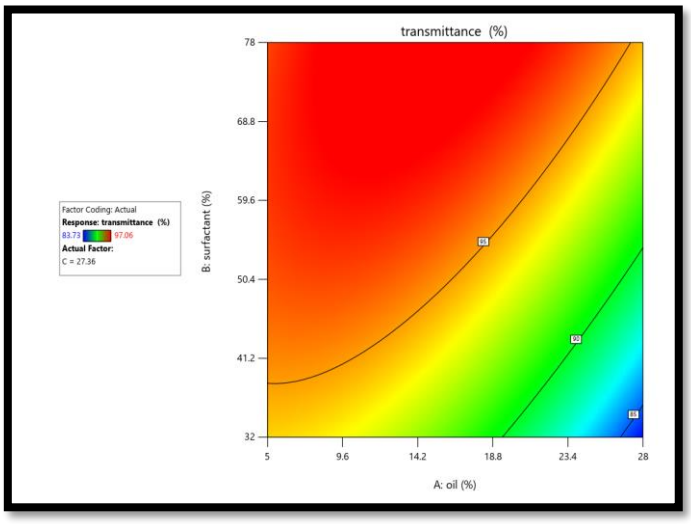


Fig. 6: Contour plot illustrating the effect of the independent formulation variables on percentage transmittance (Y_1) of the Lansoprazole-loaded SNEDDS.

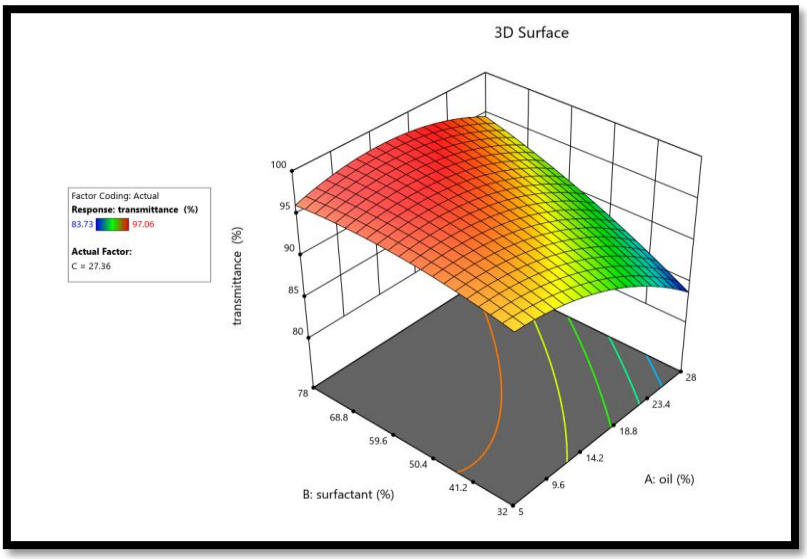


Fig. 7: Three-dimensional (3D) response surface plot illustrating the combined effect of castor oil and Tween 80 concentrations on the percentage transmittance (Y_1) of the Lansoprazole-loaded SNEDDS.

Influence of Formulation Composition Factors (X_1, X_2 and X_3) on Emulsification time [Y_2]

The emulsification time of prepared SNEDDS was found to be in range 25s (F9)–147 s (F5) as shown in Table 8. The data was fed and the following equation was generated:

$$Y_2 = +63.60 +29.38A -19.13B -22.00C -12.75AB -7.50AC +12.50BC +22.83A^2 -2.18B^2 -8.43C^2 \ldots. \text{Eqn. 2}$$

From the polynomial eqn. 2 it can be observed that unlike in the case of percentage transmittance, term 'A' (oil) has a positive impact on the emulsification time. The emulsification time increased when the oil concentration was raised as seen in the case of F5 and F15. This might be caused due to increase in the viscosity of the formulation, since the oil droplets takes longer time to break down and form a stable emulsion. Or the other situation can be hampering the dispersion and emulsification process by increasing the interfacial tension and subduing the effects of surfactant and co-surfactant. This can be clearly understood from eqn. 2, the concentration 'B' (surfactant) and 'C' (co-surfactant) has a negative impact on the emulsification time (Y_2) as seen in F3, F9 and F14. The reason for decreased response of Y_2 might be the increase in the interfacial area as surfactant act at the interfacial surface and co-surfactant synergistically to decrease the surface tension.

The 3D images demonstrate how each independent factor, such as oil and surfactant affects emulsification time. In this case A, B, C, AB, AC, BC, A^2, B^2, and C^2 are significant model terms. The predicted R^2 of 0.911 was in reasonable agreement with the adjusted R^2 of 0.9981, since the difference was less than 0.2.

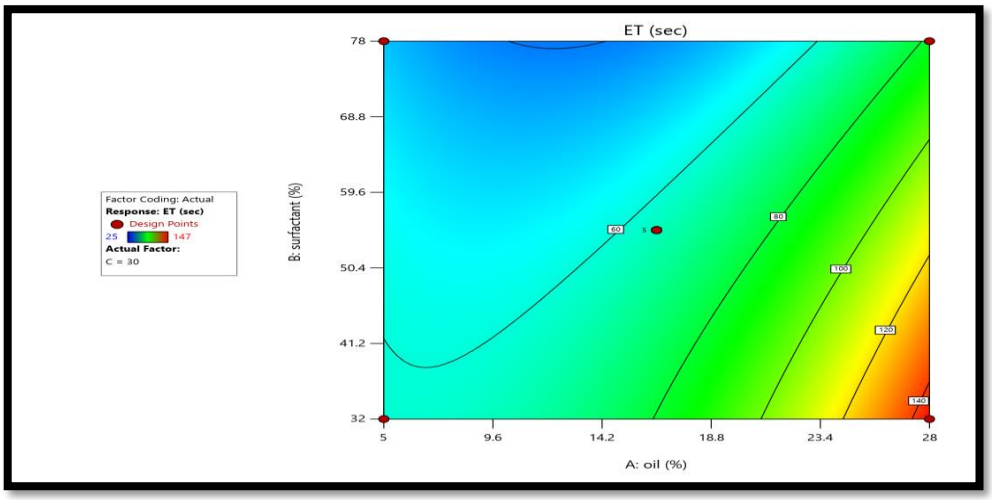


Fig.8: Contour plot illustrating the effect of the independent formulation variables on the emulsification time (Y_2) of the Lansoprazole-loaded SNEDDS.

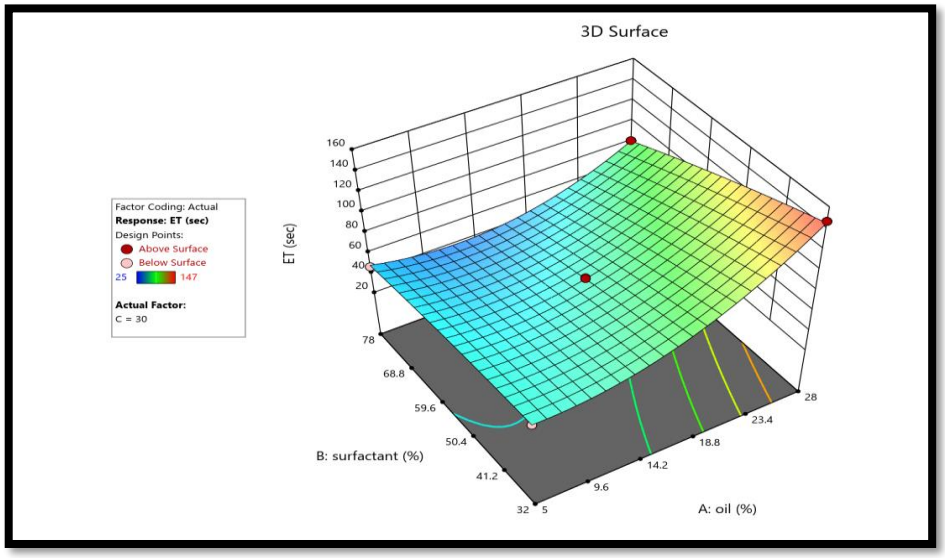


Fig.9: Three-dimensional (3D) response surface plot illustrating the combined effect of castor oil and Tween 80 concentrations on the emulsification time (Y_2) of the Lansoprazole-loaded SNEDDS.

Characterization of formulated LNS-SNEDDS

The SNEDDS of LNS were subjected to characterization based on two key parameters: percentage transmittance and emulsification. These characterization tests aimed to optimize the SNEDDS formulation. By assessing the percentage transmittance and emulsification time, the SNEDDS of LNS can be optimized to achieve desired characteristics, such as improved clarity, stability, and rapid emulsification. These characterization tests provide valuable information for fine-tuning the formulation and maximizing its performance as an effective drug delivery system.

1. **Emulsification Time:**

The rate of emulsification was taken as an important indicator to check the efficiency of self-emulsification. SNEDDS should be able to disperse completely in a short period of time (usually in >2min) under mild agitation. Each of the formulation (F1-F17) were assessed for their emulsification time, all the formulation showed rapid self-emulsification rate ranging from 25-147sec as shown in table 8.

F9 took the least amount of time to emulsify i.e. 25sec. Almost all of the formulation were graded A or B which signifies good emulsification efficiency, however some of the formulation F15 and F5 were graded C since they were slightly unclear. But they had good emulsification time so they were also considered for further testing. The reduced emulsification was due to optimum concentration of surfactant that lead to decrease in the interfacial tension. Or it might be the result of co-surfactant that enhanced the dispersibility of the formulation. The formulation were further graded according to their appearance upon dilution with water as shown in table 11.

Table 11: Visual grading and appearance of the Lansoprazole-loaded SNEDDS formulations (F1–F17) based on emulsification performance.

S.No.	Formulation No.	Grade	Appearance
1.	F1	B	Translucent/slightly bluish
2.	F2	B	Translucent/slightly bluish
3.	F3	B	Translucent/slightly bluish
4.	F4	B	Translucent/slightly bluish
5.	F5	C	Slightly bluish and translucent/unclear
6.	F6	B	Translucent/slightly bluish
7.	F7	B	Translucent/slightly bluish
8.	F8	A	Clear nanoemulsion
9.	F9	A	Clear nanoemulsion
10.	F10	B	Translucent/slightly bluish
11.	F11	A	Clear nanoemulsion
12.	F12	B	Translucent/slightly bluish
13.	F13	B	Translucent/slightly bluish
14.	F14	A	Clear nanoemulsion
15.	F15	C	Slightly bluish and translucent/unclear
16.	F16	A	Clear nanoemulsion
17.	F17	B	Translucent/slightly bluish

2. **Optimization of formulation using design expert:**

In order to optimize the SNEDDS, a number of independent variables and their impacts on the response variables were examined. The formulation with the most desirable properties was found through this analysis.

F9 demonstrated the best emulsification time of all the prepared SNEDDS formulations, with an emulsification time of 25sec. This finding show that the excipient composition in F9 was optimal for producing SNEDDS. The emulsification time of 25sec suggests that F9 was successful in achieving rapid emulsification, which denotes effective oil phase dispersion and the development of a stable emulsion. This is an advantageous quality because it enables rapid drug release and absorption after administration.

The analysis of variance (ANOVA)-generated Predicted/Actual graph gives a visual representation of how well the predicted values match the actual responses obtained during the formulation evaluation process. It enables a thorough evaluation of the predictive model's accuracy and dependability. The Predicted/Actual graph in the present case demonstrated in figure 10 that the actual responses generated during the experiment met all of the predicted values for the responses (dependent variables). This shows that the predicted values and the experimental results have a good degree of agreement.

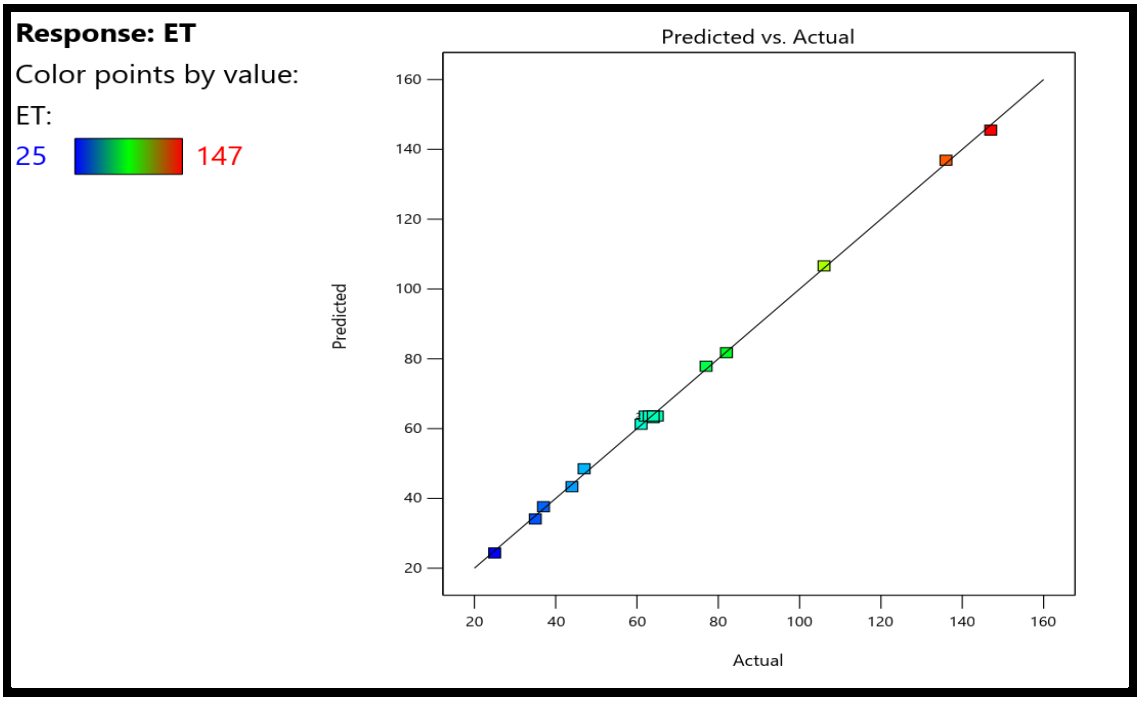


Fig. 10: Predicted versus actual values for the emulsification time (Y_2) of the Lansoprazole-loaded SNEDDS generated using the Box–Behnken experimental design.

3. **Characterization of optimized formulation:**

After carefully refining and improving the formulations, F9 stood out as the most promising option based on its outstanding performance in key aspects. As a result, it was chosen for further in-depth analysis and thorough examination to better understand its specific properties and potential applications in delivering drugs effectively.

4. **Globule size and Polydispersity index (PDI):**

The Zetasizer instrument was used to measure the globule size and polydispersity index (PDI) of F9, the chosen SNEDDS formulation. The globule size of the SNEDDS was found to be 80.1 nm,

as shown in figure 11 indicating small droplets that can enhance drug absorption. The PDI value of 0.246 suggests a reasonably uniform particle size distribution, ensuring stability and consistent performance. The combination of a small globule size and a low PDI value in F9 indicates that the formulation achieved a desirable level of homogeneity and stability.

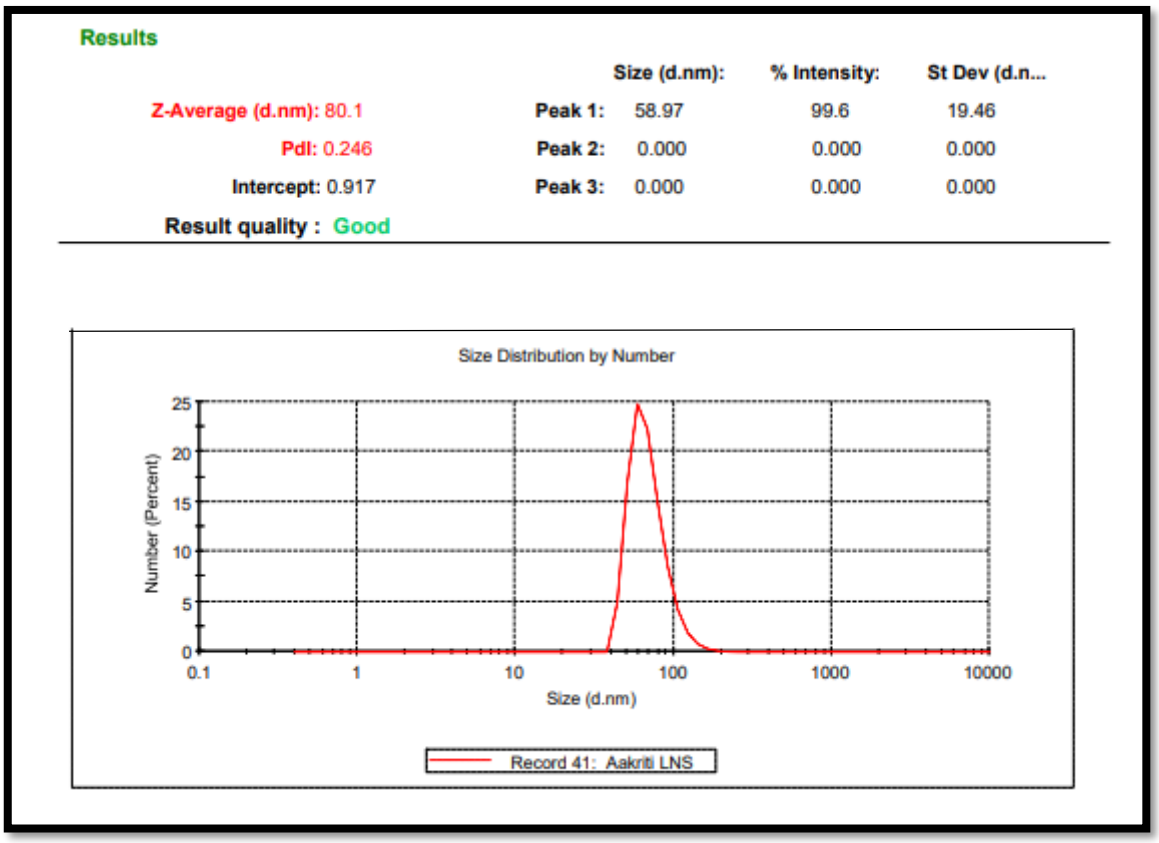


Fig. 11 Globule size distribution and polydispersity index (PDI) of the optimized Lansoprazole-loaded SNEDDS formulation (F9).

5. **Entrapment efficiency:**

The chosen SNEDDS formulation, F9, was found to have an entrapment efficiency of 89.45%. This measurement shows that the formulation successfully encapsulated a significant quantity of the drug or active ingredient. A high entrapment efficiency ensures that the drug is retained in significant amounts in the formulation, potentially increasing the drug's ability to deliver therapeutic benefits. For precise dosage and effective drug delivery, entrapment efficiency must be optimized. The 89.45% entrapment efficiency in the case of F9 suggests that the drug was successfully incorporated into the SNEDDS formulation, indicating its potential for efficient drug delivery and therapeutic applications.

6. **Cloud point Determination:**

It was determined that SNEDDS F9 cloud point was 62 ºC. It is the temperature at which the phase separation of the components of the SNEDDS formulation begins to give rise to a cloudy or turbid appearance. When F9 reaches a cloud point of 62 ºC, it means that the formulation is still stable and clear at lower temperatures. But when the temperature rises above 62 ºC, the parts

of F9 start to separate, giving the product a cloudy appearance. The cloud point is a crucial factor to take into account when storing and moving SNEDDS because it ensures the formulation's stability over a range of temperatures.

7. **Comparative drug release study of Marketed formulation and Optimized formulation**:

Optimized SNEDDS formulation (F9) was compared with the marketed product as explained in table 12 and figure 12. The dissolution values have been obtained and showed the better release of drug as compared to the marketed product in pH 0.1N HCL.

The enhanced drug release observed in the optimized SNEDDS formulation (F9) can be attributed to the lipid-based carriers and surfactants/co-surfactants present in the formulation. These components promote better drug solubility, increase the interfacial surface area available for dissolution, and facilitate uniform dispensability of drug particles in the dissolution medium [37].

Table 12: Comparative in vitro drug release profiles of the optimized Lansoprazole-loaded SNEDDS formulation (F9) and the marketed Lansoprazole tablet.

S.No.	% drug release (min)	Commercial tablet	SNEDDS F9 (%)
1.	5	0.61	86.3±0.09
2.	10	0.93	92.6±0.14
3.	15	1.52	75.5±0.33
4.	20	3.64	62.7±0.24
5.	30	9.01	52.0±0.55
6.	60	9.24	48.0±0.82

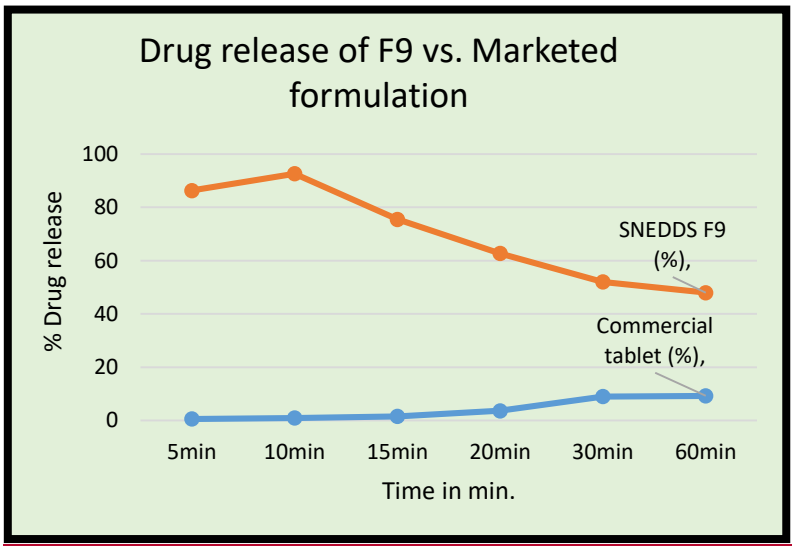


Fig. 12 Comparison of the in vitro dissolution profiles of the optimized Lansoprazole-loaded SNEDDS formulation (F9) and the marketed Lansoprazole tablet.

8. Accelerated Stability Study:

The stability of the optimized formulation (F9) was assessed on all the given parameters and the change in the optimized formulation is shown in table 13.

Table 13: . Results of the accelerated stability study of the optimized Lansoprazole-loaded SNEDDS formulation (F9).

Optimized formulation	Parameters	0 day	30th day	60th day	90th day
F9	Visual appearance	Clear solution	Clear solution	Clear solution	Clear solution
	% T	97.06	97.05	96.98	96.95
	% drug release	92.6±0.14	91.95± 0.13	90.21±0.2	86.57± 0.64

9. Conclusion

In the present study, optimized LNS SNEDDS was developed using Box-Behnken statistical experiment design which gave the optimum concentration of oil, surfactant and con-surfactant at which the maximum %T of 97.06% and minimum emulsification time of 25sec was successfully achieved. From the release study of F9 it was found that there was significant increase in the aqueous solubility of the develop LNS SNEDDS, that might lead to increase in the absorption of LNS in GIT. The FTIR and DSC test of developed SNEDDS also revealed that no interaction was seen between the components used for the development of SNEDDS Further the test also revealed that developing SNEDDS of LNS helped in increasing the kinetic stability and protected LNS from degrading in the gastric environment.

Overall, the successful formulation of SNEDDS for LNS achieved the objective of the study. These findings provide a strong basis for the conclusion that the formulated SNEDDS of LNS is a promising approach for drug delivery and could be beneficial for improving the therapeutic outcomes of LNS.

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Author Contributions

Astha Singh: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing – Original Draft.

Nikita Yadav: Methodology, Validation, Data Curation, Investigation, Writing – Review & Editing.

Komal Rao: Supervision, Resources, Project Administration, Validation, Writing – Review & Editing.

All authors have read and approved the final version of the manuscript.

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Conflict of Interest
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