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Optimization and Characterization of Self Nano Emulsifying Drug Delivery System loaded with *18- β Glycyrrhetinic acid*

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Authors' contribution

The first author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The purpose of this study was to prepare, optimize and evaluate self nano emulsifying drug delivery system (SNEDDS) containing 18- β glycyrrhetinic acid which enhances the dissolution profile or bioavailability of the drug in comparison to pure suspension of 18- β glycyrrhetinic acid.18- β glycyrrhetinic acid loaded SNEDDS having geranium oil as oil phase, tween 80 as a surfactant, and dimethyl sulfoxide (DMSO) as co-surfactant were prepared using pseudo ternary phase diagram and Box-Behnken experimental design was used to optimize the different formulations. Optimized formulations were characterized for self-emulsifying time, globule size, zeta potential, and drug release. The mean droplet size and PDI of the optimized formulation were found to be in a variation of 93.42 nm and 0.401 respectively. FTIR data showed no physicochemical interaction between excipients and drug. The encapsulation efficiency of optimised 18- β glycyrrhetinic acid SNEDDS was found 80.12±1.52% , % transmittance was found 99.34±0.134% and the viscosity of all the formulations was found 0.8872 cp. Three-dimensional response surface plots and two-dimensional contour plots of the responses across the selected factors were constructed that explained the relationship between the independent and dependent variables. Release kinetics was calculated by using KinetDS3.0. It was concluded that prepared formulations were formulated with approximately desired mean droplet size confirmed by Box-Behnken experimental design as well as properly optimized and characterized.

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Keywords: SNEDDS; box- Behnken design; optimization; 18- β glycyrrhetinic acid.

1. INTRODUCTION

Recently, an active pharmaceutical ingredient derived from natural origin has drawn attention in the pharmaceutical field day by day. Researchers, put more attention to planning novel dosage forms with these phytochemicals as they have high biological activity, satisfactory clinical efficacy, and low toxicity [1]. 18-β glycyrrhetinic acid is separated from *glycyrrhizaglabra* roots (liquorice) belonging to the family Fabaceae, which is widely used as an herbal medicine for many years. 18-β glycyrrhetinic acid shows many pharmacological effects, such as antimicrobial, antiulcer, immune-modulatory, anti-inflammatory, anti-tussive, and antiviral [2]. It also controls and prevents various skin inflammation diseases, such as atopic dermatitis and UV-induced skin photo-aging. From the literature review, it was studied that 18-β glycyrrhetinic acid is having biopharmaceutical classification system (BCS) class II, which shows low solubility and high permeability but, although having high permeability properties, these class II drugs have limited bioavailability because of low dissolution rate [3,4]. It was found in studies that drugs to be absorbed or diffusion through membranes was to be first dissolved in the physiological medium. Since these drugs are having poor solubility in media, so they cannot be absorbed properly, this results in poor bioavailability. Owing to the high permeation property, it cannot transport them to the membrane because of poor solubility in aqueous media [5]. So in this research article, SNEDDS were designed with aim to deliver topically. SNEDDS are isotropic mixture thermodynamically stable solutions of drug, oil, surfactant, and co-surfactant which, upon small agitation, generate fine droplets of water-in-oil nanoemulsions [6]. They require very low free energy for the self nano emulsification process. In this study, SNEDDS were used as the ideal carrier for the delivery of these phyto-chemical drugs for enhancing their activity and efficacy [7].

This study was performed to develop SNEDDS having 18- β glycyrrhetinic acid as a natural origin drug to show in-vitro antioxidant effect and in vivo anti-inflammatory effect and hair growth-promoting effect during animal studies in further research work that is not included in this paper. The components of 18- β glycyrrhetinic acid-SNEDDS were geranium oil, tween 80, and DMSO. Phase diagrams were shown for identifying the emulsification region. 18- β Glycyrrhetinic acid is characterized as a broad-spectrum drug [8]. 18- β glycyrrhetinic acid inhibits the formation of dihydrotestosterone (DHT), which is an androgen that is a sex hormone that contributes to the development of hair in males [9].

The most liked design of experiment for response surface studies is the Box- Behnken design applied to optimize SNEDDS loaded with 18- β glycyrrhetinic acid. Box-Behnken design is used to identify a relationship between response variables as dependent factors and quantitative experimental parameters as independent factors. The design needs three independent factors that comprise three levels. Box- Behnken design is selected because it requires fewer runs and has three-level factorial designs. That is why it is to be considered more efficient than other computational designs [10]. Compared to other response surface method designs, Box- Behnken designs require a few runs that are 13 runs in a 3- factor experimental design. That is why Box-Behnken design was applied to optimize SNEDDS of 18- β glycyrrhetinic acid. The independent variables were selected as the amount of oil (geranium oil, X1), amount of surfactant (tween 80, X2), and amount of co-surfactant (DMSO, X3). The dependent variable has globule size in nanometer (Y1), self emulsification time in sec. (Y2) and percentage drug release after 12 hours (Y3) [11]. In this study, mathematical model equations from computer simulation programming of Design Expert trial version 12 software for optimizing SNEDDS of 18- β glycyrrhetinic acid was derived [12]. The physicochemical characterization studies were done by using zetasizer, Fourier transform infrared spectroscopy (FTIR) [13].

2. MATERIALS AND METHODS

2.1 Materials

18-β glycyrrhetinic acid is obtained from hi-media laboratories private limited, Mumbai, India. Tween-80, DMSO, and geranium Oil was used of analytical grade from central drug house (P) Ltd. UV Spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan), vortex mixer (Sanjay Sc. Corporation, Delhi), cooling centrifuge (Remi elektrotechnik ltd. Vasai, India), Fourier transform infrared spectrophotometer (Shimadzu Corpn.,

Japan, IR Prestige 21), dialysis cellophane membrane (Sigma, Aldrich).

2.2 Preliminary Studies

2.2.1 Optimising diffusion rates of drug

An approach for optimizing the diffusion rate of drugs from a vehicle based on the relative polarity index or log P of the drug to the log P of the stratum corneum, a value called the penetrant polarity gap (PPG). They estimate the log P of the stratum corneum to be 0.8 and use this value along with the log P of the drug to calculate the PPG: Penetrant polarity gap = PPG = log P penetrant log- P stratum corneum. The relative polarity of the phase of the formulation in which it dissolved the active ingredient should be the magnitude of the PPG greater or less than the log P of the active ingredient [14].

2.2.2 Solubility studies

The selection of oil, surfactant, and co-surfactant is done by dissolving an excess amount of the drug. Various oil, surfactant, and co-surfactant were taken such as geranium oil, bottle guard oil, arachis oil, lemon grass oil, span 20, span 80, tween 40, tween 80, gelucire, and DMSO were screened on solubility bases using the shake flask method. In this method, excess quantity (1g) of 18-beta- glycyrrhetinic acid was dissolved in each test tube having 2 ml of excipients (oil, surfactant, and co-surfactant). These mixtures were thoroughly mixed with a vortex shaker at 37^0C. The mixture is kept for 24 h and centrifuged using a high centrifuge at 6000 rpm for 20 min. The supernatant was separated and after suitable dilution with methanol, the drug concentration was analyzed by using U-V Visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) at wavelength 267 nm [11].

2.2.3 Partition coefficient

By shake flask method: An excess amount (100mg) of the drug was dissolved in a separating funnel in octanol (50ml) and water (50ml). The shaking was done vigorously and kept to settle for 24 hrs after it, two phases were separated into separate beakers and further dilutions of water phase were done by pipette out 1ml in 100ml volumetric flasks, then absorbance was determined by UV Shimadzu 1800 [14].

Then the concentration of the drug was calculated by putting the absorbance value in the standard curve equation, i.e. y = mx + c

So by applying the formula,

$$Ko/w = \frac{Concentration\ of\ drug\ in\ Octanol}{Concentration\ of\ drug\ in\ water}$$

2.2.4 U.V. Characterization of drug

A standard stock solution of 18- β glycyrrhetinic acid was prepared by dissolving 10 mg of drug in 100 ml in phosphate buffer [pH 6.8]: methanol in 70: 30 proportion to make 100µg/ml from this pipette out 2ml, 4ml, 6ml, 8ml, and 10ml make up with phosphate buffer in 100 ml volumetric flask to make dilutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml then absorbance was taken at λ 267nm [6].

2.2.5 Fourier transformed infrared spectroscopy (FTIR)

Fourier transformed infrared spectroscopy (FT-IR) of pure drug, a combination of pure drug and oil, a combination of a drug with surfactant and co-surfactant, and physical mixture (drug, oil, surfactant and cosurfactant) were carried out using KBr disc. The spectral using Fourier transform infrared spectrophotometer (Shimadzu Corpn., Japan, IR Prestige 21) that scanned each KBr disc at 4 mm/s at a resolution of 2 cm over a wave number region of 4000–400 cm^{-1} were recorded. Both FTIR of plain drug and drug with oil, surfactant, and co-surfactant was carried out [15].

2.2.6 Construction of pseudo ternary phase diagram

The pseudo ternary phase diagrams were constructed for the identification of the concentration range of components for the formulation of nanoemulsions. The optimal concentration of oil, surfactant, and co-surfactant was determined by this for the formulation of SNEDDS. They constructed all the components to w/w %. The construction of pseudo ternary phase diagrams without incorporating drugs with the help of an online ternary plotter. The darker region in the phase diagram shows the self-emulsification area. Fig. 4 represents the phase diagram having geranium oil (oil), tween 80 (surfactant), and DMSO (co- Surfactant) at the apex of the ternary diagram. Surfactant and co-surfactant mixture (Smix) were taken in different

volume ratios that is (1:1, 1:2, 2:1). Each phase diagram was constructed by mixing oil and S mix in the ratio (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9). The ternary mixture of oil, surfactant, and co-surfactant was blended with a vortex shaker. 0.5 ml of the ternary mixture was taken and diluted to 500 ml distilled water in the beaker was gently stirred on the mechanical shaker while maintaining the temperature at 37^0 C. Emulsification takes place spontaneously and is investigated for the spreading of its droplets. This emulsion was kept on rest for 3 hrs and its transmittance was assessed by using a UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) at wavelength 267nm [16].

3. FORMULATION CONSIDERATION

3.1 Box–Behnken Experimental Design

Three factors, three levels (3^3) Box- Behnken experimental design was produced by using design experiment 12 software [17,18] was employed to plan liquid SNEDDS. The concentration of oil (geranium oil, X1), surfactant (Tween 80, X2), and co-surfactant (DMSO, X3) were taken as independent variables which have globule size (Y1) in nanometer, self-emulsification time (Y2) in seconds, and drug release (Y3) in percentage as shown in Table 1. This experimental design is a suitable approach for studying the effects of independent variables and their effect associated with dependent variables. The level of surfactant, co-surfactant, and oil was taken in a range of (16-60% w/w), (10-90% w/w), and (05-40% w/w), respectively. Weighed amount (50mg) of 18- β glycyrrhetinic acid was mixed first with oil after followed by the addition of a proper amount of surfactant. After proper mixing, the co-surfactant was added to the homogenized mixture. All the components were mixed gently using a vortex shaker at 37^0 C to get a clear homogenized mixture. The prepared liquid SNEDDS were stored tightly in the container at room temperature and were kept for further studies and formulations were recorded for any changes in turbidity or phase separation. The results obtained from responses were fitted into a 2FI model and quadratic polynomial model explained by a non-linear equation [11,19].

$$y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_1X_2+\beta_5X_2X_3+\beta_6X_1X_3 \quad (1)$$

$$y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_1X_2+\beta_5X_2X_3+\beta_6X_1X_3+\beta_7X_1^2+\beta_8X_2^2+\beta_9X_3^2 \quad (2)$$

Where y is the measured response β_0 - β_9 are coefficients of regression, X_1, X_2, and $X_3$are independent factors. Analysis of variance (ANOVA), lack of fit, and multiple correlation coefficients (R_2) tests validate the models.

3.2 Preparation of 18- β Glycyrrhetinic acid SNEDDS

18- β glycyrrhetinic acid SNEDDS formulations were prepared by adding 50 mg of 18- β glycyrrhetinic acid to the geranium oil after dissolving of the drug completely in oil added required quantity of tween 80 and DMSO to form a homogeneous mixture. The final mixtures were vortexes for 5 minutes until transparent preparations were obtained. The prepared liquid SNEDDS were placed in a tight container until used and we examined formulations for any change in turbidity or phase separation [19].

Table 1. Dependent and Independent variables

Batch No	X1: Geranium oil	X2: Tween 80	X3: DMSO	Y1:Globule Size (nm)	Y2: SET (sec)	Y3: % Drug release in 30 mins
F1	-1	-1	0	87.9	82	79.6
F2	+1	-1	0	80.43	95	92.34
F3	0	+1	-1	126.5	100	92.2
F4	0	-1	+1	60.24	50	80.12
F5	-1	0	+1	82.45	78	70.92
F6	0	+1	+1	55.46	45	75.8
F7	0	-1	-1	85.25	84	75.01
F8	0	0	0	110.9	65	70.22
F9	+1	+1	0	83.45	79	91.2
F10	+1	0	-1	120.9	80	89.5
F11	-1	0	-1	144.6	120	96.4
F12	-1	0	+1	50.24	40	86.3
F13	-1	+1	0	89.21	85	91.4

Table 2. Formulation concentration

S.No.	Independent Variables	Levels (mg)%		
		Low	Medium	High
1	X1= Conc. of oil (Geranium oil)	25	30	35
2	X2= Conc. of surfactant (Tween 80)	45	50	55
3	X3= Conc. of cosurfactant (DMSO)	10	12.5	15.0

4. CHARACTERIZATION OF SNEDDS

4.1 Droplet Size and Zeta Potential

The average droplet size, zeta potential, and polydispersity index of 13 formulations were determined using Malvern Zetasizer (Malvern Instruments, UK). Liquid SNEDDS were diluted 1000 times with distilled water and agitated gently to ensure proper distribution of fine emulsion in aqueous media. [11].

4.2 Dispersibility Studies

By dispersibility studies, self-emulsification time was determined. 1 ml of SNEDDS formulation was added drop-wise to 250 ml of Phosphate buffer having pH 6.8 with gentle agitation using USP Type II (paddle) dissolution apparatus having speed of 50 rpm at temperature 37^0 C ± 0.5^0 C. All the 13 formulations are visually observed and monitored for the formation of nanoemulsions and the time which was taken to disperse SNEDDS in buffer solution was recorded [20].

4.3 Percent Transmittance

% Transmittance of 18- β glycyrrhetinic acid SNEDDS was determined by adding 1 ml of each formulation to 100 ml of distilled water with continuous stirring and the diluted formulation was assessed by using UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) at wavelength 267nm. % transmittance can be calculated by using the formula

$\%T = I/I_0 \text{ X } 100$

I = amount of light that passes through the sample, I_0= amount of light entering the sample [21].

4.4 Drug Entrapment Efficiency

The entrapment efficiency of drug 18- β glycyrrhetinic acid in SNEDDS was calculated by the process of separation of SNEDDS and supernatant with centrifugation at 4000 rpm for 15 minutes. The 6 ml supernatant was further diluted with methanol and phosphate buffer pH 6.8 in ratio 2:1 after the amount of free drug was calculated by assessing it in UV Visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) at wavelength 267nm [22]. The formula to calculate the entrapped efficiency is

$$Entrapment\ Efficiency\% = \frac{Total\ amount\ of\ drug - Amount\ of\ free\ drug}{Total\ amount\ of\ drug} X\ 100$$

4.5 In vitro Drug Release Studies

Drug release studies of 18- β glycyrrhetinic acid SNEDDS for all 13 SNEDDS formulations were done by USP dissolution apparatus II (Paddle type) in 500 ml of phosphate buffer having pH 6.8 as dissolution medium at a speed of 50 rpm and temperature 37^0 C ± 0.5^0 C. 40 mg (4 ml) of 18- β glycyrrhetinic acid SNEDDS was placed in dialysis cellulose membrane bag. Aliquots of 5ml at a predetermined time interval was withdrawn (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min.) collected, and analyzed for 18- β glycyrrhetinic acid by double-beam U.V Visible spectrophotometer at wavelength 267 nm. To keep the sink condition 5 ml, immediately fresh dissolution medium was added to the apparatus and the drug release profile from 18- β glycyrrhetinic acid SNEDDS was observed [23,14].

5. EVALUATION OF OPTIMIZED FORMULATION

To study the effect of the composition of fresh formulation on response variables were selected and assessed for parameters or response variables such as globule size, self-emulsification time, zeta potential, PDI, % transmittance, drug release studies.

6. RESULTS AND DISCUSSION

6.1 Optimising Diffusion Rates of Drug

Penetrant Polarity Gap= PPG= |log P penetrant - log P stratum corneum|

PPG= |2.75- 0.8|= 1.95

The log P of the vehicle chosen for 18- β glycyrrhetinic acid should be 2.75+1.95 = 4.7 or higher or 2.75–1.95= 0.8 or lower. From the literature review we find, Log P value of tween 80 is 2.39, which differs from the polarity of the stratum corneum so, If the drug's polarity differs from the polarity of stratum corneum lipids, its skin penetration can be enhanced by the addition of a co-solvent that dissolves the drug and which also has a high affinity for stratum corneum lipids. So, DMSO was used as a co-solvent, having Log P -0.6, which helps in enhancing the permeation of the drug [14].

6.2 UV Characterization

UV characterization has been done for the preparation of the standard curve by making the dilutions and getting absorbance at wavelength 267 nm. The standard curve is shown in Fig. 1 by this curve sample concentration was calculated. The concentration of the sample was calculated by the absorbance value [24].

6.3 Partition Coefficient

The value of partition coefficient of 18- β glycyrrhetinic acid in n-octanol/phosphate buffer (pH 6.8) system was found to be 2.75 shown in Table 3. The log P value of 18- β glycyrrhetinic acid indicates the drug has lipophilic nature and is having good properties for the formulation of SNEDDS.

6.4 Fourier Transform Infrared Spectrophotometer

From the FTIR studies, no interaction was found between drugs and excipients. The FT-IR spectra of the drug showed peaks at 3437.15 cm^{-1} (-OH stretch), 2943.37-2866.22 cm^{-1} (-CH stretch), 1705.07 -1662.64 (C=O stretch) cm^{-1}, -OH stretch is seen at peak 2943.37 and C=O stretch is found at 1705.07 cm^{-1}, Ar C-H bend is found at 675.09 cm^{-1}, Ar C=C at 1662.64, =CH bend is found at 918.12 cm^{-1}. Drug-loaded physical mixture showed no specific physicochemical interaction, they were chemically compatible. The peaks found in it having oil, surfactant, and co-surfactant were observed and it presented all important peaks because of a functional group of drugs in the physical mixture. The major peaks of the drug at -OH stretch at 3433.29, -CH stretch at –OH stretch of acid is found 2920.23-2866.22, C=O stretch is found at 1732.08-1651.07, at fingerprint region =CH bend is found at 948.98 cm^{-1}, Ar. There was no significant difference found in the wavenumber (cm^{-1}) of the drug, broadening effect was observed in Fig. 2.

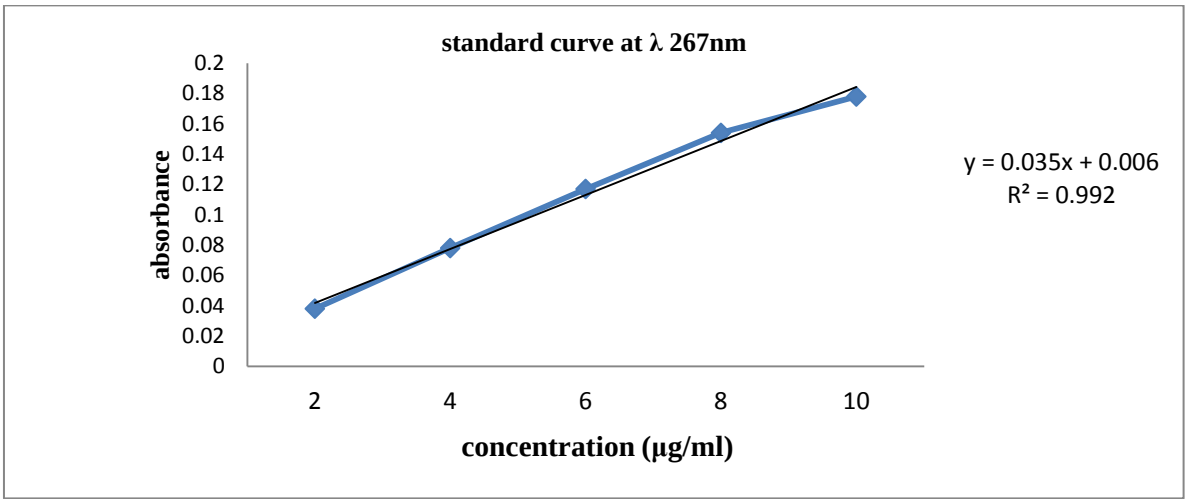


Fig. 1. Standard curve of 18- β glycyrrhetinic acid

Table 3. Drug partition coefficient

	Concentration of drugs in Octanol (mg)	Concentration of drugs in Water (mg)	K_{ow}= C $_{octanol}$/ C $_{water}$	Log K_{ow}
1	99.82 ± 0.05	0.18±0.05	565.03	2.75

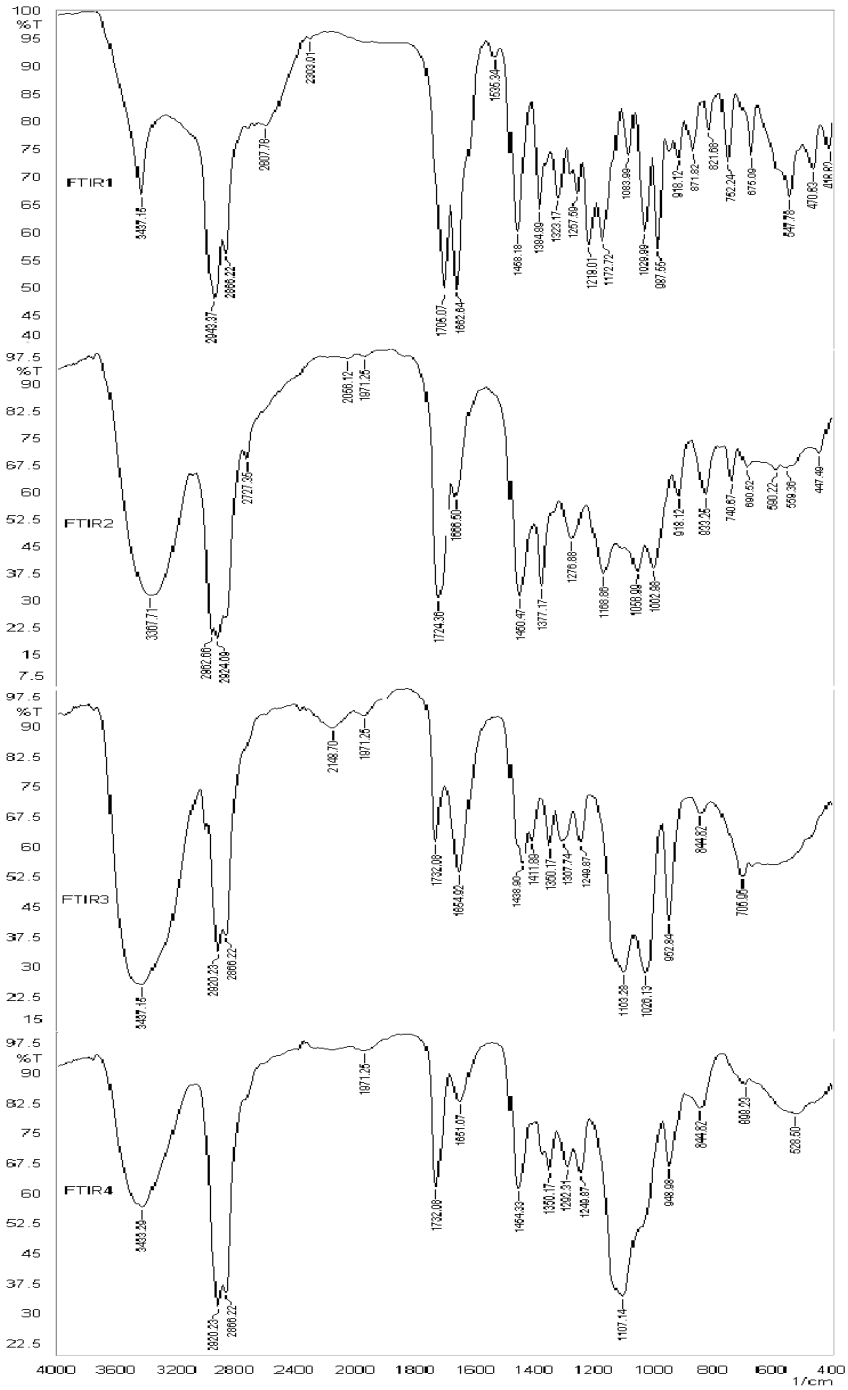


Fig. 2. FTIR1 represents FTIR of Drug, FTIR2 (a drug with oil), FTIR3 (a drug with surfactant and cosurfactant) FTIR4 (a drug with oil, surfactant and co-surfactant) represents FTIR of Physical mixture

6.5 Solubility Studies

The solubility of 18- β glycyrrhetinic acid was assessed in different vehicles are shown in Fig. 3. The excipients used in the formulation of SNEDDS should solubilise the maximum quantity of drug and possess a major self-emulsification region in the ternary phase diagram and the excipients were chosen by considering the solubility and compatibility with the drug. Different solubility of the drug in different oils/surfactants/ cosurfactant is shown in Table 4.

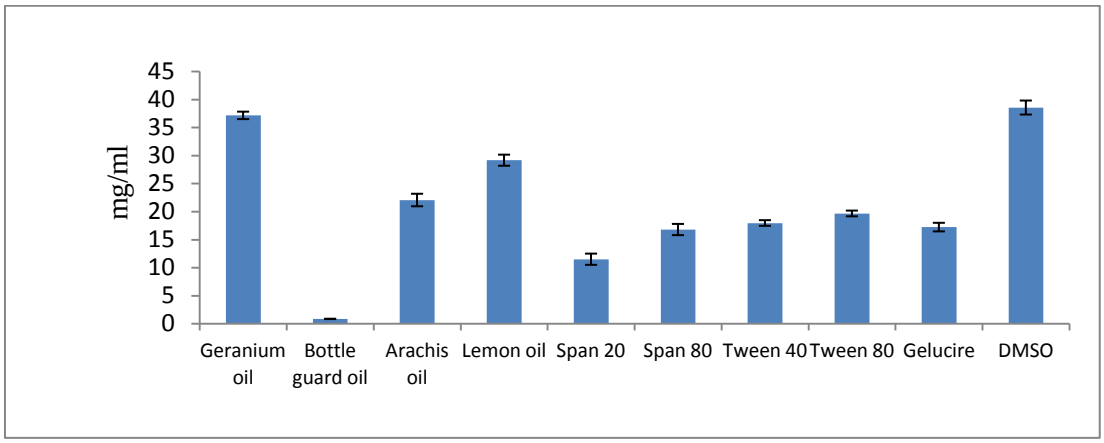


Fig. 3. The solubility profile of 18- β Glycyrrhetinic acid is assessed in different vehicles

Table 4. Different solubility of the drug in different Oils/ Surfactant/ Cosurfactant

S. No.	Oil/Surfactant/Cosurfactant	Solubility (mg/ml)
1	Geranium oil	37.5± 0.66
2	Bottle guard oil	0.83±0.032
3	Arachis oil	22.5±1.11
4	Lemongrass oil	29.16±0.99
5	Span 20	12.5±1
6	Span 80	15.8±1
7	Tween 40	17.5±0.50
8	Tween 80	19.1±0.51
9	Gelucire	17.4±0.76
10	DMSO	38.0±1.25

The components that were used for the formulation of SNEDDS solubilize the maximum amount of drug and also possess a large efficient self-emulsification region in the pseudo-ternary phase diagram. We selected vehicles that are suitable for a drug on solubilizing capacity, compatibility, and safety. Among the oil tested geranium oil shows the highest solubility of the drug that was 37.5± 0.66 mg/ml, so we chose it as an oil base. Tween 80 shows high solubility that was 19.1±0.51 mg/ml among the various surfactants and screening of different co-surfactants, DMSO was selected, which shows high solubility of 38.0±1.25 mg/ml. These studies were aimed at identifying a suitable oil, surfactant, and co-surfactant.

6.6 Construction of Pseudo-ternary Phase Diagrams

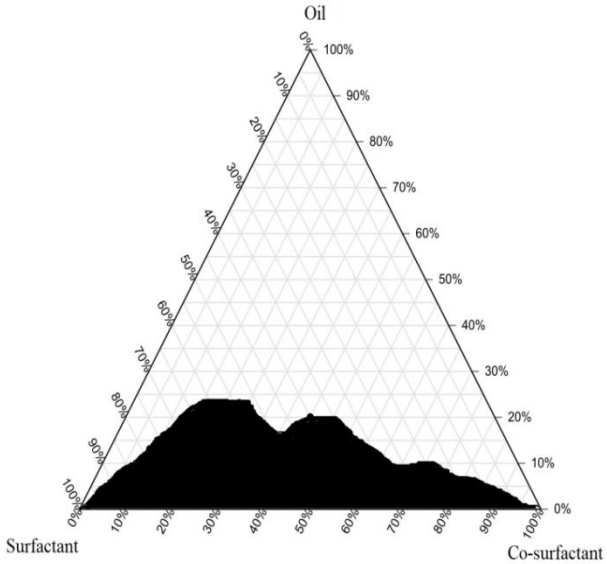
Based on preformulation studies of solubility of a drug in various vehicles, pseudoternary phase diagrams were constructed by taking geranium oil as the oil phase, tween 80 as a surfactant, and DMSO as co-surfactant. The darker region shown in Fig. 4 expresses and represents the effectiveness of the self nano emulsifying region that has visual characteristics like clarity, no phase separation, and spontaneous formation of the emulsion was observed in the formulation. So it was necessary to define the range of self-emulsification regions for oil, surfactant, and co-surfactant in Box Behnken design. The % range that was selected from the pseudoternary diagram for the formation of emulsion for the independent variables were at around 16-60% for the oil 10-90% for the surfactant, and 05-40% w/w for the co-surfactant. Pseudoternary phase diagram optimizes the three components of emulsion also it is used for screening of self dispersible formulation and to find the self emulsification region.

6.7 Box- Behnken Design Analysis

Three factors, three-level Box- Behnken design, require 13 experimental runs at 1 centre point. Experiments were performed in series on the experimental runs at different combinations of factor levels. It showed the experiment of the runs for the independent variables and their responses. Batches showed globule size (Y1) of

nanoemulsions from 50.24nm to 144.6 nm, Self emulsification time (Y2) 40-120 sec. and the percentage of drug release in 30 min (Y3) was 70.22%- 96.4%. Maximum formulations show acceptable PDI (< 0.5). PDI value over 0.5 shows aggregation in the particles. If the value of PDI is more, it shows about the polydisperse system, and if the value is less, i.e. near to zero shows about the monodisperse system. The polydisperse system has a greater tendency to aggregate compared to the monodisperse system. Fig. 5 shows the prepared formulation by employing Box- Behnken design.

All data was obtained from design experiment 13, it auto-select the fitted model type, Responses (Y1), and (Y2) were fitted to the 2F1 model, while (Y3) was fitted to the Quadratic model. ANOVA verified the significance of the Model, Lack of fit, and multiple correlation coefficients (R_2) test. Table 3 shows the result of ANOVA and Lack of Fit tests of quadratic models for all the responses. In the ANOVA test, the p values for the model (Y1), (Y2), and (Y3) were 0.1146, 0.0294, and 0.0220 respectively. The p-value for the model should be less than



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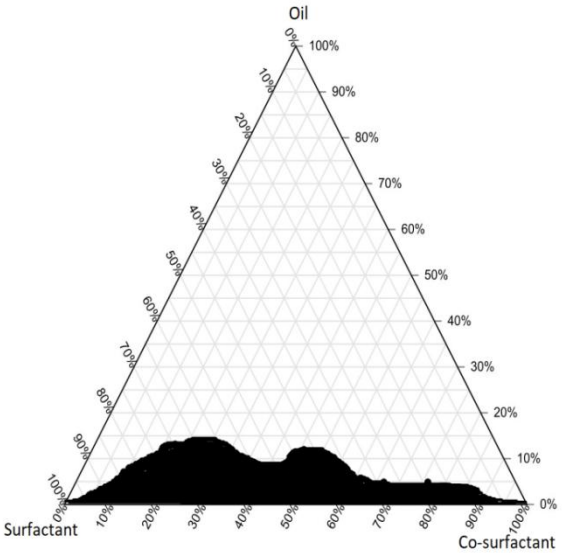

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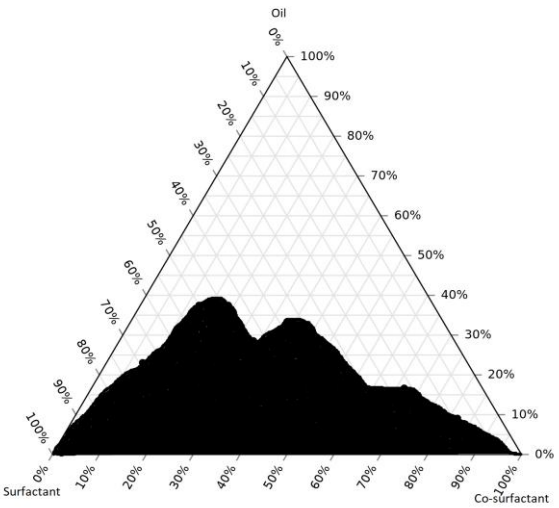

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Fig. 4. Pseudoternary phase diagram at 1:1, 1:2 & 2:1

Fig. 5. Prepared 13 SNEDDS formulation

Table 5. Particle size, zeta potential, and PDI

Batch No.	Particle size (nm)	Zeta Potential(mV)	PDI
F1	87.9±14.23	-10.03±2.34	0.378
F2	80.43± 23.34	-23.32±3.32	0.404
F3	126.5±981.5	-16.01±3.23	1.000
F4	60.24±12.45	-13.85±2.12	0.625
F5	82.45±12.45	-14.38±3.12	0.456
F6	55.46±11.61	-10.32±4.16	0.370
F7	85.25±10.43	-25.00±3.32	0.361
F8	110.9±24.61	-10.94±2.34	0.341
F9	83.45±32.21	-22.62±3.43	0.401
F10	120.9±12.43	-9.09±4.23	0.708
F11	144.6±54.23	-15.60±3.12	0.487
F12	50.24±14.56	-14.17±4.12	0.631
F13	89.21±43.21	-44.4±3.23	0.465

0.05, which shows the value is significant, but here p-value (Y1) is greater than 0.05 which shows the value of the model is not significant. So, the p-value of (Y1) is not fitted to the quadratic model and the p-value of (Y2) is fitted to the quadratic model. The variation of data is analyzed by the Lack of fit test which is also a good statistical parameter for checking the better fitness of the model. The analyzed value should be insignificant, that is p-value should be greater than 0.05, which is relative to the pure error. R2 value that is multiple correlation coefficient tests is denoting the amount of variation around the mean and its value should be near to 1.

7. CHARACTERIZATION OF SNEDDS

7.1 Particle Size and Zeta Potential

Particle size or droplet size, zeta potential, and polydispersity index (PDI) of all 13 formulations loaded with drugs were determined by Malvern Zetasizer Version 7.12 (Malvern Instruments Limited, Worcestershire, UK). 1 ml of sample from each formulation was diluted with 100 ml distilled water and agitated for proper distribution of the formulation in aqueous media. The measurements were taken in triplicate. Zeta potential is an important parameter for the characterization of the total surface charge and stability of the formulated SNEDDS.

The size of the globule of glycyrrhetinic acid SNEDDS was in the range 50.24 nm to 144.6 nm as depicted in Table 5. SNEDDS globule size is changing with changes in concentrations of oil, surfactant, and co-surfactant. The polydispersity index (PDI) is a dimensionless unit that finds the width of the size distribution and its values lie between 0 and 1. Values near 0 show a monodisperse system while higher values show a heterogeneous system. All the 13 formulations are in the PDI range 0.3 to 1.0 which shows good and average globular size of prepared formulations. Zeta potential is in the range +30 and -30mv. Combinations of the independent variables X1 (geranium oil), X2 (tween 80), and

X3 (DMSO) give different responses for the dependent variable that is globule size (Y1). It expressed a mathematical relationship for globule size (Y1) as it showed 2 FI equations as below:

Y1 = 90.58 – 9.38X1 – 4.81X2+6.09X3-14.70X1X2+13.16X1X3-32.95X2X3

The equation in terms of coded factors can make predictions about the response for given levels of each factor. By default, it coded the high levels of the factors as +1 and coded low levels as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

P-value less than 0.0500 shows model terms are significant. Values greater than 0.1000 show the model terms are not significant.

A negative predicted R2 implies that the overall mean may be a better predictor of your response than the current model. Sometimes, a higher-order model may also predict better.

Adequate Precision shows a ratio greater than 4 is desirable. Our ratio of 5.2211 shows an adequate signal. This model can use to navigate design space.

The model F value of 5.43 implies the model is significant. P-value less than 0.0500 show that the model terms are significant. Values greater than 0.1000 show the model terms are not significant.

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant and the intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around the average based on the factor settings. When the factors are orthogonal, the VIFs are 1; VIFs greater than 1 show colinearity, the higher the VIFs, the more severe the co-relation of factors. As a rough rule, VIFs less than 10 are tolerable.

The predicted R2 of 0.1749 is not as close to the adjusted R2 of 0.6887 as one might normally expect, i.e. the difference is over 0.2. This may show a large block effect or a problem with the model or data. Things to consider are model reduction, response transformation, outliers, etc.

Y2 = +77.15–11.13–13.25 +3.38 – 15.75 + 3.50 – 20.25

Adequate Precision value greater than 4 is desirable. Our ratio of 10.582 shows an adequate signal. This model can navigate the design space.

Y3 = +91.40 + 0.0937 – 6.20 – 2.42 – 2.10 – 11.60 + 6.26 – 6.81

Table 6. Analysis of Variance (ANOVA) for response surface 2FI model for Globule size

Source	Sum of squares	Degree of freedom	Mean Square	F- value	p-value
Model	7086.82	6	1181.14	2.84	0.1146
X1	704.44	1	704.44	1.70	0.2406
X2	185.28	1	185.28	.4460	0.5291
X3	296.83	1	296.83	.7145	0.4304
X1X2	864.07	1	864.07	2.08	0.1993
X1X3	692.74	1	692.74	1.67	0.2441
X2X3	4343.47	1	4343.47	10.46	0.0178
Residual	2492.46	6	415.41		
Cor Total	9579.28	12			

Table 7. Values of Regression coefficient and probability for Y1

Factor	Coefficient estimate	Fit Statistics	
Intercept	90.58	R2	0.7398
X1	-9.38	Adjusted R2	0.4796
X2	-4.81	Predicted R2	-0.4528
X3	6.09	Adeq Precision	5.2211
X1X2	-14.70		
X1X3	13.16		
X2X3	-32.95		

Table 8. Self-emulsification time

Source	Sum of squares	Degree of freedom	Mean square	F- value	p-value
Model	5167.25	6	861.21	5.43	0.0294
X1	990.13	1	990.13	6.24	0.0467
X2	1404.50	1	1404.50	8.85	0.0248
X3	91.12	1	91.12	0.5741	0.4773
X1X2	992.25	1	992.25	6.25	0.0465
X1X3	49	1	49	0.3087	0.5986
X2X3	1640.25	1	1640.25	10.33	0.0183
Residual	952.44	6	158.74		
Cor Total	6119.69	12			

Table 9. VIFs value for SET is 1 for all factors

Factor	Coefficient estimate	Fit statistics	
Intercept	77.15	R2	0.8444
X1	-11.13	Adjusted R2	0.6887
X2	-13.25	Predicted R2	0.1749
X3	3.38	Adeq Precision	8.3286
X1X2	-15.75		
X1X3	3.50		
X2X3	-20.25		

Table 10. Percentage drug release after 30 min

Source	Sum of squares	Df	Mean square	F-value	p-value
Model	955.96	9	106.22	15.85	0.0220
X1	0.0703	1	0.0703	0.0105	0.9249
X2	16.30	1	16.30	2.43	0.2168
X3	0.0325	1	0.0325	0.0049	0.9489
X1X2	154.01	1	154.01	22.98	0.0173
X1X3	23.38	1	23.38	3.49	0.1586
X2X3	17.64	1	17.64	2.63	0.2032
$X1^2$	307.50	1	307.50	45.88	0.0066
$X2^2$	89.68	1	89.68	13.38	0.0353
$X3^2$	106.12	1	106.12	15.83	0.0284
Residual	20.11	3	0.0703		
Cor Total	976.07	12			

Table 11. The model F-value of 15.85 implies the model is significan

Factor	Coefficient estimate	Fit statistics	
Intercept	91.40	R2	0.9794
X1	0.0937	Adjusted R2	0.9176
X2	-1.43	Predicted R2	NA
X3	0.0638	Adeq Precision	10.5825
X1X2	-6.20		
X1X3	-2.42		
X2X3	-2.10		
$X1^2$	-11.60		
$X2^2$	6.26		
$X3^2$	-6.81		

Table 12. Coefficient table

	Intercept	X1	X2	X3	X1X2	X1X3	X2X3	X12	X22	X33
Globule Size	90.5792	-9.38375	-4.8125	6.09125	-14.6975	13.16	-32.9525			
p- values		0.2406	0.5291	0.4304	0.1993	0.2441	0.0178			
Self Emulsification Time	77.1538	-11.125	-13.25	3.375	-15.75	3.5	-20.25			
p- values		0.0467	0.0248	0.4773	0.0465	0.5986	0.0183			
% Drug release after 30 min	91.4	0.09375	-1.4275	0.06375	-6.205	-2.4175	-2.1	-11.5987	6.26375	-6.81375
p- values		0.9249	0.2168	0.9489	0.0173	0.1586	0.2032	0.0066	0.0353	0.0284

In table p- value is shading p< 0.05 0.05≤ p < 0.1 p≥

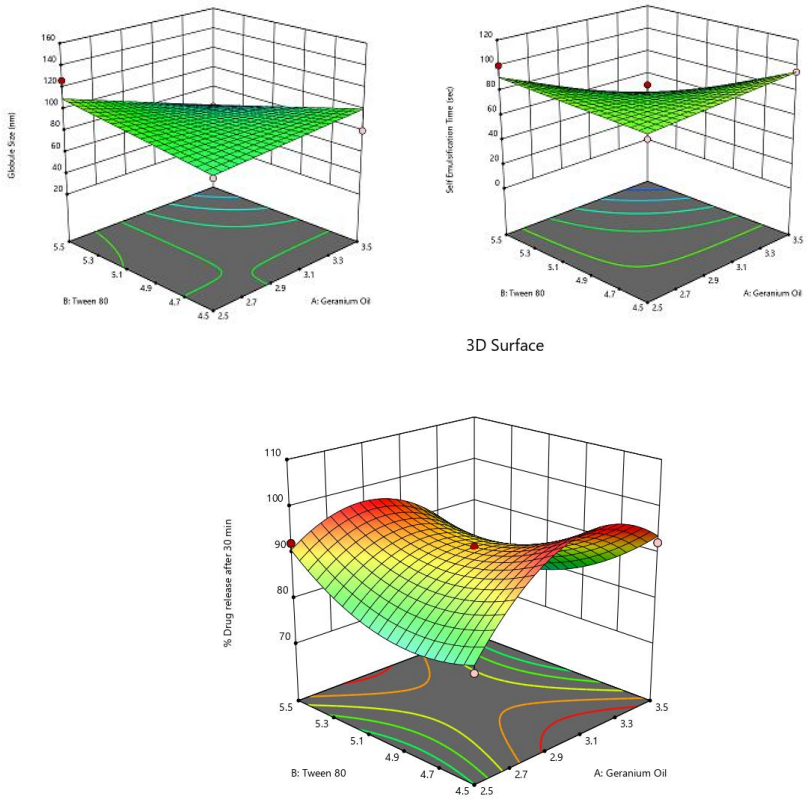

Fig. 6. Response Surface Plots represents X1 and X2 on the mid-level of X3

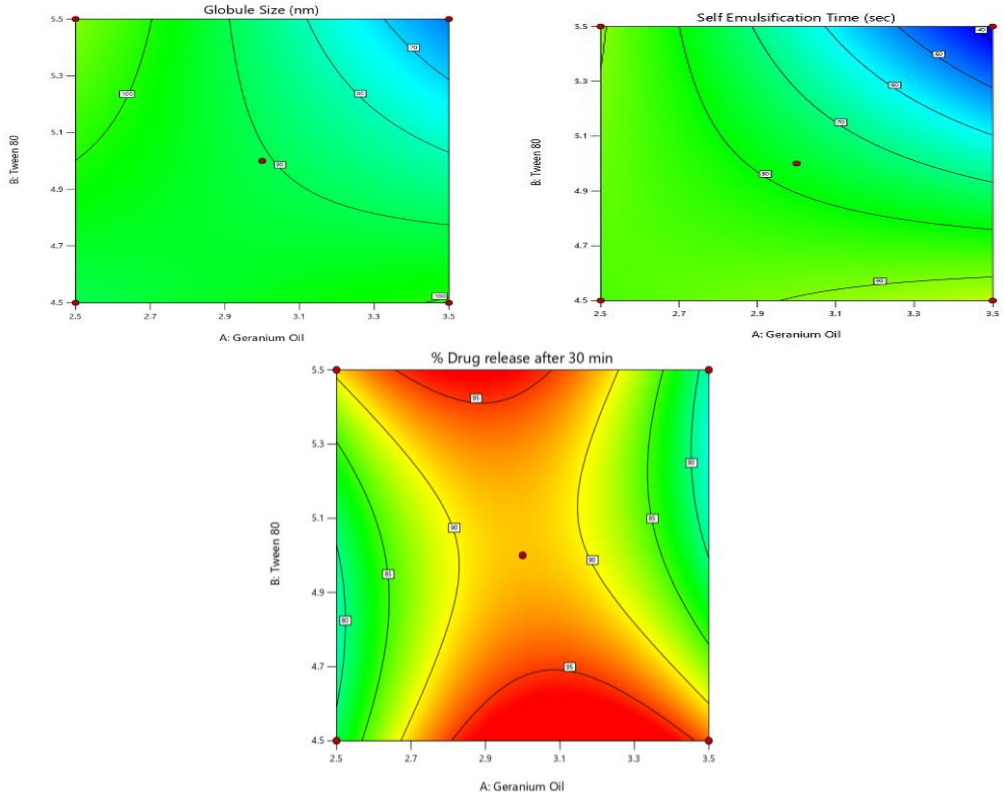

Fig. 7. Contour Plots represent X1 and X2 on the mid-level of X3

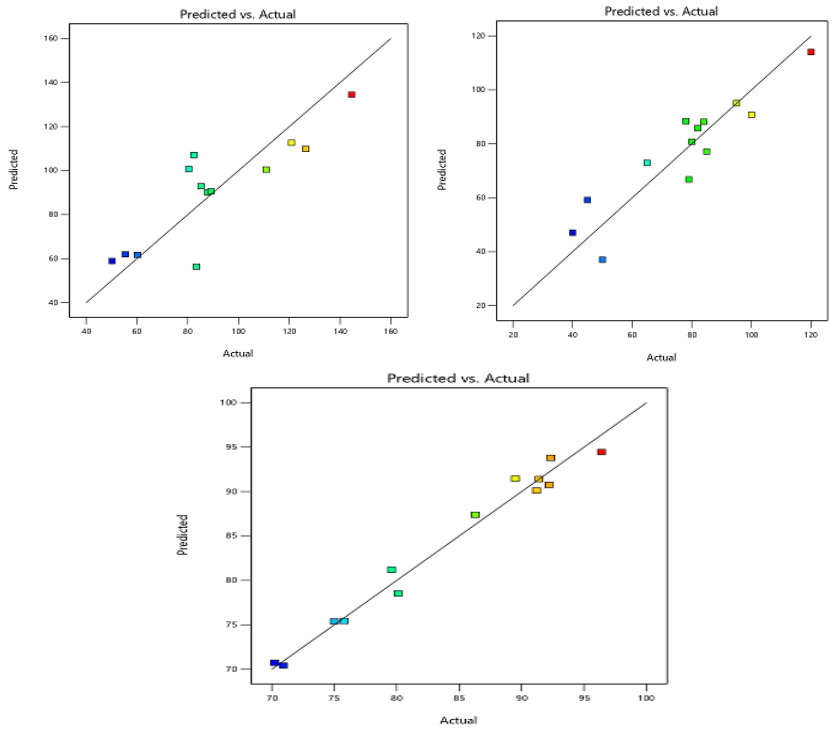

Fig. 8. Predicted versus actual graph of Globule size, Self emulsification time and Percent drug release

7.1.1 Response surface and contour plot analysis

Three-dimensional response surface plots and two-dimensional contour plots of the responses across the selected factors were constructed to further explain the relationship between the independent and dependent variables [25], as shown in Figs. 6 and 7. These types of plots are very useful for studying the interaction effects between two factors and for understanding how the effect of one factor will be influenced by the change in the level of another factor. As these types of plots can only express two independent variables at a time against the response, one independent variable must always be fixed [26]. Considering the p-value of coefficients for each independent factor of the different responses [27] in Table 12, we concluded that geranium oil, tween 80, and DMSO showed the least significant contribution to responses Y1, Y2, and Y3, respectively. Therefore, these factors were fixed as mid-values when plotting the response surfaces and contour plots. The influence considering for formulation composition factors on droplet size to be one of the most crucial factors for assessing the quality of SNEDDS. It determines the rate and extent of drug release, as well as absorption. Predicted versus actual graph of globule size, self emulsification time, and percent drug release was shown in Fig. 8.

Table 13. Confirmation table after optimization

Analysis	Predicted mean
Globule Size	90.5792
Self Emulsification Time	77.1538
% Drug release after 30 minutes	91.4

Based on optimization through Box-Behnken design, a new optimized formulation having a concentration of 4.5ml, 3ml and 1 ml of oil, surfactant, and co-surfactant respectively was prepared which have an average globule size 93.42±54.17 nm found near to mean globule size 90.57 nm that was confirmed by optimal design as shown in Table 13 having PDI 0.401 however, dual peaks in the graph and value of PDI towards 1, shows the system was heterogeneous but is selected for further studies due to having desired mean droplet size as well as good zeta potential that is -28.62 mV±3.65 shown in Fig. 9. Normally, the zeta potential value ±30mV is sufficient for stability of emulsion. The results are shown below [28], so by implying this design higher-

order responses surface were generated using fewer required runs than a normal factorial technique. Table 13 describe the confirmation table after optimization.

7.2 Self Emulsification Time of Optimized Formulation

Self-emulsification time could determine the rate of emulsification which is an important index for the assessment of the efficacy of emulsification. The SNEDDS should disperse completely after when subjected to aqueous dilution under mild agitation. Table 8 shows the self emulsification time of all the formulations. Optimized formulation FF have a self emulsification time of 76.133±0.950 [29].

7.3 Entrapment Efficiency

The encapsulation efficiency of 18- β glycyrrhetinic acid in SNEDDS was found to be 80.12±1.52%. By this, easily estimate the difference between the initial drug quantity and the free or un-entrapped quantity of drug in the supernatant concerning the total quantity incorporated in the SNEDDS preparation, so in 10 ml of SNEDDS preparation, 4 ml of SNEDDS having 40 mg of drug and 6 ml of supernatant have 10 mg of an unentrapped drug, so the entrapment efficiency of 18- β glycyrrhetinic acid in SNEDDS was found to be 80.12±1.52% [22].

7.4 Drug Release Studies

Dialysis cellulose membrane bag used for the drug release studies in USP dissolution apparatus II. 4 ml of drug-loaded SNEDDS equivalent to 40 mg was filled in a dialysis bag. Percent drug release in phosphate buffer (pH 6.8) was observed at different time intervals. The results of the study of cumulative % drug release studies through dialysis bags are shown in Fig. 10. The formulations were observed and found that F2, F3, F9, F11 and F13 show over 90% cumulative percent drug release in 30 min. The same process also analyzed an optimized formulation and had 91.8% cumulative percent drug release [30]. Fig. 10 showing the drug release studies of optimized formulation FF up to 360min.

7.5 Release Kinetics

Release kinetics of optimized formulation through dialysis bag is calculated by using KinetDS3.0 in table 14. AIC value tells about that our formulations fit which model, they consider low AIC value to be best, AIC value is 120.86 which is very low so it tells our formulation fits Korsmeyer Peppas model having R^2 value= 0.9862. Korsmeyer Peppas model was best employed in this formulation, to better characterize the drug release behavior.

$$\mathbf{Mt/M\infty = Kt^n}$$

Where Mt/M∞ is the fractional drug release in time t, K is constant for geometric and structural characteristics of controlled-release device and n is a parameter indicating the mechanism of drug release, a plot of log % drug released vs log time yields slope n, where 0.5 value of n indicate fickian diffusion, 0.5-1 or 0.45-0.89 indicates anomalous non-fickian diffusion, 0.89- 1 indicates zero-order release. Here the value of n is 0.620 indicates anomalous non-fickian diffusion.

FF formulation having n- value (diffusion exponent) 0.733, which indicates anomalous non-fickian diffusion, n- value also indicates that the geometry of swellable controlled release system is spherical [31].

7.8 Percentage Transmittance

Percentage transmittance of SNEDDS having 18- β glycyrrhetinic acid was measured by taking 1 ml of formulation into 100 ml of distilled water with stirring and then this formulation was analyzed by UV- Visible Spectrophotometer at 267 nm. This study was conducted in triplicate. It found the transparency of the material and also measures the amount of light that passes through a material and is usually reported as a percent comparing the light energy transmitted through a material to the light energy that entered the material. Value of transmittance near to 100% shows the formulation was transparent as shown in Table 15 [32].

7.10 Viscosity of Formulations

The viscosity studies tell about the SNEDDS system is physically stable. The estimated viscosity was 0.8872 cp as determined during the process of particle size analysis, and the pH was 6.8 as estimated by pH meter for all the formulations. The viscosity investigations are required for SNEDDS to physically define the system and control its stability. The viscosity of SNEDDS is crucial for their aqueous phase dispersion. Higher viscosities slow down emulsification, which might influence medication

release and bioavailability profiles in vivo. The viscosity of SNEDDS formulas increased as the concentration of oil and surfactant mixture increased, according to the results of the viscosity determination. The average viscosity of the SNEDDS after 100 times dilution with pure water, the viscosity range shrank to 0.8872 cp. All of the formulas had low viscosities, indicating that the resulting nano emulsion was of the O/W type. The SNEDDS formulas in this work recorded viscosity values that were low enough to rule out the likelihood of rapid self-emulsification [33].

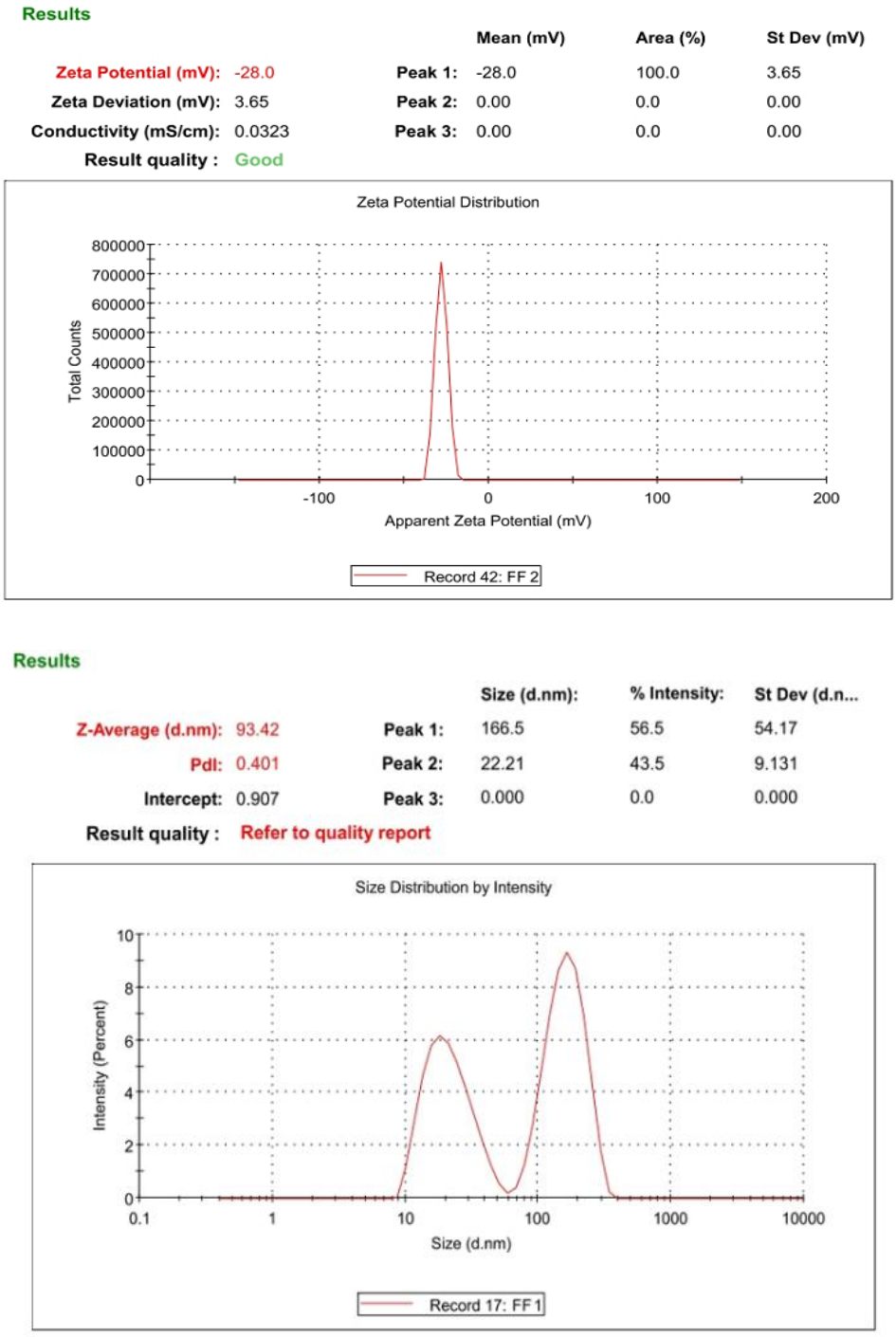

Fig. 9. Zeta potential and Particle size of optimized formulation FF

Table 14. Drug release kinetics of formulation FF and Pure drug through dialysis bag

	Parameters	Zero Order	First Order	Second Order	Higuchi	Hixon-Crowell	Korsmeyer-peppas	n- value
FF SNEDDS	**R^2**	0.4076	0.1749	0.1572	**-**2.2343	0.2916	0.9862	**0.620**
	AIC	128.24	211.58	166.64	152.00	137.31	120.86	
	R^2	0.7353	0.1983	0.1572	-1.0284	0.4688	0.9930	**0.515**
Pure Drug	**AIC**	76.88	168.185	121.96	105.39	91.34	104.15	

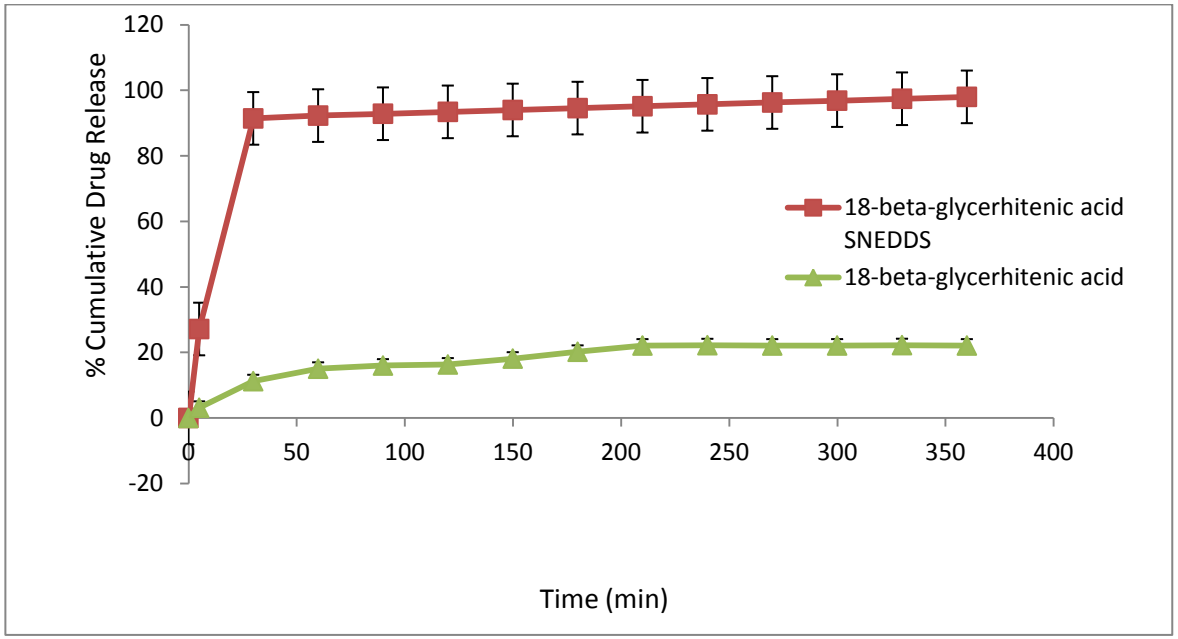

Fig. 10. Drug release studies of optimized formulation FF

Table 15. % Transmittance of all formulations

S. no	Formulations	%Transmittance
1	F1	99.53±0.092
2	F2	99.38±0.132
3	F3	99.03±0.525
4	F4	99.52 ±0.158
5	F5	99.44±0.205
6	F6	99.69±0.059
7	F7	99.20±0.069
8	F8	99.40±0.070
9	F9	99.62±0.139
10	F10	99.24±0.023
11	F11	99.32±0.115
12	F12	99.38±0.038
13	F13	99.38 ±0.137
14	FF	99.34±0.134

8. CONCLUSION

In this study, it was found that Box- Behnken experimental design optimised the formulation in fewer runs, the prepared formulation showing results in particle size, self emulsification time, and drug release studies which were almost near to the confirmation value were optimized by the design of experiment (DOE) software. In this study, the formulation of SNEDDS of 18-β glycyrrhetinic acid (FF) was done to show good dissolution profile against pure suspension of 18-β glycyrrhetinic acid which employs SNEDDS have capability to enhance solubility of poorly water soluble drug which may helps further to achieve desired bioavailability through stratum corneum. Percentage transmittance shows the formulation features, including uniformity and size of the droplets.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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