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Design, Optimization and Evaluation of Snedds Based System for Orlistat: A Quality by Design Approach With 2^3 Factorial

Amisha Vyas*[1], Anupama Diwan[1], Rupali Sharma[2]

[1]School of Pharmaceutical Sciences, Apeejay Stya University, Gurgaon, Haryana-122103, India
[2]Amity Institute of Pharmacy, Amity University, Manesar, Haryana-122413, India

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ABSTRACT

Obesity, as defined by WHO is an abnormal or excessive fat accumulation that may impair health. Bodyweight is directly proportional to the resultant impact of genetic, metabolic, environment, behaviour and culture issues. The leading cause of obesity is an energy imbalance between calories consumed and calories exhausted. To design and optimize Orlistat SNEDDS formulation by the development of SNEDDS with an appropriate quantity of oil, surfactant and co-surfactant with the factorial approach. A series of SNEDDS formulations for Orlistat was prepared based on solubility studies, pseudo ternary phase diagram and visual observation. Orlistat was added inaccurately weighed amount of oil into a screw-capped glass vial and heated in a water bath at 40ºC. The surfactant and co-surfactant were added to the oily mixture and stirred with a magnetic bar. The formulation was further sonicated for 15 minutes and stored at room temperature. From the result of evaluation parameters such as emulsification time 6±1s, % transmittance 94.01±1.5%, drug Loading 99.89%±0.56%, Polydispersity Index 0.47±0.01 and 0.211±0.02, Globulesize 99±6 nm, Zeta potential -28.12 mV and -24.5 mV, the centrifugation, Freeze-thaw cycle, Heating-cooling cycle showed no signs of phase separation, Viscosity 132.4±0m Pa.s, drug release 99.25% within 90 minutes, drug release follows Korsmeyer-Peppas model mechanism, n value was found to be 1.083 hence it can be postulated formulation F-6 followed the non-Fickian or anomalous release and P-value for factors emulsification time, % transmittance and % drug release was found less than 0.0500 and hence it was concluded formulation F-6 an optimized formulation.

*Corresponding Author

Name: Amisha Vyas
Phone: +91 9810450592
Email: amishavyas@gmail.com

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INTRODUCTION

Obesity is a state of being grossly fat or overweight. As per WHO, obesity is defined as abnormal or excessive fat accumulation that may impair health. It can be due to two reasons:

1. Excessive intake of food, salt, sugars, carbohydrates and less intake of vitamins, minerals and other nutrients;

2. Stagnant lifestyle, lack of exercise, more increase in consumption of junk food, psychological factors, increase in workload, stress level, etc.

Obesity is a significant risk factor for a long list of chronic diseases and hence requires careful attention and assessment. The assessment of obesity can be done in two ways; first, by measuring the Body Mass Index (BMI) and second, by waist circumference.

1. WHO defines The Body Mass Index the weight in kilograms divided by the square of the height in meters (kg/m^2)
2. Waist circumference is considered as a reasonable estimate of body fat, especially internal fat deposits (Vyas and Dahiya, 2015).

Management of obesity encompasses comprehensive lifestyle modifications including dietary changes, physical activity and behaviour modification, pharmacologic therapy and bariatric surgery. Lifestyle modifications often require multidisciplinary teams to ensure necessary changes are made as well as maintained but are often associated with high relapse rates. Bariatric surgery is associated with risks of perioperative mortality and operative complications and is consequently reserved for clinically severe obesity. The surgical procedures are expensive, and operated individuals require lifelong medical monitoring. Several anti-obesity agents have been approved in the past and were touted as 'magic pills' for addressing the obesity epidemic. However, many of them were subsequently found to have unacceptable risks leading to the unrestricted use/withdrawal from the market. Among the currently approved anti-obesity agents for chronic weight management, Orlistat was approved in 1999 (Kakkar and Dahiya, 2015).

Role of Pancreatic lipase

Pancreatic lipase enzyme (triacylglycerol acyl hydrolase) secreted from the pancreas is a crucial enzyme related to the dietary triglycerides absorption and catalyzes the digestion of dietary triglycerides. Among various lipases, pancreatic lipase performs the hydrolysis of 50-70% of total dietary fats. The reduction of fat absorption through pancreatic lipase inhibition is known to benefit the regulation of obesity (Kim and Shin, 2016).

Self nano emulsifying drug delivery system (SNEDDS)

These are Nanoemulsions formed from SEDDS. Self-Nano emulsifying drug delivery systems are heterogeneous dispersion soft two immiscible liquids which have a mean droplet size that falls within the Nanometric scale (20-200nm). They form microemulsions when in contact with water. The emulsions formed from SMEDDS have a mean droplet size that falls within the micrometric scale which ranges between 2-100nm. SMEDDS are thermodynamically stable (Amrutkar *et al.*, 2014). They form optically transparent emulsions. Because of the small droplet size, the surface area for absorption and dispersion is increased significantly, and it quickly penetrates the gastrointestinal tract and can be absorbed. Self-emulsifying drug delivery systems are essential for increasing the solubility of drugs.

MATERIALS AND METHODS

Materials

Caproic acid was procured from Qualikems Fine Pvt. Ltd. Vadodara, Transcutol P was procured from Lubrizol, Labrafac PG was procured from Thermo Fischer Scientific India Pvt. Ltd., Mumbai, Olive oil and Sunflower oil was procured from Thomas baker (chemicals) Pvt. Ltd. Mumbai, India, n-Octanol, PEG 90 and PEG400 was procured from SDFCL, Mumbai, Tween 80 was procured from Molychem, Mumbai, Kolliphor ER and Polysorbate 80 was procured from Qualikems Fine Pvt. Ltd. Vadodara.

Methods

Development of Orlistat SNEDDS

Here Castor oil, Sunflower oil, Olive oil, Labrafac PG were used as oil phase and Transcutol P, Tween80, Kolliphor ER, Caproic acid and Polysorbate 80, PEG 400, PEG 90 were used as surfactant and co-surfactant respectively. The compositions are given in Table 1. Orlistat was added in an accurately weighed amount of oil into a screw-capped glass vial and heated in a water bath at 40ºC. The surfactant and co-surfactant were added to the oily mixture using positive displacement pipette and stirred with a magnetic bar. The formulation was further sonicated for 15 minutes and stored at room temperature until its use in subsequent studies.

Experimental Design

A 2^3 full factorial design was selected because; an experiment may be designed to focus attention on a single independent variable or factor. An alternative approach is to study the influence of one independent variable in conjunction with variations in one or more additional independent variables. We can study not only the effects of the two independent variables separately but also how they combine to influence the dependent variable. Three- factors (X1, X2 and X3), two-level (-1, +1) design can be developed. Three-factor were evaluated each at two-level and experimental trials were performed for all eight possible combinations. Emulsification

Table 1: Composition of Preliminary Formulation Batches

S.No.	Formulation	Ingredients	Quantity
1	PF 1	Castor oil	12.30 ml
		Transcutol P	4.65 ml
		Polysorbate 80	6.17 ml
		Orlistat	120 mg
2	PF 2	Sunflower oil	2.5 ml
		Tween 80	18 ml
		PEG 400	4.5 ml
		Orlistat	120 mg
3	PF3	Olive oil	3.75 ml
		Caproic acid	8.75 ml
		PEG 90	11.25 ml
		Orlistat	120 mg
4	PF4	Labrafac PG	2 ml
		Kolliphor ER	14.4 ml
		PEG 400	3.6 ml
		Orlistat	120 mg

Table 2: Actual and coded formulation design of SNEDDS formulation

Independent Variable	Level	
	Low (-1)	High (+1)
X1: Labrafac PG	1	2
X2: Kolliphor ER	7.2	14.4
X3: PEG 400	1.8	3.6
Dependent Variable		
Y1: Emulsification time		
Y2: % Transmittance		
Y3: % Drug Release		

Table 3: Composition of Factorial Batches

Factorial Batches	Drug Orlistat	Factor 1 A: Labrafac PG 2 ml	Factor 2 B: Kolliphor ER 14.4 ml	Factor 3 C: PEG 400 3.6 ml
F1	120 mg	1	7.2	3.6
F2	120 mg	1	7.2	1.8
F3	120 mg	2	14.4	1.8
F4	120 mg	1	14.4	1.8
F5	120 mg	2	7.2	1.8
F6	120 mg	1	14.4	3.6
F7	120 mg	2	14.4	3.6
F8	120 mg	2	7.2	3.6

time, % transmittance and % Drug release were selected as dependent variables. The actual and coded formulation design of SNEDDS formulation according to factorial design (2^3) layout is shown in Tables 2 and 3.

Response Surface Methodology (RSM) is also widely employed to optimize formulations with suitable experimental design because it permits a deeper understanding of a process or product and has essential applications in establishing the robustness of that product. Full factorial designs, which have been widely used in response surface modelling and optimization. RSM was used to establish the relative importance of two or more factors and also to indicate whether or not interaction occurs between the factors and thereby affects the magnitude of the response. The data was interpreted using response surface methodology (Design Expert Software Version12, Stat-Ease, Inc.).

Refractive Index

Study Refractive index of formulations was determined at 25 $\pm$ 0.5°C using Abbe refractometer, Germany. Standardization was performed using castor oil. The electrical conductivity (σ) of the prepared formulations was determined using digital conductometer (HANNA instrument H1255, Romania) to assess the nanoemulsion structure. The measurement was made at a constant frequency of 1 Hz at ambient temperature (Kuruvila *et al.*, 2017).

Assessment of the spontaneous emulsifying properties of the developed SNEDDS was done visually. The study was performed in USP type II dissolution, one gram of each system was dropped into 500mL of distilled water with agitation by a rotating paddle at a speed of 50 rpm, and the temperature was kept at 37 ºC. Emulsification time was recorded as the time taken to obtain a clear homogenous solution. Samples were tested in triplicates. The efficiency of the self-emulsification process was judged depending on the following grading system:

1. Grade A: Nanoemulsions that are formed rapidly (within 1 min) and attain a clear or bluish appearance.
2. Grade B: Rapidly forming translucent nanoemulsions displaying a bluish-white appearance.
3. Grade C: Fine milky emulsions that are developed within 2 min.
4. Grade D: Dull, greyish white emulsions that take more than 2 min to be formed with an as lightly oily appearance.
5. Grade E: Systems that show reduced or negligible emulsification with large oil globules on their surfaces.

Systems of Grade A and Grade B were considered as nanoemulsions, and they were selected for further investigation; as they are expected to form a nanoemulsion when they are diluted with the physiological solutions. Percentage transmittance is used as an indicative measure of the optical transparency of the nanoemulsions; therefore the % transmittance of the formed emulsions was assessed at 203nm utilizing UV spectrophotometer (Shimadzu, UV-160, Japan) using distilled water as a blank (Khattab *et al.*, 2017; Abouhussein *et al.*, 2019).

Drug Loading Efficiency

For determining the Orlistat content, 1mL of SNEDDS formulae (equivalent to 20 mg of Orlistat) was diluted with methanol in a volumetric flask and mixed well by shaking or inverting the volumetric flask two to three times. Samples were prepared in triplicate and absorbance was measured after suitable dilutions at 203 nm using UV-Vis Spectrophotometer (UV/Vis spectrophotometer). The amount of Orlistat present in each formula was calculated from a calibration plot (Yoo *et al.*, 2010).

TEM analysis Transmission electron microscopic (TEM)

(Philips Tecnai 12, Netherlands) the analysis was carried out to determine the morphology of the dispersed oil droplets. 0.1ml of selected SNEDDS was diluted with 100ml of distilled water and mixed by slightly shaking. A drop of diluted SNEDDS was placed on a copper grid and was stained with phosphotungstic acid (PTA) (1% w/v) for 30 s. The excess solution was removed with a filter paper. The grid was analyzed after drying in air at room temperature.

Emulsion droplet size, Poly dispersibility Index and Determination of Zeta Potential

It is assumed that a droplet size value below 100 nm has led to the formation of SNEDDS, which are stable, isotropic and clear o/w dispersions. The droplet size of the formulations was analyzed using a Malvern Zetasizer (Nano ZS). A 0.1ml from each formulation was diluted to 20ml with purified water at 25°C, and the contents were gently stirred using a magnetic stirrer. The droplet size of the resultant emulsions was determined by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments, UK). A laser beam at 203 nm wavelength was used, and light scattering was monitored

at 25 °C at a 173 ° angle. The z-average diameter and polydispersity index (PDI) of the emulsions were derived from cumulated data by Auto measure software (Malvern Instruments, Worcestershire, United Kingdom). The emulsion stability is directly related to the magnitude of the surface charge. In conventional SNEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SNEDDS formulation was measured using a zeta meter system. The SNEDDS were diluted with a ratio 1:2500 (v/v) with distilled water and mixed with a magnetic stirrer (Bhikshapathi and Priya, 2018). Zeta-potential of the resulting microemulsion was determined using a Zetasizer (Nasr *et al.*, 2016; Sisinthy *et al.*, 2016; Kim *et al.*, 2017).

Determination of pH and Effects of pH and Dilution Ratio (Robustness to dilution)

The pH values of the Orlistat SNEDDS formulations were measured using pH meter, Jenway 3310, UK; standardized using pH 4.0, 7.0 and 10 standard buffers. It is necessary to keep a stable property of SNEDDS with various fold dilutions at different pH conditions. The optimized Orlistat-SNEDDS formulation was diluted 50, 100, and 1000 times with distilled water, pH 1.2 HCl media, pH 4.5 acetic acid buffer media, and pH 6.8 media, respectively. Then, the diluted samples were stored at room temperature for 24 h. Any changes in the physical property should be recorded, such as flocculation, precipitation, and phase transition. Besides, the changes in mean droplet size and PDI were tested to evaluate the physical stability (Shahba *et al.*, 2018; Xue *et al.*, 2018).

Centrifugation

The SNEDDS was diluted 100 times with aqua pro injection. Then, it was centrifuged using the centrifugation (Hanil MF 80) with a speed of 3500 rpm for 30 minutes. Then, the phase separation was observed visually, the presence of phase separation indicates a difference in kinetic stability in nanoemulsion resulting in emulsion system instability, such as creaming, flocculation, cracking or coalescence (Jumaryatno *et al.*, 2018).

Freeze-thaw cycle (accelerated ageing) and Heating-cooling cycle

They have involved three freeze-thaw cycles at -21 °C and 25 °C with storage at each temperature for 48 h. Formulations were centrifuged at 3000 rpm for 5 min and then observed for phase separation and drug precipitation. All formulations were diluted with distilled water (1:25), and the resulting nanoemulsions were then observed for any instability problems. Only stable formulations were selected for further evaluation and characterization. Six cycles between 4ºC and 45ºC at each temperature for not less than 48 hours were studied. The formulations that passed at this temperature without any signs of instability (creaming, cracking) were subjected to centrifugation test (Ujilestari *et al.*, 2018; Chabib *et al.*, 2017; Fotouh *et al.*, 2017).

Determination of viscosity

The viscosity of the SNEDDS formulation was measured by Brook field viscometer (DV2T) using at10 rpm. Each reading was taken after the equilibrium of the sample at the end of two minutes. The samples were repeated three times (Subramanian and Siddalingam, 2017).

***In vitro* dissolution test**

The dissolution test was performed using the USP dissolution apparatus II with 0.1 N HCl solutions (pH 1.2) with the media volume of 900mL at 37.5°C±0.5°C. The rotational speed was adjusted to 50 rpm. The selected LSP, SSPL, and SSPH filled in a gelatin capsule (equivalent to an Orlistat amount of 120 mg) were prepared and placed into a dissolution tester with a sinker. At each predetermined interval, the aliquot (5mL) of the medium was collected and filtered through a membrane filter (pore size: 0.45μm). Orlistat content was measured utilizing UV spectrophotometer (Shimadzu, UV-1600; Japan) (Tong *et al.*, 2018; Ravala and Patel, 2011).

Drug Release Kinetics

To investigate the drug release mechanism from tablets, the drug release data were analyzed with the following mathematical models and interaction of diffusion release mechanism Figure 1.

The most appropriate model was selected based on regression values (r^2) and diffusion release exponent (n). The zero-order kinetics describes the systems in which the drug release rate is independent of its concentration. The first-order kinetics describes the systems in which the drug release rate is concentration-dependent. Higuchi model describes the release of water-soluble drug from an insoluble matrix as a diffusion process based on the Fick's law and is square root time-dependent. The Hixson-Crowell cube root law describes the drug release from a system depends upon the change in surface area or diameter of particle or system and involves no diffusion mechanism. Korsmeyer-Peppas model describes the fraction of drug release relates exponentially concerning time. This model is generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than

one type of release phenomena could be involved. Drug release kinetics and best fit model for all the selected batches was found out with the help of *PCP DISSO Version 2.08* software and Microsoft Excel (Costa and Lobo, 2001; Samaha *et al.*, 2009; Yuksel, 2000).

Stability

The stability studies were conducted to determine the changes *in vitro* drug release studies, drug content, emulsion droplet size, and PDI on storage, according to ICH guidelines at 40^{o} C/75±5% RH by storing the optimized S-SNEDDS for three months. After three months, the samples were collected and analyzed for drug content, emulsion droplet size, PDI, and *in vitro* release studies. The viscosity of the selected formulation was found to be 187.5±0 mPa.s (Subramanian and Siddalingam, 2017).

RESULT AND DISCUSSION

Results

The refractive index mean values of the formulations ranged from 1.39 to 1.46. The shortest emulsification time was attained in case of formulation PF4 (6 ± 1.4 s) followed by PF1 (11 ± 1.3 s); they rapidly formed grade A nanoemulsions upon dilution. On the other hand, PF2 and PF3 showed a lower emulsification time (17± 1.5 s and 14 ± 1.2 s) forming a slightly translucent (grade B) nanoemulsion. The drug loading efficiency for all Orlistat SNEDDS formulae was found in the range of 92.37% ± 0.75% (PF1) to 99.09% ± 0.56% (PF4). The diluted preparation (nanoemulsion) possesses spherical shape oil globules with no sign of coalescence. The Polydispersity Index (PDI) of liquid SNEDDS Orlistat was found to be 0.51 ± 0.02and 0.243 ± 0.03, respectively. Globule size of freeze-dried SNEDDS was found to be 108 ± 11 nm. Zeta potential for the liquid SNEDDS and self-emulsifying Orlistat on reconstitution was found to be -31.11 mV and -28.4 mV, respectively.

Refractive Index

Refractive index is an indicator of the transparency of the formulation. The refractive index mean values of the formulations ranged from 1.39 to 1.46, which is closer to the refractive index of water (1.33) and reflects the transparency of the formulations.

It is also reported that when the refractive index is close to the refractive index of water, the formulation has per cent transmittance more than 99% and has transparent nature. Also, the results indicate that the formulations will form o/w nanoemulsion after dilution. The results are shown in Table 4.

Zero order kinetics	$Q_t = Q_o + K_o t$
First order kinetics	$\ln Q_t = \ln Q_o + K_1 t$
Higuchi square root model	$Q_t = K_H t^{½}$
Hixson-Crowell cube root model	$(Q_o)^{1/3} - (Q_t)^{1/3} = K_{HC} t$
Korsmeyer- Peppas model	$Q_t/Q_a = K_k t^n$

Q_t: amount of drug released in time t; Q_o: initial amount of drug in the dosage form; Q_a: total amount of drug dissolved when the dosage form is exhausted; K_o, K_1, K_H, K_{HC}, K_K: release rate constants; n: release exponent

Figure 1: Mathematical models for the kinetics of drug release

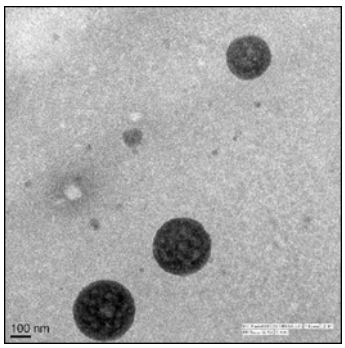

Figure 2: TEM image of formulation PF4

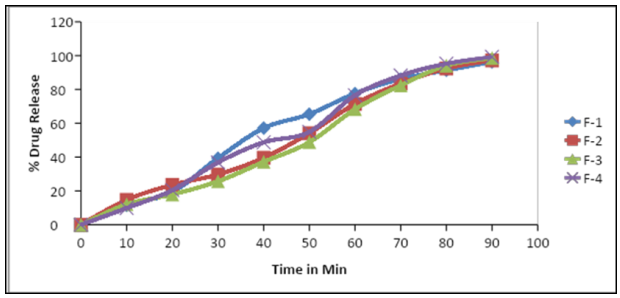

Figure 3: Dissolution profile for formulation F1-F4

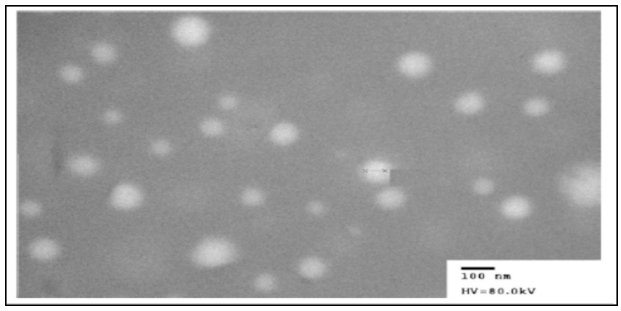

Figure 4: TEM image of formulation F6

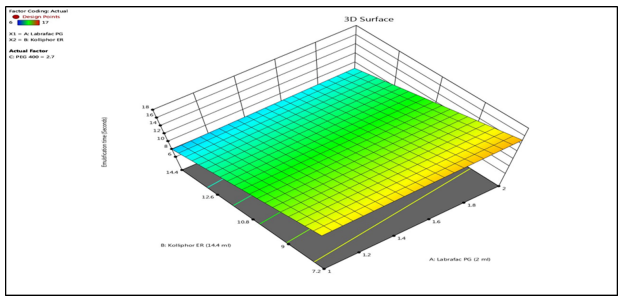

Figure 5: 3D graph for emulsification time

Table 4: Result of refractive index of blank and drug loaded Orlistat SNEDDS

S.No.	Formulations	Refractive Index	
		Blank	Drug-Loaded SNEDDS
1	PF1	1.427 ± 0.011	1.416 ± 0.014
2	PF2	1.382 ± 0.013	1.39 ± 0.018
3	PF3	1.443 ± 0.010	1.466 ± 0.012
4	PF4	1.39 ± 0.014	1.42 ± 0.017

Table 5: Table showing % Transmittance, Emulsification time and grade of emulsion formed

S.No	Formulation	% Transmittance	Emulsification time	Grade of the Formed Emulsion
1	PF1	90.15 ± 1.3 %	11 ± 1.3 s	A
2	PF2	86.73 ± 1.4 %	17± 1.5 s	B
3	PF3	89.42 ± 1.3 %	14 ± 1.2 s	B
4	PF 4	91.58 ± 1.4 %	6 ± 1.4 s	A

Table 6: Emulsion droplet size Poly dispersibility index and Determination of Zeta Potential

S.No.	Parameter	Liquid SNEDDS	Solid SNEDDS
1	Globule Size	65.3 ± 7.5 nm	108 ± 11 nm
2	Zeta Potential	-31.11 mV	-28.4 Mv
3	PDI	0.51 ± 0.02	0.243 ± 0.03

Table 7: Determination of pH and Effects of pH and Dilution Ratio (Robustness to dilution)

Formu	Water			0.1 N HCl			Buffer pH 7.4		
	50 fold	100 fold	1000 fold	50 fold	100 fold	1000 fold	50 fold	100 fold	1000 fold
PF1	Turbid	Turbid	Slightly turbid	Turbid	Turbid	slightly turbid	Turbid	Turbid	Slightly turbid
PF2	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
PF3	Turbid	Turbid	Slightly turbid	Turbid	Turbid	Slightly turbid	Turbid	Turbid	Slightly turbid
PF4	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear

Table 8: Observation of Centrifugation, Heat-Cool Cycle and Freeze-Thaw Cycle

S.No.	Replicatic	Centrifugation	Heat-Cool Cycle	Freeze-Thaw Cycle
1	1	No phase separation	No phase separation	No phase separation
2	2	No phase separation	No phase separation	No phase separation
3	3	No phase separation	No phase separation	No phase separation

Table 9: Cumulative % Drug Release for All Batches

S. No.	Time	PF1	PF2	PF3	PF4
1	0	00	00	00	00
2	10	11.4	14.7	12.1	9.85
3	20	19.3	23.6	17.9	20.44
4	30	39.4	29.7	25.7	36.85
5	40	57.1	39.6	37.2	48.72
6	50	65.4	54.1	48.8	54.88
7	60	77.6	71.4	68.3	76.5
8	70	86.2	83.5	82.4	88.21
9	80	91.3	92.4	94.1	95.22
10	90	96.78	96.77	95.75	98.82

Table 10: Observations for % Transmittance, Emulsification time and grade of emulsion formed for factorial batches

S.No.	Formulation	% Transmittance	Emulsificati	Grade of the Formed Emulsion
1	F 1	85.63± 1.3 %	13± 1.5 s	A
2	F 2	82.21± 1.7 %	16± 1.2 s	B
3	F 3	85.23± 1.1 %	11± 1.4 s	A
4	F 4	88.49± 1.6 %	10± 1.1 s	A
5	F5	78.52± 1.6 %	17± 1.2 s	B
6	F6	94.01± 1.5 %	6± 1 s	A
7	F7	90.46± 1.4 %	7± 1.3 s	A
8	F8	83.63± 1.0 %	13± 1.4 s	A

Table 11: Emulsion droplet size Poly dispersibility index and Determination of Zeta Potential for factorial batches

S.No.	Parameter	Liquid SNEDDS	Solid SNEDDS on reconstitution
1	Globule Size	58.2 ± 2.4 nm	99 ± 6 nm
2	Zeta Potential	-28.12 mV	-24.5 mV
3	PDI	0.47 ± 0.01	0.211 ± 0.02

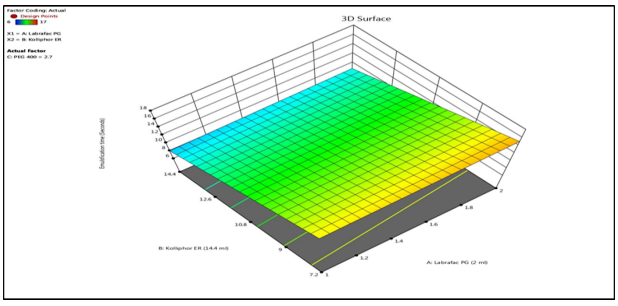

Figure 6: 3D graph for emulsification time

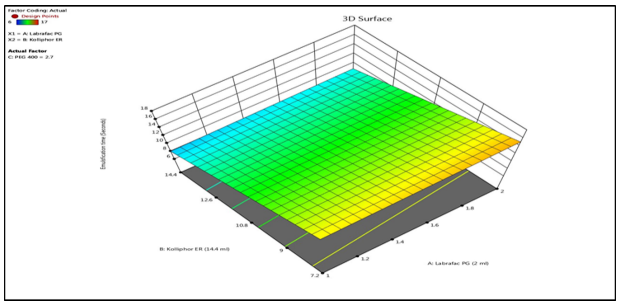

Figure 7: 3D graph for % Transmittance

Assessment of emulsification time and % transmittance

As shown in Table 5, the shortest emulsification time was attained in case of formulation PF4 (6 ± 1.4 s) followed by PF1 (11 ± 1.3 s); they rapidly formed grade A nanoemulsions upon dilution. On the other hand, PF2 and PF3 showed a lower emulsification

Table 12: Determination of pH and Effects of pH and Dilution Ratio (Robustness to dilution) for factorial batches

Formulat	Water			0.1 N HCl			Buffer pH 7.4		
	50 fold	100 fold	1000 fold	50 fold	100 fold	1000 fold	50 fold	100 fold	1000 fold
F 1	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
F 2	Turbid	Turbid	Slightly turbid	Turbid	Turbid	Slightly turbid	Turbid	Turbid	Slightly turbid
F 3	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
F 4	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
F 5	Turbid	Turbid	Slightly turbid	Turbid	Turbid	Slightly turbid	Turbid	Turbid	Slightly turbid
F 6	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
F 7	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
F 8	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear

Table 13: Observation of Centrifugation, Heat-Cool Cycle and Freeze-Thaw Cycle for factorial batches

S.No.	Replication	Centrifugation	Heat-Cool Cycle	Freeze-Thaw Cycle
1	1	No phase separation	No phase separation	No phase separation
2	2	No phase separation	No phase separation	No phase separation
3	3	No phase separation	No phase separation	No phase separation

Table 14: Cumulative % Drug Release For All Batches

S. No.	Time	F1	F2	F3	F4	F5	F6	F7	F8
1	0	00	00	00	00	00	00	00	00
2	10	9.5	11.5	12.04	9.85	12.8	9.85	11.4	7.4
3	20	17.2	21.7	17.28	20.44	19.4	20.44	25.6	19.3
4	30	35.38	25.6	21.69	36.85	39.7	36.85	42.2	26.2
5	40	51.85	37.5	35.84	48.72	51.3	48.72	54.6	39.4
6	50	62.57	52.34	51.33	54.88	65.4	54.88	64.2	51.3
7	60	71.27	69.26	69.55	76.5	78.6	76.5	77.6	63.5
8	70	85.4	80.23	80.43	88.21	83.4	88.21	88.4	82.4
9	80	92.10	90.1	89.5	95.22	87.59	95.22	91.25	88.68
10	90	96.78	96.77	95.75	98.82	93.44	99.25	95.77	93.31

Based on all the above evaluation parameters it was concluded that formulation F-6 showed optimum results and hence same batch was considered for Tem Analysis, drug release kinetic study, Stability study and In-vivo inhibition of fat absorption in rats

Table 15: Model Fitting (Average)

Kinetic Model	R	k
Zero order	0.9937	1.1797
T-test	24.999	(Passes)
First order	0.8626	-0.0353
T-test	4.822	(Passes)
Matrix	0.9275	9.2104
T-test	7.017	(Passes)
Peppas	0.9959	0.8386
T-test	31.182	(Passes)
T-test	31.182	(Passes)
Hixson-Crowell	0.9494	-0.0073
T-test	8.546	(Passes)

Table 16: Best Model Fit and n value

Best fit model- Peppas Parameters for Korsmeyer - Peppas Equation	
n =	1.0845
k =	0.8386

Table 17: Analysis of variance table for Emulsification time [Partial sum of squares - Type III]

Cor Total Source	107.88 Sum of Squares	7 Df*	Mean Square	F-value	p-value	
Model	107.38	3	35.79	286.33	< 0.0001	Significant
A-Labrafac PG	1.13	1	1.13	9.00	0.0399	
B-Kolliphor ER	78.13	1	78.13	625.00	< 0.0001	
C-PEG 400	28.13	1	28.13	225.00	0.0001	
Residual	0.5000	4	0.1250			

*Degrees of freedom

Table 18: Analysis of variance table for % Transmittance [Partial sum of squares - Type III]

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Observation
Model	165.25	3	55.08	147.07	0.0002	significant
A-Labrafac PG	19.50	1	19.50	52.06	0.0020	
B-Kolliphor ER	99.33	1	99.33	265.22	< 0.0001	
C-PEG 400	46.42	1	46.42	123.93	0.0004	
Residual	1.50	4	0.3745			
Cor Total	166.75	7				

Table 19: Analysis of variance table for % Drug Release [Partial sum of squares - Type III]

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Observation
Model	34.90	3	11.63	200.77	< 0.0001	Significant
A-Labrafac PG	23.32	1	23.32	402.58	< 0.0001	
B-Kolliphor ER	11.52	1	11.52	198.83	0.0001	
C-PEG 400	0.0512	1	0.0512	0.8837	0.4004	
Residual	0.2318	4	0.0579			
Cor Total	35.13	7				

time (17± 1.5 s and 14 ± 1.2 s) forming a slightly translucent (grade B) nanoemulsion. All the tested SNEDDS showed a high % transmittance. However, the formulation PF1 and PF4 showed more excellent % transmittance 90.15 ± 1.3 % and 91.58 ± 1.4 % respectively.

Drug Loading Efficiency

The drug loading efficiency for all Orlistat SNEDDS formulae was found in the range of 92.37% ± 0.75% (PF1) to 99.09% ± 0.56% (PF4), indicating uniform drug dispersion in formulae. It was observed that formula PF4 have the highest drug content.

TEM analysis

The surface morphology of all the SNEDDS formulations was observed using TEM after 24 h of post-dilution with water. The images are shown in Figure 2. The diluted preparation (nanoemulsion) possesses spherical shape oil globules with no sign of coalescence. The photographs also reveal the formation of stable nanoemulsion as there is no sign of drug precipitation even after 24 h post-dilution.

Emulsion droplet size Poly dispersibility index and Determination of Zeta Potential

The globule size was determined by using dynamic light scattering technique using particle size analyzer (Nanotrac R-150 ULTRA, Microtrac Inc.), and the samples were prepared by adding the freeze-dried powder to 50 ml of the distilled water in a beaker by using a magnetic stirrer. The Polydispersity Index (PDI) of liquid SNEDDS Orlistat was found to be 0.51 ± 0.02and 0.243 ± 0.03, respectively, which indicated narrow size distribution. Globule size of freeze-dried SNEDDS was found to be 108 ± 11 nm. Zeta potential for the liquid SNEDDS and self-emulsifying Orlistat on reconstitution was found to be -31.11 mV and -28.4 mV, respectively Table 6.

Determination of pH and Effects of pH and Dilution Ratio (Robustness to dilution

As explained in Table 7, the inspected formulations that were composed of Sunflower oil PEG 400 and Tween 80 in formulation PF2 and Labrafac PG, Kolliphor ER and PEG 400 in formulation PF4 exhibited no signs of any precipitation, opacity or separation for 24 h which guaranteed the stability of the formed nanoemulsions. On the contrary, formulation PF1 and PF3 which was composed of Castor oil, Transcutol P and Polysorbate 80 and Olive oil, Caproic acid and PEG 90 respectively displayed a turbid appearance when diluted to 50 and 100 fold in case of distilled water, 0.1 N HCl, and phosphate buffer (pH 7.4) and a slightly turbid when diluted to 1000 fold using those media; therefore it was excluded from further characterization.

Centrifugation

The centrifugation test was conducted to assess the SNEDDS stability after an emulsion is formed, against the gravity force. The result of centrifugation that was shown in Table 15 indicated that no phase separation occurred during the test. Centrifugation describes the gravity force that occurs on the droplets. The small size of droplets can minimize the gravity force and Brownian motion on the particles that prevent the occurrence of phase separation.

Freeze-thaw cycle (accelerated ageing) and Heating-cooling cycle

Table 8 indicates that there was no phase separation occurred in the SNEDDS formula during the heating-cooling cycle and freeze-thaw cycle test.

Viscosity Measurement

The viscosity of the selected formulation was found to be 187.5±0 mPa.s. The inclusion of PEG 400 improved self-emulsification. The lower viscosity of

SNEDDS was mainly due to the smaller droplet size.

In vitro dissolution: for preliminary batches

The dissolution profiles of ORLISTAT SNEDDS (F1 TO F4) were illustrated in Fig. B and Table 9 F4 could successfully release 98.82% of Orlistat in 90 minutes while the % of Orlistat release from PF1, PF2 and PF3 were 96.78 %, 96.77% and 95.75 %, respectively.

Results for Factorial Batches

Assessment of emulsification time and % transmittance

Emulsification time was known as an excellent importance parameter to describe the stability of the system and prepare emulsification in a gastric fluid of SNEDDS self-emulsifying characteristics. Kolliphor ER as a surfactant was found to have good solubility and better emulsification ability that allowed rapid dispersion when in contact with biological fluids. PEG 400 commonly use in nanoemulsion formulation to increase solubility and bioavailability. The clarity of microemulsion was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w microemulsion since water is external phase Formulation F6 has % transmittance value (94.01%) than other formulations. These results indicate the high clarity of microemulsion. As shown in Table 10, the shortest emulsification time was attained in case of Kolliphor ER based formulations F6 (6± 1s) followed by F7 (7± 1.3s) all other formulations showed emulsification time in between 10± 1.1s to 17± 1.2 s they rapidly formed grade A nanoemulsion upon dilution except that of formulation F2 and F5 which may be due to less concentration of Kolliphor ER and PEG 400. All the tested SNEDDSs showed a high % transmittance except that of F5 due to less concentration of PEG 400. However, the Kolliphor ER based formulations showed a greater % transmittance (94.01±1.5 %).

Drug Loading Efficiency

The drug loading efficiency for all Orlistat SNEDDS formulae was found in the range of 95.87% ± 0.24% (F1) to 99.89% ± 0.56% (F6), indicating uniform drug dispersion in formulae**.**

Statistically, it was further justified that there was no significant difference in drug content among the various formulae. It was observed that formula F6 have the highest drug content.

This may be attributed due to a higher concentration of surfactant and co-surfactant in these two formulae that possess a high solubilizing capacity of Orlistat.

Emulsion droplet size Poly dispersibility index and Determination of Zeta Potential

The globule size was determined by using dynamic light scattering technique using particle size analyzer (Nanotrac R-150 ULTRA, Microtrac Inc.), and the samples were prepared by adding the freeze-dried powder to 50 ml of the distilled water in a beaker by using a magnetic stirrer. The Polydispersity Index (PDI) of liquid SNEDDS Orlistat was found to be 0.47 ± 0.01and 0.211 ± 0.02, respectively, which indicated narrow size distribution. Globule size of freeze-dried SNEDDS was found to be 99 ± 6 nm. Zeta potential for the liquid SNEDDS and self-emulsifying Orlistat on reconstitution was found to be -28.12 mV and -24.5 mV, respectively Table 11.

Determination of pH and Effects of pH and Dilution Ratio (Robustness to dilution)

As explained in Table 12, all the inspected formulations that were composed of PEG 400 and Kolliphor ER in formulations exhibited no signs of any precipitation, opacity or separation for 24 h which guaranteed the stability of the formed nanoemulsions. On the contrary, formulation F2 and F5 which displayed a turbid appearance when diluted to 50 and 100 fold in case of distilled water, 0.1 N HCl, and phosphate buffer (pH 7.4) and a slightly turbid when diluted to 1000 fold using those media; therefore it was excluded from further characterization.

Centrifugation

The centrifugation test is conducted to assess the SNEDDS stability after an emulsion is formed, against the gravity force. The result of centrifugation that was shown in Table 13 indicated that no phase separation occurred during the test.

Centrifugation describes the gravity force that occurs on the droplets. The small size of droplets can minimize the gravity force and Brownian motion on the particles that prevent the occurrence of phase separation.

Freeze-thaw cycle (accelerated ageing) and Heating-cooling cycle

Freeze-thaw cycle test is conducted to examine the effect of heating, cooling, and centrifugation against the stability of SNEDDS formula. An emulsion tends to be stable at the temperature of 40°C- 45°C in a few hours of storage.

Heating and freezing are potential to damage or break the droplets of an emulsion. Table 13 indicates that there was no phase separation occurred in the SNEDDS formula during the heating-cooling cycle and freeze-thaw cycle test. Overall, the stability of the formulation was found to be acceptable.

Viscosity Measurement

The viscosity of the selected formulation was found to be 132.4±0 mPa.s. The inclusion of PEG 400 improved self-emulsification. The lower viscosity of SNEDDS is mainly due to the smaller droplet size.

***In vitro* dissolution**

An in vitro dissolution test is useful to evaluate the performance of the product, especially the one that contains water-insoluble and lipophilic drugs. All the formulations in oral administration route expose to an acidic condition due to the secretion of HCl in the stomach. Thus, simulated gastric fluid of pH 1.2 was used as the priority dissolution medium for dissolution tests. As shown in Table 14, F-6 showed significantly faster dissolution curves and reached the mean values of 99.25% at pH 1.2 within 90 minutes. No significant differences in dissolution behaviours among all formulations were observed. F-6 and optimized formulation showed comparably good dissolution profiles as compared to all the formulations. This rapid dissolution at the simulated gastric fluid can contribute to the enhanced solubilization and lipase inhibition activity without any help of fat intake and gastric motility. However, due to the absence of detergent-like SLS in the medium, no significant detection was possible for raw Orlistat during the test time of 90 minutes. In several previous studies, the similar dissolution test setup was used, but SLS has been included as a dissolution enhancer for its low water solubility of Orlistat. In the present study, the suggested formulations such as F-6 provided sufficient solubilization and rapid dissolution in an aqueous medium without the support of SLS.

TEM analysis

The surface morphology of the optimized SNEDDS formulations F6 was observed using TEM after 24 h of post-dilution with water. The images are shown in Figure 4. The diluted preparation (nanoemulsion) possesses spherical shape oil globules with no sign of coalescence. The photographs also reveal the formation of stable nanoemulsion as there is no sign of drug precipitation even after 24 h post-dilution. Furthermore, the globule size seen in the microscope was almost consistent with that obtained in the globule size analysis.

Drug release Kinetic Study

A kinetic parameter can be used to study the influence of formulation factors on the drug release for statistical optimization. The drug release kinetics was studied by plotting the data obtained from the *in-vitro* drug release in various kinetic models. To establish the mechanism involved in drug release from the tablets, data of percentage drug release versus log time were plotted according to Korsmeyer–Peppas equation as drug release exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight line was found to be n= 1.083. If the exponent n= 0.45, then the drug release follows the Fickian diffusion and if 0.45< n <0.85 then it is said to be non-Fickian or anomalous release. The mechanism of release for the above formulations was determined by finding the R^2 value for each kinetic model *viz.* zero-order, first-order, Higuchi, Hixson-Crowell and Korsmeyer–Peppas corresponding to the release data of each formulation. From most of the formulations, the R^2 value of Korsmeyer–Peppas model is very near to one than the R^2values of other kinetic models. Thus, it can be said that the drug release follows Korsmeyer–Peppas model mechanism, out of which the R^2=0.9959offormulationF-6wasfoundbestamongst other formulations and n value were found n = 1.083 hence it can be postulated that formulation F-6 followed non- Fickian or anomalous release. The results are shown in Tables 15 and 16.

Response Surface Methodology

The linear model obtained from the regression analysis was used to build a 3-D graph in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots presented in Figures 5, 6 and 7. Three dimensional (3-D) surface plots for the obtained responses were drawn based on the model polynomial functions to assess the change of the response surface. These plots explain the relationship between the dependent and independent variables, *i.e.,* the effects of three factors on the response at one time. The response surface analysis for Y1: Emulsification time, Y2: % Transmittance and Y3: % Drug Release was studied, which showed significant results.

The Model F-value of 286.33, 147.07 and 200.77 for Y1: Emulsification time, Y2: % Transmittance and Y3: % Drug Release implies the model is significant. Values of "P" less than 0.0500 indicate model terms are significant.

Response 1: Emulsification time

ANOVA for Response Surface Linear model Emulsification time.

Response 2: % Transmittance

ANOVA for Response Surface Linear model % Transmittance.

Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable.

Response 3: % Drug Release

ANOVA for Response Surface Linear model % Drug Release.

The "Predicted R-Squared" of 0.9954 for Emulsification Time, 0.9910 for % Transmittance and 0.9934 for % Drug release is in reasonable agreement with the "Adjusted R-Squared" of 0.9919 for Emulsification Time, 0.9843 for % Transmittance and 0.9885 for % Drug release. The probability value, *i.e.,* P-value found, was also less than 0.0500. The Predicted R^2 for Emulsification Time, % Transmittance and % Drug release is in reasonable agreement with the Adjusted R^2, i.e., the difference is less than 0.2. This model can be used to develop the design. The values are shown in Tables 17, 18 and 19.

The calculation for effect of formulation variables on Emulsification Time, % Transmittance and % Drug release.

1. Final Equation in Terms of Coded Factors: Emulsification time = +11.63+0.3750 A-3.13 B-1.88C
2. Final Equation in Terms of Coded Factors: % Transmittance = +86.02 1.56A+3.52B+2.41C
3. Final Equation in Terms of Coded Factors: % Drug Release = +96.28 1.71A+1.20B+0.0800 C

From the equation for % Transmittance, % Drug Release, and Emulsification time, the information conveyed was:

1. R2 was high, indicating the adequate fitting of the Linear Model.
2. As (positive coefficient) showed a positive sign, it also indicated that formulation F6 showed good % Transmittance, % Drug Release and Emulsification time.
3. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Stability Study

Stability study was done to see the effect of temperature and humidity on tablets. Stability data Storage conditions are below

1. The accelerated temperature of 40°C ± 2°C.
2. The accelerated temperature at 75% RH ± 5%.

The Orlistat SNEDDS were put into hard gelatin capsules as the final dosage form. The formulation (F6) was subjected to stability studies for three months. There was no significant change in the drug content, emulsion droplet size, PDI, and in vitro release studies.

It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There was no significant change in the appearance of the micro emulsifying property.

CONCLUSION

In the present study, we have successfully developed liquid SNEDDS for lipase inhibitor Orlistat for enhancement of oral solubility and bioavailability & dispense them in capsule dosage form for oral delivery. SNEDDS of Orlistat was prepared and optimized by using various parameters like droplet size, PDI, zeta potential, *in-vitro* release data. Based on the ternary phase diagram, we have selected Labrafac, Kolliphor ER and PEG 400 as oil, surfactant and co-surfactant respectively. From the result of evaluation parameters such as emulsification time 6±1s, % transmittance 94.01 ± 1.5%, drug Loading 99.89% ± 0.56%, Polydispersity Index 0.47 ± 0.01 and 0.211 ± 0.02, Globule size 99±6 nm, Zeta potential -28.12 mV and -24.5 mV, The centrifugation, Freeze-thaw cycle, Heating-cooling cycle showed no signs of phase separation, Viscosity 132.4 ± 0 mPa.s, drug release 99.25% within 90 minutes, drug release follows Korsmeyer–Peppas model mechanism, n value was found n=1.083 hence it can be postulated formulation F-6 followed the non-Fickian or anomalous release and P-value for factors emulsification time, % transmittance and % drug release was found less than 0.0500, and hence it was concluded formulation F-6 an optimized formulation. Response surface methodology factorial design method is best suitable for this study. Thus our studies exemplified the excellent use of the self-micro emulsified drug delivery system to dispense poorly water-soluble drugs by the oral route.

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Conflict of Interest

The authors confirm that there is no conflict of interest for this publication.

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