



Research Article

DESIGN AND OPTIMIZATION OF NOVEL SELF NANOEMULSIFYING FORMULATION OF ETODOLAC

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ABSTRACT

The objective of the study is to develop and optimize self nanoemulsifying drug delivery systems (SNEDDS) of Etodolac. Among the oils and surfactants studied, Phosal 53 MCT, Labrasol and PEG 400 were selected as they showed maximal solubility to Etodolac. The ternary phase diagram was constructed to find out the region forming Microemulsion. The optimized liquid SNEDDS formulation consisted of Phosal 53 MCT, Labrasol and PEG 400 as oil, surfactant and cosurfactant. Self emulsification time, % Transmittance and Relative turbidity were used as variables for optimizing the Liquid SNEDDS formulation based on Box Behnken Design. L-SNEDDS formulation was evaluated for various studies including globule size analysis, TEM and in vitro dissolution study. The results suggested that the Self emulsifying formulation can enhance the dissolution of poorly soluble drug and has a potential to enhance drug absorption and improve bioavailability of drug.

KEY WORDS: Self-emulsifying formulation, Water insoluble drug, Etodolac, Drug dissolution

INTRODUCTION

Etodolac is a selective COX-2 inhibitor type of non-steroidal anti-inflammatory drug (NSAID) used to treat various inflammatory conditions like acute pain, rheumatoid arthritis and osteoarthritis. Oral dose of etodolac is 200mg twice daily¹⁻⁴. Etodolac is safe for treating inflammatory disorders without causing gastric irritation, ulceration, or bleeding as it produces prostaglandin which is involved in cytoprotection of gastric mucosa and regulation of the renal blood flow^{3, 6}.

Etodolac belongs to BCS class II drugs with water solubility of 75mcg/ml and is practically water insoluble drug. It is having poor oral bioavailability may be because of poor solubility in water and insufficient dissolution rate. Etodolac is highly bound to plasma protein primarily to albumin (>99% bound) and undergoes virtually complete biotransformation. The metabolized product of etodolac is oxidized metabolites and acyl-glucuronides. It is well absorbed and attains maximal plasma concentrations within 1 to 2 hours in healthy volunteers with elimination half-life of 6 to 8 hours⁷⁻⁹.

Various methods for improving drug solubility and dissolution rate have been reported in literature such as particle size reduction, solid dispersion, inclusion complex formation, nanosuspension, Prodrug approach, lipid-based formulation, and self-emulsifying drug delivery system¹⁰.

Among these approaches, self emulsifying drug delivery system (SEDDS) is found to be a most promising approach to improve solubility, drug dissolution and intern Bioavailability of Drug. SEDDS is a thermodynamically stable oil-in-water emulsions formulated using isotropic mixture of oil, surfactant, cosurfactant and drug. When such SEDDS formulation added in water spontaneously forms microemulsion under gentle agitation conditions^{11, 12}. Formed microemulsion increases drug release and absorption by its surface property¹³⁻¹⁷.

Components of SMEDDS and their concentrations affect droplet size, emulsification efficiency and release property of formed microemulsion. SMEDDS are prepared by trial-and-error basis to get good region forming microemulsion. However this is time-consuming and requires a larger number of trials¹⁸.

Box Behnken Design of experiment was used as a qbd approach to optimize etodolac SNEDDS formulation. In Box Behnken Design the points are taken at the edges of boundary forming Microemulsion region and at center containing three factor experimental designs. It is preferred design as it requires fewer runs with three factor and is an independent quadratic design (contains no embedded factorial or fractional factorial design)¹⁹⁻²¹.

The objective of this study was to increase dissolution rate of water insoluble drug Etodolac by development of SNEDDS formulation. Further the interaction effect of component on variables to be studied by Box Behnken Design.

MATERIALS AND METHODS

Materials

Etodolac was obtained as gift sample from IPCA Labs, Mumbai, India, Phosal 53 MCT gifted from Lipoid, Germany, Labrasol was gifted from Gattefosse, Mumbai, India. Polythelene Glycol 400 was purchased from SD Fine, Mumbai, India. All other ingredients used were of analytical grade.

Solubility studies

Solubility studies were conducted by adding an excess amount of etodolac in a vial containing 2 ml of oils, surfactants or co-surfactant each. The mixtures were then vortex mixed, sonicated and kept at equilibrium for 72 h at 37 °C using Orbital Shaker Incubator (Labline, Mumbai, India). The mixtures were centrifuged using micro centrifuge (Bioera, Mumbai, India) at 10000 rpm for 15 min. The separated supernatant fraction was

suitably diluted with methanol and analyzed for drug concentration spectrophotometrically at $\lambda_{\max}$ 280 nm using UV-Visible spectrophotometer (LabIndia).

Construction of ternary phase diagram

A ternary phase diagram was plotted for the selected oil (Phosal 53 MCT), surfactant (Labrasol) and co-surfactant (PEG 400) Based on the results of solubility study. A series of formulations were prepared using various concentrations 10 to 60% of oil, 10 to 80 % surfactant and 10 to 80 % cosurfactant. This mixture was mixed on vortex mixture and sonicated to get clear homogeneous liquid. Each sample (100 mg) was then diluted with 100 ml of purified water and self-emulsifying performance was assessed. It was kept for observation to find any precipitation or gelling property. Such samples were rejected. Finally phase diagram were plotted and appropriate percentage of oil, surfactant and cosurfactant were selected for preparation of SNEDDS formulations containing Etodolac.

Formulation and optimization of SNEDDS using Box-Behnken design

Three factors, three level and 13 runs Box-Behnken Experimental Design using Design Expert 10.0.2.0 software (State- Ease Inc. Minneapolis, USA) was employed to optimize liquid SEDDS of etodolac. The concentration of Phosal 53 MCT (X1), Labrasol (X2) and PEG 400 (X3) were selected as independent variables, while Self emulsification time in sec (Y1), % Transmittance (Y2) and Relative Turbidity (Y3) were selected as responses as shown in Table 1. Response surface analysis was carried out to study the effect of different independent variables on the responses under study.

The liquid mixture was prepared by using specified amount of oil, surfactant and co-surfactant and stirred continuously to get homogenous liquid. Weighed quantity of Etodolac (100 mg) was dissolved in this mixture. The prepared liquid SNEDDS were stored in transparent glass bottles at room temperature until used. The formulations were recorded for any changes in turbidity or

phase separation and evaluated for Globule size and Zeta potential.

Evaluation of SNEDDS formulations

Determination of self-emulsification time

Each formulation (500 mg) was added dropwise to 500 mL of purified water in USP dissolution type II apparatus (Labindia) at temperature of 37 ± 0.5 °C with rotational speed of 50 RPM. Self Emulsification time was assessed visually and determined.

% Transmittance

Percent transmittance of Etodolac loaded SNEDDS formulations was measured using UV-visible spectrophotometer at $\lambda_{\max}$ 638.2 nm by diluting the L-SEDDS 100 times with purified water using purified water as blank.

Relative Turbidity

Relative Turbidity of Zaltoprofen loaded SNEDDS formulations were measured using Digital Nepheloturbidimeter by diluting the L-SNEDDS 100 times with purified water.

Globule size and Zeta Potential

The globule size and zeta potential of SNEDDS was determined using a photon correlation spectrometer (Zetasizer, Malvern Instruments, UK) based on laser light scattering phenomenon. SNEDDS samples, diluted 100 times with purified water and globule size Analysis were performed

Transmission electron microscopy

The morphology of the formed microemulsion was determined using transmission electron microscopy (TEM) (Jeol/JEM 2100). A drop of diluted liquid SNEDDS spread then observed using TEM.

Table 1: Variables in Box Behnken Experimental Design

Independent Variables	Levels		
	Low (-1)	Middle (0)	High (+1)
X ₁ : Amount of Oil (mg)	10	25	40
X ₂ : Amount of Surfactant (mg)	40	60	80
X ₃ : Amount of Cosurfactant (mg)	10	20	30

Table 2: Box-Behnken experimental design with measured responses

Std	Run	Factor 1 A:Oil	Factor 2 B:Surfactant	Factor 3 C:Cosurfactant	Response 1 Self emulsification Time	Response 2 % Transmittance	Response 3 Relative Turbidity
		mg	mg	mg	Sec	%	NTU
4	1	40	80	20	90.91	89.2	9.4
6	2	40	60	10	132.4	84.9	11.2
5	3	10	60	10	45.2	98.9	5.8
2	4	40	40	20	118.9	81.6	12.1
12	5	25	80	30	43.2	97.6	6.7
1	6	10	40	20	33.2	98.9	6.1
13	7	25	60	20	57.1	97.2	6.8
7	8	10	60	30	26.21	99.1	4.2
8	9	40	60	30	57.1	86.4	10.8
3	10	10	80	20	27.1	99.5	4.3
11	11	25	40	30	63.14	96.2	7.3
9	12	25	40	10	81.8	95.3	7.6
10	13	25	80	10	75.61	97.3	6.8

Table 3: Results of ANOVA

Source	df	Y 1				Y 2			
		Sum of Squares	Mean Square	F Value	p-value	Sum of Squares	Mean Square	F Value	p-value
Model	9	13093.54	1454.837	11.96352	0.032712	459.2567	51.02853	50.81679	0.004027
A-Oil	1	8951.22	8951.22	73.60831	0.003328	368.5613	368.5613	367.032	0.000311
B-Surfactant	1	453.3061	453.3061	3.727659	0.149062	16.82	16.82	16.75021	0.026374
C-Cosurfactant	1	2641.191	2641.191	21.71923	0.018643	1.05125	1.05125	1.046888	0.38153
AB	1	119.793	119.793	0.985091	0.394108	12.25	12.25	12.19917	0.039689
BC	1	792.704	792.704	6.518621	0.083712	0.4225	0.4225	0.420747	0.562791
A^2	1	47.26563	47.26563	0.388678	0.57718	0.09	0.09	0.089627	0.784195
B^2	1	53.95989	53.95989	0.443727	0.552975	48.10321	48.10321	47.90362	0.006183
C^2	1	70.88223	70.88223	0.582884	0.500733	0.223214	0.223214	0.222288	0.66947
Residual	3	24.42223	24.42223	0.200831	0.684438	0.188929	0.188929	0.188145	0.693765
Cor Total	12	364.8183	121.6061			3.0125	1.004167		

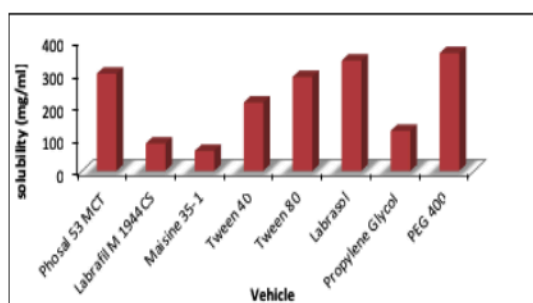


Fig. 1: Solubility of etodolac in different Vehicles

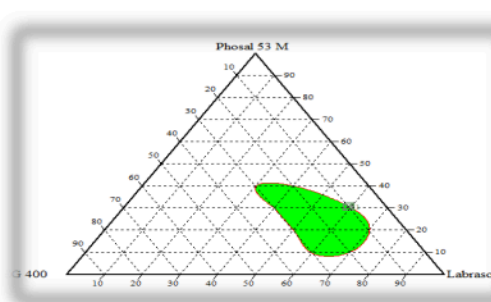
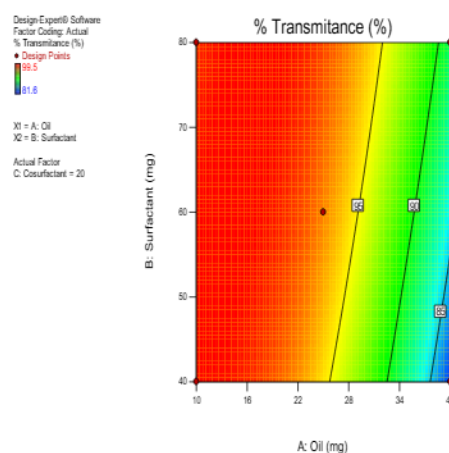
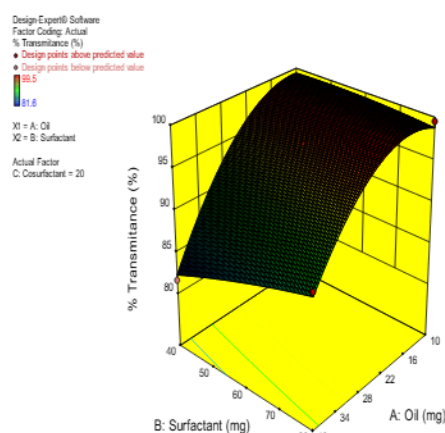
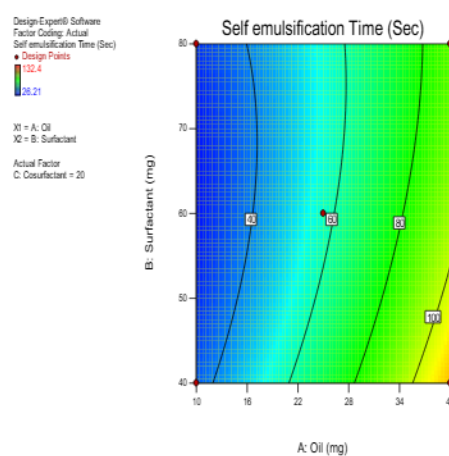
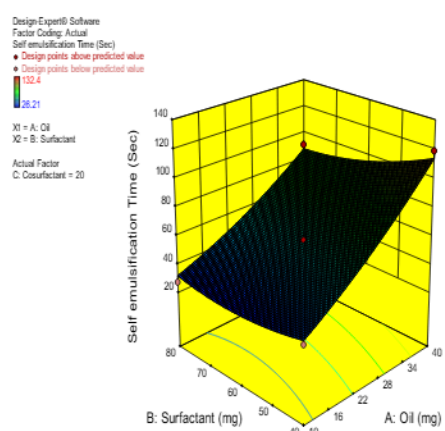


Fig. 2: Ternary phase diagram



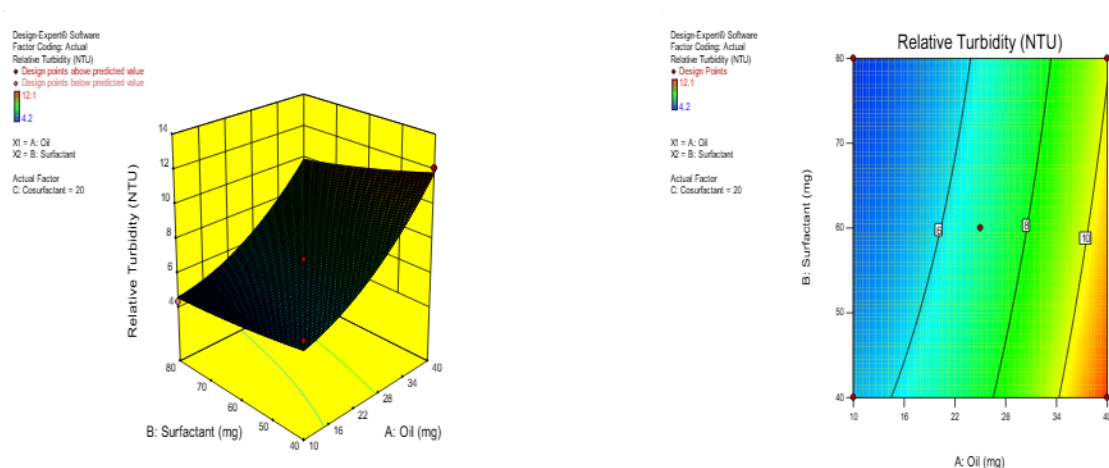


Fig. 3: 3D plot and Response Surface plot (a) Self Emulsification Time (b) % Transmittance (c) Relative Turbidity

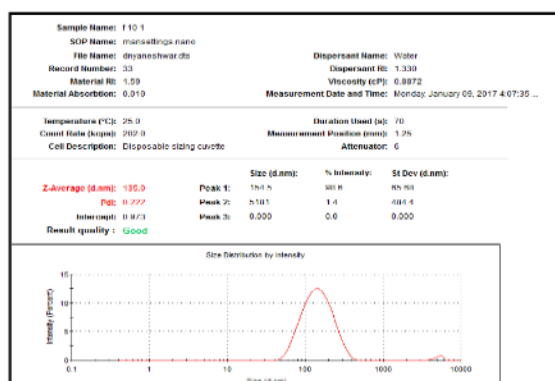


Fig. 4: Globule size of L-SNEDDS

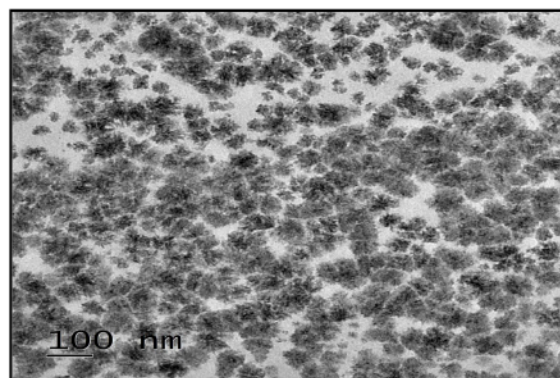


Fig. 5: TEM of Etodolac L-SNEDDS

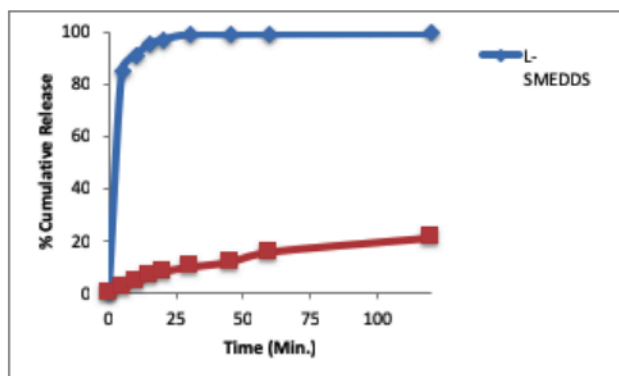


Fig. 6: Dissolution rate of Etodolac (a) 0.1 M HCl pH 1.2 (b) Phosphate buffer pH 6.8

In vitro dissolution studies

In vitro dissolution studies of Etodolac L-SNEDDS and pure drug powder were performed using USP dissolution apparatus II (Labindia) with a paddle rotation speed of 50 RPM maintained at $37 \pm 0.5^\circ\text{C}$ in 900 ml dissolution medium. Dissolution medium employed were 0.1 M HCl pH 1.2 and phosphate buffer pH 6.8. L-SNEDDS equivalent of 100 mg Etodolac were used for dissolution studies; results were compared with same quantity of pure Etodolac filled in capsules.

Samples were withdrawn at predetermined time intervals and replaced with fresh dissolution medium. The amount of Etodolac dissolved in the dissolution medium was determined by UV visible spectrophotometer at λ_{max} 278 nm. Results are averaged from three replicated experiments.

RESULTS AND DISCUSSION

Drug solubility determination

The solubility of Etodolac in various vehicles is presented in Fig. 1. Etodolac showed high solubility in Phosal 53 MCT (298.12 ± 1.38 mg/g) as compared to other oil. Labrasol showed maximum drug solubilization (338.26 ± 2.45 mg/g). Among the cosurfactant, PEG 400 exhibited highest capacity to dissolve etodolac with 361.42 ± 1.67 mg/g and thus appears to be good cosolvent for this drug. The excipients with high solubility for Etodolac were used to construct the ternary phase diagrams.

Construction of ternary phase diagrams

To ensure the spontaneous formation of microemulsion within the gastrointestinal conditions, the construction of ternary plots is considered to be important task. It is used to obtain optimal ratio of components in the areas forming microemulsion. A ternary phase diagram of SEDDS was prepared by varying the concentration of Phosal 53MCT as oil, Labrasol as surfactant, and PEG 400 as cosurfactant and is indicated in Fig. 2. The purified water was used as diluting medium. The shaded region indicates stable microemulsion region. Systems containing more than 40% oil phase were found to be out of microemulsion region, signifying the importance of surfactant for microemulsification. The systems containing higher oil content requires higher concentrations of surfactant to produce stable transparent emulsion.

Optimization of SNEDDS

Formulation of Etodolac SNEDDS was optimized by Box-Behnken experimental design with 3 independent variables at 3 different levels, to study the interaction effect of independent variables on responses under study. A total of 13 formulations were prepared and evaluated for responses like self emulsification time and % Transmittance and Relative turbidity as shown in Table 2. For all the 13 batches dependent variables, self emulsification time (Y_1), % Transmittance (Y_2) and Relative turbidity (Y_3) demonstrated wide variations from 27.1 to 132.4 S, 81.6 to 99.5% and 4.2 to 12.1 NTU respectively indicating good influence of independent variables (X_1 , X_2 and X_3) on the selected responses.

The response surface plots were shown in Fig. 3 which describes the relationship between the dependent and independent variables. The coefficients of second order polynomial equation generated using multi linear regression analysis for self emulsification, % Transmittance and Relative turbidity to establish mathematical relationship which was found to be quadratic in nature. The coefficients of the polynomials fitted well to the data, with the values of R^2 , 0.9728, 0.9934 and 0.9788 for Y_1 , Y_2 and Y_3 respectively.

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

Where, Y is the response of the dependent variables; β_0 – β_9 are the regression coefficients; and X_1 , X_2 , X_3 are independent variables.

As shown in Fig. 3 surface plots it was concluded that with increase in the concentration of oil (Phosal 53 MCT) and co-surfactant (PEG 400) the Self emulsification time increases whereas with increase in surfactant (Labrasol) and decrease in oil concentration (Phosal 53 MCT) the % Transmittance increases. All the response surfaces were fitted with quadratic polynomial models. The ANOVA results are shown in Table 3.

Using software optimization process and overlay plot from the software, level selected for X_1 , X_2 and X_3 were 22.04, 41.50 and 29.99 respectively, which gives theoretical values of 52.48 Sec, 97.21 % and 6.78 NTU for self emulsification time, % transmittance and Relative turbidity respectively. Fresh formulation was prepared using the optimum levels of independent variables. The observed values of self emulsification time and % transmittance were found to be 55.12 Sec, 96.1 % and 6.1 NTU respectively, which were in close agreement with the theoretical values.

Evaluation of SNEDDS formulations

Determination of self-emulsification time

Rate of self emulsification is important in SMEDDS as formulation should disperse completely and spontaneously form microemulsion when added to water under mild agitation. Developed formulation of SNEDDS formulation shown good emulsification within 120 s.

% Transmittance

A high value of transmittance is indicative of optical clarity (transparent system). Optimized SNEDDS formulations showed % transmittance values above 95%, confirming the microemulsification efficiency of the SEDDS.

Relative Turbidity

Low value of turbidity indicates good optical clarity. For optimized L- SNEDDS relative turbidity was found to be 6.78 ± 0.1 NTU confirming the transparency of formed microemulsion.

Globule size and Zeta potential

Emulsion globule size is considered to be a decisive factor in self-emulsification performance because it determines the rate and extent of drug release and absorption. Results show that the prepared optimized SNEDDS had globule size of 135 nm (Fig. 4). The presence of zeta potential to the tune of -25.0 mV on the globules conferred physical stability to the system.

Transmission electron microscopy

TEM image of diluted SNEDDS is shown in Fig. 5. The microemulsion droplets were appeared to be spherical with a dark background

In vitro dissolution studies

The dissolution profile of Etodolac from S-SNEDDS in 0.1 M HCl pH 1.2 and phosphate buffer pH 6.8 was compared with L-SNEDDS and the drug powder (Fig. 6). S-SNEDDS released more than 90% of drug in 20 min in both 0.1 M HCl pH 1.2 and phosphate buffer pH 6.8 media, while the pure drug showed only 8.26% and 21.36% dissolution respectively. It was observed that changes in the dissolution medium had no effect on the drug release from S-SMEDDS formulation whereas with pure Etodolac, the dissolution was faster in Phosphate buffer pH 6.8 as compared to that in 0.1M HCl. The L-SNEDDS gave dissolution above 90% in 30 min without pH influence and was significantly higher than the pure drug. This shows that the total Etodolac in L-SNEDDS could dissolve and consequently be absorbed more rapidly and completely than the pure drug in the stomach or intestine. Thus, SSEDSS was useful for improving the dissolution rate of the poorly water-soluble etodolac.

CONCLUSION

SNEDDS formulation of Etodolac using phosphatidylcholine (Phosal 53 MCT), Labrasol and PEG 400 as oil, surfactant and cosurfactant respectively was successfully developed Ternary phase diagram was plotted and region forming microemulsion was identified. Box Behnken experimental design has shown the good influence of selected independent variables on responses under study. In vitro dissolution study demonstrated that Drug dissolution rate were efficiently enhanced with developed L-SNEDDS formulation as compared to pure drug. However further study in animal need to be carried out to confirm improved therapeutic efficacy. The results of this study suggest the potential

use of developed SNEDDS formulation for the delivery of poorly water-soluble drug Etodolac.

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