Original Article

DOE Optimized Self-Nanoemulsifying Drug Delivery System (SNEDDS) Based Cilnidipine Formulations For Bioavailability Augmentation: Physical Characterization And Pharmacodynamic Assessment

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Abstract

Background: Cilinidipine (CIL), an antihypertensive agent in the BCS class II category, has poor bioavailability constraints owing to limited aqueous solubility. Self-nano emulsifying drug delivery system (SNEDDS) provides excellent solubilizing capacity to poorly water dissolvable drugs since its ternary constituents possess traits like solubilisation/ nanonization activity led by surfactant/cosolvent and. SNEDDS possesses blends of surfactants and cosolvents to inherit its dispersion behaviour in nanodroplet forms.

Objective: The present investigation aims to develop a DOE-optimized lipid-based system. For enhancement of oral bioavailability of CIL, Ternary components were screened out for higher CIL solubility, and the purpose of developing ternary phase diagrams was to feature the regions where nanoemulsion area existed.

Methods: Formulations were designed on the basis of DOE, where data modelling between independent variables (% oil, oil/six ratio and drug loading) and dependent variables (droplet size, clearity and drug solubility) under Box Behnken design was investigated and optimized.

Results: Self-nano emulsifying and solubilization capacity were produced from (SNC7) comprised of 40.4% (w/w) Ethyl Oleate as oil, 48.6% (w/w) Cremophor EL as a surfactant, and 11% (w/w) Transcutol as cosurfactant. The typical drop size and zeta potential of SNC-7 were 72.10 nm and 1.96±0.045 mv, respectively. DOE optimization showed optimized SNEDDS produced quick dissolution of CIL over its suspension. It could be attributed to better solubilization potential with enhanced interfacial activity. Pharmacokinetic data showed optimized system produced 2.5 folds enhancement.

Conclusion: DOE-based SNEDDS design improved the oral bioavailability of CIL with an approach SNEDDS-based formulation of CIL could be employed for improvised dissolution and oral bioavailability parameters.

Keywords: SNEDDS, Poor Dissolution Rate, Solubility, Nanoemulsion, Cilnidipine, Oral bioavailability.

1. INTRODUCTION

The oral administration of the drug is the most advantageous and simple method of medication. It improves patient compliance and defers the strict sterility requirement; however, the bioavailability requirement is mandatory for drug products or delivery systems from the oral route. Hence poor bioavailability of a drug should be meticulously dealt [1,2]. Though several dissolutions or solubility-enhancing technologies can be available, nanotechnology-based lipid-based delivery structures have gained considerable impetus. To provide adequate bioavailability of drug, a dosage form or delivery system design should be carefully rationale in terms of the drug's product performance. Such delivery system design has impaired the drug's poor bioavailability; it acted upon the aqueous solubility or permeability constraints [3-5]. It is evidenced that around 60% of drugs have poor water solubility. Various physiochemical strategies, including salt selection, micronization methods, surfactants based solubilisation approaches, cosolvents activity and complexation, e.g. cyclodextrin, contributed enormously to improving water solubility. Notwithstanding these methods, each drug has its own limitation; therefore, the problem can be addressed differently. With the access of nanotechnology, lipid-based drug delivery approach has been utilized extensively. [6-10]. Lipid-based drug delivery system, for example, nanoemulsions,

microemulsions, nanoemulsions, solid lipid nanoparticles (SLN), nanostructured lipid transporters (NLC), polymeric micelles, have gained significance met out the challenge of Biopharmaceutical Classification System (BCS) class II and IV [11-15]. Lipid-based drug delivery systems modify drug attributes via a selection of ternary components in its design, including surfactants or cosolvents [16-18].

To enhance drug bioavailability, drug must exhibits dissolvability in the biological fluids present in the GIT where drug abide timing in the stomach, changing the biophysical obstruction, further developing medication solubilization, debilitating stomach divider digestion, and elective courses, for example, the lymphatic vehicle of medication [19,20]. Nanoemulsions are the most unmistakable lipid-based drug conveyance frameworks as colloidal scatterings. They are optically isotropic, straightforward, thermodynamically unsound, and dynamically stable frameworks of oil, surfactant, and water with nanoscopic bead size. The capacity of nanoemulsions to work on the oral bioavailability of inadequately water-dissolvable medications has been perceived for a long time [21]. Notwithstanding, nanoemulsions in oral conveyance were restricted because of disadvantages, such as unfortunate acceptability as per their lipidic piece, processing of its parts, etc.

Besides, nanoemulsions are typically consumed in an enormous volume to accomplish the medications' restorative focus, restricting patient consistency. Also, the high water content of nanoemulsions brings about the powerlessness to be conveyed through delicate gelatin, hard gelatin, and HPMC cases. The water content in nanoemulsions would advance the hydrolysis and precipitation of medications during capacity, influencing their utility in oral conveyance [22-24].

Self-nano emulsifying drug delivery systems (SNEDDS) are nanoemulsion preconcentrates or anhydrous nanoemulsions. Upon exposure to GIT fluids, this preconcentrate mix was quickly transformed into the o/w nanoemulsions with globules of nanosize range under 200 nm [25, 26]. The fine drops of the medication broke down in the oil stage and produced improved interfacial activity for quick dissolution in the gastrointestinal liquid [27]. The augmentation in the interfacial surface region prompts drug solubilization and pervasion expansion. The nanosized SNEDDS could accomplish fast processing and medication retention in the gastrointestinal lot. SNEDDS can sidestep the basic rate-restricting disintegration step because the medication would be pre-broken down.

Accordingly, a quick beginning of activity could be accomplished [28]. The lipid transporter of the SNEDDS would prompt an addition in the lymphatic take-up of an exceptionally lipophilic medication, and the first-pass digestion could likewise be decreased. The gastrointestinal lymphatic framework is a physiological pathway for engrossing dietary lipid processing items, like long-chain unsaturated fats, fatty substances, and cholesterol esters [29, 30]. Long-chain unsaturated fats and monoglycerides are esterified into fatty substances in the enterocytes. Before the fatty substances leave the enterocytes, they are integrated into chylomicrons. The huge chylomicrons can't go through the blood vessels, however, they rather enter the lymphatic vessels, which can sidestep the hepatic first entry [31]. This speculation has been used to make sense of the ingestion of lipophilic medications. They can connect with the fatty oil of the chylomicrons in the enterocytes and go through the foundational course by means of the lymphatic course. The lipid-based conveyance framework, including the SNEDDS, can increment lymphatic medication transport [32]. The oil stage utilized in the SNEDDS detailing is a fundamentally significant part. It can work with the solubilization of a lipophilic compound and advance the lymphatic vehicle of lipophilic medications that bypass the hepatic entryway vein course. Subsequently, the chance for hepatic first-pass digestion would be diminished. Furthermore, ingesting greasy food might assist with engrossing the SNEDDS bringing about the rise of bioavailability [33-38].

In contrast with regular nanoemulsions, the SNEDDS offer superior benefits. The detailing of the SNEDDS doesn't contain water; accordingly, the synthetic and actual soundness ought to be further developed during long-haul stockpiling [39]. SNEDDS can be managed in measurements from under 25 mg to more than 2 g. These can give business practicality and patient consistency. These are plausible to produce and can be scaled [40].

Cilnidipine is indicated in the treatment of hypertension; regardless, it has exceptionally low water solubility limiting its efficacy. The primary objective of the present investigation is to improve the CIL's bioavailability using an experimentation-based approach design. DOE would assess the critical parameters in experimentation and how these control the dependent variable and outcome associated with bioavailability of the CIL.

2. MATERIALS AND METHODS

Cilnidipine (purity>98.0%) was obtained from Yarrow Chem Products, Mumbai, Maharashtra, India. Labrafac, Lauroglycol FCC, Transcutol as a bestowal from Gattefosse, France. Oleic corrosive, Ethyl Oleate, different polysorbates (Tween 80, 60, 40, and 20) and polyethylene glycol (200, 400, and 6000) were bought from Sigma-Aldrich, India. Cremophore EL was obtained from BASF Mumbai, India. Any remaining synthetics and reagents utilized in this research stood a coherent mark and were utilised without refinement.

2.1 HPLC methodology

Cilnidipine was estimated using RP-HPLC (AGILENT A1100, India) methodology methanol: water (80:20 v/v) at a stream pace of 1 ml/min with UV location at 245 nm RP-C18 section (ODS C18, 200 mm×4.6 mm, 5 µm). The segment temperature was kept up at 25ºC. The medication showed linearity in the fixation scope of 10-70 µg ml^{-1} for CIL.

2.2 Fourier transformed infrared spectroscopy

The unadulterated medications utilizing KBr pellet spectra were recorded using FT-IR (Shimadzu 8400, Japan). KBr plate was filtered at 4 mm/s at a goal of 2 cm over a wavenumber district of 4000-400 cm^{-1}.

2.3 Differential scanning calorimetry

Cilnidipine sample (50 mg) was filled in a DSC pan (Shimadzu, DSC 60TSW 60, Japan) and was retained in the sampling port. The sample was scanned in the range of 50-350ºC screening speed of 10 °C/min, taking medium as references.

2.4 Assessment of Cilnidipine Solubility

Solubility of Cil was assessed in various oils, surfactants, and cosurfactants. An excess of Cil was taken in 1mL of each excipient. The drug excipients mixture is mixed in a vortex mixer and then placed in a water bath at 37±0.5ºC for 72 hours. The mixtures were centrifuged at 3000 rpm for 15 min. Some insoluble Cil was filtered through a 0.45 µm membrane by filtration. The solubility of Cil in different excipients was measured using the HPLC method described previously. The trials acted three-fold, as addressed in Table **1**.

Table 1. Solvency of the medication in the various parts chosen for ternary phases.

Solvents	Solubility (mg/mL)
Ethyl Oleate	22.61± 1.03
Campul MCM	19.86± 0.09
Labrafac	5.26±0.33
Oleic Acid	6.11±0.96
Luroglycol FCC	3.76±0.33
Isopropyl Myristate	5.89±0.089
Tween 20	238.64±1.02
Tween 80	226.5±1.02
Captex	8.1±0.68
Cremophor EL	242.47±0.92
PEG 200	132.55±1.2
PEG 400	230.1± 1.14
Methanol	36.55±0.06
Water	0.036±0.03
Transcutol	351.06±0.99

(n=3) mean ±s.d.

2.5 Pseudo-Ternary Phase Diagram

Pseudo ternary graphs were constructed at four Smix ratios. The oil and surfactant/cosurfactant mixture at a specific weight proportion was titrated with water blended by attractive mixing until equilibrium was reached. In the SNEDDS, ethyl oleate was used as the oil; a cremophor EL was used as a surfactant and transcutol as a co-surfactant. Equal weight fractions of cremophor EL and Transcutol were taken to form Smix 1:1, 2:1, 3:1 and 4:1. The critical variable for SNEDDS is the proportion of surfactant to cosurfactant. Stage graphs at explicit surfactant proportions to cosurfactant Smix by loads 1:1, 2:1, 3:1 and 4:1 were taken. Combinations of surfactant/cosurfactant (at a particular proportion) with oil were ready at proportions 9: 1, 8: 2, 7 : 3, 6: 4, 5: 5, 4: 6, 3: 7, 2: 8, and 1: 9 by weight. The weight fraction of each component was calculated and plotted on a ternary graph by taking some of the ternary components 100% in each titration, as displayed in Fig. **(1)**.

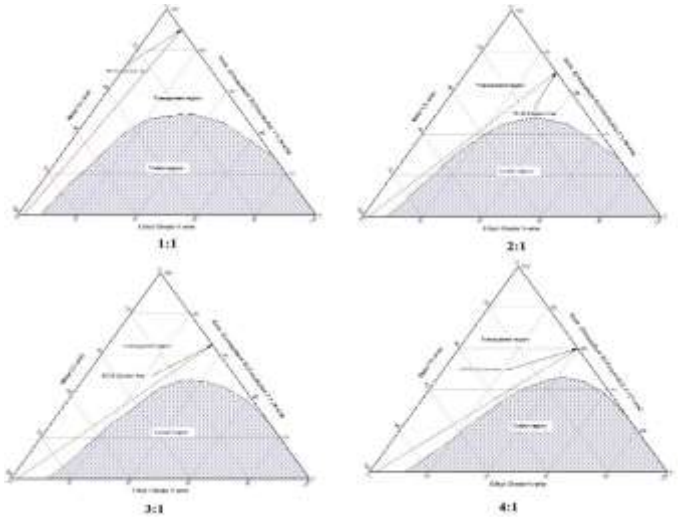

Fig. (1). Pseudo Ternary Phase diagram with Smix (1:1) (2:1) (3:1) (4:1).

2.6 Preparation of SNEDDS formulation

Thirteen different formulations were prepared by taking the amount of oil and Smix. A fixed quantity of Cilnidipine (10 mg) was taken in oil and Smix. Mixed for half an hour and vortex blending at ambient room temperature until the SNEDDS plan was acquired, as displayed in Table **2**.

Table 2. Composition of various SNEDDS formulations of Cilnidipine.

Formulation	Oil (%)	Co-Surfactant(%)	Surfactant(%)
SNC1	33.2	13.7	53.1
SNC2	43.4	17.4	39.2
SNC3	25	15	60
SNC4	26.4	16.2	57.4
SNC5	34.6	26.2	39.2
SNC6	32	9.8	58.2
SNC7	40.4	11	48.6
SNC8	28.8	23.8	47.4
SNC9	35	20	45
SNC10	37.6	14.6	47.8
SNC11	36.8	28.2	35
SNC12	30	10	60
SNC13	33.5	18.2	48.3

2.7 Box-Behnken design in formulation and optimization of SNEDDS

Three factorials, i.e. 3^3 gives 13-run in experimental Box-Behnken design utilizing Design Expert V13 programming (Stat-Ease Inc. Minneapolis, USA), were utilized to plan fluid SNEDDS. The amount of Ethyl Oleate (X1), Cremophore EL (X2) and Transcutol (X3) were chosen as free factors, as displayed in Table **3**. Conversely, droplet size (nm) Y1 and self dispersion time (sec) Y2 were chosen as reactions. Reaction surface examination was done to concentrate on the impact of various autonomous factors on the noticed responses. Coded and transformed values of Y1, Y2, and Y3 with responses X1 and X2 are given in Table **4**.

Table 3. Factors and levels with the coded and changed esteem

Factors	Levels					
	Changed Esteem			Coded value		
	High	Middle	Low	High	Middle	Low
X1(Ethyl Oleate)	40	35	25	1	0	-1
X2(Cremophore EL)	60	50	40	1	0	-1
X3(Transcutol)	25	17.5	10	1	0	-1

Table 4. Design matrix generated by **Using Box- Behnken Design** through design expert software (V13)

	INDEPENDENT VARIABLES			DEPENDENT VARIABLES	
FORMULATION	**OIL (X1)**	**SURFACTANT (X2)**	**CO SURFACTANT (X3)**	**Droplet size (nm) (Y1)**	**DISPERSION TIME (Sec) (Y2)**
SNC1	1	1	0	55.70	152
SNC2	1	-1	0	70.03	138
SNC3	-1	1	0	44.40	97
SNC4	0	1	1	21.56	58
SNC5	-1	-1	0	51.90	36
SNC6	-1	0	-1	67.75	98
SNC7	1	0	-1	72.10	136
SNC8	-1	0	1	19.20	72
SNC9	1	0	1	46.38	93
SNC10	0	-1	-1	94.40	58
SNC11	0	-1	1	58.90	22
SNC12	0	1	-1	94.05	27
SNC13	0	0	0	98.80	57

2.8 Characterization of SNEDDS Formulations

2.8.1 Dispersibility and Stability Investigations

Aqueous dispersibility and self-emulsification were assessed using USP dissolution apparatus-II. Briefly, 1 mL of each formulation was added dropwise into 250 mL of recreated gastrointestinal liquid (pH 6.8, without catalysts), kept up at 37ºC, with delicate blending utilizing tempered steel paddles pivoted at 60 rpm. Each formulation was visually assessed for emulsification, time dispersibility and physical appearance. The physical stability of the formulation has been physically evaluated by dissolution fluid after 24 hours. Formulations were steady (no precipitation), smooth, dull-white, whitish, or unsteady (showing precipitation). The stable formulation with a small particle size that passed the dispersibility test was selected for further characterization.

2.8.2 Conductivity Measurement

The electrical conductance was estimated utilizing a DELUXE conductivity meter (MS Electronics India Pvt Ltd, Harayana, India) working at 50Hz. The temperature was kept at 30 ±0.5ºC and maintained by thermostatic control. The conductance of SNEDDS was estimated by diluting it with water (1:50).

2.8.3 Rheology Test

Rheological investigations were performed on Brookfield R/S (Model PS-P) cone and plate viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA; connected axle # 50.3250; type C25-2 at shearing rates (1/s) values 25-400. The product utilized for the computations was Rheocal V2.8. Apparent viscosities (estimated time of arrival esteem) were estimated. A normal of ten readings at the timespan seconds with an all-out run season of 100 seconds was recorded at 30µ 0.5ÀC at 10 RPM. Distance between the cone and plate was kept at 0.01mm with a cone point of 1.

2.8.4 Globule Size, Size Distribution and Zeta Potential

Malvern Zetasizer (Malvern Instruments, UK) was utilized to decide droplet size, polydispersity file and zeta potential for it 0.5mL samples were diluted with double distilled water and placed in the sample holder. SNEDDS was repeatedly weakened with purified water and shaken delicately to shape a fine emulsion. The resultant emulsion was used for additional review. The upsides of z-normal breadths were utilized. Each size esteem detailed was normal of somewhere around three free estimations.

2.8.5 Refractive Index and Percentage Transmittance

Abbe's refractometer was used to determine RI samples diluted with distilled water (1:250) placed over the temperature-controlled attachment in the instrumentation in a uniform layer. Crosshair proportion was visualised at the particular knob setting on the scale reading was renowned in triplicate.

2.8.6 Drug Content Determination

Each formulation was centrifuged at 10,000 rpm for 30 minutes and carried through a 0.45 µm membrane filter. The filtrate was collected and diluted with10mL of methanol and further dilution upto 2.5 times as necessary. Analysed for the determination of drug content using the HPLC method.

2.8.7 Assessment of Thermodynamic Stability

Formulation obtained after the freeze-thraw system was exposed to 6 warming and cooling cycles by brooding them for 48h at 45ºC and 4ºC, monitored for drug precipitation, phase separation etc. Centrifugation at 18,000 rpm of nanoemulsions was exposed for 30 min at 4ºC. Stability parameters like drug assay, droplet size dispersibility and percent transmittance were analysed using the HPLC method, which was repeated after three months storage period for a long time at 37± 2ºC and refrigerated (2-8ºC).

2.8.8 In Vitro Drug Release Studies

In vitro, drug release studies of Cil drug-loaded SNEDDS and suspension were carried out in a USP-II type dissolution device (Model Disso 2000, Lab India) utilizing 0.1N HCl and phosphate buffer (pH 6.8) as 900 ml dissolution media at 50 rpm and temperature 37 ± 0.5 °C. 1mL formulation (containing 10mg equivalent of Cil) was placed in the dissolution apparatus. Aliquots of 5 ml were removed at a reasonable time (5-90 min), filtered, appropriately diluted and analysed through HPLC at λmax of 245 nm.

2.8.9 Pharmacodynamic study

Pharmacodynamics studies on selected formulations were performed in Wistar rats of 200-220 g of either sex. The institutional creature morals committee analyzed and supported every single exploratory convention. The temperature of the creatures (22±2°C) and the 12 hours light/dim cycle was kept up in customary lab settings. All creatures were kept in isolation for the multi-week before the examinations. The rodent was treated with traditional lab food and not indispensable water. One night before the test, the creatures were fasting. The creatures were prepared to remain in the holder before research. This dependable a quiet and incapable rodent during circulatory strain estimations (BP). Subcutaneous methylprednisolone acetic acid derivation (20 mg/kg/week) infusion presented hypertension for multi-week. These groups were made, containing 6 animals in each group. Group 1 was given a low dose; Group 2 had a high dose of Cil SNEDDS, respectively; Group 3 had a Cil suspension. System BP was measured using the tail-cuff method at

intervals of 0,1,2,3, and 6 hours. After the last infusion of methylprednisolone, drug organization begins at 0 min. A portion of 0.9 mg/kg in cilnidipine was given orally. An amount of 0.9 mg/kg/ml in the arrangement was taken orally utilizing cilnidipine SNEDDS, using a cannula joined to a creature's mouth.

3. RESULTS AND DISCUSSION

3.1 HPLC Condition

The dissolvable methanol: water (80:20 v/v) with a 1 ml/min stream rate. Segment RP-C18 section (ODS C18, 200 mm×4.6 mm, 5 µm). Segment temperature-25ºC. The Drug showed linearity in the fixation scope of 10-70 µg ml-1 for CIL with UV discovery at 245 nm, as displayed in Fig. **(2)**.

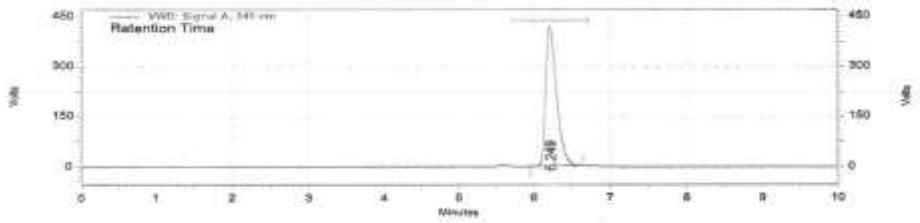

Fig. (2). HPLC Graph of Cilnidipine pure drug.

3.2 Fourier transformed infrared spectroscopy

The FT-IR spectra, Fig. **(3)** of the medication showed groups at 1346.07 (C-N Aromatic 2° amine (S)), 1376.93 (N-O (S)), 1947.75 (C=O(S)), 2780.85 (- OCH3), 3405.67 (N-H Aromatic 2° amine (S))cm^{-1}. It was seen that terrifically significant tops because of practical gatherings of medications were available in the medication. No tremendous contrast was found in the wavenumber (cm^{-1}) of the medication, and an expanding impact was noticed.

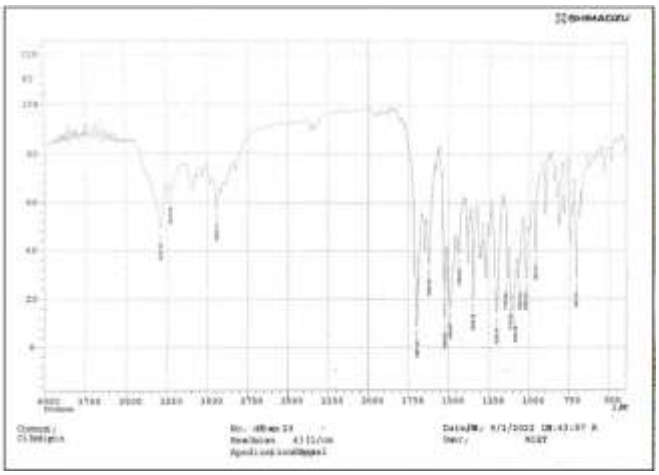

Fig. (3). FTIR of cilnidipine.

3.3 Differential scanning calorimetry studies

Fig. **(4)** shows DSC thermograms of unadulterated Cil; the thermogram of Cil showed a solitary endothermic top at 111.621 °C showed maintenance of the trademark pinnacle of Cil as well as a worth viewed as inside the given reach, for example, 108ºC-112ºC, showing that the dissolving point and in this way the virtue of the prescriptions are both affirmed.

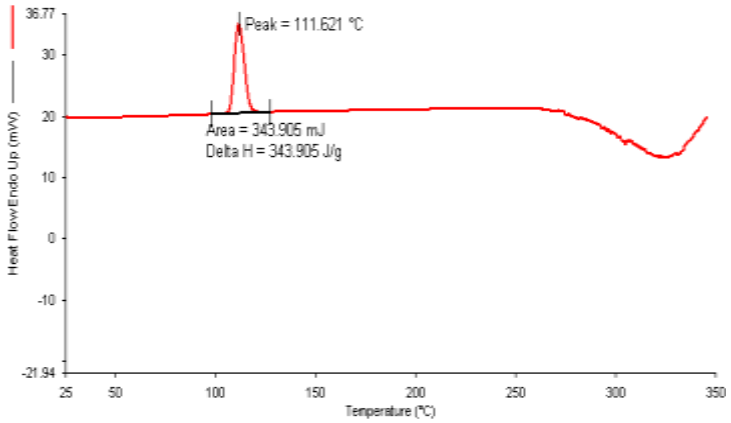

Fig. (4). DSC of Pure drug Cilnidipine.

3.4 Solubility studies

A comparative investigation of the dissolvability of Cil in different solvents is given in Table **1**. The medication shows the greatest dissolvability in Ethyl Oleate, Cremophor EL, and Transcutol, further used as oil, surfactant, and cosurfactant. The ternary stage graphs were built to decide the fixation scope of parts for nanoemulsion development. Before creating the stage outline, all aspects were changed into weight/weight percent. The more obscure district in the stage outline addresses the self-emulsification region. It was seen that the effectiveness of emulsification was improved with the guide of Ethyl Oleate, Cremophor EL, and Transcutol, among the oils, surfactants and co-surfactants, separately. They were additionally used for the development of ternary stage graphs.

3.5 Pseudo-Ternary Phase Diagram Study

Fig. **(1)** portrays the stage graph of the frameworks containing Ethyl oleateCremophore EL, Transcutol as oil, surfactant, and co-surfactant expansion because of their higher hydrophilicity properties [13]. Stable frameworks were seen with centralization of oil upto 40% w/w in the framework. It was seen that the expansion of co-surfactant, Transcutol further developed the self-emulsification property of the framework [14]. The unconstrained emulsion arrangement was not productive, with under 45% w/w of the surfactant in the system phase outlines.

3.6 Optimization of SNEDDSusing Box-Behnken design

A Box-Behnken probationary pilot with three factors & their distinct levels was utilized to concentrate on the impact on subordinate factors. 13 definitions were ready according to the trial plan and described further for reactions like bead size and self-emulsification time, as displayed in Table **4**. For every one of the 13 clumps of ward factors, drop size (Y1) and scattering time (Y2) showed wide varieties from 19.2 to 72.1 nm and 22 to 152 Seconds, separately, demonstrating the great impact of autonomous factors (X1, X2 and X3) on the chose reactions.

The connection between the reliant and free factors was additionally edified utilizing the reaction surface plot displayed in Fig. **(6)**. The numerical connections were laid out, and coefficients of second-request polynomial conditions were produced utilizing multilinear relapse investigation for globule size and self-emulsification time. The conditions were viewed as quadratic with communication terms. The polynomials' coefficients fit well with the information, with the upsides Y1 and Y2 of R^2 being 0.9962 and 0.9976.
This information was fitted to this quadratic condition (Eq. **1**):

$$Y_1 = \theta_0 + \theta_1 X_1 + \theta_2 X_2 + \theta_3 X_3 + \theta_4 X_1 X_2 + \theta_5 X_1 X_3 + \theta_6 X_2 X_3 + \theta_7 X_1^2 X_{12} + \theta_8 X_2^2 X_{13} + \theta_9 X_3^2 X_{23} \ldots\ldots\ldots.$$

Eq. **(1)**

Y_1= The reliant or estimated reaction of the reliant factors related to each element - a level mix

θ_0 = Intercept

$\theta_1 - \theta_{23}$ = The regression coefficients

$X_1 - X_2 X_4$ = The impendent factors utilized in the contemplated

Fig. **(5)** portrays a form plot of a collaboration impact between oil (Ethyl Oleate) and surfactant (Cremophore EL) on the globule size as a reliant variable. A straight expansion in the globule size was seen with expanding how much ethyl oleate and cremophore EL.

Fig. **(6)** shows the reaction surface plot, describing the expansion in the centralization of oil (Ethyl Oleate) and co-surfactant (Transcutol). All the reaction surfaces were fitted with quadratic polynomial models.

The ANOVA results are displayed in Table **5**. The F determined worth of 4.16 and 3.38 is not exactly the table worth 9.55 for Y1 and Y2.

Consequently, the precluded terms don't anticipate globule size and self-emulsification time. Utilizing the programming enhancement cycle and reaction surface plots displayed in Figs. **(5-6)**, levels chosen for X1, X2 and X3 were 40, 10 and 50 percent, separately, giving upsides of 72.10 nm and 136 sec for globule size and self-emulsification time. The plan was arranged utilizing the ideal degrees of free factors. The product's qualities were close to the SNEDDS plan (SNC7), which shows the ideal outcomes.

Table 5. Result of ANOVA

Response	Sum of Square	R^2	df	Mean Square	F Value	P Value	Model
Droplet size (Y1)	146.87	0.9976	9	16.81	4.16	<0.05	Significant
Dispersion time (Y2)	846.26	0.6654	9	97.01	3.38	<0.05	Significant

∗P < 0.05 compared to all the runs

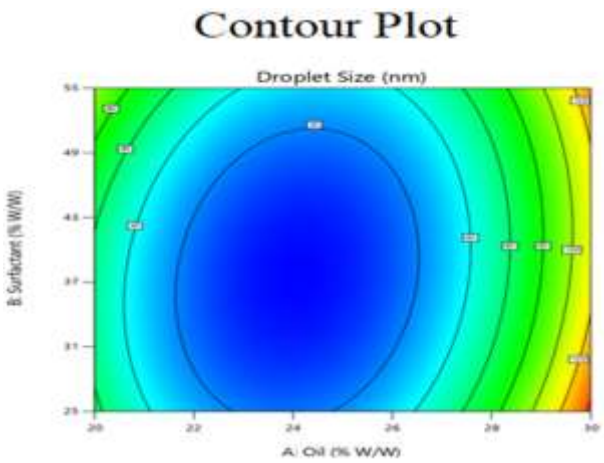

Fig. (5). Contour plot from the Box-Behnken experimental design.

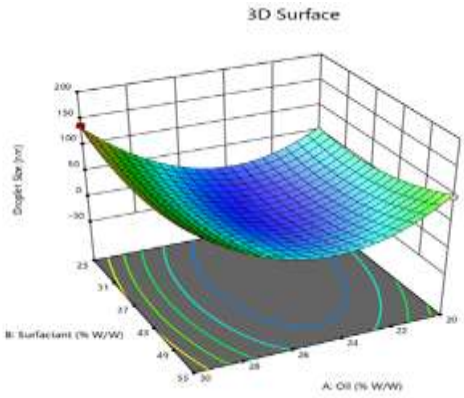

Fig. (6). 3D Surface plot from the Box- Behnken experimental design.

3.7 Characterization of SNEDDS

3.7.1 Dispersibility and Stability Investigations

In the plan of SNEDDS, various groupings of the oil stage, surfactant and co-surfactant were utilized, and scattering time relied upon synthesis, as displayed in Table **6**. The surfactant to cosurfactant proportion expanded, creating a more modest molecule size and diminished scattering time.

3.7.2 Conductivity test

The negative worth shows that oil globules are adversely charged because of a surfactant or potentially co-surfactant. Cil SNEDDS shows conductivity from 88.7-180.5 µS/cm, as shown in Table **6**. Every one of the plans has conductivity higher than 50 µS/cm, demonstrating that every detail is O/W emulsions.

3.7.3 Rheology Measurement

The rheological portrayal has been utilized in colloidal scattering frameworks, and changes within structures, either isotropic or mesophase, existed in the ternary stage diagram. The thickness of weakened SNEDDS was portrayed by the weakening addressed in Table **6**. It is recognized that the scattered stage's consistency was expanded upon the watery stage, weakening until the development of enlarged micelles. These outcomes showed frail entrapment among oil and water stages seen at the w/o framework. Water and oil stages commonly hinder the framework's thickness at the transitional stage. Dynamic consistency of SNEDDS more traps among oil and water stages occurred within sight of liquid particles. Oil ceaseless nanostructure was framed because of the progress in microstructure from an opposite micellar blend comprising S/CoS and weakened with a fluid stage. Consequently, the viscosities of each microstructure fluctuated with watery stage content.

3.7.4 Globule size, size distribution and zeta potential

These parameters of SNEDDS weakened upto 10.0ml, as displayed in fig. **(7)**. Once more, it was additionally affirmed bead size estimation was displayed in Table **6**. These outcomes acquired from bead size estimation affirmed that adding fluid stage volume to the pre-concentrate combination expands the size of microemulsion drops because of the change of converse micelle to enlarged switch micelles. The underlying variety is also recommended to start when surfactant/co-surfactant blends with the fluid stage. The drop size of the weakened preconcentrate definition of SNEDDS was in the nanosize range with a low polydispersity file. As the definition is made out of non-ionic surfactants, its zeta potential qualities stayed unaffected by the expansion of water.

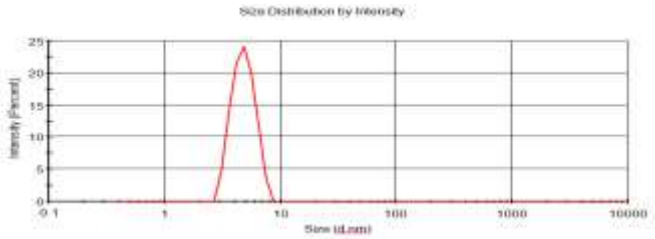

Fig. (7). Size distribution (nm) of SNEDDS Cilnidipine (SNC7)

3.7.5 Refractive index and Percentage Transmittance

The refractive index and percentage transmittance of different plans are displayed in Table **6**. Design (SNC1, SNC2, SNC7) has RI similar to water (1.333), and all transparent formulations, as well as bluish dispersibility, have % transmittance >97%. The data prove the transparency of the system.

3.7.6 Drug Content Determination

It was calculated by HPLC, an optimized formulation of SNEDDS, which was 99.97 ± 1.02%, confirming the precision of the dosage in the formulation.

Table 6. Physical Evaluation of the SNEDDS formulations

Formulations	Dispersibility (Dilution to 250mL)	Conductivity (µS/cm)	Rheology (mPa.S)	PDI	Zeta Potential (mV)	Refractive Index	% Transmittance
SNC1	TRANSPARENT	171.3±0.21	5.32±0.62	0.236±0.01	0.58±0.19	1.394±0.017	99.80±0.37
SNC2	TRANSPARENT	178.9±0.32	5.44±0.38	0.367±0.001	0.0209±0.22	1.392±0.015	99.64±0.36
SNC3	BLUISH	155.6±0.74	6.25±0.62	0.324±0.003	-0.73±0.239	1.425±0.021	98.37±0.92
SNC4	TRANSPARENT	110.5±0.12	7.34±0.54	0.383±0.001	-1.95±0.072	1.428±0.024	54.76±2.01
SNC5	BLUISH	155.4±0.52	6.08±0.34	0.308±0.007	-0.82±0.21	1.419±0.02	98.83±0.88
SNC6	BLUISH	168.2±0.24	6.37±0.42	0.372±0003	-0.75±0.34	1.454±0.028	97.62±0.96
SNC7	TRANSPARENT	90.3±0.22	7.22±0.32	0.214±0.005	1.96±0.045	1.393±0.018	99.89±0.32
SNC8	BLUISH	162.2±0.26	6.26±0.66	0.366±0.004	-0.66±0.035	1.430±0.025	96.07±0.54
SNC9	TRANSPARENT	180.5±0.25	5.19±0.18	0.226±0.001	0.0292±0.024	1.396±0.019	99.78±0.36
SNC10	TURBID	91.8±076	7.26±0.48	0.371±0.003	-2.80±0.046	1.453±0.027	50.48±2.52
SNC11	TURBID	92.6±0.36	6.94±0.36	0.386±0.003	-1.81±0.041	1.407±0.017	48.37±2.78
SNC12	TURBID	88.7±0.48	7.82±0.44	0.401±0.003	-2.63±0.032	1.435±0.021	62.35±1.86
SNC13	TURBID	95.2±0.62	6.86±0.47	0.365±0.003	-1.74±0.036	1.438±0.022	47.82±3.02

(n=3) mean ±s.d.

3.7.7 Thermodynamic Stability Profile

The different formulations are checked for time-subordinate actual changes like medication precipitation. HPLC investigation for cilnidipine was completed to look at the substance dependability of the medication inside SNEDDS. Chosen plans were saved for a long time and tracked down soundness upon capacity.

3.7.8 In Vitro Drug Release Studies

The dissolution study of half-hour and 90 minutes was led at the same time in 0.1N HCl and phosphate buffer (pH 6.8). All plan investigations are done and contrasted with Cil- suspension. The consequence of in-vitro drug discharge concentrates on cilnidipine SNEDDS, exceptionally SNC7, showed a higher level of delivery than CIL Suspension, as displayed in Fig (**8**).

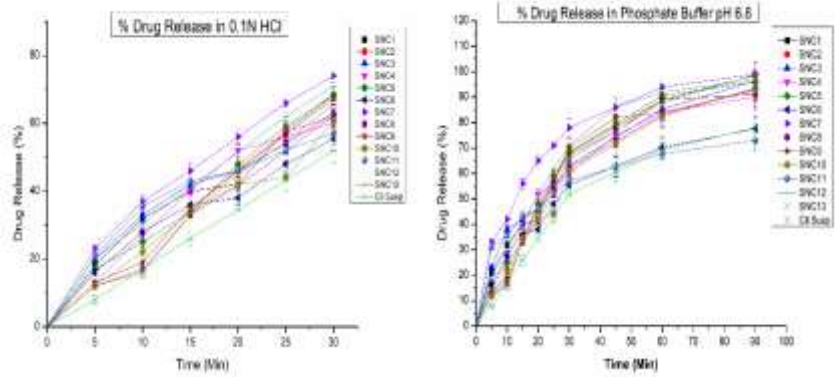

Fig. (8). Graph for drug release in different media.

3.7.9 Pharmacodynamic study

However, the SNEDDS (SNC7) showed a more remarkable improvement in its bioavailability than durg in suspension form, as shown in Table **7**. A similar difference can be drawn from systolic and diastolic activity reduction compared with the control, as shown in Fig **(9).** The difference in hypersensitivity activity between the optimized formulation vs control can be marked on the basis of the higher solubility of CIL in SNEDDS formulations.

Table 7. Pharmacodynamic study of Cil SNEDDS (SNC7) and Suspension.

	Low Dose			High Dose			Cil Suspension		
Time (Hr)	**Systolic BP**	**Mean BP**	**Diastolic BP**	**Systolic BP**	**Mean BP**	**Diastolic BP**	**Systolic BP**	**Mean BP**	**Diastolic BP**
0	152	139	126	150	140	130	155	144	132
1	140	125	110	122	106	90	150	138	125
3	132	115	98	115	98	82	146	129	112
6	122	104	86	110	94	79	140	117	95

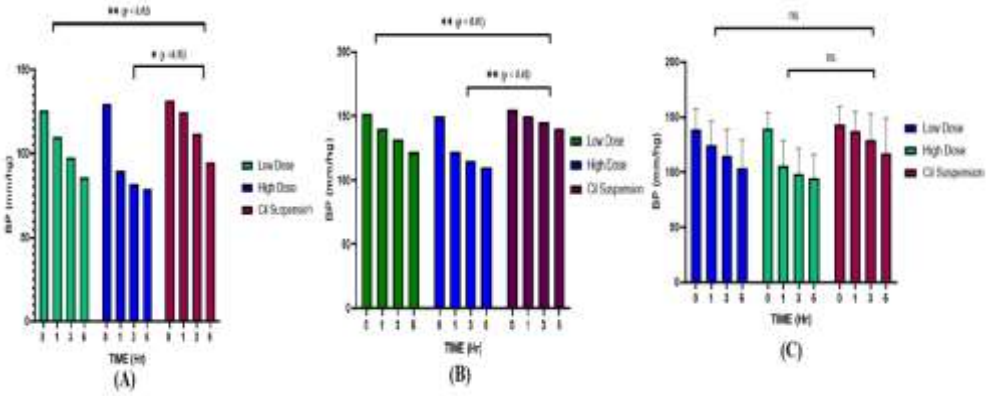

Fig. (9). (A) Diastolic data (B) Systolic data (C) Combined Blood pressure values before and after drug treatment (n = 6 rats/group). ∗P < 0.05 compared to the group; #P < 0.05 compared to the groups.

CONCLUSION

Cilnidipine (CIL) is a BCS class II drug with low bioavailability traits, and its SNEDDS based system was developed using the DOE approach. Oil, surfactant and co-surfactant were selected on the basis of maximal drug solubility in the respective ternary components identified as Ethyl Oleate, Cremophor EL and Transcutol as oil phase, surfactant/ cosurfactant (Smix). Aqueous dilutable areas were identified from ternary phase diagrams drawn at Smix ratios of 1:1, 2:1, 3:1, and 4:1. Box Behnken modelling was devised on independent variables to affect the response. The composition variable of ternary components was located from the ternary phase diagrams to design Thirteen SNEDDS formulations. Post design, the software-based response analysis showed that the optimal values of Ethyl Oleate, Cremophor EL and Transcutol were 40, 50 and 10% w/w, respectively, matching SNC-7. Formulations were characterized for dispersibility and stability, conductivity, rheology, zeta potential, RI, transmittance, and drug content. In vitro CIL release was maximal in SNC7. Thermodynamic stability of formulations was assessed and found stable when kept under storage conditions. Optimized formulation was investigated for pharmacodynamic studies conducted on wistar rats at low, high doses and compared with CIL oral suspension as control. SNC-7 formulation produced significant antihypertensive activity over control ($p<0.001$) at both levels. SNEDDS-based CIL formulation optimized using DOE improved dissolution characteristics and produced a significant difference in pharmacodynamic activity over control.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Reg. No. RKGIT/IAEC/2021/06 has been approved by IAEC of Raj Kumar Goel Institute of Technology (Pharmacy), Ghaziabad U.P. 201003, India, dated 10/09/2021.

HUMAN AND ANIMAL RIGHTS

Animals were used for this research investigation under Reg. no. RKGIT/IAEC/2021/06, dated 10/09/2021.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS:

Not applicable.

FUNDING
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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