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Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system

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ABSTRACT

The present study deals with the development and characterization of self-nanoemulsifying drug delivery system (SNEDDS) to improve the oral bioavailability of poorly soluble third generation calcium channel blocker lercanidipine (LER). Solubility of the LER was estimated in various oils, cosurfactants and surfactants which were grouped into two different combinations to construct pseudoternary phase diagrams. Various thermodynamic stability and dispersibility tests were performed on the formulations from phase diagram. After constructing phase diagram of two different combinations NL-I and NL-II, the effect of cosurfactants on the nanoemulsifying area was studied and the effect of number and length of hydrophobic alkyl chains of cosurfactant in its emulsification capacity was proved. Percentage transmittance, emulsification time, viscosity and droplet size analysis were used to characterize optimized formulations. The optimized formulation composed of Cremophor EL (45% wt/wt), (13.5% wt/wt) Caproyl 90 with (1.5% wt/wt) Transcutol® HP as per limits of inactive ingredients guidelines of FDA and Maisine oil (10% wt/wt). The mean droplet size in selected nanocarrier system was 20.01 nm. The *in vitro* dissolution profile of LER SNEDDS was found significant in comparison to the marketed LER (Zanidip) tablet and pure drug in pH 1.2, 4.5 and 6.8 buffers. Empty hard gelatin capsule shells were filled using Pfizer's Licap technology and charged on stability conditions of 30 °C/65% RH, 40 °C/65%RH and 50 °C/75% in glass bottles where no significant degradation ($p > 0.05$) was observed in 3 months. The results indicate that SNEDDS of LER, owing to nanosized, has potential to enhance the absorption of drug.

1. Introduction

Lercanidipine is chemically, 2-[(3,3diphenylpropyl) methylamine]-1,1-dimethylethylmethyl 1, 4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridinedicarboxylic ester (Fig. 1). It is a new third generation amphiphilic drug which belongs to the well-known pharmacological active compound series classified as 1,4-dihydropyridine calcium channel blockers [1,2].

This drug is used in hypertension treatments, based on its selectivity and specificity on the smooth vascular cells [3]. Lercanidipine is a third-generation dihydropyridine calcium channel antagonist, which blocks calcium entry into L-type calcium channels present in smooth muscle cells, thereby, causing peripheral vasodialation and a reduction in blood pressure [4]. After absorption, oral lercanidipine undergoes extensive first-pass metabolism, with approximately equivalent amounts of an oral dose eliminated in the urine and the faeces as metabolites therefore generating mainly inactive metabolites [4]. This molecule corresponds to a new molecular design in which its liposolubility has been increased to obtain a long action [5]. It is an amphypathic drug which is transported quickly across the cellular barrier, arriving inside to both hydrophilic and hydrophobic sites in spite of its highest solubility in the lipophilic bilayer [5]. Literature suggests single dose of 10 and 20 mg of LER has mean half-lives of 2.8 and 4.4 h in humans, respectively [6]. After oral administration, LER is completely and erratically absorbed from the gastrointestinal tract [6]. However, absolute bioavailability is reduced to approximately 10% because of extensive first pass metabolism to inactive metabolites as undergone by other drugs under the class dihydropyridines of calcium channel blockers [7]. These pharmacokinetic parameters make LER a suitable candidate for development of SNEDDS formulation to enhance oral bioavailability, avoiding first pass metabolism by getting absorbed through lymphatic pathway.

Lipid based formulations represents a unique solution to delivery of poorly soluble compounds. A lipid dosage form typically consists of one or more drugs dissolved in a blend of lipophilic excipients such as triglycerides, partial glycerides, surfactants or co-surfactants [8]. Among the lipid-based systems, the self-microemulsifying drug delivery system (SMEDDS) is a promising technology to improve the rate and extent of absorption of poorly

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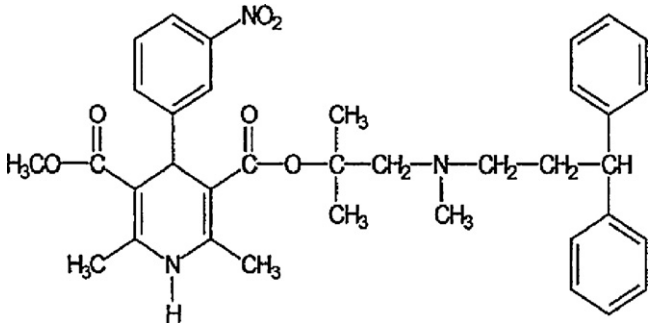

Fig. 1. Chemical structure of lercanidipine.

water-soluble drugs [9]. Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of drug, lipids and surfactants, usually with one or more hydrophilic co-solvents or co-emulsifiers [10]. Hydrophobic drugs can be dissolved in these systems, enabling them to be administered as a unit dosage form for per-oral administration [11]. When such a system is released in the lumen of the gastrointestinal tract, it disperses to form a fine emulsion (micro/nano) with the aid of GI fluid [11]. This leads to in situ solubilization of drug that can subsequently be absorbed by lymphatic pathways, by passing the hepatic first-pass effect [11].

Extensive survey of literature and patent databases did not reveal any SNEDDS formulation developed of LER for improving bioavailability. The present investigation was aimed at developing SNEDDS for the delivery of LER. SNEDDS of LER with globule size <100 nm were successfully developed as also shown by images of TEM. Characterization of optimized formulation, *in vitro* evaluation and stability studies of LER formulation was performed which are presented in this investigation. The oral formulations of LER are rapidly metabolized and incompletely absorbed, limiting its use in hypertension. This enhances need to develop a formulation which offers quick dissolution and complete absorption in order to yield improvement in bioavailability and therapeutic efficacy of LER.

Thus, the objectives of the present study were to develop and characterize an optimal self emulsifying drug delivery system formulation of lercanidipine to avoid first pass metabolism of drug thus enhancing oral bioavailability and to study the effect of cosurfactant combinations on nanoemulsifying area in phase diagram.

2. Material and methods

2.1. Materials

Lercanidipine was received as a gift sample from Glenmark Pharmaceuticals Limited, batch number (A22026013) (Mumbai) and certified to contain (99.81% purity). Excipients were chosen based on their functionality, widespread commercial use, solubility with drug and biological properties. Compatibility with various Surfactants, Cosurfactants and Oils were examined. All excipients were US Pharmacopeia/National Formulary grade. The following materials were donated by Gattefosse (Mumbai, India) and were used as received: Labrafac CM10 (C8–C10 polyglycolized glycerides), Labrasol (Caprylo Caproyl macrogolglycerides), Maisine 35-1 (glyceryl monolinoleate), Lauroglycol 90 (propylene glycol monolaurate), Labrafil M1944 CS (Oleoyl macrogoglyceride), Labrafac PG (propylene glycol caprylate/caprate), Transcutol® HP (Diethylene glycol monoethyl ether), Pluro oleique (Polyglyceryl oleate), Caproyl 90 (Propylene glycol monocaprylate) and Capmul (Glyceryl mono or dicaprate). Cremophor RH 40 (Polyoxyl 40 hydrogenated castor oil) and Cremophor EL (polyethoxylated castor oil) were obtained from BASF (Mumbai). Tween 80 (polyoxyethylene sorbitan monooleate) and PEG (Polyethylene glycol) 400 were bought from Merck (Mumbai, India). LR grade castor oil and isopropyl myristate were also used. Deionized water was obtained in the laboratory, using ionic interchanged columns Milli-Q (Millipore). HPLC grade methanol from (Fisher Scientific, Mumbai) was used as received for analysis of bulk drug and formulation on HPLC. Empty hard gelatin capsule shells (size 2) were filled using Pfizer's Licap technology.

2.2. Screening of excipients

The solubility of lercanidipine was ascertained in oils, surfactants, and cosurfactants. An excess amount of LER was added in 2 mL of the selected lipophile in stopperred vials and mixed with the help of vortex mixer (Nirmal International, Delhi, India). These vials were then kept at $25 \pm 1\,^{\circ}C$ in an isothermal shaker (Nirmal International, Delhi, India) for 72 h. The resulting samples were centrifuged at 3000 rpm for 15 min (REMI International, Mumbai, India). The supernatant was filtered through a 0.22 μm filter. The concentration of LER in the supernatant was then quantified by using in house validated HPLC method with UV detector at 240 nm.

2.3. HPLC analysis of LER

The solubility of LER in various excipients was determined by a validated in-house HPLC method. The HPLC apparatus consisted of Agilent HPLC (1120 series) binary pump system and UV detector (Switzerland) equipped with a column compartment with temperature control and an on-line degasser. Data collection and integration was accomplished using EZChrom Elite software. A C18 reverse phase column [(Agilent TC C18 (2), 250 mm × 4.6 mm), particle size 5 μm, Agilent, Switzerland] equipped with a guard column of same packing material was used for the study. Mobile phase consisted of Methanol/Millipore Water at (90:10 v/v) at 1.2 mL/min flow rate, detection at 240 nm with retention time at 5.53 min (Fig. 2). The same method was used in stability studies of formulation kept for 3 months by carrying out assay of drug content in formulation.

2.4. Excipient compatibility studies

On the basis of solubility studies it was found that lercanidipine has high solubility in surfactants (Cremophor EL, Labrasol), cosurfactants (Caproyl 90, Lauroglycol 90 and Transcutol) and in oils (Maisine oil, Labrafil M1944CS). Thermal and non thermal techniques were used to detect any interaction between drug and selected excipients. Samples from stopperred vials containing excess drug loaded excipients were subjected to Differential Scanning Calorimetry (DSC) Pyris 6 DSC, Perkin Elmer (Software pyris series) and Fourier transform infrared spectroscopy (FTIR) (Shimadzu, Japan) and thermograms were obtained.

2.5. Phase diagram studies

Based on solubility studies, one combination (NL-I) comprises of Cremophor EL as surfactant, Capryol 90 with Transcutol® HP (90:10 wt/wt) as cosurfactant and Maisine as the oil phase. Other combination (NL-II) comprises of Labrafil M1944CS, Labrasol and Lauroglycol 90 as the oil, surfactant and cosurfactant respectively. Double distilled water was used as the aqueous phase. The pseudo-ternary phase diagrams were constructed by titration of homogenous liquid mixtures of oil, surfactant and cosurfactant with water at room temperature. Surfactant and cosurfactant were mixed (Smix) in different volume ratios (1:1, 1:2, 1:3, 2:1, 3:1 and 4:1). The ratios were chosen such as by increasing concentration of surfactant with respect to cosurfactant and vice versa. For every phase diagram, oil and specific Smix ratio was mixed in volume

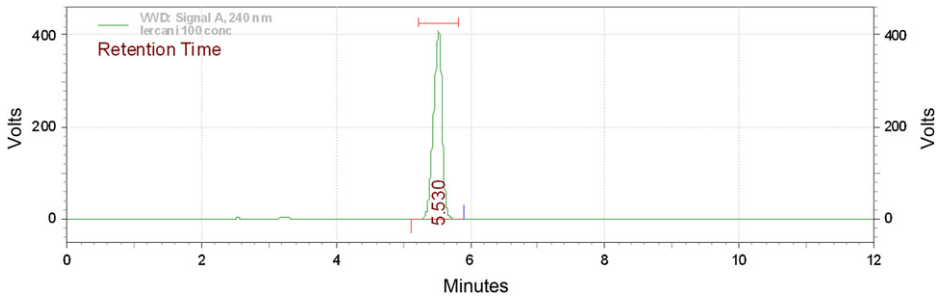

Fig. 2. Typical chromatogram for standard lercanidipine (100 μg/mL): peak (tR = 5.530 min) in the experimental conditions, At UV 240 nm; flow rate 1.2 mL/min; temperature 25 °C, mobile phase methanol: Water (90:10, v/v).

ratios ranging from 1:9 to 9:1 in sixteen ratios like 1:9, 1:8, 1:7, 1:6, 1:5 1:4, 1:3.5, 1:3, 3:7, 1:2, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Water titration method was employed to construct the phase diagrams. After each aliquot addition of aqueous phase, physical state of the mixture was marked on a pseudo ternary phase diagram using PCP triangle excel sheet where one axis symbolized the aqueous phase, one axis corresponded to the oil and the third indicated the Smix. Phase diagrams were also constructed in the presence of drug, using drug-enriched oil as the oil phase, to observe the effect of drug addition on the nanoemulsion boundary.

2.6. Selection of combination (NLI or NLII)

Formulations were chosen, from each of the constructed phase diagram of nanoemulsifying region so obtained, such that amount of oil in each formulation should be able to dissolve 10 mg of drug easily as recommended dose of LER is 10 mg/day Charde et al. [12].

From nanoemulsion region of same Smix ratio in phase diagram, 4 formulations were selected from each group (NL-I and NL-II) and subjected to following studies to select best group from two combinations for further studies and characterization:

1. *In vitro characterization of the formulation using optical microscopy*: Nanoemulsion formation and appearance of globule size was assessed under microscope (Olympus) at 10×. Microscopic images of two formulations (NLI and NL-II) are shown in Fig. 3.
2. *Globule growth studies*: 0.5 g of each formulation (NLI and NL-II) was dispersed in 250 mL of water. The sample was withdrawn at 0 and 6 h for microscopic examination and absorbance was measured at 400 nm (Table 1).
3. *Heating and cooling cycle*: 5 g of each formulation (NLI and NL-II) was filled in amber colored glass vials. Samples were subjected to 6 cycles (40 °C for 24 h in incubator and then at 4 °C for 24 h in refrigerator (Table 2).
4. *Interim stability studies*: Interim stability was carried out for one month (40 °C/75%RH) in each formulation (NLI and NL-II). Physical appearance and % drug content was observed after 1 month (Table 3).

2.7. Thermodynamic stability studies

Formulations were chosen from different Smix of selected combination and subjected to further thermodynamic studies.

2.7.1. Centrifugation study

In this study formulations were centrifuged at 5000 rpm for 30 min and the formulations were then checked instability such as phase separation, creaming or cracking. The formulations that did not show any signs of instability were chosen for heating-cooling cycle.

2.7.2. Heating and cooling cycle

Heating cooling cycle so performed involved six cycles between 4 °C and 40 °C with storage at each temperature for not less than 48 h. The formulations that passed at these temperatures without undergoing any creaming or cracking were chosen for Freeze thaw stress test.

2.7.3. Freeze thaw cycle (accelerated ageing)

Freeze thaw cycle involved three freeze thaw cycles at temperatures between −21 °C and +25 °C with storage at each temperature for not less than 48 h. Centrifugation was performed at 3000 rpm for 5 min. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected dispersibility study.

2.7.4. Dispersibility studies

Dispersibility studies were performed to evaluate the efficiency of dispersibility of oral SNEDDS. 300 mg of each formulation containing 10 mg of LER was added to 30 mL of distilled water and 0.1 N HCl in a standard USP XXII dissolution apparatus II (Labindia, Delhi, India). Paddle speed was adjusted to 50 rpm and the temperature was maintained at 37 ± 0.5 °C (Pouton CW, 1997). The formulations were visually evaluated using the grading system as reported (Table 4).

Formulations were chosen from Smix ratio, having the least Smix concentration and those passing the dispersibility test in distilled water as well as in 0.1 N HCl were given Grade A (Table 5).

2.8. Drug content

Weighed amount of formulations were assayed to determine the drug content. The weighed samples were dissolved in methanol and stirred by vortex mixer. The solutions were diluted to follow Lambert beer law. The solutions filtered, using Whatman filter paper was estimated spectrophotometrically (UV, Shimadzu, Japan) at 240 nm using standard curve.

2.9. Characterization of SNEDDS

SNEDDS have been classified in wide varieties of techniques. The primary means of self emulsification assessment is visual evaluation [11]. Percentage transmittance, droplet size analysis, polydispersity index, viscosity, zeta potential, emulsification time and drug content were used to characterize all the selected formulations and optimize the results. The results help in successful commercialization of any product.

2.9.1. Percentage transmittance

Since appropriate definition of microemulsion is “a system of water, oil, and surfactant (or amphiphile) which is a single optically isotropic and thermodynamically stable solution”. There-

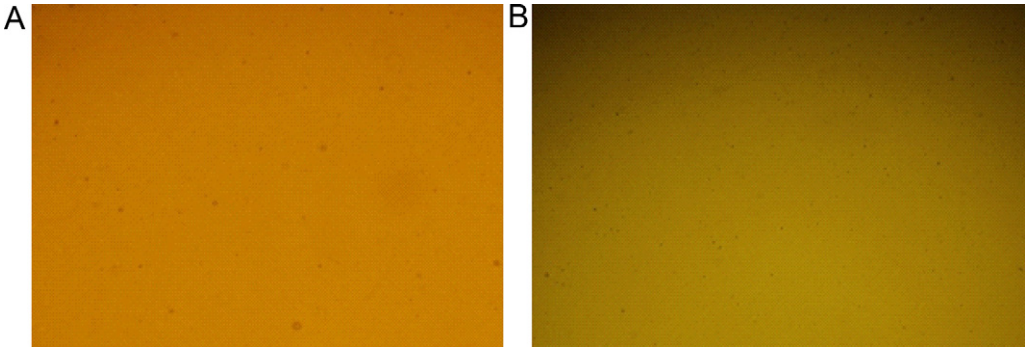

Fig. 3. Optical microscopic images of formulation (A) NL-II (Labrasol, Lauroglycol 90 and Labrafil M 1944CS Oil) and (B) NL-I (Cremophor EL, Capryol 90 with 10% Transcutol and Maisine oil).

Table 1
Evaluation of thermodynamic stability of the formulation after 0 and 6 h of two different groups NL-I and NL-II ($n = 4$).

Sample No.	Composition	Microscopic observation			Absorbance at 400 nm	
		Initial	6 h	Size rating	Initial	6 h
NL-I	Cremophor EL, Capryol 90 with 10% Transcutol, Maisine Oil	Extra fine globules	No globule size growth, No crystallization	XF	0.176	0.182
NL-II	Labrasol, Lauroglycol 90, Labrafil M 1944CS	Large globules	No globule size growth, No crystallization	L	0.488	0.482

Table 2
Observations after 6 heat and cool cycles of two different groups NL-I and NL-II ($n = 4$).

Sample No.	Composition	Crystal growth	Color appearance
NL-I	Cremophor EL, Capryol 90 with 10% transutol, Maisine Oil	No crystal growth	Light yellow
NL-II	Labrasol, Lauroglycol, Labrafil M 1944CS	No crystal growth	Brownish color

Table 3
Interim stability studies for one month at40 °C/75%RH. Physical appearance and % drug content was observed after 1 month ($n = 4$).

Sample No.	Initial		1 Month (40 °C/75% RH)	
	Physical appearance	Drug content (%)	Physical appearance	Drug content (%)
NL-I	Slight yellowish	100.9	Slight yellowish	100.4
NL-II	Slight yellowish	101.1	Dark brown	88.7

fore, to meet the isotropic parameter percentage transmittance studies is important. The optical clarity of SNEDDS formulations was measured spectroscopically upon dilution. Percentage transmittance was determined using Shimadzu UV spectrophotometer (Shimadzu, Japan). 300 mg of the formulation containing 10 mg of LER was diluted 100 times using double distilled water and analyzed at 500 nm using double distilled water as blank.

2.9.2. Emulsification time

The emulsification time of SNEDDS formulation was assessed on USP II dissolution apparatus (DS 8000, Labindia, India). Each formulation (300 mg) was added dropwise to 500 mL of distilled water maintained at 37 ± 0.5 °C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm. The emulsification time was assessed visually as reported by Khoo et al. [13].

2.9.3. Determination of zeta potential

Zeta potential was measured by photon correlation spectroscopy using Zetasizer (Nano ZS, Malvern Instruments, UK) equipped with 4.0 mW He–Ne red laser (633 nm) which measures the potential ranged from −120 to 120 V. SNEDDS formulation

Table 4
Observation table of dispersibility study.

S. No	Observation	Visual aspect	Grade
1.	Nanoemulsion formation in less then 30 s, which is clear and transparent, high spreadability	Bluish tinge	A
2.	Nanoemulsion formation in less than 1 min slightly less transparent, less clear	Bluish white tinge	B
3.	Nanoemulsion turbid in nature formed in less than 2 min.	Milky white tinge	C
4.	Nanoemulsion devoid of or minimal emulsification In 4-5 min with nonuniform distribution of oil droplets	Dull white tinge	D

Table 5
Dispersibility study of 16 formulations selected from group NL-I which passed thermodynamic stability test.

Oil	Smix	Water	Distilled water	0.1N HCl	Inference
1:1(Smix)					
10	50	40	Grade A	Grade A	Passed
15	40	45	Grade C	Grade C	Failed
3:1					
10	55	35	Grade A	Grade A	Passed
10	60	30	Grade A	Grade A	Passed
10	65	25	Grade A	Grade A	Passed
15	50	35	Grade A	Grade A	Passed
15	55	30	Grade B	Grade B	Failed
15	60	25	Grade A	Grade B	Failed
4:1					
10	55	35	Grade A	Grade A	Passed
10	65	25	Grade A	Grade A	Passed
10	70	20	Grade B	Grade A	Failed
10	75	15	Grade A	Grade A	Passed
15	55	35	Grade B	Grade B	Failed
15	60	25	Grade B	Grade C	Failed
15	65	20	Grade A	Grade A	Failed
15	70	15	Grade B	Grade B	Failed

(300 mg) was diluted 100 times using double distilled water and analyzed for zeta potential measuring instrument. All measurements were carried out at 25 °C (Table 6).

2.9.4. Viscosity

The viscosity of the prepared SNEDDS formulations was determined as such without dilution by Searle type R/S-CPS Plus Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) using spindle # C 50-1 at 25 ± 0.5 °C. The software used was RHEO3000. 600 μL of the formulation was used for viscosity determination. Controlled stress rate was studied to get important information of flow behaviour with change in speed of spindle (rpm) and shear strain at a temperature of 25 ± 0.5 °C. Wait time for the operation was 60 min. Viscosity of formulation with respect to variation of Smix and oil phase is shown in Table 6.

2.9.5. Droplet size analysis (particle size distribution)

SNEDDS formulation (300 mg) containing 10 mg of LER was diluted to 100 mL with distilled water in a flask and was mixed gently by inverting the flask. The particle size so formed was determined by dynamic light scattering (DLS) technique using Zetasizer (Zetasizer Ver. 6.01, Malvern Instruments, UK) using He–Ne red laser, 4.0 mW, 632.9 nm; temperature, 25 °C; refractive index, 1438; or with adjustment if needed. All measurements were done in triplicate using disposable polystyrene cuvettes (Malvern Instruments, UK) (Table 7).

2.9.6. Transmission electron microscopic analysis

Transmission electron microscopic (TEM) analysis was done to determine the shape of the dispersed oil droplets. SNEDDS was diluted with distilled water at 1:200 and mixed by slightly shaking. A drop of diluted SNEDDS was applied to a 300 mesh copper grid and was left for 1 min. After this the grid was kept inverted and a drop of phosphotungstic acid (PTA) (2% w/v) was applied to the grid for 10 s. Excess of PTA was removed by absorbing on a filter paper and the grid was analyzed using the JEM-2100F (JEOL, USA) operated at 200 kV operated with AMT image capture engine software.

2.10. In vitro drug release study

The quantitative estimation of release was perfomed by *in vitro* dissolution study of optimized SNEDDS formulation of LER, which was determined using US Pharmacopeia XXIV dissolution apparatus II (DS 8000, Labindia, India). The paddles were rotated at 100 rpm. The SNEDDS formulations were put in hard gelatin capsules (2 sizes). The dissolution vessels contained 500 mL of media of pH 1.2, 4.5 and 6.8 buffer solutions as dissolution medium maintained at 37 ± 0.5 °C. A 5 mL sample was withdrawn at 5, 10, 15, 20, 30, 45 min. The withdrawn sample was replenished with 5 mL of fresh blank media. The withdrawn samples were filtered and analyzed for LER content using validated HPLC method at 240 nm. Optimized formulation release was compared with that of plain LER and marketed tablet in multimedia to evaluate the solubility enhancement by SNEDDS.

2.11. Stability studies

The optimized SNEDDS formulation were put into empty hard gelatin Licap capsules (size 2) kept in glass bottle and subjected to stability studies at different temperatures and ambient humidity conditions 30 °C/65% RH, 40 °C/65%RH and 50 °C/75% RH. Sam-

Table 6
Oil used: (MAS) Maisine Oil, surfactant used: (CEL) Cremophor El, cosurfactant used: (C 90) Caproyl 90 with Transcutol (TC) (90:10), aqueous phase: double distilled water. PDI indicates polydispersity index, cP indicates centipoises, % *T* indicates Percentage transmittance, SD (standard deviation) and LER indicates lercanidipine ($n = 4$).

Composition (mg)						Drug Content (%)	Emulsification time (s)		Zeta potential	Viscosity(cP)	PDI
Code	LER	CEL	C90	TC	MAS	Mean (SD)	Mean (SD)	% T^a		Type of Flow	
F1	10	124.9	112.4	12.4	50.0	84.28(0.078)	26.56(2.65)	56.77	−10.26	Newtonian, 14	0.325
F2	10	173.0	51.9	5.76	57.6	92.80(0.089)	38.14(3.61)	49.35	−12.25	HershelBulkley, 143	0.275
F3	10	190.4	57.1	6.34	46.1	101.93(0.024)	45.26(1.96)	72.11	−12.20	HershelBulkley, 161	0.185
F4	10	192.8	57.8	6.42	42.8	102.65(0.015)	56.25(1.21)	76.98	−11.89	HershelBulkley, 182	0.157
F5	10	195.0	58.5	6.50	40.0	100.13(0.056)	57.36(2.19)	81.22	−11.56	HershelBulkley, 182	0.261
F6	10	203.0	45.7	5.07	46.1	98.56(0.065)	60.41(2.30)	65.28	−21.62	HershelBulkley, 189	0.451
F7	10	208.0	46.8	5.20	40.0	99.89(0.036)	60.83(2.52)	66.85	−20.90	HershelBulkley, 217	0.519
F8	10	211.7	47.7	5.29	35.3	97.58(0.069)	67.25(2.71)	68.53	−24.63	HershelBulkley, 243	0.568

Table 7
Particle size distribution as evaluating parameter of various formulations ($n = 3$).

Formulations	Particle size distribution (μm)		PDI	Z-average (d nm)	Precipitation
	D10	D90			
F1	0.046 ± 0.006	0.210 ± 0.002	0.325	97.79	Stable
F2	0.018 ± 0.008	0.232 ± 0.056	0.275	123.8	Stable
F3	0.025 ± 0.025	0.194 ± 0.056	0.185	62.3	Stable
F4	0.018 ± 0.005	0.061 ± 0.075	0.157	20.01	Stable
F5	0.019 ± 0.002	0.173 ± 0.004	0.261	110.8	Stable
F6	0.209 ± 0.052	0.022 ± 0.004	0.451	125	Stable
F7	0.019 ± 0.006	0.256 ± 0.058	0.519	135.2	Unstable
F8	0.292 ± 0.048	0.017 ± 0.023	0.568	158.9	Unstable

ples were charged to stability chambers (Thermolab, Mumbai, India) in glass bottles with humidity and temperature control. They were withdrawn at specified time intervals of 0, 30, 60 and 90 days for clarity, drug content, viscosity and particle size analysis. The drug content analysis was carried out using validated HPLC method at 240 nm over a period of 3 months under accelerated conditions.

3. Results and discussion

3.1. Solubility studies

Solubility of drug in excipients plays an important role in determining stability of formulation, as many formulations undergo precipitation before undergoing in situ solubilization. Therefore screening of appropriate oil is primary requirement of SNEDDS development. Various long chain, medium chain and synthetic triglycerides of different HLB values were employed for determination of solubility of LER. The self emulsifying nanoemulsion should be isotropic, monophasic and must be having good solubilizing capacity to incorporate dose of drug in minimum volume of formulation [9]. Higher solubility of drug in oil leads to lowers requirement of surfactant and cosurfactants, which minimizes the toxic effect of surfactants. Developed HPLC method explained before was used for solubility analysis of drug in excipients. High log P value 5.98 of LER assures high lipophilicity of drug as is also shown in results from solubility studies which are reported in Fig. 4. As seen from the figure, in oil phase Maisine 35-1 (52.14 ± 2.13 mg/mL) and Labrafil M 1944CS (43.57 ± 0.4 mg/mL) showed the highest solubilization capacity for LER, followed by Cremophor EL (20.01.1 ± 1.4 mg/mL) and Labrasol (50.32 ± 0.3 mg/mL) in surfactants in multicomponent phase system. Among various cosurfactants screened, Lauroglycol 90 (44.04 ± 2.1 mg/mL), Caproyl 90 (43.57 ± 0.2 mg/mL) and Transcutol (71.42 ± 0.5 mg/mL) showed good solubilization of drug. Compatibilities studies were performed to make sure selected vehicles not interfere with drug using developed analytical method of the drug. DSC thermograms of drug, excipients and their physical mixtures showed no definite interaction between the drug and selected excipients (Fig. 5) show DSC of drug alone. All the major peaks of drug and excipients were retained in DSC thermograms as shown in figure at almost same temperature with negligible change in enthalpy values. FTIR data also showed presence of all the peaks of drug indicating no interaction as shown of the Drug alone FTIR in Fig. 6. DSC thermogram of LER showed a distinct melting exotherm of drug at 198.078 °C with an enthalpy value of 1132.489 J/g.

3.2. Multicomponent phase diagram studies

Ternary phase diagrams were constructed to identify the self-microemulsifying region and to select a suitable concentration of oil and surfactant for the development of SNEDDS. These phase diagram plays important role in studying phase behaviour of the prepared nanoemulsions. A simple ternary phase diagram comprises of oil, water, and Smix, each corner in the phase diagram represents 100% of that particular component. The surfactant is mixed with oil phases at various ratios, and the mixture is titrated with aqueous phase at an increment of 9–95%. The oil phase is varied from 5% to 20%. The isotropic clear regions are identified by optical observation after formation of monolayer around emulsion droplets which reduces the interfacial tension, augmenting interfacial area and minimizing the destabilizing effect because of gain in dispersion entropy [24]. The cosurfactant helps to achieve two prerequisites of nanoemulsion formation it helps in keeping the film flexible, fluid, and tightly packed. Non-ionic or zwitterionic surfactants are often considered for pharmaceutical applications and nanoemulsion formulation since these are less toxic and less affected by pH and ionic strength changes [14]. Thus, for present study, Cremophor EL and Labrasol were used as surfactants hav-

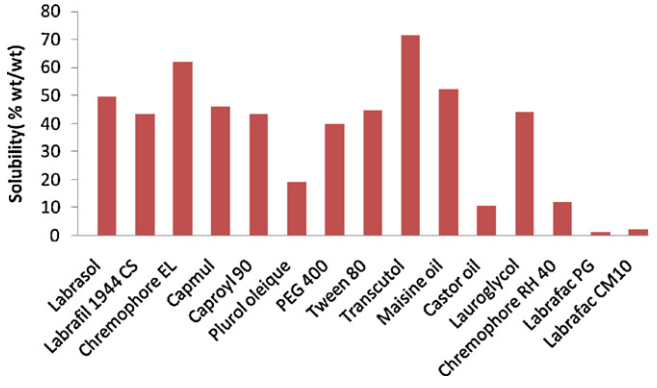

Fig. 4. Solubility of lercanidipine in various components. PEG indicates polyethylene glycol.

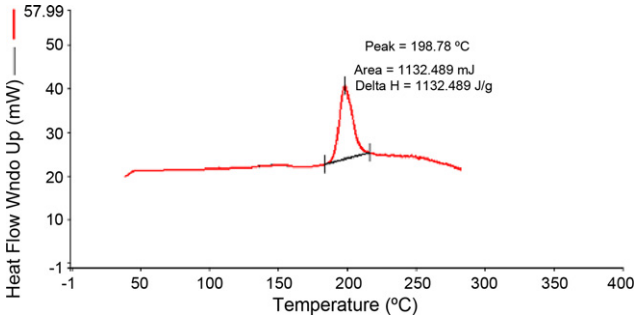

Fig. 5. DSC thermogram of lercanidipine.

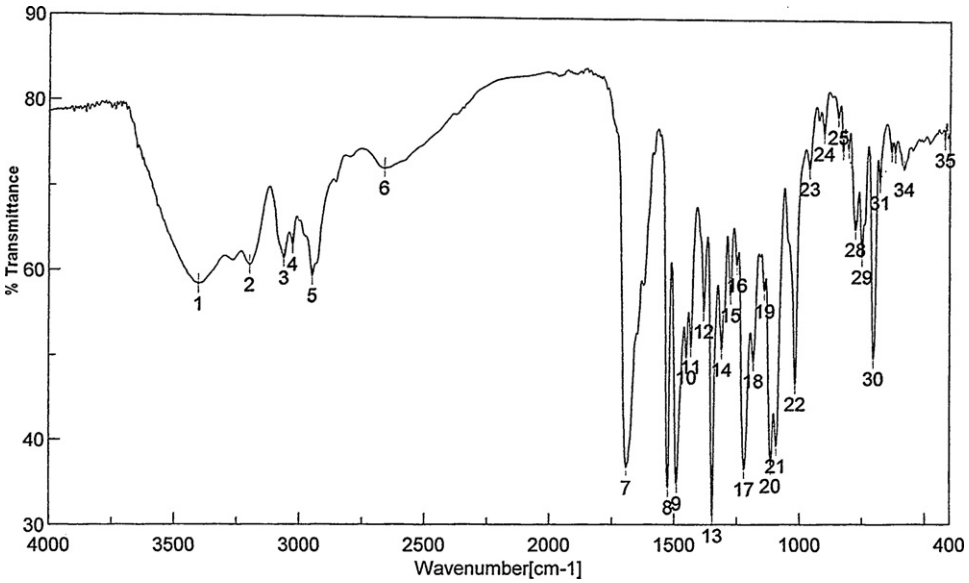

Fig. 6. FTIR of lercanidipine.

ing high HLB value of 14. As both the surfactants in two different groups have same HLB value, this neutralizes the effect of surfactants in nanoemulsifying area by reducing the interfacial energy required for nanoemulsion formation. Addition of cosurfactant which acts as a second amphiphile plays a vital role in reducing interfacial tension and formation of mechanical barrier to coalescence. Therefore for present study, Caproyl 90 with Transcutol (90:10 wt/wt) and Lauroglycol 90 of same HLB value were used as cosurfactant in two different combinations. A more comprehensive study was carried out in the current research for improved elucidation of the association between the phase behaviour of the mixture and nanoemulsifying area. Transcutol was used as per limitations in the inactive ingredients guidelines of FDA. Two cosurfactants were used in NLI as was observed during experiment, more oil could be emulsified therefore high nanoemulsion region was observed.

After studying the above two phase diagrams (Figs. 7 and 8). The formulation NL-I showed high nanoemulsifying area as compared to NLII. As required for SNEDDS development, surfactants of high HLB value have high self emulsifying capability in aqueous phase therefore both Cremophor EL and Labrasol used had high HLB value of 14 therefore surfactant selection does not leads to difference in emulsifying area in two combinations. Similarly among cosurfactants, Lauroglycol 90 in NL-II and Caproyl 90 in NL-I have approximately same HLB value though there is difference in length of carbon chain which is C_{12} in Lauroglycol 90 and C_8 in Caproyl 90 which leads to difference in emulsification capacity and reducing the interfacial tension close to zero which is property of ideal cosurfactant. As number and length of hydrophobic alkyl chains increases, molecular volume increases [15]. Lauroglycol 90 and Capryol 90 are propylene glycol mono- and diester of lauric acid and caprylic acid, respectively. Though both oils are monoesters of respective fatty acids, lauric acid has longer chain length than caprylic acid which limits emulsification capacity of Lauroglycol 90. Therefore in this study the effect of cosurfactant on nanoemulsifying area can be easily observed. Cosurfactants improve emulsification of surfactants by penetrating interfacial surfactant monolayer; their performance is affected by their structure and chain length [16,17]. Results obtained indicated that apart from HLB value and type of surfactant, other factors such as structure and relative length of hydrophobic chains of cosurfactant had influence on microemulsification and therefore nanoemulsifying area.

3.3. Selection of combination (NL-I or NL-II)

The nanoemulsifying area of two selected group was compared. Selection of formulation was also done keeping in concern over stability issues. Two formulations were selected from both the groups and subjected to optical microscopy, globule size growth using UV spectroscopy in visible region, Heating and cooling cycle and Interim stability studies. Microscopic observations show that formulations NL-II had large globule size whereas NL-I showed comparatively extra fine globule size (Fig. 3). NL-I showed low absorbance initially and after 6 h as compared to NL-II, indicating smaller globule size. Interim stability study and heating and cooling cycle showed that formulations NL-II changed their color from yellowish to dark brown and drug content also reduced to 87%. Formulations NL-I showed no color change and drug content remained same after 1 month. Therefore group NL-I was selected for optimization of SNEDDS formulation.

3.4. Thermodynamic stability

The main difference between emulsions and nanoemulsions is kinetic stability, reflecting the thermodynamic stability of the two systems. SNEDDS system undergoes in situ solubilization to form nanoemulsion system, and it should have stability such that it does not undergo precipitation, creaming or cracking. However, in many cases, prolonged storage might cause the drug to precipitate from the microemulsion; seed crystals start to appear and might grow to large crystalline materials that will precipitate out at the bottom of the vessel. Therefore to check the stability, formulation was exposed to centrifugation study, heating and cooling cycle and freeze thawing cycle. Sixteen formulations passed the test from NLI group and were further tested for dispersibility.

3.5. Dispersibility studies

As SNEDDS systems are released in the lumen of the gastrointestinal tract, it disperses to form a fine emulsion (micro/nano) with the aid of GI fluid. Thus, it is important that formed nanoemulsion

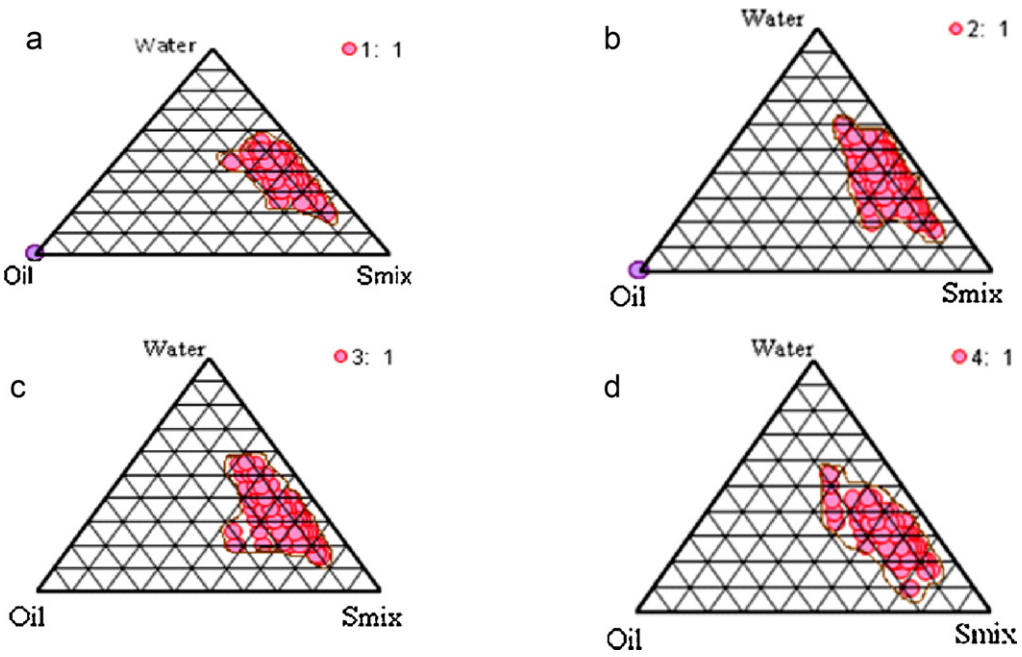

Fig. 7. Pseudoternary phase diagrams involving Capryol 90:Transcutol (90:10), Maisine 35-1 and Cremophor El as the cosurfactant, oil and surfactant respectively. Ratio of surfactant to cosurfactant in (a) is 1:1, (b) is 2:1, (c) is 3:1 and (d) is 4:1. Dotted area shows oil in water nanoemulsion region.

does not undergo precipitation following phase separation with infinite dilution in the GI fluids. It is observed more prominently with drugs having poor aqueous solubility or nanoemulsion which undergoes phase transition. To avoid such a situation, dispersibility studies in double distilled water and in 0.1 N HCl was vital. Formulations passing the dispersibility test in both the media in grade A were considered to pass the dispersibility test (Table 5). Since these formulations were certain to form nanoemulsion upon dilution in the aqueous environment, these were selected for further study. Eventually, eight formulations from various Smix ratios which passed dispersibility tests were selected for further study of globule size analysis, viscosity assessment, zeta potential and *in vitro* release studies.

3.6. Drug content studies

In all the eight selected formulations drug content was found highest in Smix ratios of 3:1, irrespective of less % wt/wt of oil used in F3, F4 and F5 formulations. Drug content was observed between 84 and 102.65% in the formulations. This helps in selecting the size of hard gelatin capsules based on volume of SNEDDS formulation to be filled incorporating 10 mg of LER.

3.7. Characterization of SNEDDS

Nanoemulsions have been characterised in wide varieties of techniques. Nanoemulsions comprises of various structures and

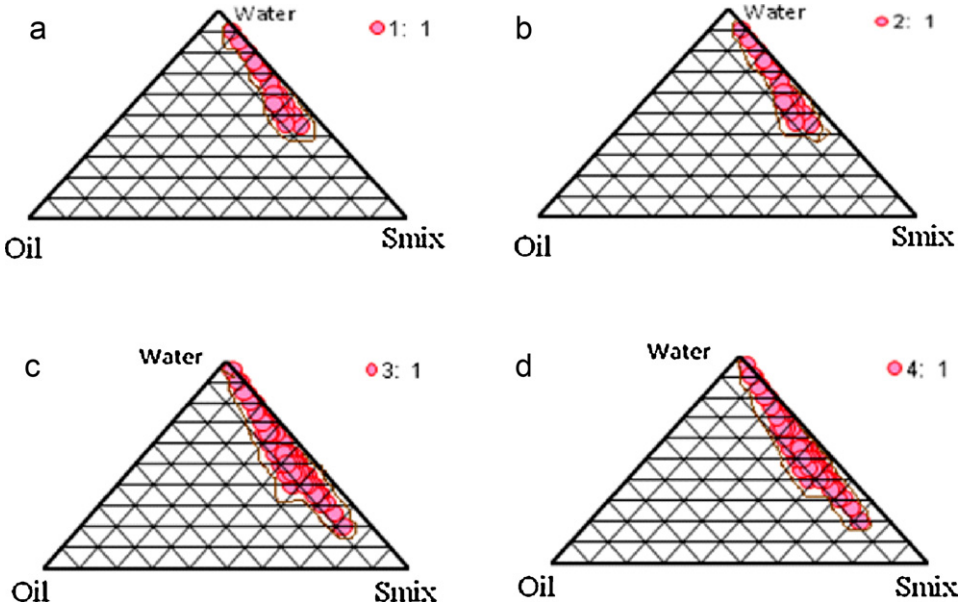

Fig. 8. Pseudoternary phase diagrams involving Lauroglycol 90, Labrafil M 1944CS and Labrasol as the cosurfactant, oil and surfactant respectively. Ratio of surfactant to cosurfactant in (a) is 1:1, (b) is 2:1, (c) is 3:1 and (d) is 4:1. Dotted area shows oil in water nanoemulsion region.

different components such as vesicles which are needed to be characterised by different techniques which makes easy and successful pharmaceutical applications of these formulations [18].

3.7.1. Percentage transmittance

The percentage transmittance of the eight selected optimised formulation was determined. Significant difference ($p < 0.001$) was observed among the percentage transmittance of formulation (F1–F6). As the value closer to 100% is formulation which is isotropic in nature therefore, optimized formulations of F3–F5 from Smix ratio of 3:1 gave maximum percentage transmittance (Table 6). As SNEDDS form nanoemulsion in GIT, it meets with patient acceptability but isotropic nature of formulations or percentage transmittance closer to 100% gives an indication of globule size in nanometer range. The droplet size of the emulsion is a crucial factor in self-emulsification performance, because it determines the rate and extent of drug release as well as absorption [19,20]. Thus, the formulation has the capacity to undergo enhanced absorption and thus ability to have increased oral bioavailability.

3.7.2. Emulsification time

The rate of emulsification is an important parameter for the assessment of the efficiency or spontaneous emulsification of formulation without aid of any external thermal or mechanical energy source. Formulation should disperse completely and quickly when subjected to aqueous dilution under mild agitation of GIT due to peristaltic activity. It has been reported that self emulsification mechanism involves the erosion of a fine cloud of small droplets from the monolayer around emulsion droplets, rather than progressive reduction in droplet size [21]. The ease of emulsification was suggested to be related to the ease of water penetration into the colloidal or gel phases formed on the surface of the droplet [22,23]. When the content of Caproyl 90 decreased from 112.4 to 45.7 mg, the emulsification time increased from 26.56 to 60.41 (Table 6). This might be because of high viscosity imparted by Cremophor EL which overshadows the effect of low viscosity of Caproyl 90 and increases the free surface energy of system thereby increasing the emulsification time with increase in content of surfactant.

3.7.3. Zeta potential measurement

Emulsion droplet polarity is also a very important factor in characterizing emulsification efficiency [19]. Zeta potential is the potential difference between the surface of tightly bound layer (shear plane and electroneutral region of the solution). The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability i.e. the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized. Negative values of zeta potential of the optimized formulations indicated that the formulations were negatively charged and therefore gives indication of stable system. Formulations F1, F3 and F4 are most stable formulations (Table 6).

3.7.4. Viscosity

Viscosity studies are necessary for SNEDDS to characterize the system physically and to control its stability. The viscosity of the optimized formulations was determined and the values are shown in Table 6. Flow behaviour of the formulations was studied and it was found that F1 formulation followed Newtonian flow

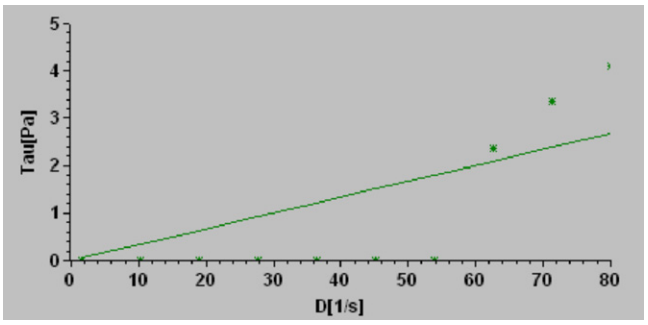

Fig. 9. Newtonian flow behaviour of F1 SNEDDS formulation with linear plot between shear stress with unit Tau (Pa) and shear rate with unit 1/s. Pa represents Pascal.

due to high content of Caproyl 90 which has viscosity close to zero (Fig. 9). Whereas other formulation F2 to F8 followed HershelBulkley flow and as observed the value of n (flow index) in equation was >1 therefore all followed shear thickening behaviour (Fig. 10).

3.7.5. Droplet size analysis

The droplet size of the emulsion is important factor in SNEDDS formulation, as this determines the rate and extent of drug release as well as absorption. The droplet size distribution of various formulations is given in Table 7. A decrease in the content of the oil phase (Maisine oil) resulted in a proportional decrease in particle size, because of the simultaneous increase in the Smix proportion. Increasing the Smix ratio led to a decrease in mean droplet size from formulation F1 to F5 this is probably explained by stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil–water interface. But, as we move from F6 to F8 mean droplet size increases which could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the high surfactant concentration and leading to ejection of oil droplets into

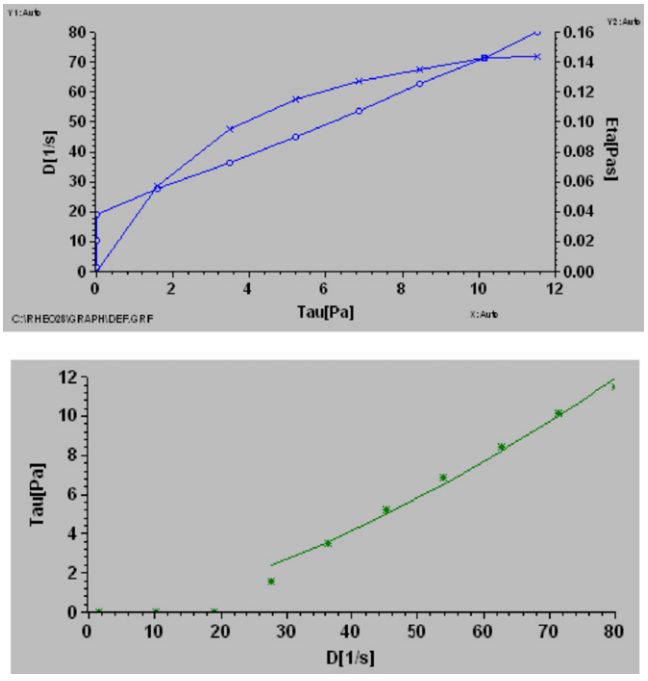

Fig. 10. HershelBulkley equation followed by optimized SNEDDS formulation F4. It follows shear thickening behaviour as we obtain non linear plot between shear stress and shear rate.

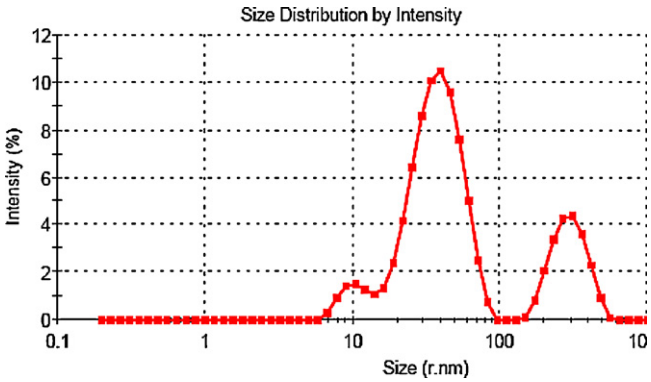

Fig. 11. Particle size distribution of lercanidipine SNEDDS formulation F3 at 24 h post dilution with distilled water using Malvern Zetasizer.

the aqueous phase. From Table 7, it can be seen that F3 formulation has size of 62 nm (Figs. 11 and 12) and formulation F4 has the smallest particle size 20.01 nm (Fig. 12) which is highly significant ($p < 0.001$) in comparison to other formulations of the group.

3.7.6. TEM analysis

TEM images of F3 and F4 after 24 h of post dilution in distilled water are shown in Fig. 12. It could be seen that spherical micelles were formed with no signs of coalescence even after 24 h of post dilution. The nanoemulsion droplets emerged as dark and the surroundings were found to be bright. Furthermore, no signs of drug precipitation were observed inferring the stability of formed nanoemulsions. Closer analysis of TEM images reveals that each globule is surrounded by a thick layer. This indicates the formation of monolayer around the emulsion droplets, reducing the interfacial energy, and forming a barrier to coalescence.

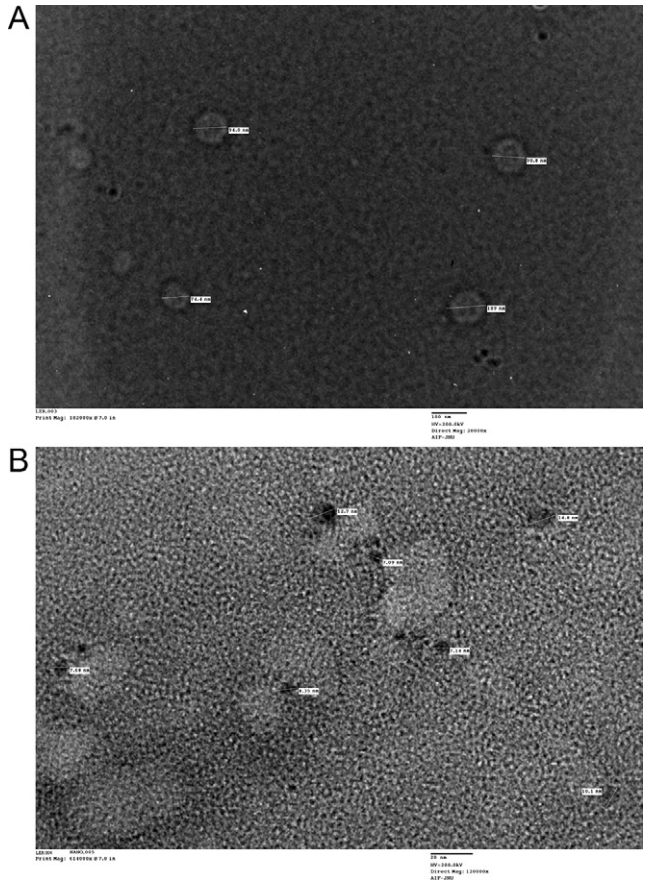

Fig. 12. TEM images of (A) F3 and (B) F4 after 24 h post dilution in distilled water.

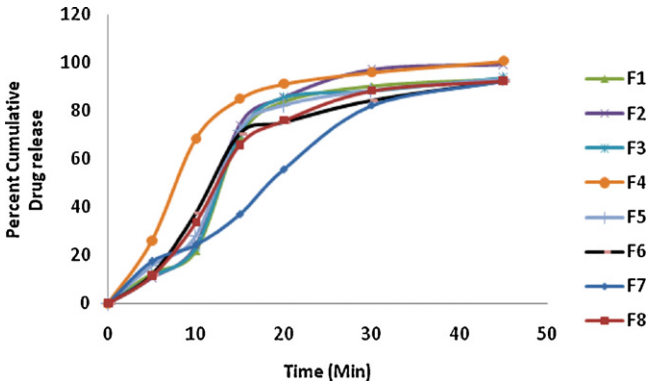

Fig. 13. Dissolution profile of lercanidipine (mean percent cumulative release. $n = 3$) from nanoemulsion formulations F1 to F8 in which F4 showed 100.7% release.

3.8. In vitro release studies

Drug release from the LER SNEDDS formulations F1 to F8 was as shown in Fig. 13. As shown the F4 provided the highest release of 100.7% among all the formulations F1–F8. This could be attributed to the small globule size of 20.01 nm, high percentage transmittance (76.98), low polydispersity index and negative zeta potential in case of F4 nanoemulsion formulation so formed after in situ solubilisation as also confirmed by TEM studies, which provided large surface area for the release of drug and thus permitting faster rate of drug release. The release from optimized and selected (F4) LER SNEDDS formulation was found to be significantly higher ($p < 0.001$) as compared with that of plain lercanidipine (Fig. 14). Thus, this greater availability of dissolved lercanidipine from the SNEDDS formulation could lead to higher absorption and therefore higher oral bioavailability. It was also seen that changes in the dissolution medium (buffer pH 1.2, 4.5, and 6.8) has significant effect on the drug release from either plain lercanidipine or the SNEDDS formulation (Fig. 14). The release was higher in pH 1.2 as compared to release in other pH. This is because of the presence of ester ionizable group and thus its solubility and dissolution is pH-dependent. The release was also evaluated from marketed "Zanidip tablet" (10 mg) in multimedia and was observed significantly high of developed SNEDDS formulation (see Fig. 15).

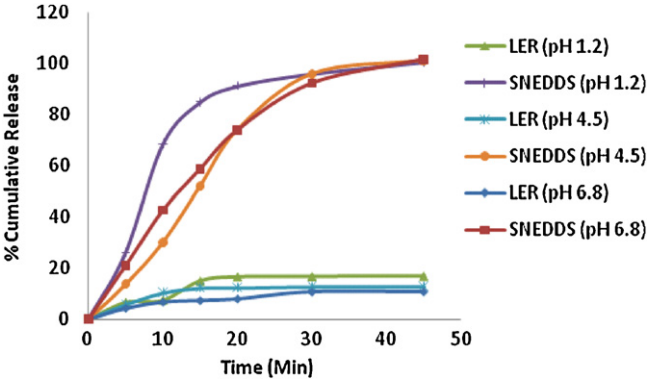

Fig. 14. Comparative results of percent cumulative release from plain lercanidipine and the selected F4 SNEDDS formulation in different dissolution media; $n = 3$. LER indicates plain lercanidipine; SNEDDS, self-nanoemulsifying drug delivery system.

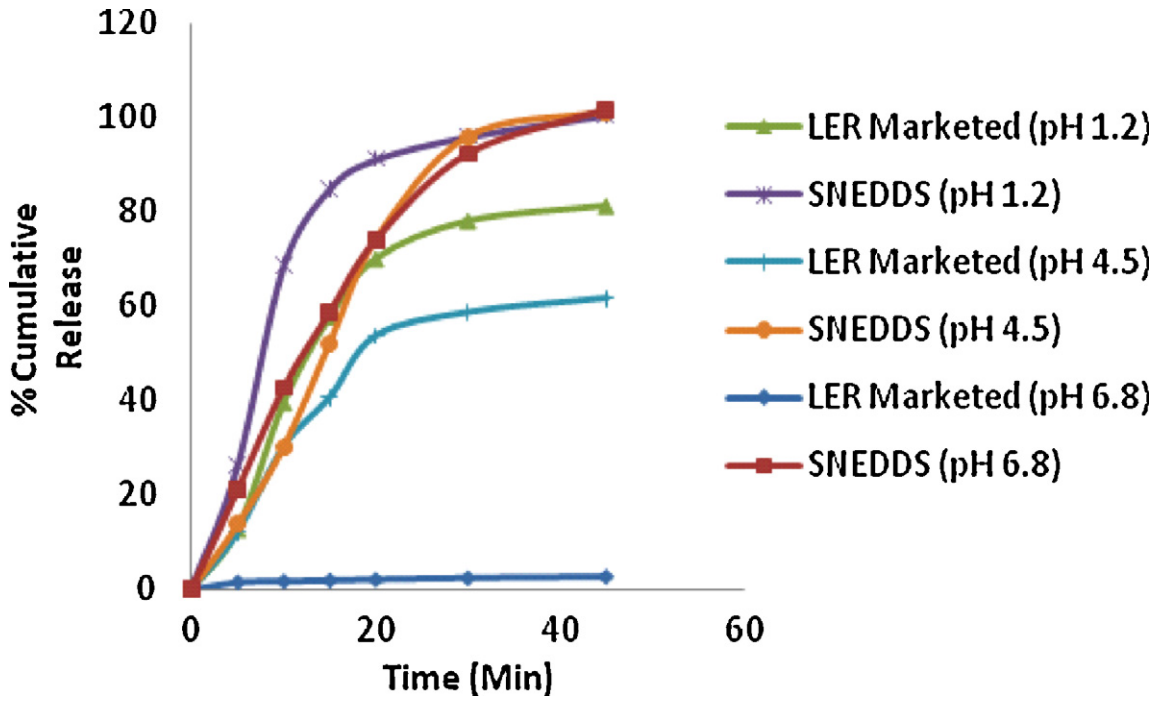

Fig. 15. Comparative results of percent cumulative release from Marketed Zanidip formulation of lercanidipine of 10 mg dose and the selected F4 SNEDDS formulation in different dissolution media; $n = 3$. LER Marketed indicates Zanidip tablet; SNEDDS, developed self-nanoemulsifying drug delivery system.

Table 8
Percent drug remaining, viscosity, droplet size (±SD, $n = 3$) and percentage transmittance in SNEDDS (F4) stored at elevated temperatures (30/65% RH, 40/65% RH and 50/75% RH °C) for 3 months.

Time (days)	Temperature/RH	% Drug remained	Mean droplet ± SD (nm)	Mean viscosity ± SD (cP)	Percent transmittance
0	30 °C/65%	100	62.15 ± 8.12	182.1 ± 0.77	77.1
30	30 °C/65%	99.56	62.12 ± 9.32	182.3 ± 0.80	77.12
60	30 °C/65%	99.24	63.56 ± 9.42	182.4 ± 0.76	77.12
90	30 °C/65%	99.04	63.65 ± 8.98	182.4 ± 0.84	77.13
0	40 °C/65%	100	62.15 ± 8.12	182.1 ± 0.77	77.1
30	40 °C/65%	99.62	62.13 ± 8.96	182.2 ± 0.81	77.21
60	40 °C/65%	99.43	64.21 ± 9.21	182.2 ± 0.80	77.22
90	40 °C/65%	99.15	63.12 ± 9.31	182.4 ± 0.81	77.19
0	50 °C/75%	100	62.15 ± 8.12	182.1 ± 0.77	77.1
30	50 °C/75%	99.52	64.12 ± 9.73	181.5 ± 0.81	77.13
60	50 °C/75%	98.43	64.85 ± 9.34	181.4 ± 0.82	77.14
90	50 °C/75%	97.86	62.26 ± 9.12	181.6 ± 0.83	77.15

3.9. Stability studies

During stability studies, droplet size, viscosity, drug content and percent transmittance were determined at 0, 30, 60, and 90 days. As can be seen from Table 8, these parameters were slightly varied with respect to time but the changes in the observed parameters were not found to be statistically significant ($p > 0.05$). Stability studies at 30 °C/65% RH, 40 °C/65%RH and 50 °C/75% RH predicted highest degradation of 0.97% of lercanidipine in the optimized F4 formulation at 50 °C and 0.90% at other conditions at the end of 90 days.

4. Conclusions

A SNEDDS formulation of lercanidipine containing Cremophor EL (45% wt/wt), Caproyl 90 (13.5% wt/wt) with Transcutol® HP (1.5% wt/wt) and Maisine oil (10% wt/wt) was successfully developed and optimized based on globule size in nanometer range, minimum polydispersity, lower viscosity, lower surfactant concentration thus minimizing toxicity issues, higher solubility and therefore increased dissolution rate. Results from the determination of zeta potential also established stability of the formulation which was reconfirmed in stability studies at different temperatures. After Phase diagram studies on two different combinations NL-I and NL-II, it was confirmed that structure and chain length plays a vital role in the performance of second amphiphile (Cosurfactant). Results obtained indicated that apart from HLB value and type of surfactant, other factors such as structure and relative length of hydrophobic chains of cosurfactant had influence on microemulsification and therefore nanoemulsifying area. Also pseudoternary plot corroborated the effect cosurfactants on the nanoemulsifying area. The optimized F4 formulation owing to nanosized showed higher percent cumulative release as compared to the pure drug as well as with marketed formulation of LER "Zanidip". Results from the stability studies at 30 °C/65% RH, 40 °C/65%RH and 50 °C/75% indicated stability of the optimized formulation as there was no significant change in the observed physical parameters. The present study confirmed that the developed SNEDDS formulation was superior to commercial formulation with respect to *in vitro* dissolution profile and could be used as a possible nanocarrier system to deal with poorly soluble calcium channel

blocker (LER) and later on to enhance bioavailability because of droplet size in nanometers.

Acknowledgements

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