RESEARCH ARTICLE

Investigation of the Effect of Variable Components on the Preparation and *In-vitro* Evaluation of Lacidipine as an Oral Nanoemulsion Dosage Form

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Received: 17th April, 2021; Revised: 13th June, 2021; Accepted: 17th July, 2021; Available Online: 25th September, 2021

ABSTRACT

Nanoemulsion (NE) is one of the prevalent techniques that has been utilized to take care of the solvency issues of numerous drugs. Lacidipine (LCDP) is a calcium-channel blocker with low water solubility and low bioavailability. LCDP was planned as a NE using oleic acid as an oil phase, tween 80 and tween 60 as surfactants and ethanol as a co-surfactant. Nine equations were readied, and various tests performed to ensure the stability of the prepared NEs, for example, thermodynamic stability, droplets size, polydispersity index, zeta potential, conductivity measurements, determination of pH, transmittance percentage (%T), drug content estimation, filter paper test, dilution test, viscosity measurements and drug release by *in-vitro* study. The selected formula exposed to advance examination which is Atomic force microscope (AFM).

Results of characterization showed that LCDP NE (F-4) using oleic acid, tween 80, ethanol and DDW in a ratio of (10:60:30) was selected as the best formula, since it have excellent thermodynamic stability with a droplets size of 16.56 nm, low PDI 0.105 , zeta potential (-7.80 mV), efficient electrical conductivity 0.163 ms/cm,with acceptable pH value (5.5), high percentages of transmittance (99.768), higher percent of drug content (99.23%), rapid spreadability of over filter papers,dilution test revealed the high physical stability of the LCDP NE with acceptable viscosity, and complete release of the drug after (30 minutes) with significantly higher ($p < 0.05$) dissolution rate in comparison with pure drug powder. The Atomic force microscope (AFM) of the selected formula (F-4) confirmed spherical small particle size without aggregation.

Keywords: Nanoemulsion, Oleic acid, Tween 80 and tween 60

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.65

How to cite this article: Dahash RA, Rajab NA, Almajidi YQ. Investigation of the Effect of Variable Components on the Preparation and *In-vitro* Evaluation of Lacidipine as an Oral Nanoemulsion Dosage Form. International Journal of Drug Delivery Technology. 2021;11(3):1031-1036.

Source of support: Nil.

Conflict of interest: None

1. INTRODUCTION

The liquid systems are the most diverse group of dosage forms, accommodating many therapeutic agents with different physicochemical properties.[1] New technology in pharmaceutical industries confronted with helpful creation frameworks, nanoemulsion (NE) are very hopeful dosage forms that have been used and an area of interest in the formulation field since nanotechnology has significant benefits in the delivery of NE is a colloidal drug delivery system. It is kinetically stable composed of two immiscible phases with suspended particles of one phase dispersed in the other phase.[2]

Lacidipine (LCDP) is a calcium channel blocker that is White to off-White Crystalline powder. Its molecular weight is 455.54 and structural formula (3,5-diethyl-4-{2-[(1*E*)-3-(tert-butoxy)-3-oxoprop-1-en-1-yl] phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) is a dihydropyridine calcium channel antagonist.[4] Lacidipine is mainly used for the prevention and treatment of hypertension. It is practically insoluble in water, sparingly soluble in dehydrated alcohol; freely soluble in acetone and dichloromethane.[5]

MATERIALS AND METHODS

Materials

LCDP was obtained from Baoji Guokang Bio-Technology Co., Ltd, China, Oleic acid was purchased from Thomas baker (chemicals), Ltd. India, tween 20 was obtained from Himedia, India. The tween 60 was purchased from Avonchem, England, and tween 80 from Riedel-De-Haen, Germany. Ethanol was obtained from Sigma-Aldrich, Germany, and Hydrochloric acid was obtained from Thomas Baker, India. Deionized water was purchased from J. T. Baker, The Netherlands. All other chemicals were of analytical grade.

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METHODS

Characterization of LCDP

Determination of LCDP Melting Point

LCDP melting point was measured by utilizing a capillary tube method announced by the United States Pharmacopoeia (USP). The temperature recorded was the temperature at which LCDP converted to the liquid state.[6]

Construction of Pseudo-ternary Phase Diagrams

Deionized water (DDW)was used as an aqueous phase, surfactant, and co-surfactant mixture (Smix) were mixed at different ratios (1:3, 1:2, 1:1, 2:1, and 3:1).[7]

The homologous mixtures of SMIX and oil were titrated with water separately by adding 0.5 mL of water (drop by drop) in each step at room temperature under gentle stirring (~500 rpm) and was optically observed. The water amount at which the transition occurs (from transparency to turbidity endpoint) was derived from the volume measurements. The monophasic samples should be pointed out in the phase diagram when the solutions were transparent and clear. The emulsion area is the region covered by monophasic solution points.[8,9]

Preparation of LCDP NE

According to pseudo-ternary phase diagrams, different o/w NE formulations (Table 1) were prepared to utilize the Smix and oil ratios.

Primary LCDP emulsion was prepared by dissolving (2 mg) of the drug in the selected oil, magnetic stirrer used. The selected Smix was added slowly in a fixed proportion until a clear solution was obtained, the DDW was added slowly with continuous stirring (~500 rpm) at room temperature until the formation of a transparent emulsion. The emulsions prepared ultrasonicated using a 20 kHz sonicator for 10 minutes to produce minimal particle size NEs.[10,11]

LCDP NEs Characterization Lacidipine Nanoemulsions Characterization

Visual Transparency

Optical observation for NE formulas was determined under the good light source for transparency and ease of flowability.[12]

Thermodynamic Study

It includes the following tests:

Centrifugation Study

In this study, LCDP nano-formulations were centrifuged at 5000 rpm for 30 minutes and then checked for instability such as cracking, separation, and creaming. The formulations that did not show any signs of instability were selected for the heating-cooling cycle.[13]

Heating-cooling Cycles Test

The stability of NE relies on the variation of temperature was examined by the heating-cooling cycle. Nano-formulations were subjected to six cycles between refrigerator temperature 5°C and at 50°C storage at each temperature for not less than 48 hours, the only formulation that remained stable at these temperatures was subjected to freeze-thaw cycle.[14]

Freezing-thawing Test

Formulations were exposed to two different temperatures (-21°C) and (25°C), utilizing a refrigerator and the time for each temperature not less than 24 hours. This test was used to indicate accelerated stability of LCDP formulations.[15]

Particle Size

The particle size of NE was demonstrated by analyzing the fluctuations in light scattering due to Brownian motion of the particle utilizing dynamic light scattering technique (Zetasizer Nano ZS).[16]

Polydispersity Index Measurement (PDI)

The measurement of (PDI) was done to get information about the uniformity of the droplet size. The PDI value closer to 1 indicates a wide droplet size range, while the lower PDI value (near zero) indicates a monodisperse droplet population.[17,18]

Transmittance Percentage (%T)

NEs were checked by using turbidity test. Taking 2 mL of each NE formula (F1–F9) and measuring the absorbance at 650 nm (light wavelength) using UV/Vis spectrophotometer, the distilled water was used as a blank.[19,20]

RESULTS AND DISCUSSION

Characterization of LCDP

Determination of LCDP Melting Point

The LCDP melting point was 179°C, which is inconsistent with the reported data (178°C) and proves the drug powder purity.[21]

Table 1: Composition of the LCDP NE

F-Code	*LCDP %w/v*	*Oleic acid*	*Surfactant*	*Co-surfactant*	*Smix ratio*	*Smix % w/w*	*DDW %w/w*
F-1	0.02	10	Tween 80	Ethanol	1:3	60	30
F-2	0.02	10	Tween 80	Ethanol	1:2	60	30
F-3	0.02	10	Tween 80	Ethanol	1:1	60	30
F-4	0.02	10	Tween 80	Ethanol	2:1	60	30
F-5	0.02	10	Tween 80	Ethanol	3:1	60	30
F-6	0.02	10	Tween 60	Ethanol	1:2	60	30
F-7	0.02	10	Tween 60	Ethanol	1:1	60	30
F-8	0.02	10	Tween 60	Ethanol	2:1	60	30
F-9	0.02	10	Tween 60	Ethanol	3:1	60	30

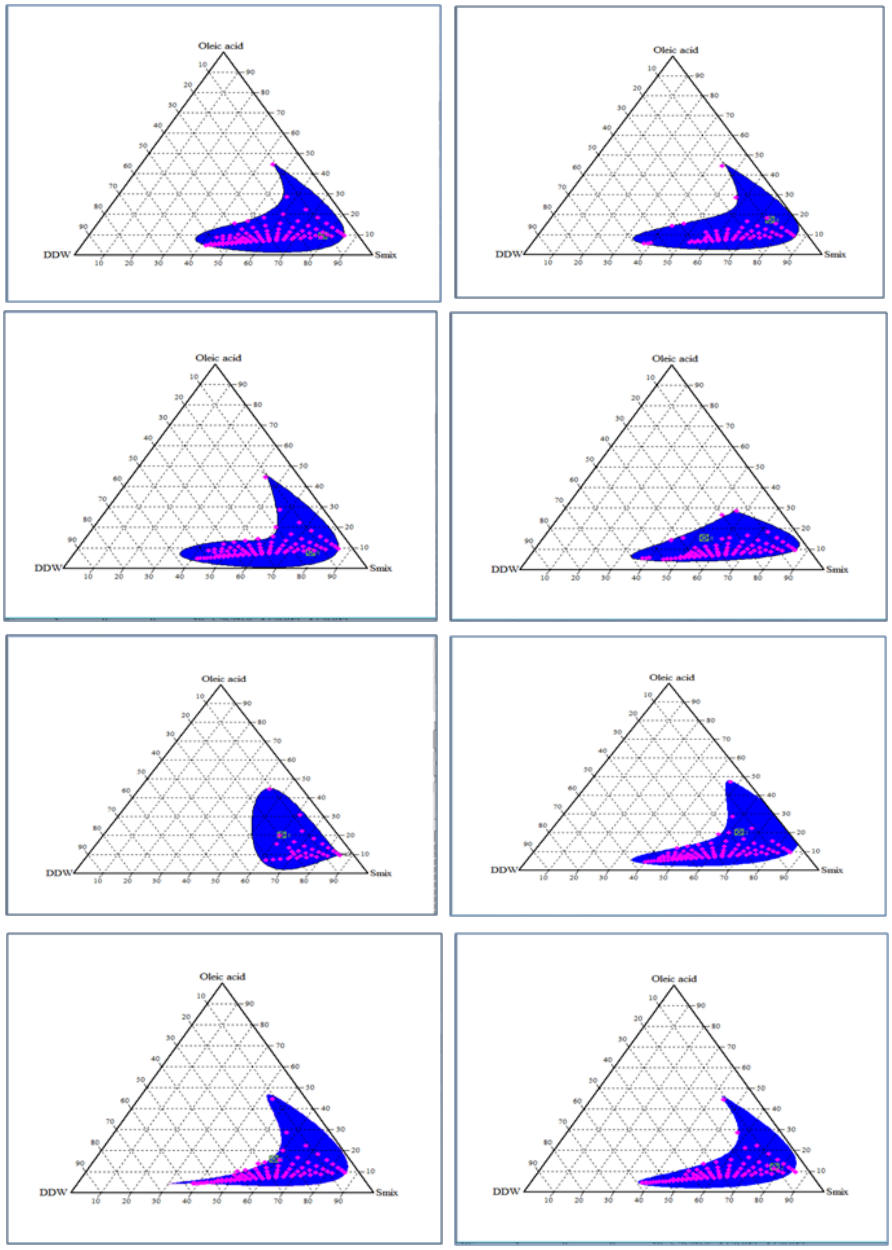

Figure 1: Pseudo-ternary phase diagrams showing the (o/w) nanoemulsion (colored area) regions of oleic acid at different Smix ratios

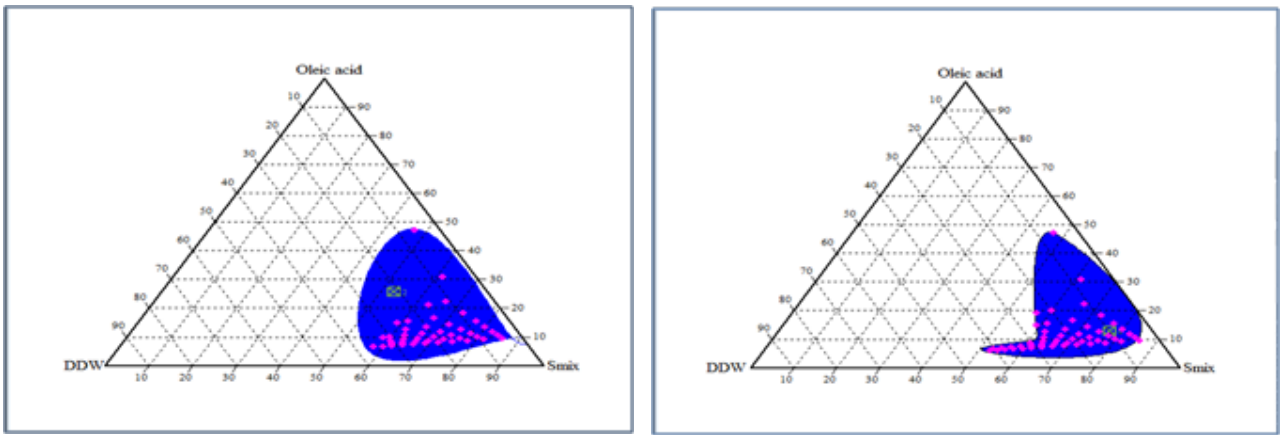

Figure 2: Pseudo-ternary phase diagrams showing the (o/w) nanoemulsion (colored area) regions of oleic acid at different Smix ratios

Construction of Pseudo-ternary Phase Diagrams

Pseudoternary phase diagrams were constructed to examine the formation of o/w NEs using four components: oil, surfactant, co-surfactant, and aqueous phase. In these diagrams, the shaded area indicates the presence of good nano emulsifying activity of the formulated NEs and perfect interaction among the oil, Smix, and DDW Figures 1 and 2 showed the pseudo-ternary phase diagram for the o/w NEs using oleic acid as an oil phase, tween 80 and tween 60 as a surfactant and ethanol as a co-surfactant.

HLB value of tween 80 and tween 60 are 15 and 14.9, respectively, so they are hydrophilic surfactant and more soluble in aqueous phase favoring for the formation of o/w NEs according to Bancroft's rule which state that the phase in which the surfactant is more soluble represents the continuous phase and determine the type of NE produced (o/w) or (w/o).[22]

Preparation of Lacidipine Nanoemulsion

Eighteen formulas were prepared according to pseudo ternary diagrams and were subjected to characterization.

Figure 3: Lacidipine nanoemulsions(F1-F9) visual transparency

Table 2: Results of thermodynamic stability studies for lacidipine nanoemulsions

F-code	*Centrifugation test*	*Heating-cooling cycles*	*Freeze-thawing cycles*
NE-1	Pass	Pass	Pass
NE-2	Pass	Pass	Pass
NE-3	Pass	Pass	Pass
NE-4	Pass	Pass	Pass
NE-5	Pass	Pass	Pass
NE-6	Pass	Pass	Pass
NE-7	Pass	Pass	Pass
NE-8	Pass	Pass	Pass
NE-9	Pass	Pass	Pass

Table 3: Particle size measurements for LCDP nanoemulsions

F-code	*Particle size (nm)*	*F-code*	*Particle size (nm)*
F-1	481	F-6	324.2
F-2	312.3	F-7	302.5
F-3	243.6	F-8	209
F-4	16.56	F-9	193.4
F-5	193.4		

Lacidipine Nanoemulsion Characterization

Visual Transparency

Figure 3 showed that all the prepared NE formulas (F1–F9) were optically clear since the small droplet size causes weak light scatters (more transparent).[23,24]

Thermodynamic Study

NEs are thermodynamically stable systems, formed of a particular concentration of oil, Smix and DDW with no phase separation or creaming, small droplet size prevents any flocculation, enabling the system to remain dispersed with no separation,[25,26] and the results were revealed in the Table 2.

Droplet Size

Regarding particle size, the results illustrated that when the surfactant concentration increased, the particle size reduced.

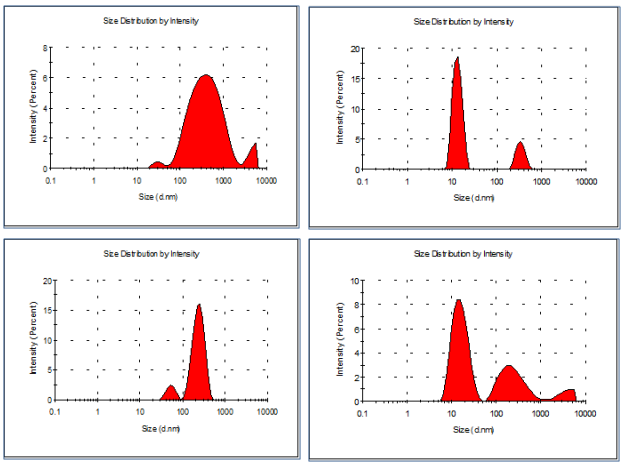

Figure 4: Particle size distribution of LCDPNEs

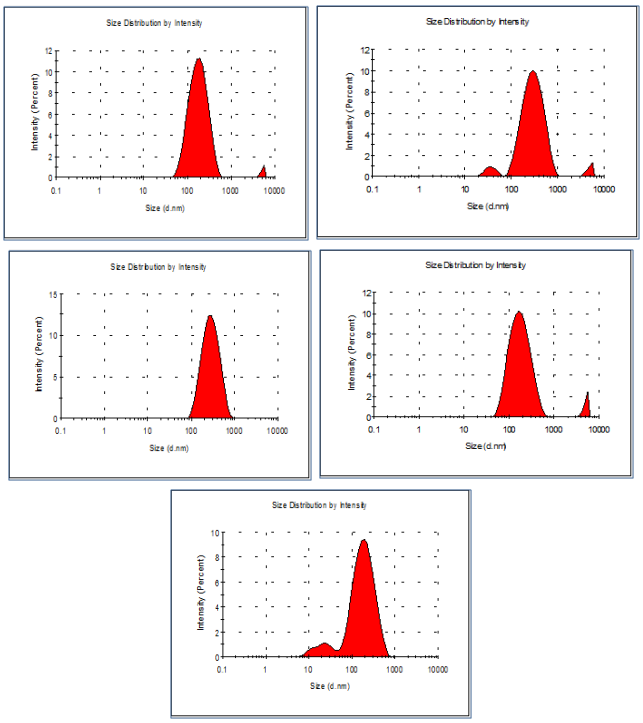

Figure 5: Particle size distribution of LCDPNEs

Since this high surfactant concentration decreases surface tension and stabilizes newly developed surfaces during homogenization and smaller particles. These results may also be due to the accumulation of surfactant molecules at the interface provides better stabilization against droplet aggregation and helps in lowering the flocculation rate and greater penetration of the oil phase in the hydrophobic region of the surfactant leads to reduce the droplet size.[27,28]

Table 3 showed that F-5 had a particle size more significant than that of F-4; by increasing surfactant concentration, droplet size increased due to the depletion-flocculation mechanism of adsorbed surfactant. When the surfactant concentration increase, it forms micelle in the continuous phase instead of orientation on the particle surface, so the continuous phase between some droplets moves to them, which causes the depletion of the continuous phase between the drops. Consequently, the aggregation takes place, and the particle size increases.[29,30] Figures 4 and 5 show the droplet size measurement of the LCDP NEs (1-9).

This index represents the homogeneity and uniformity of the particles in the formulations and width of the size distribution. PDI refers to the quality of a polydispersity index and is not stable. The low value of PDI (0.08–0.7) is considered desirable for uniform distribution, stability, and high dispersion,[31,32] Table 4 showed that all NE formulations have PDI less than 0.7 of droplet size.

Transmittance Percentage (%T)

Percentages transmittance of NEs demonstrates that all formulas were translucent, clear, and convey the light easily since the values of percentage transmittance were closer to 100 % since the reducing droplet sizes to the nanoscale led to higher transparency, as revealed in Table 5.[33,34]

Table 4: Polydispersity index for LCDP formulations

F-code	*PDI*	*F-code*	*PDI*
F-1	0.492	F-6	0.399
F-2	0.377	F-7	0.249
F-3	0.369	F-8	0.455
F-4	0.105	F-9	0.379
F-5	0.379		

Table 5: Percentage of transmittance (%T) of LCDP nanoemulsions.

F- code	*Absorbance*	*%T*
F-1	0.0032	99.26
F-2	0.0201	95.477
F-3	0.0213	95.213
F-4	0.0098	97.768
F-5	0.0116	97.364
F-6	0.0081	98.152
F-7	0.0105	97.611
F-8	0.0048	98.90
F-9	0.0178	95.984

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