Formulation and Investigation of Lacidipine as a Nanoemulsions
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Abstract
Lacidipine (LCDP) is a calcium-channel blocker with low aqueous solubility and bioavailability. Nanoemulsion (NE) is one of the popular methods that has been used to solve the solubility problems of many drugs. LCDP was formulated as a NE utilizing triacetin as an oil phase, tween 80 and tween 60 as surfactants and ethanol as a co-surfactant. Nine formulas were prepared, and different tests performed to ensure the stability of the NEs, such as thermodynamic stability, particle size, polydispersity index, zeta potential, dilution test, conductivity test, drug content, viscosity and in-vitro drug release. Results of characterization showed that LCDP NE (F-5) using triacetin, tween80 , ethanol and DDW in a ratio of (10:60:30)  was selected as the best formula, since it has excellent thermodynamic stability with a particle size of 13.42, low PDI 0.243 , zeta potential (-14.5mV), good dilution without drug precipitation , efficient electrical conductivity 0.241ms/cm , higher percent of drug content (99.14%) with acceptable viscosity, and complete release of the drug after (30 min.) with significantly higher (P<0.05) dissolution rate in comparison with pure drug powder.

The selected formula (F-5) subjected to further investigations as drug and excipient compatibility study by Fourier transform infrared spectroscopy (FTIR) and high performance liquid chromatography (HPLC) and Atomic force microscope (AFM).

The outcomes of the (FTIR) explain that the distinctive peaks for LCDP were displayed the same functional group's band with very slight shifting, which suggests the presence of hydrogen bonding. This indicates that there was no interaction between LCDP and other NE components, as the FTIR analysis results confirmed that there was no change in retention time and no extra peaks reported. Therefore, these excipients were found to be compatible with LCDP.

In conclusion, the NE was found to be an efficient method to enhance the solubility and dissolution rate of drugs that have poor water solubility (lipophilic drugs).

Key word: Lacidipine, Triacetin, Tween 80, Tween60 , Nanoemulsion.
Introduction

Oral delivery of drugs is regarded as the optimal route to achieve therapeutic and prophylactic effects against various diseases, especially chronic conditions. It may have poor bioavailability as a hurdle, leading to challenges for pharmaceutical manufacturers to design delivery system(s) that can provide improved pharmacokinetic profiles and hence therapeutic responses (1,2).

The term nanoemulsion (NE) refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. Their size varies from 10 to 1000 nm. (3,4).

Lacidipine (LCDP) is a dihydropyridine calcium-channel blocker developed for oral administration. It used in the treatment of hypertension and atherosclerosis and possessed an antioxidant effect (5). Chemical name of LCDP is (E)-4-[2-[3-(1,1-Dimethylethoxy) -3-oxo-1-propenyl] phenyl]-1,4-dihydro -2,6-dimethyl -3,5-pyridine dicarboxylic acid diethyl ester. The molecular weight (455.5) and pka 7.5 , log P (octanol/water) is 5.0, the powder is a white to pale yellow powder, melt at 17°C (6,7), and the chemical structure is shown in figure(1) (8).

Figure 1. The chemical structure of LCDP

Lacidipine poorly absorbed from the gastrointestinal tract after oral doses and undergoes extensive first-pass metabolism the bioavailability has been reported to be 2 to 10%. The rate limiting step for drug absorption in this class is dissolution (9).

This study aims to prepare LCDP as a nanoemulsion to improve its dissolution rate.

Materials and Methods

Materials

Lacidipine was obtained from Baoji Guokang Bio-Technology Co., Ltd, China, triacetin was purchased from Hyper-Chem LTD CO, China, tween 20 obtained from HiMedia Chemicals, India, tween 60 was obtained from Avonchem, England, tween 80 was purchased from Riedel-De-Haen, Germany, Ethanol was purchased from Sigma-Aldrich, Germany, Hydrochloric acid was purchased from Thomas Baker, India. All other chemicals were of analytical grade.

Methods

Characterization of LCDP

Differential scanning calorimetry analysis (DSC)

The DSC study was performed for the pure drug to evaluate the thermotropic properties and thermal behavior of the LCDP.

Approximate weighed samples (about 3 mg) were put in sealed aluminium pans and warmed at a scanning rate of 10°C/min. (11).

Saturation solubility study of LCDP

Saturated solubility of LCDP was estimated in various oils, surfactants, co-surfactants and dissolution media.

The measurement of solubility was done as follows: The excess amount of LCDP was added to (5 mL) of each selected individual oils, surfactants and co-surfactants contained in stoppered vials separately, then shaken utilizing a water bath shaker at 25±1°C for 72 hours to prepare a saturated solution. After accomplishing the equilibrium, the mixtures were centrifuged at 3000rpm for 15min, followed by filtration through a 0.45-micrometer millipore filter. Samples were suitably diluted with ethanol and analyzed by UV/Vis spectrophotometer at λ max of LCDP. The measurements were done in triplicate (12,13).

Construction of pseudo-ternary phase diagrams

The aqueous titration method was utilized to construct the pseudo-ternary phase diagram. Based on the solubility studies, triacetin was selected as an oil phase, tween 80 and tween 60 were selected as surfactant and ethanol was selected as a co-surfactant, and deionized water (DDW) used as an aqueous phase. The oil: surfactant:co-surfactant (Smix) mixed at different ratios ranging from (1.9 to 9:1). Smix ratios was 1:3, 1:2, 1:1, 2:1and 3:1 for Smix (tween 80/ethanol) and 1:2, 1:1, 2:1 and 3:1 for Smix tween 60/ethanol (14,15).

Preparation of LCDP nanoemulsion

Different o/w NE formulations (table 1) were prepared using the Smix and oil concentrations according to pseudo-ternary phase diagrams; primary LCDP emulsion was prepared via dissolving (2 mg) of the drug in the selected oil. The magnetic stirrer used to ensure complete mixing then the selected Smix added slowly in a fixed ratio till the clear solution was obtained.
DDW added dropwise to the clear solution with the continuous stirring at (~500 rpm) at room temperature until the formation of a clear emulsion. After that, the prepared emulsions were ultrasonicated via utilizing a 20 kHz sonicator for 10 min (16, 17).

Table 1. Composition of LCDP Nanoemulsion

| NE-F | Triacetin %W/W | Surfactant | Co-surfactant | Smix ratio | Smix % W/W | DDW %W/W |
|------|----------------|------------|---------------|------------|------------|-----------|
| F-1  | 10             | Tween 80   | Ethanol       | 1:3        | 60         | 30        |
| F-2  | 10             | Tween 80   | Ethanol       | 1:2        | 60         | 30        |
| F-3  | 10             | Tween 80   | Ethanol       | 1:1        | 60         | 30        |
| F-4  | 10             | Tween 80   | Ethanol       | 2:1        | 60         | 30        |
| F-5  | 10             | Tween 80   | Ethanol       | 3:1        | 60         | 30        |
| F-6  | 10             | Tween 60   | Ethanol       | 1:2        | 60         | 30        |
| F-7  | 10             | Tween 60   | Ethanol       | 1:1        | 60         | 30        |
| F-8  | 10             | Tween 60   | Ethanol       | 2:1        | 60         | 30        |
| F-9  | 10             | Tween 60   | Ethanol       | 3:1        | 60         | 30        |

LCDP nanoemulsion characterization

Visual transparency
Optical observation for NE formulas was intent utilizing good light source for transparency and flow ability (18).

Thermodynamic study
I. Centrifugation study: In this study, formulas were centrifuged at 5000 rpm for 30 min and then checked for instability such as phase separation. The formulations that did not show any signs of instability were chosen for heating-cooling cycle (19).

II. Heating-cooling cycles test: Stability of NE depends on the variation of temperature was studied by heating-cooling cycle. Formulations subjected to six cycles between refrigerator temperature 5 °C and at 50 °C storage at each temperature for not less than 48 hours (20).

III. Freezing–thawing test: This test was done by exposing the formulations for two different temperatures which are (-21°C) and (25°C) using refrigerator and the time for each temperature not less than 24 hours (21).

Droplet size measurement
The droplet size of NE was established by analyzing the fluctuations in light scattering due to the Brownian motion of the particle utilizing dynamic light scattering technique (Zetasizer Nano ZS, Malvern, UK) (22).

Polydispersity index measurement (PDI)
The estimation of the (PDI) gives information about the uniformity of droplet size within the formulated NE. The lower PDI value (near zero) indicates a monodisperse droplet population (23).

Zeta potential measurement (ζ – potential)
The droplet charge (zeta potential) of the NEs was determined to utilize dynamic light scattering technique, Zeta potential was believed to be sufficient for ensuring the physical stability of NEs (24).

Dilution test
The aqueous dilution test was performed, one mL of each NE formulas (F1-F9) diluted to 50 mL, 100 mL and 500 mL with distilled water at 25°C with constant stirring at 50 rpm and observed visually for turbidity, clarity and phase separation (25).

Conductance measurement
The o/w NEs are highly conducting since the water is the external phase, whereas w/o NEs are not conducting as they have water in the internal phase (26).

Drug content estimation
Accurately 10 mL of each NE formula which contains (2 mg) was diluted with ethanol to the sign (100 mL) in a volumetric flask and subjected to the centrifugation for 15 minutes (3000 rpm), then filtered using 0.45 μm filter syringe and suitably diluted. Determination of the contents of LCDP NEs via utilizing UV/Vis spectrophotometer at the selected λ max (27, 28).

Viscosity measurement
Determination of viscosities determines whether the system is o/w or w/o emulsion (29).
**In vitro drug dissolution study**

The *in vitro* release of LCDP loaded NE occurs using USP dissolution apparatus type–II. Dialysis bag (Molecular cut off 12000Da) was utilized, ten ml of each formula which contain 2mg of LCDP was put in the bag, and this bag was immersed in 500 ml of dissolution medium. The rotation speed was 50 rpm, and the dissolution medium was 0.1N HCl with 1% tween 20 at 37 ± 0.5 °C. Samples (5 ml) were withdrawn at a regular time intervals (5, 10, 15, 20, 30, 40, 50 and 60 min) from the dissolution medium and the samples then filtered by using through a 0.45 μm filter syringe and were analyzed by UV/Vis spectrophotometer at the λ max. of the drug.

**Selection of the optimum formula**

The choice of the optimum formula was accomplished, and this achieved according to the globule size analysis, PDI, zeta potential measurements, electrical conductivity, viscosity, drug content and in vitro release studies.

**Evaluation of the selected LCDP optimum formula**

**Drug and excipient compatibility study by FTIR**

To demonstrate any possible interaction between the drug and the utilized excipients in the selected formula. Samples were mixed with potassium bromide and pressed in the form of a disc; FTIR spectroscopy analyzed the disc from 4000–400 cm⁻¹.

**Validation of the HPLC method**

HPLC method used in the investigation and to determine the possible interactions between oil, drug and other excipients. A waters HPLC system used which was equipped with a SPA-20A detector. The system was controlled through Breez software. The mobile phase which consisted of acetonitrile: water (65:35%v/v) with a flow rate of 1mL/min at ambient temperature and the injection volume was 10 μL.

The detective wave length was set at 239 nm. The mobile phase was filtered through (0.45μm) in millipore solvent filtration apparatus before use.

**Atomic Force Microscopy (AFM) Study**

The AFM is capable of scanning the surfaces in controlled environmental conditions and can measure the particle size of nanoparticles accurately. AFM confirmed the size and surface morphology of LCDP nanoparticles after drying of the selected formulations. Droplets of optimized formulas were deposited on freshly cleaved mica and dried 15 minutes in the oven by the droplet evaporation technique. Particle size, 3D-dimension graph, and a histogram of particle size distribution were obtained.

**Results and Discussion**

**Differential scanning calorimetry analysis (DSC)**

Pure LCDP powder showed a characteristic endothermic peak at (185.80 °C), such sharp endothermic peak signifies that drug used was in a pure crystalline state and it was near the reported one, as shown in figure 2.

**Saturation solubility study of LCDP**

As demonstrated in the table (2), higher solubility of LCDP was in triacetin while the lower solubility was in liquid paraffin. To ensure the drug is solubilized form, triacetin was utilized in the formulations, no precipitation of drug will occur since lipophilic drugs can be easily present in a solubilized form. Regarding surfactants, tween 80 and tween 60 were chosen as a surfactant to obtain a one-phase clear solution. Ethanol was found to have a higher solubilizing capacity for LCDP.
Table 2. Saturation solubility study of LCDP

| Oil                  | Solubility (mg/ml) | SD     |
|----------------------|-------------------|--------|
| Coconut oil          | 32.139            | ±0.65  |
| Corn oil             | 15.224            | ±0.27  |
| Grape seed oil       | 4.165             | ±0.32  |
| Lavender oil         | 6.175             | ±0.48  |
| Liquid paraffin      | 1.281             | ±0.89  |
| Oleic acid           | 58.126            | ±0.95  |
| Olive oil            | 16.366            | ±0.65  |
| Sunflower oil        | 6.156             | ±0.85  |
| Triacetin            | 63.137            | ±0.78  |
| **Surfactant**       |                   |        |
| Propylene glycol     | 18.221            | ±0.53  |
| Tween 80             | 41.156            | ±0.78  |
| Tween 60             | 39.187            | ±0.85  |
| Tween 20             | 35.043            | ±0.89  |
| **Co-surfactant**    |                   |        |
| Ethanol              | 65.164            | ±0.72  |
| Methanol             | 52.189            | ±0.96  |
| PEG200               | 46.232            | ±0.26  |
| PEG400               | 38.562            | ±0.87  |

Construction of pseudo-ternary phase diagrams

Figures (3 and 4) showed the pseudo-ternary phase diagram for the o/w NEs using triacetin as an oil phase, tween 80 and tween 60 as a surfactant and ethanol as a co-surfactant.

Figure 3. Pseudo-ternary phase diagram o/w emulsion diagram using triacetin, tween 80: ethanol in different ratios.
Figure 4. Triangular co-ordinate o/w emulsion diagram using triacetin, tween60: ethanol in different ratios.

**LCDP NE characterization thermodynamic study**

NEs are thermodynamically stable systems, formed of particular concentrations of oil, Smix and DDW with no phase separation and no cracking or creaming. Small droplet size prevents any flocculation, enabling the system to remain dispersed with no separation (40), as shown in table (3).

**Table 3. Results of thermodynamic stability studies For LCDP 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                  |

**Droplet size**

Table (4) showed the results of droplet size measurement. The results illustrated that when the concentration of surfactant increased the particles size reduced since this high surfactant concentration decreases surface tension and stabilizes newly developed surfaces during homogenization and production of smaller particles (41). Figures 5 and 6 show the droplet size measurement of the LCDP NEs formulas 1-9.

**Table 4. Particle size measurement for LCDP nanoemulsion.**

| F-Code | Mean particle size (nm) | F-Code | Mean particle size (nm) |
|--------|-------------------------|--------|-------------------------|
| F-1    | 345.7                   | F-6    | 304.3                   |
| F-2    | 258.8                   | F-7    | 220.6                   |
| F-3    | 205.5                   | F-8    | 15.05                   |
| F-4    | 13.42                   | F-9    | 13.47                   |
| F-5    | 13.42                   |        |                         |
**Polydispersity index measurement (PDI)**

PDI refers to the quality of the dispersion; this index represents uniformity and homogeneity of the particles in the NEs, as revealed in table (5) \(^{(35)}\).

**Zeta potential measurement (ζ – potential)**

Zeta potential governs the degree of repulsion between adjacent, similarly charged, dispersed particles. When the nonionic surfactants adsorb onto the nanoscale droplets, they lowering the zeta potentials and preserve stability \(^{(42)}\). Table (6) and figures 7 and 8 show the values of the zeta potential of formulas 1-9.

### Table 5. The Polydispersity index of LCDP nanoemulsions

| F-Code | PDI  | F-Code | PDI  |
|--------|------|--------|------|
| F-1    | 0.581| F-6    | 0.261|
| F-2    | 0.269| F-7    | 0.362|
| F-3    | 0.370| F-8    | 0.410|
| F-4    | 0.381| F-9    | 0.186|
|        | 0.234|        |      |

### Table 6. Zeta potential of LCDP nanoemulsions

| F-code | Zeta potential (mV) | F-code | Zeta potential (mV) |
|--------|---------------------|--------|---------------------|
| F-1    | -3.84               | F-6    | -3.04               |
| F-2    | -5.4                | F-7    | -10.8               |
| F-3    | -9.62               | F-8    | -11.8               |
| F-4    | -6.45               | F-9    | -3.84               |
| F-5    | -14.5               |        |                     |
Dilution test

Dilution test confirmed the high physical stability of the LCDP NE under dilution with water. In less than 1 minute, all NE formulas (F1-F9) showed clear and fine bluish NE indicating o/w type, proved that they are maintaining the nanosized character and could be diluted in GI fluids without drug precipitation \(^{(43)}\).

Conductance measurement

Electrical conductivity had a potent relation with the type or nature of the external phase of NE. Higher values for electrical conductivity indicate higher conductivity of water \(^{(44)}\). The results in a table (7) showed higher conductivity values.

| F-Code | σ(ms/cm) | F-Code | σ(ms/cm) |
|--------|----------|--------|----------|
| F-1    | 0.267    | F-6    | 0.123    |
| F-2    | 0.143    | F-7    | 0.156    |
| F-3    | 0.154    | F-8    | 0.0691   |
| F-4    | 0.176    | F-9    | 0.105    |
| F-5    | 0.241    |        |          |

Drug content estimation

All NEs formulas agreed with the requirements of the British Pharmacopeia range (95%-105%) as shown in table (8), which indicated that high content uniformity and revealed the adequacy of the preparation method \(^{(45)}\).
Table 8. Drug content of LCDP formulations (mean ±SD, n=3).

| F- code | % Drug content | SD   |
|---------|----------------|------|
| NE-1   | 99.14          | ±0.81|
| NE-2   | 96.29          | ±0.21|
| NE-3   | 98.03          | ±0.56|
| NE-4   | 96.15          | ±0.32|
| NE-5   | 99.14          | ±0.68|
| NE-6   | 99.26          | ±0.42|
| NE-7   | 99.16          | ±0.26|
| NE-8   | 96.03          | ±0.97|
| NE-9   | 97.48          | ±0.46|

**Viscosity measurement**

From figure (9), it was demonstrated as the concentration of the surfactant increased, the viscosity increased this may be due to entrapping of the water molecules in cross-linking surfactants chains and also highest surfactant concentration would make the dispersion medium more rigid, as well as formulas that contain tween 60 has a higher viscosity than that contain tween 80 since tween 60 has a higher molecular weight than tween 80 (46,47).

The results also showed that the viscosity decreased as the rotation speed increased (shear rate) indicating the pseudoplastic (shear thinning liquids) flow of the preparation (48).

**In vitro drug dissolution study**

The release of the drug from all NEs formulations was found nearly 100% at the end of 60 min.; higher the dissolution, faster the absorption, and hence quicker and higher the drug action can be obtained by smaller the particle size of a drug in the dosage forms (49). Figure (10) demonstrated that the release of LCDP from the formulas that contain tween 80 as a surfactant was higher than that contain tween 60 which could be explained by the smaller droplet size of formulas containing tween 80 as compared to that formula which contains tween 60 leading to a higher rate of dissolution. The higher HLB value of tween 80, which is 15 enhanced the continuous distribution and solubilization of the incorporated lipophilic drug within the system (50,51).

**Selection of the optimum formula**

After studying the characterization of prepared LCDP NEs (F1-F9), it was found that (F-5) is selected as the best formula that is characterized by a low particle size (13.42), low polydispersity index (PDI) (0.234), zeta potential (-14.5), good spreadability on the filter paper, efficient electrical conductivity (0.241 ms/cm), good pH value (5.9), good percent of light transmittance (99.10), accepted viscosity, higher drug content percent (99.14) and higher dissolution rate. The optimized formula would be subjected to further studies.

**Drug and excipient compatibility study by FTIR**

FTIR is an extremely powerful technique in discovering and evaluating any possible chemical interaction between LCDP and any excipient during NEs preparation.
FTIR spectra of pure LCDP powder showed characteristic peaks which are: 3348.78 cm\(^{-1}\) due to (N–H) stretching vibration, 3109.65–2976.59 cm\(^{-1}\) corresponding to (\(\cong\)C–H) stretching, 2930.31 cm\(^{-1}\) due to aliphatic (C–H) stretching, 1674.87 cm\(^{-1}\) for ester (C=O) stretching, 1495.53 cm\(^{-1}\) and 1451.17 cm\(^{-1}\) due to aromatic −C=C stretching, 1372.10 cm\(^{-1}\) corresponding to aliphatic C–H bending, 745.35 cm\(^{-1}\) for disubstituted ortho benzene stretching and 982.55 cm\(^{-1}\) for C–N stretching. The FTIR spectrum of pure LCDP and selected formula (F-5) displayed the same functional groups band with very slight shifting, which suggests the presence of hydrogen bonding \(^{52,53}\). Figures 11 and 12 showed the FTIR spectra of the LCDP and the selected formula (F-5) respectively.

![Figure 11. The FTIR spectrum of LCDP.](image)

![Figure 12. FTIR spectrum of the selected formula (F-5).](image)

Validation of the HPLC method

The chromatograms of Pure drug LCDP and LCDP in the selected formula (F-5) there was no change in retention time, and no extra peaks reported. Therefore, these excipients were found to be compatible with LCDP \(^{54}\). Figures (13 and 14) showed the Chromatograms of LCDP, triacetin, tween80 ethanol and F5 respectively.

![Figure 13. Chromatograms of pure LCDP and triacetin, respectively.](image)
Atomic Force Microscopy (AFM) Study

AFM is capable of scanning and measures the properties and characteristic of the surfaces. With the high accuracy of the AFM, it is possible to determine the dimensions of nanoparticles with high reliability. The morphological analysis and particle size of formula F5 performed by AFM were close to spherical in shape and smooth surface, as shown in figure 15.

Continue figure 13. Chromatograms of pure Lacidipine and triacetin, respectively.

Figure 14. Chromatograms of tween80 and F-5 respectively
Conclusion
After discussing the previously obtained data, it is easy to deduce the following points:

1. All the NE formulas prepared with triacetin as an oil phase, tween 80 and tween 60 as a surfactant and ethanol as a co-surfactant with different Smix ratios provided a significant increase (P<0.05) in the dissolution rate compared to pure drug powder.

2. The formula (NE-5) with triacetin oil and mix (tween80: ethanol) in a ratio of (3:1) was selected as an optimum formula.

3. The compatibility studies (FTIR and HPLC) for the selected formula revealed no specific interactions between LCDP and other excipients.

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