Lisinopril is an ACE inhibitor drug indicated in hypertension, myocardial infarction, and heart failure. It is taken orally and used as an antihypertensive drug in the treatment of hypertensive patients. It shows action by inhibiting ACE as well as renin-angiotensin-aldosterone. The mechanism of Lisinopril is worked by Inhibiting angiotensin-converting enzymes. ACE is responsible for the conversion of angiotensin I to angiotensin II; angiotensin ii is responsible for the constriction of blood vessels. Therefore, Lisinopril is a good choice to reduce hypertension by inhibiting the ACE [2]. It is chemically described as [N2-{(S)-1-carboxy-3-phenylpropyl}-L-lysyl]-L-proline dehydrate [1]. It is a small molecule with a molecular weight of 405.4879 g mol⁻¹ and molecular formula C21H31N3O5. It can take orally 10-80 mg daily and the average bioavailability is 25%. As a result, developing a new Lisinopril dosage form with increased oral bioavailability is necessary. Because most of the drugs are degraded by the gastric environment and degraded by the lymphatic system.

Phospholipids are the major structural components of cell membranes and have a significant role in signal pathways like inflammation, damage and cell injury, and drug transportation. PL can change polarity and improve the therapeutic potency of certain molecules with low oral bioavailability. In this study, Lisinopril was formed into a novel drug-phospholipid complex with increased Lisinopril lipophilicity, presumably to achieve long-term stability in the oil phase.

Nano emulsions for hydrophilic drugs such as proteins and peptides have previously been used as a delivery system. In the aqueous phase, hydrophilic drugs are typically found in either W/O or O/W Nano emulsions. For O/W macro emulsion, when drugs are delivered and diluted by gastrointestinal (GI) fluids in vivo, the drug may leak into environmental aqueous media. When a W/O micro emulsion is mixed with a significant volume of GI fluid, phase
inversion occurs often in vivo, resulting in drug leakage into the environment aqueous media. Furthermore, owing to the oily nature of the formulation, patient compliance is a problem for W/O Nano emulsion. Therefore to resolve this drawback of the previous formulation, a self-Nano emulsifying drug delivery system came into existence. There are so many potential advantages of self-Nano emulsifying drug delivery system such as 100 percent drug entrap capacity minimize dissolution step formation of ultrafine droplet size result in increased absorption surface area and thus increased n bioavailability.

Self-Nano emulsion drug delivery systems (SNEDDSs) have recently emerged as innovative colloidal systems with excellent thermodynamic stability and effective absorption properties. O/W Nano emulsion may develop spontaneously in the waters as an isotropic mixture of oil, surfactants, and co-surfactant. The resulting Nano emulsion is a thermodynamically stable system of extremely tiny droplet sizes, ensuring effective oil droplet absorption.

Therefore, the aim of this study was to develop a novel drug phospholipid complex was designed to develop SNEDDSs for Lisinopril delivery, in which phospholipid complexes were formulated to significantly improve Lisinopril liposolubility and greatly promote Lisinopril integration into SNEDDS [3].

2. Materials and methods

2.1. Materials

Lisinopril (90.3%) was purchased from Yucca Enterprises Pvt. Ltd, Mumbai, India. Linseed oil, Peppermint oil, Olive oil, Cinnamon oil, Almond oil, Sesame oil, Orange oil, and Ashwagandha oil were purchased from Veda oil, New Delhi, India. Soy lecithin, Capmul MCM C8, Capryol 90, Oleic acid, tween 80, tween 20, span 20, propylene glycol, PEG 400, and PEG 200 were purchased from Sigma Aldrich, India.

2.2. Preparation of Lisinopril-phospholipid complex

Lisinopril-phospholipid complex was prepared by the method reported earlier by Jinyang Shen et al with slight modification. Briefly, Lisinopril (5mg) was weighed and dissolved in ethanol (50 ml) containing 50mg of soy-lecithin in a container. The mixture was heated gently, stirred with a magnetic agitator, and kept on water bath at 50°C for 15 minutes. The viscous mass was rotary evaporated (rotary evaporator) and the final product possesses thick consistency and is removed and stored in airtight containers at room temperature.

2.3. Characterization of LPC Complex

2.3.1. FTIR Characterization

The FTIR spectra of Lisinopril, phospholipids and Lisinopril-phospholipid Complex were taken in the KBr pellet using the Shimadzu Fourier transformed infrared (FT-IR) spectrophotometer. The drug and potassium bromide mixture were mixed in a ratio of 1:100 using a mortar pestle and then compressed for 1 minute for each drug separately [4]. The loaded sample was placed in the spectrophotometer, where they were scanned at 4000 to 400 cm1 at a resolution of 4 cm1, and the spectrum was interpreted [5].

2.3.2. Thermal characterization

To detect thermodynamic properties, a differential scanning calorimeter (Diamond DSC, PerkinElmer Instruments, USA) was employed to take thermograms of Lisinopril powder, soya lecithin, and LPC. The samples have been sealed into a crimp cell and heated at a rate of 50°C/min from 10°C to 350°C [6].

2.3.3. Determination of Conductometry

The conductivity measurements were performed with a digital conductivity meter having a cell constant of 1.071 cm⁻¹. The measurement of conductivity was generated by alternating current. The solution's temperature was maintained by water flowing into the observed solution. In this first measure the conductivity of solvent, subsequently, a set quantity of the drug-phospholipids complex of Lisinopril is introduced into the beaker and adds water through the micropipette and attains the conductance of solution. Same procedure was done with the (drug/tween/ethanol), (complex/ethanol/tween), (water/drug), and (phospholipids/ethanol/tween) [7].

2.3.4. Determination of Partition Coefficient
The partition coefficient was used to observe the partitioning behavior of drugs in a hydrophilic and lipophilic environment. The nature of a drug as aqueous or non-aqueous was determined by the shake flask method [8]. To maintain the drug and complex in unionized form water was used as an aqueous phase and n-Octanol was used as a non-aqueous phase. In brief, the shake flask method has defined the nature of a drug that is aqueous or non-aqueous. Water was used as an aqueous stage and n-octanol as a non-aqueous phase for maintaining the drug and the complex in the unionized form. In short, in an Eppendorf tube for the pre-saturation of the two stages, n-Octanol 500μL and distilled water 500μL were added and shaken for 30 minutes (not using the drug) into the Eppendorf tube. Drug and complex were then added to the mixture and shaken for 3 hours separately. The final mixture was kept for one hour to separate two layers (the aqueous layer and oily layer). Pipette 500 μL from the aqueous layer into a 5ml volumetric flask, fill to 5 ml with distilled water, and analyze at 218 nm for Lisinopril against a similarly treated blank using a UV spectrophotometer. A 500µL sample was taken from the oil layer and kept at room temperature for 6-7 hours. Following evaporation, distilled water up to 5 mL was used for dilution of the remaining solution and a UV spectrophotometer was used to analyze the solution concentration against a similarly treated blank. Calculation of the partition coefficient by the following formula.

\[
\text{Partition Coefficient} = \frac{\text{concentration of drug in the organic phase}}{\text{concentration of drug in the aqueous phase}}
\]

(3.1)

2.4. Formulation, optimization and evaluation of DPC-Self-nanoemulsifying drug delivery system

2.4.1. Selection of excipients

The selection of oil was dependent on the solubility of the drugs since a load of a drug depends on the solubility of the oil [9]. Oil tends to retain the drug solubilized or drug precipitation results in the creation of unstable Nanoemulsion. Solubility of Lisinopril-phospholipid complex in various oils (peppermint oil, linseed oil, olive oil, cinnamon oil, almond oil, sesame oil, orange oil, and ashwagandha oil), surfactant (tween 20, tween 60, tween 80), co-surfactant (propylene glycol 600, propylene glycol, propylene glycol 400, Transcutol P, propylene glycol 200) was determined by adding an excess amount of Lisinopril-phospholipid complex in 1 ml of the selected vehicles in 2 ml Eppendorf tube and allowed to mix using a vortex mixer. The clarity of the resulting combinations was assessed visually. Based on miscibility with specified oil, surfactants and co-surfactants were selected. Miscibility tests were carried out by combining a selected oil with a surfactant or co-surfactant in a 1:1 ratio. For around 10 minutes, the system was agitated. The resultant mixtures have been physically distinctly observed [9]. The transparent mixtures were taken into consideration.

2.4.2. Construction of pseudo ternary phase diagram

Pseudo-Ternary phase diagrams were plotted using Kumar and its associates' procedure to describe the ratio of surfactant-co-surfactant in Nanoemulsion [10]. The rising concentration of surfactant as regards the co-surfactant and increasing co-surfactant concentrations concerning the surfactant preparation have been combined with surfactants and co-surfactants with a particular ratio of volume (1:1, 2:1, and 1:2). The surfactant and co-surfactant mixture is named Smix. For the design of the pseudo ternary process diagrams, oil, and different smix ratio is combined into several different combinations of volumes (1: 15, 1:7.5, 1:3 and 1:1.5). The pseudo ternary phase diagram was prepared using an aqueous titration process. For both Smix and oil ratios, slow titration with water was done. After adding water to the volume ratio, visible observations were made and recorded as:

1) Nano emulsion (if the system has been obtained with clarity and simple flow);

2) Nano gel (if transparent gel was obtained)

3) Emulsion (if milky or cloudy appearance was obtained or phase separation was obtained)

4) Emulgel (if milky gel was obtained)

2.5. Preparation of L-PC based SNEDDS

Based on the solubility of L-PC in common oil materials and miscibility of oil with surfactant and co-surfactant, the
system (peppermint oil/tween80/polyethylene glycol 400/water) was selected as the LPC-SNEDDS. In detail, the oil phase (Peppermint oil), surfactant (Tween 80), and co-surfactant (Polyethylene glycol 400) were mixed at a mass ratio of 0.5:1:1. L-PC (1:10) was added in an oil/surfactant/co-surfactant mixture, and then the mixture was shaken by magnetic stirring for over 30 min until a clear appearance was observed. Next, the resulting uniform mixture was added to distilled water dropwise at 37 °C under stirring (200 rpm). Finally, L-PC based SNEDDS with a clear and translucent appearance were obtained.

2.5.1. Selection of formulation from phase diagram
Six formulations have been selected from the phase diagram on the basis of least oil, least surfactant, higher smix, higher oil and least smix. The Formulation I, containing the least oil and smix ratio is 1:1. Formulation II contains least surfactant and the smix ratio is 1:2. Formulation III containing least cosurfactant and smix ratio is 2:1. Formulation IV contains higher smix and the ratio is 1:1. Formulation V contains higher oil and the smix ratio is 2:1 and the last formulation VI contains least smix in ratio 1:1.

Table 1. Selected formulation from phase diagram.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Phospholipid +oil (1:2) (mg)</th>
<th>Tween 80 (mg)</th>
<th>Peg 600 (mg)</th>
<th>Ratio (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>200</td>
<td>750</td>
<td>750</td>
<td>1:1</td>
</tr>
<tr>
<td>F2</td>
<td>250</td>
<td>400</td>
<td>800</td>
<td>1:2</td>
</tr>
<tr>
<td>F3</td>
<td>250</td>
<td>800</td>
<td>400</td>
<td>2:1</td>
</tr>
<tr>
<td>F4</td>
<td>400</td>
<td>1750</td>
<td>1750</td>
<td>1:1</td>
</tr>
<tr>
<td>F5</td>
<td>500</td>
<td>1400</td>
<td>700</td>
<td>2:1</td>
</tr>
<tr>
<td>F6</td>
<td>200</td>
<td>325</td>
<td>325</td>
<td>1:1</td>
</tr>
</tbody>
</table>

2.6. Optimization of L-PC based SNEDDS formulations.

2.6.1. Robustness to Dilution
Robustness of DPC- SNEDDS to dilution was studied by diluting it 50, 100 and 1000 times with distilled water. The diluted nanoemulsions were stored for 24 h and observed for any signs of phase separation or drug precipitation [11].

2.6.2. Refractive index
The refractive index (RI) is a useful instrument for determining the presence of transparent formulation. A refractometer is used to test the system's RI by placing a drop of the solution on a slide and comparing it to water, which has a RI of 1.333. If the system's RI is similar to the RI of water, the formulation is transparent [12]. The RI is also used to determine the formulation's thermodynamic stability. The negligible changes in the RI during the several time points of storage would indicate that the SNEDDS have a consistent structure and thermodynamic stability.

2.6.3. Percentage transmittance
The percentage transmittance of the system is determined following the dilution of the formulation at 638 nm wavelength by a UV spectrophotometer and using the water as blank. If the percentage transmittance value were closer to 100%, the formulation would indicate a clear and transparent nature [13].

2.6.4. Cloud Point Measurement
The temperature at which the Nano emulsion breaks is known as the cloud point. The cloud point will examine the integrity of the GI tract of the SNEDDSs. Formulations are diluted with distilled water and Put on a water bath with steadily increasing temperature [14]. SNEDDSs should have a cloud-point of more than 37°C; otherwise, the drugs could be obstructed because cloudy emulsion affects the absorption of components in SNEDDS formulations by dehydration.

2.7. Characterization of optimized formulation

2.7.1. Droplet size and PDI
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Zetasizer was used to measure the size distribution and size of a droplet of optimized Nano emulsion (Zetasizer ver.8.00.4813) [15].

2.7.2. Zeta potential
Surface charge of Nano emulsion was estimated by measuring the electrophoretic mobility. Measurements were carried by zeta potential measuring equipment (Zetasizer-1000 HAS, Malvern Instruments, UK) [15].

2.7.3. Percentage transmittance
For calculating the transmitting percentage the spectrophotometer UV (U.V 1700 SHIMADZU) has been used. Formulation (1 ml) was taken and diluted with water, the transmission percentage was observed in triplicate at 638 nm[16].

2.7.4. Refractive index
A few drops of the formulation were put on a slide, and the refractive index was calculated in triplicate using an Abbe refractometer (Nirmal International, India) [12].

2.7.5. Electro conductivity
Electro conductivity of the resultant system was measured by an electro conductometer (Conductivity meter 305, Systronic). For the conductivity measurements, the tested DPC-SNEDDS were prepared with distilled water. At 25±1o C, readings were recorded in triplicate.

2.8. Ex-vivo permeability studies
For ex-vivo permeability study, a male Swiss albino rat was sacrificed and ileum were isolated and washed to clear the lumen content. Drug-phospholipid complex based SNEDDS diluted and filled in the intestine. An equivalent amount of Lisinopril suspension was used for comparison. Both ends of tissues were properly tied to prevent leaks and placed in beakers containing 75ml of medium, with a continuous stream of air with gentle stirring at a temperature of 37±2 °C. At regular intervals, samples were taken from the receptor phase and analyzed using spectrophotometry. All the experiments were performed in triplicate[11].

2.9. Pharmacodynamic study
In order to assess the blood pressure of the albino rats, a tail-cuff method (BIOPAC, CA, USA) was taken to determine the antihypertensive activity of developed formulation. The antihypertensive effect of formulation was investigated using a fructose-induced hypertension model[19].Following confirmation of blood pressure induction, the drug was administered by oral feeding tube at a dose of 1 mg/kg of Lisinopril. The animals were placed into four groups, each with six animals. Group I was considered to be an ordinary control group, group II was regarded as a hypertensive control group, group III suspension of medicines and group IV optimised LPC-SNEDDS formulation. The tail cuff method was used for measurement of systolic (SBP) blood pressure and diastolic (DBP), utilising a non-invasive technique at an interval of 2 hours (BIOPAC, CA, USA).

3. RESULTS
3.1. Characterization of Lisinopril Phospholipid Complex
3.1.1. Determination of FTIR[17]
The interaction between Lisinopril and phospholipids was studied using Fourier transform infrared spectroscopy (FT-IR). Figure 1 (a, b) show the IR spectra of Lisinopril and phospholipid, respectively). Figure 1(c) shows the IR spectrum of the Lisinopril–Pc complex had obvious change[18]. The FTIR Spectra of a drug with phospholipid was compared with the standard FTIR Spectrum of a pure drug. The presence of a characteristic peak at around 3553 cm-1 is assigned to the stretching vibrations of an O–H band of water. The peak at 3002 cm-1 is caused by aromatic C–H stretching vibrations, whereas the peaks at 2963 and 2925 cm-1 are caused by asymmetric C–H stretching vibrations. The carbonyl stretching of the tertiary amide group and/or scissoring NH2 vibration are responsible for the peak at around 1657 cm-1; the aromatic ring mode is responsible for the peak at 1610 cm-1; and the asymmetric carboxylate and/or ring mode of the aromatic group are responsible for the peaks at 1569 and 1545 cm-1. The peak

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at 1453 cm⁻¹, and 1391 cm⁻¹ caused by the CH₂ scissoring vibration and the symmetric carboxylate respectively. Phospholipid show a characteristic peak at 3449 cm⁻¹ due to hydroxyl stretching, 2922 cm⁻¹ due to C-H stretching of long chain fatty acid, 1196 cm⁻¹ due to P=O stretching band and 1091 cm⁻¹ due to P-O-C stretching. The Lisinopril phospholipid complex showed a change of peak in comparison to the drug. The IR of the complex showed a shift in the O-H band of water from 3553 cm⁻¹ to 3544 cm⁻¹. There is also a slight change in peak of tertiary amide group from 1657 cm⁻¹ to 1652 cm⁻¹ in the drug phospholipid complex. In the complex spectra the phospholipid peaks were similarly not observed at 1196 and 1080 cm⁻¹. These modifications suggested that Lisinopril and phospholipids formed a complex via hydrogen bonding between the Lisinopril –OH group and the phospholipids P=O group.

![Figure 1](image1.png)

**Figure 1.** (a) FTIR spectrum of Lisinopril, (b) FTIR spectrum of Phospholipid, (c) FTIR spectrum of Lisinopril-Phospholipid Complex.

### 3.1.2. Determination of Differential scanning calorimetry (DSC)

The DSC is a quick, accurate way to screen drug-excipient compatibility, providing full detail on potential interactions. DSC is an advanced and reliable process. The changes in DSC peak is caused by the removal of the endothermic peak, appearance of new peak and changes in the peak shape and onset [18]. Fig- 2(a) Shows the DSC thermograms of pure Lisinopril, Fig- 2(b) Shows the DSC thermograms of phospholipid, and Fig- 2(c) Shows the DSC thermograms of Lisinopril–phospholipid complex. The thermogram of Lisinopril showed two peaks, first at 114.355 °C and the second peak at 183.088 °C. The second peak (183.088 °C) which is very sharp. Thermograms of phospholipids show two distinct peaks; the first (45.8555 °C) is mild and is due to the hot movement of the polar head group of phospholipids. The second peak (146.813 °C) is very sharp and tends to be the result of a phase shift from gel to fluid. The thermogram of the Drug phospholipid complex exhibits two different peaks one at 49.070°C and another one at 133.497 °C. Which is depart from the peaks of the individual components of the complex.

![Figure 2](image2.png)

**Figure 2.** (a) DSC curve for Lisinopril, (b) DSC curve for phospholipid, (c) DSC curve for Lisinopril–phospholipid complex.

### 3.1.3. Determination of Conductometry

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The electrical conductive behaviour of nanoemulsion systems is strongly correlated with their specific structures. In the nanoemulsions containing lisinopril and complex, the electrical conductivity slightly increased in comparison to free complex. If a small amount of water is added into nanoemulsion, the electrical conductivity of the system will change. The result shows that Lisinopril was bounded with phospholipid and the complex was successfully formed.

![Figure 3. Determination of Conductometry](image)

3.1.4. Determination of Partition Coefficient
The increase in solubility could be related to the phospholipid amphipathic character after the synthesis of L-PC. As po/w as the log P, lipophilicity is generally quantified. The L-PC po/w was considerably higher than the free Lisinopril. The L-PC po/w (LogP = 0.637 ± 0.031) was around 10 times the L-PC powder (Log P = -2.043 ± 0.0032) approximately. A negative logP value indicates that the molecule is closely related to the aqueous phase (it is more hydrophilic). The improved lipophilicity is probably associated with Lisinopril's interaction between phospholipids and polar groups, which disguised Lisinopril's hydrophilic groups.

3.2. Formulation, development and characterization of DPC- self-nanoemulsifying drug delivery system
3.2.1. Excipient selection
Amongst oils, Lisinopril phospholipid complex showed highest solubility and compatibility with peppermint oil therefore this oil was selected as oil phase. Peppermint oil is mainly compounded by menthol, menthon and other minor components such as menthofuran, 1, 8-cineole and limonene. It has the ability to improve emulsification potential because it can easily penetrate the oil-water interface and interact with the surfactant.

Tensile surfactants have often been utilised to minimise interfacial energy during the manufacturing of Nanoemulsions, because the surface molecules adsorb at the interface between the oil and water leading to the creation of droplets that have the surfactant coat. In the present study, on the basis of miscibility with oil, surfactant selection was carried out. Amongst surfactants, tween 80 showed better miscibility in comparison to others. Results of a miscibility study showed that tween 80 was miscible with peppermint oil. In present work, the selection of co-surfactant was done on the basis of tween 80 miscibility with peppermint. Tween 80 showed better compatibility in PEG 600 in comparison to other co-surfactants (Table -2). Results of miscibility study showed that PEG 600 was miscible with Tween 80 and peppermint oil.

<table>
<thead>
<tr>
<th>Oil phase</th>
<th>Surfactant</th>
<th>Co-surfactant</th>
<th>Miscibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>Cremophor</td>
<td>polyethylene glycol 400</td>
<td>Phase separation</td>
</tr>
</tbody>
</table>

Table 2. Selection of excipients on the basis of miscibility.
3.2.2. Construction of pseudo ternary phase diagrams
Pseudo-ternary phase diagrams are important for evaluating optimal oil, surfactant and Co-surfactant concentrations or concentration ranges needed to create an efficient microemulsion/nanoemulsion. Pseudo-ternary phase diagrams at ambient temperature were built to produce a concentration area that could lead to a greater area of nanoemulsion. The chosen phase schedules consisted of peppermint (oil), tween 80 (surfactant) and polyethylene glycol 600 (co-surfactant). On the basis of observation of clear system, pseudo ternary phase diagrams were plotted. For each S_{mix} ratio separate phase diagrams were plotted (figure 4). In S_{mix} ratio 1:1, the area of self-Nano emulsion was less as compared to the area of drug phospholipid complex based self-Nano emulsion same with the S_{mix} ratio 1:2. In S_{mix} ratio 2:1, the area of drug phospholipid complex based self-Nano emulsion was drastically greater than the area of self-Nano emulsion. Effect of increased amount of co-surfactant with respect to surfactant (1:2 and 1:3) was also observed. It was observed that on increasing the co-surfactant amount there was decrease in Nano emulsion region.

Figure 4. Pseudo ternary phase diagrams of self-Nano emulsion(a, b, c) composed of peppermint oil, Tween 80, water and PEG 600 and phospholipid complex- self-Nano emulsion (d, e, f) composed of phospholipid, peppermint oil, Tween 80, water and PEG 600; at S_{mix} 1:1, 1:2, 2:1 respectively.

3.3. Optimization of DPC- SNEDDS formulations.
3.3.1. Robustness to Dilution
DPC-SNEDDS formulations have been subjected to various folds of dilution in order to simultaneously imitate the conditions in vivo, where the formulation is subjected to incremental dilution, which could affect the drug release profile and cause the drug to precipitate at higher dilutions, significantly slowing its permeation or absorption. As a result, robustness dilution was observed at 50, 100, 250, and 1000-fold dilution with distilled water. No cloudiness, separation or precipitation revealed all the dispersions resulting, which confirmed their robustness for 24 hours of various dilution volumes. It also indicates that the in vivo drug release profile is likely to be standardized if the formulation is gradually diluted.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Surfactant</th>
<th>Co-surfactant</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linseed</td>
<td>Tween 20</td>
<td>polyethylene glycol 600</td>
<td>Turbid</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Tween 20</td>
<td>polyethylene glycol 600</td>
<td>Turbid</td>
</tr>
<tr>
<td>Clove</td>
<td>Tween 60</td>
<td>polyethylene glycol 200</td>
<td>Turbid</td>
</tr>
<tr>
<td>peppermint</td>
<td>Tween 80</td>
<td>polyethylene glycol 600</td>
<td>Clear</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Transcutol p</td>
<td>Propylene glycol</td>
<td>Phase separation</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Solutol HS</td>
<td>Propylene glycol</td>
<td>Turbid</td>
</tr>
<tr>
<td>olive</td>
<td>Tween 80</td>
<td>polyethylene glycol 400</td>
<td>Turbid</td>
</tr>
</tbody>
</table>
3.3.2. Refractive index
Refractive index shows the isotropic nature of DPC-SNEDDS. Refractive index of all formulations are written below in the table.

Table 3. Refractive index of formulation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Water(µl)</th>
<th>F-1</th>
<th>F-2</th>
<th>F-3</th>
<th>F-4</th>
<th>F-5</th>
<th>F-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1-A</td>
<td>0</td>
<td>1.463</td>
<td>1.443</td>
<td>1.453</td>
<td>1.454</td>
<td>1.458</td>
<td>1.452</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>1.453</td>
<td>1.441</td>
<td>1.449</td>
<td>1.446</td>
<td>1.447</td>
<td>1.448</td>
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<tr>
<td>C</td>
<td>200</td>
<td>1.435</td>
<td>1.438</td>
<td>1.44</td>
<td>1.426</td>
<td>1.439</td>
<td>1.436</td>
</tr>
<tr>
<td>D</td>
<td>300</td>
<td>1.429</td>
<td>1.418</td>
<td>1.431</td>
<td>1.417</td>
<td>1.429</td>
<td>1.421</td>
</tr>
<tr>
<td>E</td>
<td>500</td>
<td>1.428</td>
<td>1.415</td>
<td>1.428</td>
<td>1.413</td>
<td>1.422</td>
<td>1.419</td>
</tr>
<tr>
<td>F</td>
<td>600</td>
<td>1.412</td>
<td>1.412</td>
<td>1.419</td>
<td>1.41</td>
<td>1.412</td>
<td>1.411</td>
</tr>
<tr>
<td>G</td>
<td>700</td>
<td>1.388</td>
<td>1.41</td>
<td>1.416</td>
<td>1.395</td>
<td>1.402</td>
<td>1.398</td>
</tr>
</tbody>
</table>

3.3.3. Percentage transmittance
Percent transmittance indicates the clarity of DPC-SNEDDS and is related to droplet size. Smaller the droplet size more is the transmittance of DPC-SNEDDS. Percentage transmittance for prepared batches was in the range of 75.00 to 97.84 % indicating that prepared Nano emulsions were transparent and clear except F2. Transmittance is inversely proportional to oil concentration. It is a well-known fact that more oil concentration favors formation of large droplet size.

3.3.4. Cloud Point Measurement
Six DPC-SNEDDS formulations' cloud point values were calculated and compared. All formulations demonstrated cloud point at extremely high temperatures (37 °C is the baseline), indicating stability at physiological temperatures encountered in GIT.

3.4. Characterization of optimized formulation
3.4.1. Droplet size and PDI
DPC-SNEDDS exhibits droplet size in the Nano range with low values of polydispersity (PDI). The droplet sizes and PDI of the optimized Nano emulsion was 232.0 ± 2.32 nm and 0.438 ± 0.055, respectively which are shown in figure-5. Droplet size of formulation plays a key role in deciding the rate and extent of drug release. It is one of the important parameters for oral delivery of drugs as Nano size droplets have more surface area. This area favors more permeation of Lisinopril phospholipid complex through GIT membrane resulting in more drug release from Nano emulsion thus leading to increased bioavailability. PDI value represents uniformity of droplet size within formulation. More value of PDI indicates lower uniformity of droplet size in formulation. PDI higher than 0.50 represents broader size distribution on the other hand value below it indicates narrow size distribution. Optimized DPC-SNEDDS had low PDI size thus showed the narrow size distribution.
3.4.2. Zeta potential
Zeta potential indicates stability of DPC-SNEDDS. The zeta potential value of ± 30 mV for the dispersion system represents a considerable space between loaded droplets avoiding the possibility of the coalescence of droplets within the DPC SNEDDS. Optimized formulation zeta potential values of –1.11 ± 0.12 mV have been determined. The obtained results suggest a more stable formulation. Negative charge on the DPC-SNEDDS is possibly due to free fatty acids present in the oil phase used.

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3.4.3. Percentage transmittance
Percent transmittance indicates the clarity of DPC-SNEDDS and is related to droplet size. Percentage transmittance for optimized formulation was 97.84 % indicating that prepared DPC-SNEDDS were transparent and clear.

3.4.4. Refractive index
Refractive index shows the isotropic nature of DPC-SNEDDS. Refractive index of optimized formulation was near to the refractive index of water, which shows that optimized formulation was clear and transparent.

3.4.5. Electro conductivity
In order to confirm the nature of formulations, electro conductivity of the optimized formulations was calculated. Compared with other formulations, Formulation F6 has significantly differed in electric conductance. The optimized formulation conductivity was 0.664 mS/cm.

3.5. Ex-vivo permeability studies
The permeability of any formulation in the gastrointestinal tract is a key factor in the absorption and bioavailability of the drug. Hence, the ex vivo permeability of Lisinopril in the intestine from DPC-SNEDDS and plain drug suspension studies have been performed. The cumulative % permeation of drug from rat intestine is shown in Fig. 11. It was observed that the permeation of the drug was enhanced from the LPC- SNEDDS, in comparison to drug permeation from Lisinopril suspension indicating that permeability of drug was increased after formulating LPC-SNEDDS. In the case of LPC- SNEDDS, about 100% of the drug was permeated from the intestine, whereas in the case of Lisinopril suspension, only 54.9 percent was penetrated from the intestine. It can be noted that LPC-SNEDDS has enhanced the permeation of the drug from the intestines, which achieved our aim of increasing intestinal permeability, to increase Lisinopril's bioavailability. Enhanced Lisinopril permeability of LPC-SNEDDS can be due to the uptake mechanism of SNEDDs oil droplet such as passive diffusion, pinocytosis or endocytosis, and its Nano metrical droplet size that provides a large drugs and permeation interface.
Pharmacodynamic study
In this study tail cuff method are used for the monitoring of spontaneous and induced hypertensive blood pressure. Figure 12 shows the findings of pharmacodynamic investigation comparing LPC-SNEDDS to Lisinopril suspension with normal and hypertensive control. The result shows the change in systolic (SBP) and diastolic (DBP) in hypertensive induced rat model. SBP and DBP values were significantly lower in LPC-SNEDDS treated rats compared to Lisinopril suspension treated animals. The drug suspension group had the lowest SBP of 176.31 ± 16.04 mm Hg and the lowest DBP of 119.36 ± 12.01 mm Hg, whereas, the LPC-SNEDDS formulation performed better, lowering blood pressure by up to 154.46 ± 12.10 mm Hg in the SBP and 105.62 ± 10.46 mm Hg in the DBP. One day of following treatment the blood pressure of animals in prepared formulation maintained below hypertensive BP, whilst the hypertensive condition of the dose of the drug suspension was regained by the group of animals. It was obvious that LPC-SNEDDS enhanced oral medication absorption via selective lymphatic uptake[20]. The complete investigation clearly specifies the superiority of the proposed formulations over drug suspension and thus demonstrates that Lisinopril bioavailability via LPC-SNEDDS can be improved via lymphatic route.

Conclusion

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LPC-SNEDDS was developed in this study to solve the limitations of Lisinopril, due to its low permeability. The Lisinopril phospholipid complex are prepared by solvent evaporation method. We observed furthermore that SNEDDS technology might enhance Lisinopril Phospholipid complex (LPC) dissolution and GI absorption through simple preparation. LPC-SNEDDS was formulated and optimized by mixing both phospholipid complex and SNEDSS into a beaker. The Optimized LPC-SNEDDS formulation showed 232 nm droplet size, 0.438 PDI, -1.11 mV Zeta potential and 0.664 mS/cm electro conductivity. DPC-SNEDDS of Lisinopril showed better permeability of drug in comparison to the orally administered Lisinopril suspension. The pharmacodynamic performance confirmed the higher antihypertensive activity of LPC-SNEDDS as compared to drug suspension. These results lead us to believe that LPC-SNEDDS is a viable drug delivery system that can give a practical and effective solution to the challenge of developing drug with limited water solubility and poor systemic bioavailability.

Acknowledgments

The authors are very grateful to Dr. Anoop Kumar, Professor, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology and Mr. Pankaj Pal, Assistant Professor, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology for providing the opportunity to do research work complete in M.I.E.T, Meerut.

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