Formulation and in-vitro drug Released Mechanism of CNS Acting Venlafaxine Nanostructured Lipid Carrier for Major Depressive Disorder

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ABSTRACT
Venlafaxine (VLX) is a first line, dual acting and unique antidepressant drug which belong to the class of serotonin and norepinephrine reuptake inhibitors. Oral administration of VLX has many adverse effects, poor bioavailability due first-pass hepatic metabolism and low permeability shows poor antidepressant action in the brain. The aim of this present study was to formulate Venlafaxine Nanostructured lipid carrier (VLX-NLC) and deliver directly into the brain through intranasal route. VLZ-NLC were prepared by Melt-Emulsification-Ultrasonication process and characterized by particle size, polydispersity index, zeta potential, Encapsulation efficiency and analyzed by differential scanning calorimetry (DSC), X-ray diffraction (XRD), transmission electron microscope (TEM). The VLX-NLC was also evaluated for in-vitro drug release and ex-vivo sheep nasal mucosa permeation. The mean particle size of VLX-NLC was between 155 and 293 nm in diameter with entrapment efficacy was up to 74.13%. TEM gave confirm of spherical nature of NLC. The DSC result shows a sharp peak at 208ºC corresponds to melting peak hence confirm the peak of Venlafaxine and X-ray diffraction 2θ scattered angle represent crystallinity nature of pure Venlafaxine whereas VLX-NLC reveals decrease intensity of peak which indicates amorphization of VLX due to solubilization in lipid. In vitro studies exhibit initial burst release and afterward prolong release, ex vivo permeation through nasal sheep mucosa showed 66.45 % of drug diffused in 24 h from VLX-NLC.

Key words: Venlofexine, Nanostructured lipid carrier, Antidepressant.

INTRODUCTION
Depression is a major depressive disorder which common, serious mental illness and prevents a person from functioning usually. It is related to the high mortality rate. However pathology of mental illness is not known but have a deficiency of norepinephrine and serotonin called as monoamines in the brain may cause depression. Symptoms including irritability, fatigue, Worry and restlessness, experiences of nervousness, impaired Concentration and sleep disturbance. The brain is a delicate organ. Delivery of drugs into the brain is a challenge in the management of neurological disorders due to the presence of blood-brain barrier (BBB). Blood-Brain Barrier is a specialized system of capillary endothelial cells that protects the brain from Entry of harmful neurotoxic substances in the bloodstream while Supplying to the brain with the required nutrients for proper function and obstacle to the delivery of active drug constituent into the Central Nervous System (CNS) for disease treatment. Intranasal delivery of drugs results in high blood levels as compared to intravenous route. Intranasal (IN) drug delivery is needle-free, non-invasive, painless, self and alternative administration route to targeted drugs directly to the brain via...
the olfactory region to achieve faster and higher level of drug absorption by passing the blood-brain barrier shows a rapid onset of action, potential for nose to CNS delivery and no first-pass metabolism.9,10,11. Venlafaxine HCL (VLX) is (1-[2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl] cyclohexanol hydrochloride) a first line, bicyclic phenethylamine derivative of dual action unique antidepressant drug used to treat depression. Its molecular weight is 313.87 with a chemical formula of (C17H27NO2HCl). Structurally it differs from other Antidepressant which belongs to the class of serotonin and norepinephrine reuptake inhibitors (SNRI).12 VLX is commercially available in the market as capsules immediately released tablet and controlled release tablets form. VLX is commonly used as oral anti-depressants; nevertheless oral administration of VLX has so many adverse effects such as deliberate onset of action, side effects like a headache, tachycardia, increased blood pressure, dry mouth, dizziness, fatigue, sexual dysfunction and low bioavailability.13 Approximately 92 % of VLX absorbed by the oral administration but it undergoes extensive first-pass metabolism in the liver to des-methyl venlafaxine active metabolite. Only 12.6 % is remaining in systemic circulation, hence shows the low onset of action. The solubility of VLX is more than 500 mg in one ml of water and log P value 0.43 which representing hydrophilic nature of drug thereby limiting its permeability and shows poor antidepressant effect in the brain14,15,16. The elimination half-life of Venlafaxine is 4-5 h, therefore, needs to frequent administration of VLX to maintain an effective therapeutic concentration into the brain. The efficacy depends on the continuous availability of the antidepressant drug in a brain. Therefore, sustained release is required to maintain the steady-state levels which are narrow in case of conventional as well as parenteral remedies.17 Nanostructured Lipid Carrier (NLC) is the new second generation of solid lipid nanoparticles which consist of the mixture of various solid and oil lipid resulting matrix lipid particles. NLC offers an increased amount of payload of drugs and minimized drug expulsion during storage due to the use of oil lipid over solid lipid nanoparticle. They are biodegradable, biocompatible with Generally Recognized as Safe (GRAS) status, solid at room and body temperature18,19.

The aim of this present study was to deliver venlafaxine HCL into the brain by administering it through the intranasal route in lipid Nano particulate form. VLX-NLCs were developed in a mixture of solid and oil lipids, drug concentration, surfactant and co-surfactant ratio with stirring speed and ultra-sonication time. With the aid of solid and oil lipids were compared with particle size distribution, polydispersity index, zeta potential and drug encapsulation efficiency. The finished products were analyzed by dynamic light scattering (DLS), differential scanning calorimetry (DSC), X-ray diffraction (XRD), transmission electron microscope (TEM), In vitro release studies and Ex-vivo drug diffusion.

**MATERIALS AND METHODS**

Venlafaxine was obtained as a gifted from Ranbaxy research lab Gurgaon (India), Solid-lipid: Geleol pellet (Glyceryl palmitostearate), Gelucire 50/13 (stearoyl macrogol-32 glyceride), Compritol 888 (Glyceryl behenate), Glycerol Monostearate (monosteric acid ester of glycerol) and Precirol ATO 5 (Glycerol stearate) were obtained by Gattefosse France as a free gift sample. Oil Lipid: Medium chain triglyceride (MCT) such as Capryol 90 (propylene glycol monocaprylate), Miglyol 808, CapteX 200 P, Maisine (glycerol monolinoelae), Capmul MCM, Capryol PGMC (propylene glycol II monacrylate) obtained from ABITEC, Mumbai as a free gift sample. Pluronic F127 was from Sigma-Aldrich. Sodium taurocholate selected as bile salt from S D fine Mumbai. Potassium dihydrogen phosphate, sodium hydroxide (NaOH) was purchased from Merck Mumbai. Dialysis bag MW 12,000 Da Himedia Mumbai India. Millipore water freshly purified (Milli-Q Gradient A10 system, Elix 0.22µ m), freshly prepared double distilled methanol was whenever required. All the reagents and chemicals used of analytical grade.

**Screening of lipids for NLC formulation**

**Solid lipid**

Screening of solid Lipid was determined by adding an excess amount of Venlafaxine in 2 g of melted various solid lipids in controlled temperature Remi water bath shaker, Mumbai at 75 °C mixed thoroughly with a vortex mixer (Remi CM 101). The total amount of Venlafaxine solubilized in solid lipids was determined visually by initial saturation.

**Oil lipid**

Screening of oil Lipid was determined by shaken flask method. The excess amount of venlafaxine was added in 2 g of various oil lipids in screw cap glass bottle. All the bottles were covered with an aluminium foil to avoid entry of vapours in the bottles and placed in thermostat wrist hand water bath shake (Remi Model RSB12), Mumbai for 24 h at 40°C. The supernatant liquid was filtered. After filtration diluted a drop of supernatant In double distilled methanol solubility was analyzed by...
UV-Visible spectrophotometer (Shimadzu UV/1800) at λ 276 nm.\textsuperscript{20,21}

**Melt-Emulsification-Ultrasonication method followed by lyophilization**

The melt-emulsification-ultrasonication process was used for the preparation of Venlafaxine nanostructured lipid carrier (VLX-NLC). In NLC preparation lipid phase and aqueous phases were separately prepared. Accurately weight amount of Glyceryl monostearate as solid lipid and Capmul MCM as oil lipid in a ratio of 7:3 w/w were heated at 5 to 10ºC above the melting point of the solid lipid that is 75ºC to prevent recrystallization of lipid during the process. The drug was added in the melted lipid to dissolve. Meanwhile, separately aqueous surfactant phase was prepared in Millipore water (Milli-Q Gradient A10 system Elix 0.22µ m) in that stearic surfactant Pluronic F127 and sodium taurocholate (bile salt) maintain temperature 75ºC with continuous starring on electronic magnetic stirrer (2 MLH Remi Magnetic stirrer), Mumbai. The aqueous surfactant solution was added slowly drop by drop with the help of borosilicate glass syringe to lipid phase under magnetic stirrer at 5 to 10ºC above the melting point of the solid lipid. The obtained coarse emulsion was further treated with a probe-type sonicator (Probe ultrasonicated Bandelin sonoplus) in a water bath at 75ºC for 15 min at 40 amplitude. Finally, prepared nanostructured lipid carrier was cooled down at room temperatures on a magnetic stirrer (4 EMS Electromagnetic stirrers) in which lipids get re-crystallized to form Venlafaxine loaded nanostructured lipid carrier.\textsuperscript{22,23}

**Freeze-drying of NLC dispersion**

VLX-NLCs dispersions were lyophilized to obtain formulation in dry form. The obtained VLX-NLC was frozen using freezer at −20ºC for overnight, VLX-NLCs were transferred to the lyophilizer at the temperature at −70ºC for 48 h. After lyophilization VLX-NLC lyophilized form was collected from lyophilizer and was subjected to physicochemical characteristics, in-vitro, ex-vivo and in vivo studies.\textsuperscript{24}

**Particle size (PS) and polydispersity index (PDI) measurement**

The average particle size (PS) and polydispersity index (PDI) of VLX-NLCs were determined by Photon correlation spectroscopy (PCS) at the detection angle of 90ºC at 25ºC. The zeta potential was analyzed by a Nano-ZS Zeta Sizer (Malvern Instruments, Malvern, UK Nano size) at 25ºC after proper dilution with double distilled water. Each measurement was made in duplicate.\textsuperscript{25,26}

**Drug Excipient Compatibility Studies**

Compatibility studies of drug and solid or liquid Lipids material were evaluated to check whether any chemical interaction between them during the formulation of NLC was recorded by Fourier Transform Infrared Spectroscopy FTIR Spectrometer (Shimadzu FTIR-8400 S CE) using KBr pellets (400-4000) with a scanning speed of 2 min/sec with normal slit.

**Differential scanning calorimetry (DSC) analysis**

The crystalline state of drug samples was recorded with differential scanning calorimetry (DSC). For DSC measurement, accurately weight amount 5 mg of drug samples into aluminium pans and then hermetically sealed with aluminium lids. The thermograms of samples were obtained at a scanning rate of 10ºC/min at a different temperature range between 0 and 400ºC in a nitrogen atmosphere. The thermal measurement of physical mixture of VLX-Lipid was also carried by differential scanning calorimetry.\textsuperscript{27}

**Transmission electron microscope (TEM)**

The morphological observation of optimized VLX-NLC was carried out by Transmission electron microscope operated at voltages ranging from 100 to 200 kV. A placing a drop of the sample which was diluted with double distilled water and spread on a 200-mesh copper grid coated with carbon membrane and negatively stained with 2% phosphotungstic acid for 30 s. The grid was dried at room temperature and then observed by TEM.\textsuperscript{28}

**X-ray diffraction (XRD)**

A crystalline structure of the VLX-NLC was investigated using an X-ray diffractometer (Rigaker Geiger flex, Japan). Aqueous NLC dispersions were lyophilized before to the XRD measurement. Diffractrograms were performed from the initial angle 2θ =10º to the final angle 40º with a Cu Kα radiation source. Samples were placed in the glass sample holders and scanned from 2ºC to 80ºC with a scan angular speed 2h/min at 35 kV operating voltage and 40 mA current. The diffraction spectra were recorded.\textsuperscript{21}

**Entrapment efficiency of VLX**

The amount of drug incorporated in NLC was determined after separation of the free drug and lipids from the formulation. The 3ml volume of venlafaxine nanostructured lipid carrier suspension was transferred to the small centrifuge tubes were kept in ultracentrifuge
(Beckman Coulter Germany). The nanostructured lipid carrier was centrifuged at 70,000 rpm for 20 min. The 1 ml of supernatant liquid was collected and diluted with an appropriate volume of water. The amount of drug was determined by UV-Visible spectrophotometer (Shimadzu UV/1800) at λ 276 nm. The % encapsulation Efficiency (EE) was calculated by the following equations, respectively. All measurement was performed in triplicate.  

\[
\% \text{ EE} = \frac{W_{\text{TOTAL}} - W_{\text{FREE}}}{W_{\text{TOTAL}}} \times 100
\]

**In vitro drug release study**

*In vitro* drug release from Venlafaxine and all the batches of VLX-NLC were performed in 250 ml phosphate buffer solution (PBS) pH 6.4 using paddle Method and vessel assembly from USP type II eight station Dissolution apparatus (Electrolab-TDL-08L). The aqueous VLX-NLC (equivalent to 5 mg Venlafaxine) was placed in dialysis membrane bag (cellophane tube Himedia MW cut off 12,000 Da) which was previously clean, washed and removes impurities. The membrane bag was soaked overnight in PBS pH 6.4. The two ends of dialysis bag were sealed with thread to avoid leakage. The membrane was immered in a vessel containing 250 ml PBS pH 6.4 thermostatically controlled water bath maintained at 37±1°C, rpm of the paddle was set at 50. At regular time intervals (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h) sample was withdrawn and immediately equal volume of fresh buffer solution was replaced. The concentration of drug was analyzed using UV–Visible spectrophotometer (Shimadzu UV/1800) at a wavelength of λ 274 nm. The release studies were performed in triplicate.  

**Determination of drug Released Kinetic**

The data obtained after In-Vitro released of selected NLC formulation was determined by zero order, first order, Higuchi matrix, and Peppas Korsmeyer models.  

**Ex vivo drug release study**

*Ex vivo* drug diffusion study was performed on nasal tissue specimen of sheep for predicting the ex-vivo drug release characteristics. Freshly excised sheep nasal tissue specimen was collected from local slaughter, immediately dipped in freshly prepared phosphate buffer solution (pH 6.4). The bony cartilage was removed from the mucosal membrane, nasal tissue was isolated, washed and stabilized under phosphate buffer solution (pH 6.4). The tissue was stabilized in donor and acceptor compartments under phosphate buffer solution (pH 6.4). In *ex vivo* diffusion study 1ml Venlafaxine pure drug solution was introduced (2 mg ml-1) on freshly stabilized nasal mucosa tissue which placed in the donor compartment. The receptor compartment was filled with 50 ml phosphate buffer solution (pH 6.4). The stirring speed was 200 rpm with a magnetic stirrer at 37±5 °C. In the case of Venlafaxine nanostructured lipid carrier and the lyophilized VLX-NLC equivalent amount to 2 mg of Venlafaxine was reconstituted with 1 ml phosphate buffer solution (pH 6.4). Aliquot 2ml of Samples at specific time intervals (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h) and immediately replaced an equal volume of fresh buffer solution (pH 6.4). The amount of drug diffusion was analyzed using a UV–Visible spectrophotometer (Wenser) at wavelength λ 274 nm and % drug diffusion was calculated based on the standard calibration curve of Venlafaxine.  

**Stability Investigation**

As per the stability of NLC, concerned lyophilized VLX-NLCs was placed in screw cap glass bottle which covers with aluminium foil stored in refrigerator temperature approximately 4-8 °C and room temperature at 25 °C under for 1-3 month. After 1-3 month NLC lyophilized formulations were analyzed for particle size and EE and then compare with new formulations.  

**Statistical analysis**

All experiments were performed in triplicate and data were reported as mean ± SD (standard deviations) in Microsoft office excel.  

**Results and discussion**

**Lipid Screening**

The various solid lipid material eg., Geleol pellet (Glyceryl palmitostearate), Gelucire 50/13 (stearoyl macrogol-32 glyceride), Compritol 888 (Glyceryl behenate), Glycerol Monostearate (monosteric acid ester of glycerol) and Precirol ATO 5 (Glyceryl distearate) and Medium chain triglyceride (MCT) such as Capryol 90 (propylene glycol monacrylate), Miglyol 808, Captex 200 P, Maisine (glycerol monolinoleae), Capmul MCM, Capryol PGMC (propylene glycol II monacrylate) are used as for screening of lipid in NLC production. The selection of lipid was done carefully as per the solubility of drug in lipids. Figure 1a and 1b showed that a higher amount of VLX could be solubilized in Glycerol monostearate and Capmul MCM gave 179.5 mg/g and 516 mg/g. Therefore solid lipid Glycerol monostearate and oil lipid Capmul MCM was selected on the base of higher solubility of drug for the NLC development. By visual examination of blending a Glycerol monostearate and Capmul MCM in ratio of 70:30 was found that no any oil droplets were seen on glass slid under microscope.
The solubility data of various solid and oil lipid was shown in Table 1. The selection of surfactant such as Pluronic F127 and sodium taurocholate (bile salt) was selected on the basis of Hydrophilic-lipophilic balance (HLB) values and possess physicochemical properties which suitable for nose to brain drug delivery system, Inhibit P-glycoprotein and multidrug resistance-associated protein efflux, enhancing the permeability of drug across the nasal mucosa and BBB.

**Formulation and characterization of Venlafaxine nanostructured lipid carrier**

VLX-NLCs were prepared by melt emulsification ultrasonication followed by lyophilization. In this present study, Glyceryl monostearate was used as solid lipid and Capmul MCM was used as oil lipid. Pluronic F127 was used as Stabilizer and sodium taurocholate (Bile salt) as co-surfactant. All NLC formulation was prepared at 75 °C to stay the solid lipid remains in the liquid state throughout the production process and do not recrystallized during the process. The composition of the formulation as shown in Table 2. The particle size distributions of VLX-NLC was found to be 199.6 nm shown in Figure 2. The mean particle size of the all VLX-NLC dispersion was varied between 155 and 293 nm with a polydispersity index value of all was below 0.7, representing narrow and uniform particle size distributions depending on the concentration of Pluronic F127, Sodium taurocholate and stirring speed. VLX-NLC 4 was chosen as best VLX-NLC formulation because of optimum mean particle size, PDI and zeta potential of were 213±4.3, 0.49±0.015, -35 mV and respectively. The selected VLX-NLC with zeta potential was as shown in Figure 3. A favorable concentration of Pluronic F127 which leads to decreasing the particle size of NLC. Hence, the concentration of Pluronic F127 (1% w/v) was selected. However, a decrease in particle size with increased concentration of surfactant was used. Polydispersity index is determining particle homogeneity varies from 0 to 1. Closer the value of zero polydispersity is better the homology between the particles sample. The particle size of Venlafaxine NLC formulation was further confirmed by TEM. The surface charge of the particles was measured by Zeta potential (ZP) which indicates the degree of repulsion and predicts the physical stability of the NLC dispersions. Zeta potential of Venlafaxine dispersion was -34. The decreases in zeta potential due to the presence of a free drug in the NLC dispersion which is anionic in nature whereas used of cationic surfactant in formulation ensuring in enhanced positive zeta potential. Owing to Hydrophobic nature of the solid lipids and oil. It is difficult to encapsulate and preserve hydrophilic drugs in the lipid matrix. However, to overcome this problem, variety of the formulation excipients was found to be crucial. Arpana Patil Gadhe and Varsha Pokharkar have improved the loading of hydrophilic drug Montelukast in Nanostructured Lipid Carrier. The Encapsulation Efficiency of

| Table 1: Solubility studies of Venlafexine in various solid and Oil lipids. (n=2) |
|---------------------------------|----------------|----------------|----------------|----------------|
| Solid Lipids                   | solubility (mg) | Oil Lipids      | solubility (mg) |
| Glyceryl Monostearate          | 179.5           | Captex 355      | 152            |
| Stearic Acid                   | 154             | Capryol 90      | 396            |
| Compritol 888                  | 70              | Capmul MCM      | 516            |
| Precirol ATO 5                 | 74.5            | Capmul PG 8     | 509.5          |
| Gelucire 50/13                 | 75.5            | Captex 200 P,   | 60.9           |

| Table 2: Formulation Composition of VLX- NLC |
|---------------------------------------------|-----------------|-----------------|----------------|-----------------|----------------|
| Batches                                     | GMS (mg)        | Capmul MCM      | Drug (mg)      | F127 (%)        | STC (%)         | Stirring Speed |
|                                             |                  |                 |                | (mg)            | (mg)            | rpm/35min      |
| VLX-NLC 1                                   | 280             | 120             | 10             | 250             | 125             | 1000           |
| VLX-NLC 2                                   | 280             | 120             | 10             | 500             | 250             | 1000           |
| VLX-NLC 3                                   | 280             | 120             | 10             | 250             | 250             | 2000           |
| VLX-NLC 4                                   | 280             | 120             | 10             | 500             | 125             | 2000           |
| VLX-NLC 5                                   | 280             | 120             | 10             | 250             | 250             | 2000           |
| VLX-NLC 6                                   | 280             | 120             | 10             | 500             | 125             | 1000           |
| VLX-NLC 7                                   | 280             | 120             | 10             | 500             | 250             | 2000           |
| VLX-NLC 8                                   | 280             | 120             | 10             | 250             | 125             | 2000           |
hydrophilic VLX showed maximum solubility in Glyceryl Monostearate and Capmul MCM was found to be 174 mg/g and 559 mg/g. The loading of a drug was found to be 27%. The addition of solid lipid (Glyceryl Monostearate), Oil lipid (Capmul MCM) MCT increases in encapsulation efficiency. The % EE can be improved by improving the amorphous nature of particle resulting in enhanced stability and continuous release of a drug. The mean particle size (PS), polydispersity index (PDI), Zeta potential (ZP), % Entrapment efficiency (EE) and Drug loading (DL) of VLX-NLC are shown in Table 3. Each value represented the mean ± SD (n=3).

**Fourier transforms infrared spectroscopy (FTIR)**

Figure 4 shows FTIR spectrum of VLX, VLX-NLC and Physical mixture of drug-lipid. The characteristic peak at 3350.35 cm⁻¹ of O-H, 1614.42 cm⁻¹ due to aromatic C=H stretching, 1514.12 cm⁻¹ due to C-O stretching, 1178.51 cm⁻¹ due to C-N stretching, 1043.49 cm⁻¹ due to C-O-C stretching at, 1732.08 cm⁻¹ due to C=O stretching of an important functional group. These characteristic stretching bands were a little different after pre-formulation study and illuminating no Chemical interaction between the VLX and lipids material during the formulation of NLC was observed in IR pattern.

**Differential scanning calorimetry (DSC) analysis**

The DSC thermograms of pure drug exhibited a single sharp endothermic peak at 208.52°C, related to melting point and representing crystalline nature of the pure drug. The physical mixture of Venlafaxine also showed a peak at 193.87 °C on same thermal behaviour. The sharp peak at 208°C corresponds to melting peak ofVenlafaxine, which is not present in the thermogram of VLX-NLCs shown in Figure 5. This indicates either Venlafaxine is completely solubilized in the lipid matrix or transformation to amorphous form when compare

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**Table 3: The mean PS, PDI, Zeta potential, % EE and in-vitro drug released.**

<table>
<thead>
<tr>
<th>Batch no</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>E.E. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLX-NLC 1</td>
<td>196.8±4.3</td>
<td>0.446±0.053</td>
<td>-43.6±0.74</td>
<td>67.94±4.2</td>
</tr>
<tr>
<td>VLX-NLC 2</td>
<td>115.6±0.35</td>
<td>0.616±0.006</td>
<td>-34.32±1.2</td>
<td>64.19±3.07</td>
</tr>
<tr>
<td>VLX-NLC 3</td>
<td>250.8±4.4</td>
<td>0.439±0.016</td>
<td>-32.7±0.9</td>
<td>70.0±3.61</td>
</tr>
<tr>
<td>VLX-NLC 4</td>
<td>213.7±3.49</td>
<td>0.49±0.015</td>
<td>-36.6±2.3</td>
<td>75.97±2.10</td>
</tr>
<tr>
<td>VLX-NLC 5</td>
<td>289.4±5.2</td>
<td>0.479±0.009</td>
<td>-31.02±2.1</td>
<td>73.48±4.8</td>
</tr>
<tr>
<td>VLX-NLC 6</td>
<td>258±6.9</td>
<td>0.455±0.057</td>
<td>-49.4±1.9</td>
<td>69.65±6.11</td>
</tr>
<tr>
<td>VLX-NLC 7</td>
<td>73.41±2.33</td>
<td>0.633±0.10</td>
<td>-33.35±0.08</td>
<td>66.03±6.94</td>
</tr>
<tr>
<td>VLX-NLC 8</td>
<td>229.6±3.71</td>
<td>0.271±0.016</td>
<td>-42.04±2.6</td>
<td>74.13±5.19</td>
</tr>
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</table>

**Table 4: Kinetic analysis of optimized VLX-NLC dispersion.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Square root of time</th>
<th>Log time</th>
<th>% Drug released</th>
<th>Fraction drug released</th>
<th>Log fractioned drug released</th>
<th>% drug remaining</th>
<th>Log % drug remaining</th>
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</thead>
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<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>100</td>
<td>2.00</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.00</td>
<td>6.7217</td>
<td>0.067</td>
<td>-1.173</td>
<td>93.27</td>
<td>1.969</td>
</tr>
<tr>
<td>2</td>
<td>1.41</td>
<td>0.30</td>
<td>18.3838</td>
<td>0.183</td>
<td>-0.737</td>
<td>81.62</td>
<td>1.911</td>
</tr>
<tr>
<td>3</td>
<td>1.73</td>
<td>0.47</td>
<td>25.2004</td>
<td>0.252</td>
<td>-0.598</td>
<td>74.8</td>
<td>1.873</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.60</td>
<td>33.7304</td>
<td>0.337</td>
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<td>1.821</td>
</tr>
<tr>
<td>5</td>
<td>2.36</td>
<td>0.69</td>
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<td>0.456</td>
<td>-0.341</td>
<td>54.34</td>
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<tr>
<td>6</td>
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<td>56.0271</td>
<td>0.560</td>
<td>-0.251</td>
<td>43.98</td>
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<tr>
<td>7</td>
<td>2.64</td>
<td>0.84</td>
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<td>31.87</td>
<td>1.503</td>
</tr>
<tr>
<td>8</td>
<td>2.83</td>
<td>0.90</td>
<td>78.6654</td>
<td>0.786</td>
<td>-0.104</td>
<td>21.34</td>
<td>1.329</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>0.95</td>
<td>80.9437</td>
<td>0.802</td>
<td>-0.095</td>
<td>19.06</td>
<td>1.280</td>
</tr>
<tr>
<td>10</td>
<td>3.16</td>
<td>1.00</td>
<td>83.2403</td>
<td>0.832</td>
<td>-0.079</td>
<td>16.76</td>
<td>1.224</td>
</tr>
<tr>
<td>12</td>
<td>3.46</td>
<td>1.07</td>
<td>85.5453</td>
<td>0.855</td>
<td>-0.068</td>
<td>14.46</td>
<td>1.160</td>
</tr>
<tr>
<td>24</td>
<td>4.89</td>
<td>1.38</td>
<td>89.5337</td>
<td>0.895</td>
<td>-0.048</td>
<td>10.47</td>
<td>1.019</td>
</tr>
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Table 4a: Co-efficient correlation of optimized VLX-NLC Dispersion.

<table>
<thead>
<tr>
<th>Released Profile model</th>
<th>Regression Value($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Order</td>
<td>0.958</td>
</tr>
<tr>
<td>First order</td>
<td>0.974</td>
</tr>
<tr>
<td>Higuchi Model</td>
<td>0.854</td>
</tr>
<tr>
<td>Peppas Model</td>
<td>0.417</td>
</tr>
</tbody>
</table>

Table 5: Stability profile of optimize VLZ-NLC.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>4-8°C Month</th>
<th>25°C Month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>EE (%)</td>
<td>71.97</td>
<td>71.43</td>
</tr>
<tr>
<td>PS(nm)</td>
<td>199.1</td>
<td>199.1</td>
</tr>
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</table>

Figure 1 A: Solubility profile of Venlafaxin in solid lipid.

Figure 1 B: Solubility profile in different Oil lipid.

Figure 2: Particle size distribution of optimized VLZ-NLC.

Figure 3: Zeta potential of optimized VLX-NLC.

Figure 4: FTIR spectra of venlafazine, bulk lipid, drug lipid mixture.

X-ray diffraction (XRD) investigation to VLX alone. DSC profile of NLCs was investigated and analysis made up of Glyceryl monostearate and portion amount Capmul MCM in the ratio of 70:30 used in NLC formulation influences the crystallinity of Glyceryl monostearate hence shows broadening of the peak.
The crystallinity study of Venlafaxine (VLX), VLX-PM (physical mixture) and VLC-NLC formulation (lyophilized) was performed by X-ray diffraction. Figure 6 shows XRD of Venlafaxine, VLX-PM (physical mixture) and VLX-NLC formulation (lyophilized). The diffractograms of Venlafaxine show the sharp peak in 2θ scattered angle representing crystallinity nature of Venlafaxine whereas physical mixture and VLX-NLC formulation show decrease intensity of the peak of Venlafaxine which indicates amorphization of Venlafaxine.

**Transmission electron microscope (TEM)**

TEM images of optimized VLX-NLC formulation which represented the particles size were spherical in nature. The diameter of the VLX-NLC formulation was small 200 nm shown in Figure 7. TEM provides two-dimensional morphological images and capability to detect the size of the sample in the nanometer range. TEM is capable to form the images with higher resolution than SEM. The size difference obtained by TEM and DLS mainly came from the different sample preparation processes and different principles. The DLS did not ‘measure’ the particle sizes but detected the light scattering effects which were used to calculate the particle size. The diameter from DLS would be a little bigger than that observed from TEM.

**In-vitro drug release assay**

The Invitro drug release study of Venlafaxine from NLC formulation was studied using dialysis bag method and was compared with Venlafaxine drug solution. In vitro drug release profile of VLX-NLC was carried out in phosphate buffer solution (pH 6.4) due to partial solubility in the buffer solution which was published in the previous literature. The % drug was released within 2h from pure venlafaxine solution was found to be 93%, whereas 89.5 percentage cumulative drug was released in 24 h from Venlafaxine nanostructured lipid carrier indicating prolonged released, shown in Figure 8. It was concluded that the released rate from nanostructured lipid carrier could be significantly prolonged the released of Venlafaxine and observed that the rate of release of drug was decreased with a particle size of NLC was increased.

**Mechanism of drug released**

The data were obtained from in-vitro drug release studies were fitted to kinetic model such as zero order, first order, Higuchi square root of time and Korsmeyer-Peppas model.
model to know the mechanism of drug release from the VLX-NLC was found to be 0.958, 0.974, 0.8541 and 0.417, respectively were shown in Table 4, and Figure 9,10,11,12 respectively.

**Ex-vivo diffusion studies**

The ex-vivo drug permeation studies were performed in sheep nasal mucosa for the VLX solution, Lyophilized VLX-NLC and VLX-NLC dispersion. *Ex-vivo* drug release data of pure drug shows 89% drug release at 8 h (pH6.4), VLX-NLC exhibit 73.27 % drug release and VLX-NLC suspension plotted graph 76.82% drug diffused versus time (h) shown in Figure 13 and permeation data was given in Table 6. The pKa value of VLX is 9.4, thus at nasal mucosa pH (5.5-6.5), a large amount of the drug remains in protonated form and so it is transported through tight junctions opened by sodium taurocholate. Therefore, enhance in permeation through nasal mucosa due to an involvement of paracellular transport through tight junctions.31, 32

**Stability profile of VLX-NLC**

The stability study was performed for finally optimized formulations at 4-8°C and 25°C for the period of three months. The increasing mode of particle size (PS) and polydispersity index (PI), and decreasing trend drug encapsulation efficiency (EE) were observed with storage time at 25°C. Stability study profile indicated that the
formulations (VLX-NLC) have remained stable for a period of more than three months at normal temperature as shown in Figure 14. Hence VLX-NLC can be considered as stable formulations.36

SUMMARY AND CONCLUSION
In India Depression is a most common chronic, potentially debilitating psychiatric disorder. Depression is known as major mood disorder. Various types of antidepressants drugs are used considering their effects on neurotransmission and neurotransmitters. The Venlafaxine are taken for study which is selective serotonin reuptake inhibitors (SSRI) drug used for as mood stabilizing properties and used in treatment of psychotic depression. Therefore, the aim and objective of the present study were developed and prepared VLX-NLCs by Melt emulsification and Ultrasonication methods. The obtained VLX-NLC were characterized by Photon correlation spectroscopy (PCS), Transmission electron microscope, particle size, zeta potential, drug loading, Encapsulation Efficiency, X-ray diffraction, in-vitro drug release studies and stability study. The in-vitro release from VLX-NLCs and mechanism of kinetic. The release rate mostly depends on the drug, solid and liquid lipid interactions. The ex-vivo permeation studies showed controlled and prolonged released. The prepared NLCs were checked for their stability in 4-8°C and 25°C conditions for 3 months and the stability showed that the prepared NLCs of venlafaxine hydrochloride are stable formulations.

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COMPETING INTERESTS
The authors declare that they have no any competing interests.

CONFLICT OF INTEREST
The authors declare that they have no conflict interests.

ABBREVIATIONS USED

REFERENCES

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PICTORIAL ABSTRACT

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