Research Article

Lercanidine Hydrochloride Nanocrystals: Impact of Shear and Milling on Particle Size Reduction to Enhance Dissolution and Saturation Solubility

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The aim of the present investigation was to study impact of shear and milling on particle size reduction to improve the dissolution characteristic and saturation solubility of a poorly water-soluble drug Lercanidine hydrochloride (LER) by preparing nanocrystals through wet media milling method. A set of experiments were carried out by trying different size of zirconium oxide beads, amount of beads, concentration of stabilizer, concentration of drug, stirring speed and stirring time by keeping one parameter constant against other parameters. In Plackett–Burman (PB) screening design independent variables selected were size of beads (mm) (X₁), amount of beads (gm) (X₂), conc. of stabilizer (%w/v) (X₃), conc. of drug (%w/v) (X₄), stirring speed (rpm) (X₅) and stirring time (hr) (X₆). A Mean particle size (Y₁), saturation solubility (Y₂) and % DR₅min (Y₃) were selected as the dependent variables. Significance F was less than 0.05 for particle size, saturation solubility and % DR₅min which indicated model is statistically significant. R square was found larger than 0.9, which is a very good fit indicating that over 90% of the variation in the response is explained by the model. It was seen that %DR₅min increases as the concentration of polymer, concentration of drug, stirring speed and stirring time increases because of an increased surface area due to particle size reduction achieved. Saturation solubility increased as the loading of beads, stirring speed and stirring time increases because of an increased surface area due to particle size reduction. It was concluded that presented media milling method was successfully improve dissolution and saturation solubility of lercanidine by nanocrystal approach.

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INTRODUCTION:
The study was aimed to screen the important independent factor to improve the dissolution characteristics and saturation solubility enhancement of a poorly water-soluble drug Lercanidipine hydrochloride (LER) by preparing nanocrystals through wet media milling method. Lercanidipine hydrochloride belongs to chemical class 1,4–dihydropyridine. It is used as an antihypertensive agent. It acts by blocking the calcium channels of smooth muscles. This results in peripheral vasodilatation and reduction in blood pressure.\[1\] Chemically it is 2(3, 3- diphenylpropyl) (methyl) amino- 1,1-dimethylethyl methyl 2, 6- dimethyl-4- (3-nitrophenyl)-1, 4-dihydropyridine-3, 5- dicarboxylate hydrochloride. Lercanidipine hydrochloride is fall into BCS Class II category which is practically insoluble in water due to its high lipophilicity. Because of high lipophilicity and low solubility, lercanidipine hydrochloride shows only 10 % of oral bioavailability.\[2\]
The most challenging task for researchers and formulators of industry is to formulate poorly water soluble drugs. Dissolution is the rate limiting step for most of the pharmaceutical formulations. Drugs with poor aqueous solubility will mainly exhibit dissolution rate limited absorption and drugs with poor membrane permeability will exhibit permeation rate limited absorption.\[3\]
Nanocrystals is a new carrier free colloidal drug delivery system with particle size in nano range and is considered as a viable drug delivery strategy to develop formulation of the poorly soluble drugs.\[4\] The use of drug nanocrystals is one of the formulation approach to increase the solubility and dissolution rate of poorly water soluble drugs in any route of administration like oral, iv, pulmonary, ophthalmic, dermal and targeted drug delivery system.\[5\]
Generally, the strategies to prepare drug nanocrystals could be classified into “top-down” and “bottom-up” techniques. Top-down technologies refer to mechanic attribution of coarse drug powder including media/Bead/pearl milling and high pressure homogenizations.\[6\]

MATERIALS AND METHODS
Materials
Lercanidipine hydrochloride was obtained from (Alembic Pharmaceuticals, Gujarat, India) and Zirconium oxide beads were obtained from (Synco Industries Limited, Gujarat) as a gift sample. Distilled water used as a formulation vehicle. Methanol used in study was of analytical grade. Polymers used in this study were meet high standards.

Methods
Preparation of standard curves
The calibration curves were prepared in methanol and 0.01 M HCl. Required aliquots of stock solutions were transferred to series of 10 ml volumetric flask and volume is made up to the mark with respective dilution medium. For methanol concentration series of 4 -18 μg/ml and for 0.01 M HCl 6-20 μg/ml was prepared. The absorbance of all the solutions were measured against blank in double beam UV Vis Spectrophotometer (UV-1800, Shimadzu, Japan).\[7\]
Preparation of LER loaded nanocrystals (NCs)
Lercanidipine nanosuspension were prepared by wet media milling method. The glass vial capacity (20ml) was used as model milling chamber. The coarse suspension was prepared by dispersing drug and polymer in glass vial using distilled water (10ml batch size) as vehicle. The solution was sonicated by Ultrasonicator (Remi instrument, Mumbai, India) for 10 min to allow mixing. Then zirconium oxide beads were added to glass vial as milling agent. The milling carried out by placing vial on the magnetic stirrer (Remi instrument, Mumbai, India) with polygon magnetic stirring bar (Ø 8.0 mm) for preselected rpm and time period. After that suspension was collected by pipette and beads were rinsed with distilled water and rinsing was combined with milled suspension. Finally suspension was lyophilized using Lyophilizer to convert it into solid state and evaluation studies carried out for both nanosuspension and lyophilized drug powder. All the formulation parameters were selected based on preliminary studies.
Preliminary Screening of formulation parameters and their ranges by OFAT (one factor at a time)

To achieve nanosized particles, set of experiments were carried out by trying different size of zirconium oxide beads (1.0, 2.4 and 3.7 mm), amount of beads (5, 10 and 15gm), stabilizers (PVP K30, poloxamer 407, poloxamer 188, HPMC, HPC and PVA cold ), conc. of stabilizer (0.1 – 1.0 %w/v), conc. of drug (0.5 – 2.5 %w/v), stirring speed (800 – 1400 rpm) and stirring time (4 – 28 hr) by keeping all parameters constant against any one parameter. The parameters levels were selected on the basis of average particle size and PDI of the Lercanidipine nanosuspension.

Plackett-Burman screening design (selection of critical parameters)

Based on preliminary study, it was found that presented method includes many formulation variables which have an influence on the formulation. For Evaluation of many preparation variables usually we preferred Plackett - Burman (PB) design, which has been frequently used for screening the large number of factors as shown in Table 1. In Plackett– Burman screening design independent variables selected were size of beads (mm) (X₁), amount of beads (gm) (X₂), conc. of stabilizer (%w/v) (X₃), conc. of drug(%w/v) (X₄), stirring speed (rpm) (X₅) and stirring time (hr) (X₆).[8-9] An average particle size (Y₁), saturation solubility (Y₂) and %drug release after 5min(%DR₅min) (Y₃) were selected as the dependent variables. Statistical analysis was carried out by regression analysis and ANOVA. Design Expert® 12 (Stat-Ease Inc., USA) software was used to set experiments. Pareto chart were constructed using Minitab® statistical software. A polynomial Eq. (1) was successfully used to evaluate the responses against independent variable. The polynomial equation with coded variables and coefficients can be written as below:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_n X_n \] ........................................(I)

Where, Y is dependent variable(responses), \( \beta_0 \) is arithmetic mean of twelve runs and \( (\beta_1, \beta_2) \) is the estimated coefficient for relative factor (X₁, X₂,...).

The main effects (X₁ and X₂) signify average result of altering one factor at a time from its lowest to highest value.

| Table 1: Layout of levels in plackett–burman design |
|---------------------------------|--------|--------|
| Independent Factors(unit)       | Level(+1) | Level(-1) |
| X₁ Size of beads (mm)           | 2.4    | 1.0    |
| X₂ Amt. of beads (mm)           | 10     | 5.0    |
| X₃ Stabilizer (%w/v)            | 0.75   | 0.5    |
| X₄ Conc. of drug (%w/v)         | 1.5    | 1.0    |
| X₅ Stirring speed (rpm)         | 2000   | 1400   |
| X₆ Stirring Time (hr)           | 24     | 16     |

Characterization of Lercanidipine Nanocrystals

Mean particle size and particle size distribution

The particle size and size distribution was performed using Zetatrac (Microtrac Inc. USA). The prepared nanosuspensions (~ 5ml) were suspended with distilled water individually. The suspension was directly placed into sample cell with the help of dropper. Prior to measuring particle size samples were sonicated for 20-30 sec to make sample uniform. Each sample was analyzed for particle size and PDI. The average particle size was determined in terms of d (0.9) nm.[10]

Zeta potential (ζ) determination

The prepared nanosuspensions (~ 5ml) were suspended with distilled water individually. The suspension was directly placed into sample cell with the help of dropper. Prior to measuring samples were sonicated for 20-30 sec to make sample uniform.[11] Zetatrac measures the particle size based on Dynamic Light Scattering (DLS) principle. In a constant electric field particles moves from cathode to anode which was internally fitted in sample holder, at a constant velocity. The charge and zeta potential of suspended particles were determined by velocity of particle in electric field. The charged particles were oscillate with
high frequency in generated alternate current field. This phenomenon result in a measurement of zeta potential of particles.

**Determination of saturation solubility**

Excess amount of samples were added to distilled water and placed into 10 ml capped glass vial to avoid any changes due to evaporation and subjected for solubility study. Then glass vial containing mixtures was shaken for 3 days on Shaker Incubator (Tempo Instrument & equipment, India). Shaker temperature maintained at 37 °C. After that sample was centrifuged in ultracentrifuge and supernatant was collect and filter through 0.45 micron syringe filter. The filtered samples were analyzed using UV-Visible spectrophotometer at $\lambda_{max}$ 242.6 nm after appropriate dilutions. Experiments were carried out in triplicate and mean and standard deviation was calculated and reported. \[12\]

**In Vitro Dissolution Studies**

Dissolution studies were carried out in 900 ml 0.01 N HCl (pH 2.4) at 37 °C at 50 rpm (Paddle method, Electrolab Dissolution Tester TDT-06P, USP). 20 mg of pure LER and its equivalent formulations were added to dissolution medium, and 5 ml of sample was withdrawn at 5, 15, 30, 45, and 60 min and replaced with fresh media. The solutions were filtered with whatman filter paper (0.22 μm) and read against blank using UV Visible spectrophotometer. The apparatus were Electrolab Dissolution Tester TDT-06P Method : USP type II apparatus (paddle)

Dissolution medium: 0.01N HCl
Volume of DM : 900 ml
Temperature : 37°C ± 0.5°C
Speed : 50 rpm

**Lyophilisation of nanosuspension**

An optimized Lercanidipine nanosuspension was lyophilized to convert it into solid state to enhance its stability to a longer period of time in dry powder state. The properties of drug remain intact because lyophilisation include removal of water content without adding excess heat to the product. Cryoprotectant; Mannitol (1% w/v) was used for lyophilization of lercanidipine nanosuspension. Mannitol and freshly prepared nanosuspension was mixed in a glass vial. The sample was shaken on sonicator bath until the cryoprotectant was dissolved completely. The prepared sample was lyophilized subsequently using a lyophilizer. Mannitol offers great flow ability to dried powder. Dried powder can be used for further solid state characterization after easy reconstitution.

**Solid state characterization of lyophilized lercanidipine nanocrystals**

**Fourier Transform Infrared Spectroscopy**

Lercanidipine Hydrochloride, PVA Cold, physical mixture LER and PVA cold (1:0.5) and lyophilized nanocrystals were compared using Fourier transform infrared spectroscopy ( CARY 630, Agilent Technologies) which precisely measures the amount of light absorbed by the sample. This absorbance creates a unique spectral fingerprint that is used to identify the molecular structure of the sample with the help of unique Michelson interferometer.

**Scanning Electron microscopy**

A scanning electron microscope is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography of the sample. SEM images of pure LER and LER loaded lyophilized nanocrystals were included to study surface morphology.

**Drug content**

The drug content was determined by dissolving equivalent (~20mg) quantity of LER nanosuspension in methanol. The solution was, stirred sufficiently to dissolve the drug, and centrifuged at 5000 rpm for 15 min. The supernatant was collected, diluted and measured at 236 nm using UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) against blank. The drug content was performed in triplicate.
RESULTS AND DISCUSSION

Calibration curve
LER showed λmax 236 nm in Methanol and 242.6 nm in 0.01 N HCl. Linearity equation for methanol was y = 0.04653 x + 0.00821; R² = 0.99950 and for 0.01M HCl it was found y = 0.04161 x + 0.03496; R² = 0.99690. The calibration curve was obtained by UV Prob software as per Fig. 1a and 1b.

![Calibration curve](image)

**Fig. 1. Calibration curve of lercanidipine in (a) methanol (b) 0.01N HCl**

Preliminary studies:
In order to obtained nanosized particles and decide levels of each formulation parameter (independent variable) preliminary studies were done and results of the studies are reported in Table 2. The effect all possible variables studied individually as follows:

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<th>Parameters (unit)</th>
<th>Batch code</th>
<th>Levels</th>
<th>Particle size(nm)</th>
<th>PDI</th>
<th>Zeta</th>
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Milling Time (hr)  
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**Effect of size of beads**  
In media milling diameter of the milling beads play a significant role in the particle size reduction. The results of experiment by using different sized beads confirmed that in case of small sized beads particle size and PDI value showed marked reduction. The results might be credited to availability of more contact points between drug particle and milling beads. In present study beads having diameter (Ø 1.0mm) was employed. While choosing size of beads; ease of removal of beads from suspension and handling during study also considered owing to slippery and smooth surface of beads.

**Effect of beads loading (suspension to bead ratio)**  
Three batches were prepared by varying the suspension to bead ratio. Suspension to bead ratio of 1:0.5 showed the lowest particle size reduction due to less contact between drug particles and milling beads, 1:1.5 ratio showed higher particle size due to aggregation of particles, whereas 1:1 ratio showed better result as compared to the other ratios (Table 2). The aspect using beads with higher weight, improve the milling efficiency. A higher amount of beads achieve more contact points, impact, compression and shears forces between the drug particles and milling agents.

**Effect of stabilizers**  
A suitable stabilizer has to be added to stabilize the nanosuspension. Stabilizers added to make the interfacial tension at the nanoparticle – medium interface close to zero allowing for stability of the nanoparticle dispersion. Formulations were prepared by varying the type of stabilizer at a constant weight ratio of 1:0.5 of drug to stabilizer. The prepared nanosuspensions were evaluated for particle size, PDI and zeta potential. An efficient particle size reduction was seen with all the stabilizers which was shown in Figure 2. In case of PVA Cold (non ionic), reduction of particle size was highest. It has been reported that Zeta potential values in the range of 25 mV to 30 mV in either charge consider a stable formulation.\[13\]
Effect of concentration of stabilizer

Concentration of stabilizer plays a critical role in nanosuspension formulation to make it stable for long time. In present method (0.12, 0.25, 0.50, 1.00%w/v) stabilizers concentration were tried. The findings of trial suggested that at lower concentration of stabilizer particle size get decreased but zeta potential value was not enough to make suspension stable. As amount of stabilizer not enough to cover the surface of nanoparticle as a result of this particles formed agglomeration after some period of time. Particle size decrease with increase in concentration of PVA Cold up to 0.5%w/v. Further at 1.0%w/v particle size and PDI values were increased. With this results concentration of stabilizer was found critical parameter.

Effect of concentration of drug

To study the effect of drug concentration different formulation having concentration (0.5 - 2.5%w/v) were prepared. From result it was observed that at lower drug loading particle size reduction was less due to reduction of milling material in milling chamber results into poor mixing. At 1.0 %w/v; particle size was less as compared to 0.5% w/v. Further increase in drug loading there was marked increase in particle size this could be explained by aggregation of particles at higher drug concentration.\(^{[14]}\) It was also observed that at higher drug concentration drug particles were deposited at the wall of glass vial and remain isolate from milling process so their particle size reduction was not achieved. Preparations with 100 mg/ml drug concentration were found to be optimum for PVA-based formulations.

Effect of stirring speed and stirring time

Stirring speed and stirring time are crucial factor in media milling technique. At lower rpm particle size was not reduced as desired because less attrition and collison between drug particles and milling beads whereas at higher rpm particle size increases due to aggregation. Therefore moderate rpm showed uniform particle size distribution as per Fig. 3. In media milling, milling speed with an appropriate rpm would be acceptable. A higher rotation speed leads to abrasion and chipping as opposed to crushing of the particles this will results in increased particle size. Therefore optimum results achieved by moderate stirring speed. At 1400 rpm highest particle size reduction was observed (Table 1). Milling time had also an important effect on particle size. Milling time show mixed effect on particle size reduction. According to Fig. 4 Particle size reduction was increased when time of stirring increased up to 24 hrs, after that particle size was increased due to aggregation of particles.
Optimization of experimental design statistical analysis

In Plackett – Burman (PB) screening design independent variables selected were size of beads (mm) ($X_1$), amount of beads (gm) ($X_2$), conc. of Stabilizer (%w/v) ($X_3$), conc. Of drug(%w/v) ($X_4$), stirring speed (rpm) ($X_5$) and stirring Time (hr) ($X_6$). An average particle size ($Y_1$), saturation solubility ($Y_2$) and % $D_{5\text{min}}$($Y_3$) were selected as the dependent variables. The obtained data were reported in Table 3 and subjected to regression analysis. The polynomial equation (Eq. 2 - 4) relating the responses ($Y_1$, $Y_2$ and $Y_3$) were transformed in actual value as below:

\[
\text{Particle Size} = -1647.36 + 15.28 X_1 + 1.46 X_2 + 2314.44 X_3 - 953.00 X_4 \\
+ 1.24 X_5 + 30.53 X_6 \tag{2}
\]

\[
\text{Saturation solubility} = 28.54 - 0.17 X_1 - 0.22 X_2 + 10.44 X_3 + 6.30 X_4 \\
+ 0.01 X_5 + 0.97 X_6 \tag{3}
\]

\[
\text{% DR } s_{\text{min}} = -8.41 - 1.07 X_1 - 0.12 X_2 + 16.21 X_3 + 12.50 X_4 \\
+ 0.01 X_5 + 0.90 X_6 \tag{4}
\]
The coefficients in Eq. (2)–(4) represent the respective quantitative effect of the independent variables ($X_1$ to $X_6$) on the response variables (PS, SS and % DR$_{5\text{min}}$).

**Table 3: Plackett-burman screening design for lercanidipine nanosuspension**

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Size of beads (mm)</th>
<th>Amt of beads (gm)</th>
<th>Conc. of Stabilizer (% w/v)</th>
<th>Conc. of drug (% w/v)</th>
<th>Stirring Speed (rpm)</th>
<th>Stirring Time (hr)</th>
<th>*Mean Particle Size ± SD</th>
<th>*Saturation solubility ± SD (µg/ml)</th>
<th>*% DR$_{5\text{min}}$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LER1</td>
<td>2.4</td>
<td>10</td>
<td>0.50</td>
<td>1.5</td>
<td>2000</td>
<td>24</td>
<td>1528.0 ± 9.85</td>
<td>91.27 ± 0.74</td>
<td>62.23 ± 0.33</td>
</tr>
<tr>
<td>LER 2</td>
<td>1.0</td>
<td>10</td>
<td>0.75</td>
<td>1.0</td>
<td>2000</td>
<td>24</td>
<td>2660.0 ± 7.55</td>
<td>91.49 ± 0.89</td>
<td>61.96 ± 0.92</td>
</tr>
<tr>
<td>LER 3</td>
<td>1.0</td>
<td>5</td>
<td>0.75</td>
<td>1.5</td>
<td>1400</td>
<td>24</td>
<td>1046.3 ± 9.71</td>
<td>84.83 ± 1.53</td>
<td>58.63 ± 0.41</td>
</tr>
<tr>
<td>LER 4</td>
<td>2.4</td>
<td>10</td>
<td>0.50</td>
<td>1.5</td>
<td>2000</td>
<td>16</td>
<td>789.0 ± 8.54</td>
<td>78.93 ± 0.73</td>
<td>52.45 ± 0.46</td>
</tr>
<tr>
<td>LER 5</td>
<td>2.4</td>
<td>5</td>
<td>0.75</td>
<td>1.0</td>
<td>2000</td>
<td>24</td>
<td>2099.0 ± 3.61</td>
<td>87.01 ± 2.46</td>
<td>58.96 ± 1.02</td>
</tr>
<tr>
<td>LER 6</td>
<td>2.4</td>
<td>5</td>
<td>0.50</td>
<td>1.5</td>
<td>1400</td>
<td>24</td>
<td>786.3 ± 7.77</td>
<td>84.99 ± 0.22</td>
<td>55.12 ± 0.25</td>
</tr>
<tr>
<td>LER 7</td>
<td>1.0</td>
<td>5</td>
<td>0.50</td>
<td>1.0</td>
<td>2000</td>
<td>16</td>
<td>1661.7 ± 6.43</td>
<td>80.96 ± 0.23</td>
<td>49.63 ± 0.51</td>
</tr>
<tr>
<td>LER 8</td>
<td>1.0</td>
<td>10</td>
<td>0.50</td>
<td>1.0</td>
<td>1400</td>
<td>24</td>
<td>819.7 ± 6.51</td>
<td>74.14 ± 0.79</td>
<td>43.26 ± 1.00</td>
</tr>
<tr>
<td>LER 9</td>
<td>1.0</td>
<td>10</td>
<td>0.75</td>
<td>1.0</td>
<td>1400</td>
<td>24</td>
<td>1509.7 ± 5.03</td>
<td>75.08 ± 1.50</td>
<td>44.21 ± 1.35</td>
</tr>
<tr>
<td>LER 10</td>
<td>2.4</td>
<td>10</td>
<td>0.75</td>
<td>1.5</td>
<td>1400</td>
<td>16</td>
<td>922.0 ± 8.89</td>
<td>76.41 ± 0.89</td>
<td>52.98 ± 0.87</td>
</tr>
<tr>
<td>LER 11</td>
<td>1.0</td>
<td>5</td>
<td>0.75</td>
<td>1.5</td>
<td>2000</td>
<td>16</td>
<td>1705.3 ± 8.74</td>
<td>83.58 ± 1.19</td>
<td>55.63 ± 0.68</td>
</tr>
<tr>
<td>LER 12</td>
<td>2.4</td>
<td>5</td>
<td>0.50</td>
<td>1.0</td>
<td>1400</td>
<td>16</td>
<td>886.0 ± 8.72</td>
<td>72.43 ± 1.18</td>
<td>42.65 ± 0.77</td>
</tr>
</tbody>
</table>

**Effect of independent variables on particle size**
According to linear equation (2) size of beads, amount of beads, conc. of stabilizer, conc. of drug, stirring speed and stirring time has favourable effect on particle size while concentration of drug has inverse relationship with average particle size. The ANOVA results showed that concentration of stabilizer, stirring speed and stirring time significantly ($P = .05$) influence the average particle size while other factors are insignificantly different from zero at the 95.0% confidence level. The regression coefficient ($R^2$) for particle size indicates 94.48 % of variability around the mean which was given in Table 4. Pareto chart (Fig. 6) also exposed that conc. of PVA Cold, conc. of drug and stirring speed had maximums standardized effect on particle size at 95% confidence interval. These parameters were found critical for nanosuspension formulation by media milling method.

**Effect of independent variables on saturation solubility**
According to equation (3) conc. of stabilizer, conc. of drug, stirring speed and stirring time has favourable
effect on saturation solubility while size of beads and
amount of beads have inverse relationship with
saturation solubility. According to Table 4, ANOVA
results showed that stirring speed and stirring time
significantly (P= .05) influence the average particle
size while other factors are insignificantly different
from zero at the 95.0% confidence level. The
regression coefficient for particle size indicates 93.46
% of variability around the mean. Saturation solubility
increases as the loading of beads, stirring speed and
stirring time increases because of particle size
reduction achieved will results in an increased surface
area and.

Effect of independent variables on %DR$_{5\text{min}}$
According to equation (4) conc. of stabilizer, conc. of
drug, stirring speed and stirring time has favourable
effect on %DR$_{5\text{min}}$. The ANOVA results showed that
stirring speed, stirring time and concentration of drug
significantly (P= .05) influence the average particle
size while other factors are insignificantly different
from zero at the 95.0% confidence level. The
regression coefficient for particle size indicates 96.24
% of variability around the mean which was given as
R square value in Table 4. According to Fig. 5 almost
40% of drug release from the formulation within 5 min
which is higher than pure LER. The size reduction of	particle leads to an increased surface area and thus
according to the Noyes–Whitney equation the
dissolution velocity is increased.[15]

<table>
<thead>
<tr>
<th>Independent Factors</th>
<th>Ave. Particle Size coefficient</th>
<th>Ave. Particle Size $P$</th>
<th>Saturation Solubility coefficient</th>
<th>Saturation Solubility $P$</th>
<th>% DR 5min coefficient</th>
<th>% DR 5min $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>b0</td>
<td>-1647.36</td>
<td>0.138</td>
<td>28.54</td>
<td>0.048</td>
<td>-8.41</td>
<td>0.386</td>
</tr>
<tr>
<td>X$_1$ Size of beads (mm)</td>
<td>15.28</td>
<td>0.904</td>
<td>-0.17</td>
<td>0.911</td>
<td>-1.07</td>
<td>0.390</td>
</tr>
<tr>
<td>X$_2$ Amt. of beads (mm)</td>
<td>1.46</td>
<td>0.967</td>
<td>-0.22</td>
<td>0.608</td>
<td>-0.12</td>
<td>0.713</td>
</tr>
<tr>
<td>X$_3$ Conc. of Stabilizer (%w/v)</td>
<td>2314.44</td>
<td>0.018</td>
<td>10.44</td>
<td>0.244</td>
<td>16.21</td>
<td>0.052</td>
</tr>
<tr>
<td>X$_4$ Conc. Of drug(%w/v)</td>
<td>-953.00</td>
<td>0.037</td>
<td>6.30</td>
<td>0.172</td>
<td>12.50</td>
<td>0.011</td>
</tr>
<tr>
<td>X$_5$ Stirring speed (rpm)</td>
<td>1.24</td>
<td>0.007</td>
<td>0.01</td>
<td>0.012</td>
<td>0.01</td>
<td>0.007</td>
</tr>
<tr>
<td>X$_6$ Stirring Time (hr)</td>
<td>30.53</td>
<td>0.206</td>
<td>0.97</td>
<td>0.011</td>
<td>0.90</td>
<td>0.006</td>
</tr>
<tr>
<td>R$^2$</td>
<td>0.9448</td>
<td>0.9346</td>
<td>0.9624</td>
<td></td>
<td>0.01047</td>
<td></td>
</tr>
</tbody>
</table>

*F-value less than 0.05 considered significant model
**Solid state characterization**

**FTIR Spectroscopy**

Lercanidipine Hydrochloride, PVA Cold, physical mixture LER and PVA cold (1:0.5) and lyophilized LER nanocrystals were compared using Fourier transform infrared spectroscopy. Pure lercanidipine showed the strong characteristic peaks at 1680 cm⁻¹ due to the stretching of C=O group, 1500 cm⁻¹ due to the stretching of N-O group, 3180 cm⁻¹ due to the stretching of O-H group, 1440 cm⁻¹ due to the bending of O-H group. The FTIR spectra showed (Fig. 7) that characteristic peaks at 3300 cm⁻¹ due to the stretching of O-H might be due to presence of water. All other major peaks present in formulation (LER NC) were similar to pure drug.
Scanning Electron Microscopy

SEM images of pure drug powder and lyophilized Ler nanocrystals samples illustrated in Fig 8a and 8b. As depicted the pure lercanidipine showed cubic-like shaped crystals, after milling process with beads there was a considerable change in shape of drug particles. The prepared drug nanocrystals exhibited regular and flat shape with sharp edges.
CONCLUSION
LER nanocrystals were prepared by wet media milling method. Plackett–Burman screening design implicated to identify significant parameters that affects particle size, saturation solubility and %drug release. From this study it was concluded that among all independent parameters; concentration of stabilizer, concentration of drug, stirring speed and stirring time were found to affect the dependent variables; average particle size, saturation solubility and %DR\textsubscript{5min}. R\textsuperscript{2} values were larger than 0.9, which indicate that over 90% of the variation in the response is explained by the model. The particle size reduction, improved saturation solubility and drug release could offer an efficient drug delivery strategy. Least average particle size can be obtained by increasing the amount of beads, stirring speed and stirring time. Similarly, saturation solubility was increased by increasing stirring speed and stirring time. % DR\textsubscript{5min} increases as the concentration of polymer, concentration of drug, stirring speed and stirring time increases because of an increased surface area and particle size reduction.

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ETHICAL APPROVAL
It is not applicable

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