MODIFICATION AND CHARACTERIZATION OF LOVASTATIN CRYSTALS

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ABSTRACT
Lovastatin is an anti-cholesteremic drug used in the treatment of hypertension. Being a BCS class II drug, it has poor solubility and dissolution rate. Thus the aim of present study was to modify the crystals of lovastatin in the presence of additives to improve solubility, dissolution rate and other physicochemical properties. The lovastatin crystals were prepared using solvent evaporation method in the presence of additives such as PVP-K30, PEG-4000 and Poloxamer 407. The modified crystals of lovastatin were characterized by Scanning electron microscopy, FT-IR spectroscopy, Differential scanning calorimetry and X-ray diffractometry. Also the modified crystals were evaluated for solubility, dissolution rate and other physicochemical properties and compared with commercial lovastatin. The modified crystals exhibit the difference in the size and shape when compare to commercial lovastatin indicate the habit modification. The FT-IR spectra of modified crystals in the presence of additives showed no difference in the characteristic peaks compared to commercial lovastatin. DSC data indicate the decrease in the melting endotherm of modified crystals indicate the polymorphic changes. The XRD spectra of modified crystals in the presence of additives showed decrease in number of peaks indicate the polymorphic changes. The Modified crystals showed improved solubility and dissolution rate.

KEYWORDS: Crystals, Lovastatin, Solubility, FT-IR, DSC, Dissolution rate.
INTRODUCTION
Different physiological and formulation factors are responsible for the bioavailability of drug from the dosage form. One of the most important physical factors, which affect the bioavailability and therapeutic efficacy of drug, is the existence of active ingredients in various crystal forms having different internal structure and physical properties.[1]

It is quite common for the same material to crystallize in different arrangements of the molecules in the crystal. This phenomenon is referred to as polymorphism and it happens for elements, pharmaceuticals, biological compounds and proteins. ‘Polymorphism’ is the ability of a substance to exist as two or more crystalline phases that have different arrangements or conformations of the molecules in the crystal lattice. It Essentially means that in different polymorphs, the same molecule exists in different ways. If this difference is because of packing, it is termed as packing polymorphism and if it is due to difference in conformation, it is called conformational polymorphism. As a result of polymorphism, molecules have different arrangements in the unit cell of its crystal and thus display different physical properties.[2]

Polymorphism can cause variations in melting point, density, stability and drug solubility as these properties depend on the escaping tendency of the molecules from a particular crystalline structure.[3] As a rule, for a drug that have the highest order of crystallinity is the most stable form, exists in multiple polymorphic forms, i.e. with the least amount of free energy, and consequently, possesses the highest melting point and the least solubility. By controlling the crystallization process, amorphous or metastable forms of drugs possessing high free energy can be forcibly created. Polymorphs can vary in melting point. Since the melting point of the solid is related to solubility, so polymorphs will have different solubility’s. Generally the range of solubility differences between different polymorphs is only 2-3 folds due to relatively small differences in free energy.[4]

MATERIALS AND METHODS
Lovastatin obtained as a gift sample from Watson Pharmaceuticals Goa, India. PVP K-30, PEG-4000 was purchased from SD Fine Chemical Mumbai. Poloxamer 407 was obtained from Balaji Chemicals, Mumbai. All the solvents used are of analytical grade and purchased from Qualigens, Mumbai.
Crystallization in presence of additives
Lovastatin (200mg) was dissolved in 5 ml of methanol at 65°C under reflux on water bath containing additives with concentration of 1% W/V. The solution was then filtered through Whatmann filter paper and the filtrate was kept at room temperature for 24 hours to afford well defined crystals of Lovastatin. Then the crystals were spread on a Petri plates and dried over in a vacuum desiccators for 2 days. Finally the crystals were stored in well closed container for the further studies. The different additives such as PVP-K30, PEG-4000 and Poloxamer 407 were employed at a concentration of 1% W/V, to study the effect of additives on the crystallization.[5]

Characterization of crystals
Scanning electron microscopy
Photomicrographs of the modified crystals of Lovastatin were obtained by (JEOL 5400, Japan) scanning electron microscope. Samples were mounted on a metal stub with an adhesive and coated with gold ions for 5-6 minutes under vacuum.[6]

Infrared spectroscopy
All the modified crystals and pure drug itself were scanned and recorded in the range of 400-4000 cm⁻¹ by using Infrared spectrophotometer, (Brooker, Alfa-T, Germany). The crystals were triturated with dried potassium bromide using mortar and pestle. The mixture after grinding into fine powder was kept uniformly in suitable die and compressed into a pellet form by using hydraulic press. The resultant pellet was mounted in a suitable holder in the FTIR spectrophotometer.[7]

Differential scanning calorimetry
After calibration, thermograms were obtained by heating all the samples (5 mg) of pure drug and its modified crystals at a constant heating rate of 10°C/min with chart speed of 50/100 mL/min under an atmosphere of nitrogen. The exact peak temperatures, melting point and heat of fusion were automatically calculated. The temperature range for the scan was 30°C to 300°C for all the samples.[8]

Powder X-ray diffraction spectroscopy
X-ray diffraction pattern of Lovastatin and its crystals in presence of additives were obtained using the X-ray diffractometer (Bruker, D8 Advance, Germany) at 50 kV, 34 mA and a
scanning rate of 0.02°C/min at the diffraction angle 2θ over the range of 3°C to 35°C using Cu (as anode) radiation of wavelength 1.5406 Å.[9]

Solubility studies
The solubility of Lovastatin in different solvents was determined by using a super solubility method. A known volume (10 ml) of each solvent was placed in a screw capped bottles to which 25 mg of solute was added. The solution was shaken for some time and the approximate visual solubility of the drug in solvents was determined by noting that whether the solution remains clear or not.

The solubility of Lovastatin and its modified crystals in presence of additives were determined by adding excess solid (100 mg) to 20 ml media i.e. distilled water taken in a well stopper iodine flask. The samples were stirred for 48 hours with the help of a magnetic stirrer at 400 rpm with hot plate at 37.5°C. After 48 hours of stirring, an aliquot (1 ml) of the samples were withdrawn with the help of graduated pipette, filtered through Whatmann filter paper, appropriately diluted and absorbance was measured spectrophotometrically (Shimadzu, UV-1800, Japan.) at 238 nm.[10]

Dissolution studies
Dissolution studies of Pure drug (Lovastatin) and modified crystal was carried out using USP dissolution testing apparatus type 2, (Paddle type) (Electrolab dissolution tester TDT-081, India.). The drug and crystals were filled in capsule and place into the dissolution medium. The Paddle was rotated at the speed of 50 rpm and the dissolution media (500 ml Phosphate Buffer 6.8 pH) was maintained at temperature 37.5± 0.5°C. After specific time intervals, 5 ml of aliquot was withdrawn and replaced with fresh and equal quantity of dissolution medium. The samples were suitably diluted and absorbance was measured at 238 nm using UV spectrophotometer[11] (Shimadzu, UV-1800, Japan).

RESULT AND DISCUSSION
Solubility of drug
The solubility of Lovastatin was check in the different solvent on the basis of polarity of solvent. These results showed the suitability of solvent for the crystallization. Crystallization was carried out using different solvent to check the effect on morphology of the crystals of Lovastatin but no significant changes occur, therefore the another method was tried for the
crystal modification of Lovastatin in presence of hydrophilic additives. This method produces the significant changes in the crystal morphology.

Lovastatin was obtained as a crystalline pure drug from the supplier and hence it was used as such for the preparation of crystal forms. It showed good solubility in Dimethylformamide and Acetonitrile. It showed solubility in ethanol, methanol, Acetic Acid, 1-Propanol, 2-Propanol, Ethyl Acetate, Methylene Chloride, Diethyl Ether and Toluene. It was practically insoluble in distilled water, n-hexane. It showed freely soluble in Acetone, Chloroform, Dimethyl Sulfoxide. Thus crystals were prepared using methanol as a solvent of choice because of its easy availability and cheaper in cost. Lovastatin was recrystallized in methanol by simple solvent evaporation method with presence of additives like hydrophilic polymers such as PVP K30, PEG 4000 and Poloxamer 407 with 1% W/V quantity.

These newly formed crystals in presence of additives along with the pure drug were subjected to further characterization and various evaluation tests such as solubility, dissolution, compaction etc.

**Characterization of crystals**

**Scanning electron microscopy**

The crystal morphology of pure drug (Lovastatin) and modified crystals obtained in presence of additives were studies by scanning electron microscopy. The SEM showed that the pure drug (Lovastatin) was having irregular size and shape of crystals (Fig No 1(a)).

The modified crystals of Lovastatin with PVP K30 showed the needle shaped crystals. This is due to the effect of solvent on the crystal habit of the Lovastatin (Fig No 1(b)). The modified crystals of Lovastatin with PEG 4000 (Fig No 1(c)) and poloxamer 407 (Fig No 1(d)) had also changed the size and shape (crystal habit) of crystal. The crystals obtained in the presence of PEG 4000 and Poloxamer 407 has long and rod shaped crystals.

SEM results conclude that the crystals obtained in the presence of additives showed the morphological changes and confirms the variation in the crystal habit, which indicate the influence of additives on crystallization of Lovastatin.

The changes in morphology of Lovastatin crystals could be due to variations in face dimensions or the appearance or disappearance of some faces. Under certain conditions of
crystallization, one set of crystal faces may be induced to grow faster than others, or the growth of another set of faces may be retarded.

![Fig No 1(a): Pure drug (Lovastatin)](image1)

![Fig No 1(b): Lovastatin with PVP K30](image2)

![Fig No 1(c): Lovastatin with PEG 4000](image3)

![Fig No 1(d): Lovastatin with Poloxamer 407](image4)

**FT-Infrared spectroscopy:** All the crystals including pure drug, and modified crystals showed characteristic peaks shown in Fig. No. 2(a)-(d).

Characteristic peaks of Lovastatin appeared at 3015.2 cm\(^{-1}\) (C=C stretching), 3538 cm\(^{-1}\) (O-H stretching), 1221.0 cm\(^{-1}\) (C-O-C stretching), 1066.1 cm\(^{-1}\) (C-O stretching) and 1380.7 cm\(^{-1}\) (C-H bending), 2958.0 cm\(^{-1}\) (C-H stretching) were observed.

FTIR spectra of pure drug when compared with the modified crystals obtained in the presence of polymer shows slight or no change in their characteristic peaks, and also there is no polymorphic modifications indicated by the FT-IR spectroscopy.

These results indicated that there is no Chemical interaction between pure drug and additives when formed as crystals with the help of methanol as a solvent.
Table No.1: FTIR data of pure drug and modified crystals

<table>
<thead>
<tr>
<th>Modified Crystals</th>
<th>C≡C stretching</th>
<th>C=O stretching</th>
<th>O-H stretching</th>
<th>C-O-C stretching</th>
<th>C-O stretching</th>
<th>=C-H bending</th>
<th>C-H bending</th>
<th>C-H stretching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>3015.2</td>
<td>1703.3</td>
<td>3538.3</td>
<td>1221.0</td>
<td>1066.1</td>
<td>632.9</td>
<td>1380.7</td>
<td>2958.0</td>
</tr>
<tr>
<td>Modified crystal with PVP K30</td>
<td>2963.0</td>
<td>1701.4</td>
<td>3540.7</td>
<td>1222.7</td>
<td>1041.5</td>
<td>747.3</td>
<td>1372.5</td>
<td>2963.0</td>
</tr>
<tr>
<td>Modified crystal with PEG 4000</td>
<td>3015.2</td>
<td>1703.5</td>
<td>3542.3</td>
<td>1217.3</td>
<td>959.8</td>
<td>579.8</td>
<td>1348.0</td>
<td>2884.8</td>
</tr>
<tr>
<td>Modified crystal with Poloxamer 407</td>
<td>3007.0</td>
<td>1703.0</td>
<td>3538.3</td>
<td>1221.5</td>
<td>959.8</td>
<td>628.8</td>
<td>1376.6</td>
<td>2926.5</td>
</tr>
</tbody>
</table>

Fig No. 2: FTIR Spectra of pure drug (Lovastatin) with modified Crystals.

**Differential scanning calorimetry**

The DSC data for drug (Lovastatin) and the modified crystals are shown in Fig.3(a)-(d). It should be noted that the DSC thermo grams of all modified crystals showed significant changes due to crystal modification.

The DSC curve showed that Lovastatin appeared on sharp endothermic peak at about 174.8°C corresponding to its melting point. However the modified crystals with PVP K30, PEG 4000 and Poloxamer 407 showed the shift of endothermic peak towards lower temperature at
172.8°C, 153°C and 165.5°C Respectively. Shift of endothermic peak towards lower temperature indicates the decrease in the melting point of drug in crystals. This decreased melting point accounts for increased solubility of drug.

Table No. 2: DSC data of pure drug and modified crystals.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Modified crystals</th>
<th>DSC data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pure drug(Lovastatin)</td>
<td>Melting point (°C)</td>
</tr>
<tr>
<td>2</td>
<td>Modified crystals with PVP K30</td>
<td>Heat of fusion (J/g)</td>
</tr>
<tr>
<td>3</td>
<td>Modified crystals with PEG 4000</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Modified crystals with Poloxamer 407</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Modified crystals with Poloxamer 407</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Modified crystals with Poloxamer 407</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Modified crystals with Poloxamer 407</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Modified crystals with Poloxamer 407</td>
<td></td>
</tr>
</tbody>
</table>

Fig No. 3: DSC of Pure drug (Lovastatin) with modified Crystals.

Powder X-ray diffraction: The X-ray diffraction pattern of pure drug and modified crystals were studied by X-ray diffractometer and shown in Fig.4 (a)-(d). The X-ray diffraction pattern of pure drug has more number of peaks when compared to modified crystals in presence of additives.

XRD spectra of modified crystals with PVP K30 have decreased number of peaks but increase in intensity was observed. The XRD spectra of modified crystals with PEG 4000 and Poloxamer 407 have almost similar number of peaks but there was increase in the intensity of peaks. The differences in the relative intensities of their peaks may be attributed to differences in the crystal size and habits of modified crystals, which may be attributed to the different solubility of modified crystals.
Solubility studies: The solubility study showed that the pure drug Lovastatin (crystalline) was the least soluble (3.07 µg/ml) and the crystals grown in presence of PEG 4000 as a hydrophilic polymer was the most soluble (6.58 µg/ml) with 2.14 fold increase in solubility. The order of increasing solubility is Sample A< Sample B<Sample D< Sample C (3.07<3.22 < 4.81< 6.58 µg/ml).

The increase in the solubility of crystals in the presence of additives could be due to melting of hydrophilic polymers which are associated with the crystals when compared to pure drug Lovastatin.

Table No. 3: Solubility data of Lovastatin and modified crystals.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Crystal code</th>
<th>Solubility (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pure drug (Lovastatin)</td>
<td>3.07</td>
</tr>
<tr>
<td>2</td>
<td>Modified crystal with PVP K30</td>
<td>3.22</td>
</tr>
<tr>
<td>3</td>
<td>Modified crystal with PEG 4000</td>
<td>6.58</td>
</tr>
<tr>
<td>4</td>
<td>Modified crystal with Poloxamer 407</td>
<td>4.81</td>
</tr>
</tbody>
</table>

Dissolution studies
The release profiles of Formulations of pure drug and modified crystals with PVP K30, PEG 4000 and Poloxamer 407 showed cumulative percentage drug release 24.2, 51.33, 90.53, 69.73 respectively at the end of 90 min. This difference is may be due to orders of crystallinity and solubility. The highest dissolution rate was found for the modified crystals with PEG 4000. The modified crystals with PVP K30 and Poloxamer 407 also showed a good improvement in the dissolution rate. The improvement in dissolution rate could be due to hydrophilic polymer bonding interaction.
4. CONCLUSION
Lovastatin crystals were modified in the presence of additives. These modified crystals have improved the solubility and dissolution rate compared to commercial lovastatin. Instrumental data also supports the habit modification of Lovastatin.

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