ENHANCING SOLUBILITY AND DISSOLUTION OF LOVASTATIN BY FREEZE DRYING TECHNIQUE

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Accepted Date: 24/09/2014; Published Date: 27/10/2014

Abstract: Lovastatin, an hypolipidemic agent drug, exhibits poor water solubility, dissolution and flow properties. Thus, the aim of the present study was to improve the solubility and dissolution rate of Lovastatin by preparing crystals by freeze drying technique. Lovastatin crystals were prepared by freeze drying using chloroform and water (70:30) as solvents system to enhance solubility and dissolution rate. The prepared crystals containing Lovastatin were evaluated for in vitro dissolution and solubility. The prepared formulations were characterized by scanning electron microscopy, differential scanning calorimeter, X-ray diffraction and Fourier transform infrared spectroscopy. Dissolution profile of the freeze dried crystals was compared with its recrystallized sample and pure sample. The samples were stored in stability chamber to investigate their physical stability. Freeze dried crystals exhibited decreased crystallinity and the solubility and dissolution of the Lovastatin crystals were significant improved compared with its recrystallized and pure sample of Lovastatin. In stability test, the release profile of the freeze dried crystals was almost unchanged as compared with the freshly prepared freeze dried crystals stored at 40°C and 75% relative humidity for 3 month. Hence this technique can be used for formulation of tablets of Lovastatin by direct compression with directly compressible tablet excipients.

Keywords: Lovastatin, Hypolipidemic agent
INTRODUCTION

Lovastatin belongs to the class of statins, used for lowering cholesterol (hypolipidemic agent) in those patients suffering with hypercholesterolemia. It was the first statin approved by the FDA. It’s a prodrug and inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase). It’s a poorly soluble drug, with a shorter half life of 1.1-1.7 h and less than 5% bioavailability11. The concept of use of above three techniques has emerged from the desire to provide patients with more conventional means of taking their medication.

Lovastatin (LOV) belongs to the class of cholesterol lowering drugs and is the first clinically used statin. It is a prodrug which lowers the cholesterol level through reversible competitive inhibition of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, an enzyme involved in biosynthesis of cholesterol. It is available as conventional and extended release tablets, but its low aqueous solubility (4 ‘ 10–4 mg mL–1) finally escorts it to low oral bioavailability (less than 5 %). In addition, it undergoes extensive first pass metabolism; as a consequence of hepatic extraction it leads to low and variable availability of the drug to the general circulation. Therefore improvement in aqueous solubility of LOV is the foremost aim (1, 2).

Previous researchers have made attempts to improve the aqueous solubility of LOV by preparing solid-lipid nanoparticles (1), solid dispersions using modified locust bean gum as carrier (3), methylated beta-cyclodextrin (4) complex and floating microspheres (5). Self-microemulsifying formulation of LOV was reported in literature but was not accompanied with pharmacodynamic support (2). There is a necessity to develop a formulation that would offer rapid dissolution of LOV and improve its bioavailability and finally therapeutic efficacy.

Formulation and manufacture of solid oral dosage forms, and tablets in particular, have undergone rapid change and development over the last several decades. One of the most revolutionary technologies is that of direct compression. Direct compression is economical, facilitates processing without the need of moisture, heat and involves small number of processing steps. In direct tableting method, it is necessary to increase flowability and compressibility of the bulk powder in order to retain a steady supply of powder mixture to the tableting machine and sufficient mechanical strength of the compacted tablets [6]. In addition to increasing efficiency of the manufacturing process it is also important to increase bioavailability of the drug by improving the solubility of the bulk drug powder.

Freeze drying is one of such techniques to improve the micromeritic properties and dissolution of drug [7]. Therefore, several solubilization techniques were applied and reported to enhance the aqueous solubility of mfenamic cid, formation of Solid Dispersions of Lovastatin with crospovidone (8), formation of Lovastatin capsule with sodium lauryl sulphate (9), The Fast-dissolving mucoadhesive micro-particulate containing piroxicam (10).
The formation fast dissolving tablet of piroxicam acid has been proposed (11,12). However, in terms of sales value, sales volume and number of products available on the market, freeze drying (lyophilisation) method has been the most successful (13).

The objective of the present study was to prepare freeze dried crystals of Lovastatin using freeze drying technique and was evaluated for solvents residual and DSC, FT-IR, XRD, and SEM analysis were performed to determine the physicochemical properties of the freeze dried crystals and compare with recrystallized sample and pure drug and determined the solubility and dissolution characteristics of the Lovastatin freeze dried crystals and investigate their physical stability in a climate chamber at 400°C and 75% relative humidity (RH) for 90 days.

**MATERIALS AND METHODS**

Lovastatin was obtained as a gift sample from Biocon Limited, Bangalore, India. All chemicals and buffers used were of analytical grade.

**Preparation of freeze dried crystals of Lovastatin:**

Lovastatin (5g) was dissolved in 100 ml of chloroform and water (70:30) heated at 45°C until a clear solution was obtained. Above resulted solution is shifted to 100 ml glass bottle and then transferred to a ultra low freezer at -40°C and kept in the freezer for 24 hr. the frozen drug solution were placed in a lyophilizer for 72 hr using a Freeze Dryer (IISHIN Lab. Co. Ltd. Korea) with a condenser temperature of -40°C and a pressure of 7×10^-2 mbar followed by a secondary drying at 25°C for 24 hr. The resulted crystals were kept in a desiccator’s room temperature until further experiment.

**Recrystallization of Lovastatin (RS)**

Lovastatin (5 gm) was dissolved in 100 ml chloroform and water (70:30) heated at 45°C and cooled down to room temperature with occasional stirring. The crystals of Lovastatin were collected by filtration and were dried at 45°C for 12 hours.

**Determination of residual solvents in freeze dried crystals by gas chromatography**

GC studies were carried out on SHIMADZU model 2014 (Shimadzu Technologies, Japan) coupled with a split/split less injector, operated in a split-mode and FID. The computer with GC solutions software has been used to control the gas chromatograph. Rtx-5 capillary column (cross bond 5% diphenyl/95% dimethyl polysiloxane) with a length of 30 meters and an internal diameter of 0.25 mm was used throughout the study.

**Differential scanning calorimetry (DSC)**

A DSC study was carried out to detect possible polymorphic transition during the crystallization process. DSC measurements were performed on a DSC DuPont 9900, differential scanning calorimeter with a thermal analyzer.
Fourier transform infrared (FTIR) spectroscopy

The FTIR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8033 (USA). About 2 mg of the pure drug, recrystallized and freeze dried crystals were used separately. Pure drug, freeze dried crystals and recrystallized samples were dispersed in KBr powder and the pellets were made by applying 6000 kg/cm² pressure.

X-ray analysis

X-Ray powder diffraction patterns were used to detect possible polymorphic transition during the crystallization process. X-Ray powder diffraction patterns were obtained at room temperature using a Philips X’ Pert MPD diffractometer, with Cu as anode material and graphite monochromator, operated at a voltage of 40 mA, 45 kV.

Scanning electron microscopy (SEM)

Scanning electron microscopic (Joel- LV-5600, USA, with magnification of 250x) photographs were obtained to identify and confirm spherical nature and morphological characters of the crystals.

Mechanical Properties

Tensile strength of freeze dried crystals was determined by compressing 500 mg of crystals using hydraulic press at different kg/cm² for 1 min. The compacts stored in desiccator for overnight to allow elastic recovery. The thickness and diameter were measured for each compact. The hardness of each compact was then measured using Pfizer hardness tester. The tensile strength (σ) of the compact (kg/cm²) was calculated using following equation.

\[ \sigma = \frac{2F}{\pi Dt} \]

Where, F, D and t are hardness (kg/cm²), compact diameter (cm) and thickness (cm), respectively.

Solubility studies of crystals

The solubility of Lovastatin freeze dried crystals in water and pH 7.2 Phosphate buffer was determined by taking excess quantity of freeze dried crystals and adding to screw capped 50 ml glass vials filled with water. The vials were shaken for 24 hours on mechanical shaker. The solution was filtered through Whatmann filter paper No.1 and the drug concentration was determined spectrophotometrically at 238 nm.

Dissolution studies of crystals

The dissolution of Lovastatin pure sample, freeze dried crystals and recrystallized sample was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). Dissolution medium (900ml) consisted of 7.2 Phosphate buffer was used and 10 ml of dissolution medium
was withdrawn at every 10 min interval for 1 h. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 238 nm.

**Determination of the physical stability**

To determine the physical stability of freeze dried crystals, a stability study of prepared Microspheres was carried out at 25°C and 60% relative humidity for 6 months according to the ICH guidelines. The spherical agglomerates were packed in high density polyethylene (HDPE) container and placed in stability chamber. The samples were withdrawn at the interval of 0, 1, 3 and 6 months and evaluated for appearance, characterization by FT-IR and dissolution release and compared with initial results.

**RESULT AND DISCUSSION:**

A solvent system involved a chloroform and water for a drug. The selection of these solvent depends on the miscibility of the solvents and solubility of the drug in individual solvents. Chloroform is miscible in any proportion with water.

Recrystallization of Lovastatin was done to find out the changes in crystal lattice, being induced by solvents, can influence the physicochemical properties of the substance. Hence the mechanical, micromeritic and dissolution properties of spherical crystals were compared with pure sample and recrystallized sample.

Recrystallization of Lovastatin was carried out using same solvent composition as was used for freeze drying. Based upon high solubility of Lovastatin in chloroform, high viscosity and crystal morphology, chloroform determined to be suitable freeze drying medium for Lovastatin because of its high solubility in THF (1gm/14ml). The controlling of residual chloroform was needed though. Chloroform is a toxic organic solvent based on their concentration and has little detriment to human body. Therefore, the low level of both chloroform in the freeze dried crystals should not be harmful to both animal and human.

Gas chromatography results confirmed that there were below detection of chloroform in the freeze dried crystals against the ICH limit i.e. 60 [14,15]. The low level of chloroform in the freeze dried crystals results from its ability to form high surface area crystals and from the fact that the intermolecular forces among both chloroform molecules is not as strong as those of water. This allows chloroform to sublime more completely and easily than water.

The DSC thermograms showed a sharp endothermic peak for all the Lovastatin crystals. This one step melt might be due to only one crystal form of the Lovastatin formed during the freeze drying process, thus indicating that Lovastatin did not undergo any crystal modification. The temperature range of the endothermic peak of all the Lovastatin crystals lies in the range of 169 to 173.2 °C (Fig. 1). In DSC curve, pure Lovastatin had a sharp endothermic peak at 173.2°C with enthalpy of 174.42 J/g that corresponded to the melting point of Lovastatin. Melting points
show slight variation as the nature of the crystals might have been affected by the solvent. The melting endotherm for freeze dried Lovastatin was 169°C with decreased enthalpy of (164.43J/g) indicating decreased crystallinity of Lovastatin in freeze dried crystals [1, 2].

![Freeze dried crystals vs Recrystallized Sample vs Pure drug](image)

**Fig 1 Shows DSC Spectrum of different samples of Lovastatin**

Infrared spectra of Lovastatin commercial recrystallized and freeze dried crystals showed characteristic peaks at 3541 cm⁻¹ (alcohol O-H stretching), 3015 cm⁻¹ (olefinic C-H stretching), 2965 cm⁻¹ (methyl C-H asymmetric stretching), 2929 cm⁻¹ (methylene C-H asymmetric stretching), 2866 cm⁻¹ (methyl and methylene C-H asymmetric stretching), 1725, 1699 cm⁻¹ (lactone and ester carbonyl stretch) 1459 cm⁻¹ (methyl asymmetric bend), 1381 cm⁻¹ (methyl symmetric bend), 1262 cm⁻¹ (lactone C-O-C asymmetric bend), 1220 cm⁻¹ (ester C-O-C asymmetric bend), 1073 cm⁻¹ (lactone C-C symmetric bend), 1055 cm⁻¹ (ester C-O-C symmetric bend), 970 cm⁻¹ (alcohol C-OH stretch) and 870 cm⁻¹ (trisubstituted olefinic C-H). From Fig. 1, it was observed that there were no significant changes in the position of characteristic peaks. Spectrum of recrystallized Lovastatin was slightly different from pure sample in the region of wave number between 3350 and 3300 cm⁻¹. This suggests that the recrystallized Lovastatin from the mixture of water, isopropyl acetate and THF has a different crystalline form than its crystalline form in pure sample and in freeze dried (Figure-2). Specific changes in IR spectra are not very clear, could be due to variations in the resonance structure, rotation of a part of a molecule or certain bonds. Alteration could be due to minor distortion of bond angles, or even a result of the presence of solvents of crystallization (16, 17).
Fig 2 Shows FT-IR Spectrum of different samples of Lovastatin

X-Ray diffraction was used to analyze potential changes in the inner structure of Lovastatin crystal during the formulation of freeze dried crystals. The characteristic peak of the Lovastatin appeared in the 2θ range of 10–40°, indicating that the unprocessed Lovastatin was a crystalline material. All the samples showed similar peak positions (2θ) in X-ray diffraction, formation of different polymorphs of Lovastatin was ruled out. However relative intensities of XRD peaks were modified (Fig. 3). The relative intensities of freeze dried crystals reduced much lower than pure Lovastatin. This could be attributed to the markedly different crystal habits of the samples. Therefore the relative abundance of the planes exposed to the X-ray source would have been altered, producing the variations in the relative intensities of the peak or may be due to differences in crystal sizes (1, 2). The pure drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The X-ray diffraction of the RS of drug showed the peak corresponding to the crystalline drug molecules present in the RS, although their intensity was lower than pure drug due to the differences in crystal sizes. The X-ray diffraction pattern of the freeze dried crystals showed that Lovastatin peak intensity was much lower than the pure drug and RS samples of Lovastatin (18, 19). This could be due the increasing the wettability of freeze dried crystals. These results could explain the observed enhancement of solubility and dissolution of Lovastatin in freeze dried crystals.
In SEM study showed that crystals of pure sample are of the smallest size (7-16 μm) and they have irregular shapes. Recrystallization crystals with intermediate size (11-23 μm) which had rod like shapes. The resultant freeze dried crystals had a smooth surface covered with numbers of small crystals (average particle size 257 nm) (Fig. 4).

Freeze dried crystals exhibited superior compressibility characteristics compared to conventional drug crystals (Fig. 5). It could be due to the fact that during the process of compression fresh surfaces are formed by fracturing crystals. Surface freshly prepared by fracture enhanced the plastic inter particle bonding, resulting in a lower compression force required for compressing the freeze dried crystals under plastic deformation compared to that of single crystal[10, 11].
Freeze dried crystals showed increased solubility than the pure sample in water and increased nearly more than threefold higher (0.0637 mg/ml) than pure Lovastatin (0.0226 mg/ml). The higher solubility of Lovastatin from freeze dried may be due to the reduction in particle size and increased wettability of Lovastatin in freeze dried crystals [12, 13].

The dissolution profiles of Lovastatin (fig. 6) exhibited improved dissolution behavior for freeze dried crystals (62%) than pure sample (32%). The reason for this faster dissolution could be linked to the better wettability and reduction in particle size of the freeze dried. The amount of drug dissolved in 60 min greatly varied for Freeze dried crystals.

![Graph showing tensile strength of different samples of Lovastatin](image1)

**Fig 5 Tensile strength of different samples of Lovastatin**

**Figure 6 Dissolution profiles of different samples of Lovastatin**

![Graph showing dissolution profiles](image2)
The best way to guarantee stability is by maintaining their physical state and molecular structure. The results of the stability study of prepared freeze dried crystals of Lovastatin stored at 25°C and 60% relative humidity for 3 month is presented in table 4. The influence of physical stability on the prepared crystals was investigated. Prepared freeze dried crystals of Lovastatin were stable and complied with all the properties when compared to initial results of prepared microspheres of Fenofibrate.

<table>
<thead>
<tr>
<th>Testing interval</th>
<th>Description of Drug</th>
<th>FT-IR Study</th>
<th>XRD Study</th>
<th>Dissolution Study (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample name: Lovastatin freeze dried crystals</td>
<td>Storage condition: 25°C /60% RH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>White to off white</td>
<td>As standard</td>
<td>As standard</td>
<td>99.60±0.011</td>
</tr>
<tr>
<td>1 month</td>
<td>Complies</td>
<td>Complies</td>
<td>Complies</td>
<td>98.39±0.040</td>
</tr>
<tr>
<td>2 month</td>
<td>Complies</td>
<td>Complies</td>
<td>Complies</td>
<td>99.28±0.027</td>
</tr>
<tr>
<td>3 month</td>
<td>Complies</td>
<td>Complies</td>
<td>Complies</td>
<td>99.89±0.013</td>
</tr>
</tbody>
</table>

CONCLUSION
Freeze dried crystals of Lovastatin were prepared by freeze drying technique. Freeze dried crystals exhibited decreased crystallinity and improved mechanical properties. DSC FT-IR and XRD studies showed that there is no change in the crystal structure of Lovastatin during the crystallization process i.e., polymorphism has not occurred. The dissolution of the freeze dried crystals was improved compared with recrystallized sample and pure Lovastatin sample. Stability study showed that prepared freeze dried crystals was stable for 3 month. Hence this technique can be used for formulation of tablets of Lovastatin by direct compression with directly compressible tablet excipients.

ACKNOWLEDGEMENTS
The authors are thankful to Biocon Limited Bangalore, India for the gift samples of Lovastatin.

REFERENCE


