FORMULATION AND EVALUATION OF NANOPARTICLES OF HMG-CoA REDUCTASE INHIBITOR

Vinod Ramani*1, Sachin Chauhan1, Jibin Joshi2, Tejas Ghelani1, Gajanan Deshmukh1, Seth AK1, Jignesh Patel1, Merin Philips3, Rajdeep Gupta4

1 Sumandeep Vidyapeeth University, Department of Pharmacy, Baroda, India
2 Malik Deenar College of pharmacy, Kassaragod, India
3 Sri Ramakrishna Institute of Pharmacy, Coimbatore, India
4 Sidmack Labs. Pvt. Ltd.

ABSTRACT

Poor water solubility has been attributed to almost half of the 150,000 new molecular entities (NMEs) synthesized annually by pharmaceutical companies, and is also claimed to reduce the performance of more than 10% of successfully marketed drugs. Nanocrystal technology has become the fastest selling drug in the transplant market. Nanotechnologies offer new ways to address these drug delivery challenges and are being applied in a wide range of healthcare settings. Simvastatin is a poorly soluble lipid-lowering agent. Its Water solubility is very low, approximately 30 µg/mL and poorly absorbed from the gastrointestinal (GI) tract. This work is an attempt to overcome the poor solubility and dissolution rate of simvastatin by using Nanosuspension technology. PVP and Tween 80 with Soybean Lecithin were used at different ratios as the surfactants. The formulations were done by Emulsion-solvent evaporation method followed by freeze-drying. The formulated nanoparticles were subjected to characterization studies like Particle size analysis, X-ray diffraction studies, Differential Scanning Colorimetry, Scanning electron microscopy and UV analysis. The dissolution test of tablets containing the nanometric drug flakes revealed that, within 30 minutes, 89.76% (w/w) of the simvastatin in the tablet was dissolved. In comparison, the dissolution test of the conventional tablets revealed that under these testing conditions only 45.97% (w/w) simvastatin was dissolved. This result demonstrates the significant advantage of simvastatin nanoparticles over the conventional particulate drug and the feasibility of the proposed method.

Keywords: Simvastatin, Solubility, Nanocrystal, Dissolution rate, Nanosuspension.

INTRODUCTION

Simvastatin is a poorly soluble lipid-lowering agent which is used for the treatment of primary hypercholesterolemia. When given orally, Simvastatin (a lactone) undergoes hydrolysis and is converted to the β, δ-dihydroxy acid form, a potent competitive inhibitor of 3-hydroxyglutaryl-CoA reductase the enzyme that catalyzes the
rate-limiting step of cholesterol biosynthesis.\textsuperscript{[1]} Water solubility of Simvastatin is very low, approximately 30 µg/mL.\textsuperscript{[2]} It is practically insoluble in water and poorly absorbed from the gastrointestinal (GI) tract. Therefore, it is very important to introduce effective methods to enhance the solubility and dissolution rate of drug, substantially leading to its bioavailability. Improvement of the aqueous solubility in such a case is a valuable goal that leads to enhancing therapeutic efficacy. It is reported that the \textit{absolute bioavailability of simvastatin is 5\% after a 40 mg oral dose}.\textsuperscript{[3]}

This work is an attempt to overcome the poor solubility and dissolution rate of simvastatin by using Nanosuspension technology. PVP and Tween 80 with Soybean Lecithin were used at different ratios as the surfactants. The formulations were done by \textit{Emulsion-solvent evaporation method followed by freeze-drying}. The formulated nanoparticles were subjected to characterization studies like Particle size analysis, X-ray diffraction studies, Differential Scanning Colorimetry, Scanning electron microscopy and UV analysis.

\textbf{MATERIALS AND METHODS}

Simvastatin obtained as gift sample (Sangrose laboratories Pvt. Ltd, Kerala, India), PVP (Loba Chemie, Mumbai, India), Soybean Lecithin (Himedia Laboratories, Mumbai, India), Ethanol (Changshu Yangyuan Chemicals, China), Lactose Monohydrate (Chemdays chemicals, Ahmedabad), Powdered Cellulose- Direct compressible grad (Degussa corporation), Crospovidone (Loba Chemie, Mumbai, India), Tween 80 (Suvidhinath lab, Vadodara, India) All other reagents and chemicals used were of analytical reagent grade.

\textbf{FORMULATION OF SIMVASTATIN NANOPARTICLES} \textsuperscript{[4,5,6]}

Simvastatin nanoparticles were prepared by \textit{Microemulsion-solvent evaporation} method at temperature (23±3°C) by adding the surfactants (PVP, Tween-80 and soybean lecithin) in the different ratio (as per table 1) to the Ethanol solution to create an oil (organic) phase. Simvastatin was also added to the oil phase in drug-loaded microemulsions. Afterward the oil phase was mixed with the distilled water and magnetically stirred at until optically transparent systems were formed. The resulted nanoparticles were centrifuged and freeze dried for the complete removal of solvents.
DETERMINATION OF $\lambda_{\text{max}}$\textsuperscript{[7]}

A stock solution of 1 mg/ml of Simvastatin was prepared by dissolving 100 mg of drug in small quantity of ethanol and sonicated for few minutes and diluted with 100 ml of phosphate buffer (pH 6.8). The stock solution was serially diluted to get solution in the range of 20µg/ml and $\lambda_{\text{max}}$ of the solution was found out by scanning from 200 - 400 nm.

DETERMINATION OF CALIBRATION CURVE\textsuperscript{[7]}

A stock solution of 1 mg/ml of Simvastatin was prepared by dissolving 100 mg of drug in small quantity of methanol and sonicated for few minutes and diluted with 100 ml of phosphate buffer (pH 6.8). The stock solution was serially diluted to get solutions in the range of 10-50 µg/ml and $\lambda_{\text{max}}$ of the solution was found out. The absorbance of the different diluted solutions was measured in a UV-Visible spectrophotometer at 238 nm. A calibration curve was plotted by taking concentration of solution in X axis and absorbance in Y axis and correlation coefficient ‘$r$’ was calculated.

DETERMINATION OF DRUG CONTENT

A 50mg of the prepared formulations were weighed and dissolved in minimum quantity of Ethanol and made up to 100ml with phosphate buffer (6.8 pH). From the aliquots 1 ml was taken and diluted to 10 ml with the buffer and absorbance was taken in UV-Visible spectrophotometer at 238 nm. From the absorbance total drug content in the batches were calculated.

SATURATION SOLUBILITY STUDIES\textsuperscript{[8,9,10]}

Saturation solubility studies were conducted as per the method.\textsuperscript{[1]} Weighed amount of Simvastatin (pure drug) and the nanoparticles equivalent to 10 mg of the drug were separately introduced into 25 ml stoppered conical flasks containing 10 ml phosphate buffer (6.8 pH). The sealed flasks were agitated on a rotary shaker for 24 hrs at 37° C and equilibrated for 2 days. An aliquot was passed through 0.45 µm membrane filter and the filtrate was suitably diluted and analyzed for drug content on a UV Spectrophotometer.

CHARACTERIZATION OF NANOPARTICLES

PARTICLE SIZE ANALYSIS
Size of the formed drug particles was measured by dynamic laser light scattering diffractometer (Nanotrac, Ultra) at SASTRA University, Thanjavur. Before analysis, the drug suspension was diluted by deionized water to 0.2 mg/ml.

**X-RAY DIFFRACTION STUDY**[^11]

X-ray diffraction analysis was employed to detect the crystallinity of the pure drug and the formulations, which was conducted using a XRD-6000 diffractometer (Shimadzu, Japan) at CIF, Pondicherry University. The powder was placed in a glass sample holder. CuK radiation was generated at 30mA and 40 kV. Samples were scanned from 5° to 50° with a step size of 0.02° and the scan speed was 3° min^{-1}

**DIFFERENTIAL SCANNING COLORIMETRY (DSC)**[^12]

Differential scanning colorimetry (DSC) was conducted on Diamond DSC Calorimeter at CIF, Pondicherry University. The samples were equilibrated at 20° C for half hour and then heated to 220° C at 10° C/ml in a N₂ atmosphere.

**SCANNING ELECTRON MICROSCOPY (SEM)**

Particle morphology was observed using scanning electron microscopy (SEM), JSM-6390 (JEOL, Japan) at SASTRA University, Thanjavur. The samples and an appropriate amount of pure drug were fixed on an SEM stub using double-sided adhesive tape and coated with Pt at 50 mA for 6 min through a sputter-coater (KYKY SBC-12, Beijing, China). A scanning electron microscope with a secondary electron detector was used to obtain digital images of the samples at an accelerating voltage of 20 kV.

**PREPARATION OF SIMVASTATIN TABLETS**[^4]

The lyophilized product equivalent to 10 mg of simvastatin was weighed and mixed with 75 mg each of Lactose monohydrate and powdered cellulose.5 mg of crospovidone was added and triturated carefully in mortar and pestle. The tablet was prepared by using direct compression method. Formula for the 1 tablet of simvastatin has been given in table 2.

**IN-VITRO DISSOLUTION STUDIES**[^13]
Parameters
Instrument : USP Dissolution apparatus
Type : Paddle method
Medium : 900 ml Phosphate buffer pH 6.8
Temperature : 37± 0.5°C
RPM : 50
Testing time : 30 min
Amount withdrawn : 1 ml
\( \lambda_{\text{max}} \) : 238 nm
Sample : Tablets of Pure drug and Nanoparticles

Pure drug tablets and tablets prepared with flakes (Nanoparticles) were placed in the dissolution flask. Dissolution studies were conducted as per the parameters.

**TABLE 1: FORMULATION SCHEME**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug Simvastatin (mg)</th>
<th>Surfactant used</th>
<th>Ethanol (ml)</th>
<th>Distilled water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>50</td>
<td>Tween 80: PVP(1:1)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F2</td>
<td>50</td>
<td>Tween 80: PVP(1:2)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F3</td>
<td>50</td>
<td>Tween 80: PVP(1:3)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F4</td>
<td>50</td>
<td>Tween 80: soybean lecithin(1:1)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F5</td>
<td>50</td>
<td>Tween 80: soybean lecithin(1:2)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F6</td>
<td>50</td>
<td>Tween 80: soybean lecithin(1:3)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F7</td>
<td>50</td>
<td>PVP: soybean lecithin(1:1)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F8</td>
<td>50</td>
<td>PVP: soybean lecithin(1:2)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>F9</td>
<td>50</td>
<td>PVP: soybean lecithin(1:3)</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>
TABLE 2: COMPOSITION OF 10 MG SIMVASTATIN (1 TABLET)

<table>
<thead>
<tr>
<th>Tablet composition</th>
<th>Lyophilized product</th>
<th>Lactose monohydrate</th>
<th>Powdered cellulose</th>
<th>Crospovidone</th>
</tr>
</thead>
<tbody>
<tr>
<td>By weight (mg)</td>
<td>50</td>
<td>75</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td>By percentage</td>
<td>24%</td>
<td>37%</td>
<td>37%</td>
<td>2%</td>
</tr>
</tbody>
</table>

TABLE 3: CALIBRATION CURVE DATA OF SIMVASTATIN IN 6.8 PH PHOSPHATE BUFFER AT 238 NM.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance at 238 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>10</td>
<td>0.210</td>
</tr>
<tr>
<td>20</td>
<td>0.415</td>
</tr>
<tr>
<td>30</td>
<td>0.612</td>
</tr>
<tr>
<td>40</td>
<td>0.809</td>
</tr>
<tr>
<td>50</td>
<td>1.038</td>
</tr>
</tbody>
</table>

Figure 1
Calibration curve of simvastatin
TABLE 4: SIMVASTATIN NANOPARTICLES ASSAY DATA

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Surfactant</th>
<th>Theoretical drug content</th>
<th>Assayed drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amount (mg)</td>
<td>Expressed in %</td>
</tr>
<tr>
<td>F1</td>
<td>Tween 80: PVP(1:1)</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>F4</td>
<td>Tween 80: soybean lecithin(1:1)</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>F7</td>
<td>PVP: soybean lecithin(1:1)</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLE 5: SATURATION SOLUBILITY DATA OF BATCHES COMPARED WITH PURE DRUG

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Formulation</th>
<th>Amount per ml (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Simvastatin</td>
<td>74.2</td>
</tr>
<tr>
<td>2</td>
<td>F1</td>
<td>163.8</td>
</tr>
<tr>
<td>3</td>
<td>F4</td>
<td>198.5</td>
</tr>
<tr>
<td>4</td>
<td>F7</td>
<td>275.3</td>
</tr>
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</table>

TABLE 6: PARTICLE SIZE DATA OF THE FORMULATIONS

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Average (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>343</td>
</tr>
<tr>
<td>F4</td>
<td>220</td>
</tr>
<tr>
<td>F7</td>
<td>113</td>
</tr>
<tr>
<td>Simvastatin (pure drug)</td>
<td>28.3 µm</td>
</tr>
</tbody>
</table>
Figure 2
Particle Size Distribution for Pure Drug Simvastatin

Figure 3
Particle Size Distribution for F1

Figure 4
Particle Size Distribution for F4
Figure 5
Particle Size Distribution for F7

Figure 6
XRD Pattern of Pure Drug (Simvastatin)
Figure 7
XRD Pattern of Simvastatin Nanoparticles Prepared With Surfactants PVP And Soya Lecithin (1:1)

Figure 8
DSC Thermogram of Pure Drug (Simvastatin)
Figure 9
DSC Thermogram Simvastatin Nanoparticles

Figure 10
SEM Image of Raw Simvastatin Particles
Figure 11
SEM Image of Simvastatin Nanosuspension

Figure 12
SEM Image of Simvastatin Nanoparticle After Freeze Drying
Figure 13
Comparative Cumulative Release of Nanoparticles and Pure Drug Formulation

TABLE 7: CUMULATIVE % RELEASE FOR SIMVASTATIN 10 MG TABLETS
OF PURE DRUG AND DRUG FLAKES

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Time in mins</th>
<th>Absorbance at 238 nm</th>
<th>Concentration in 1 ml(µg/ml)</th>
<th>Amount in 900 ml (mg)</th>
<th>Cumulative % release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug tablet</td>
<td>10</td>
<td>0.215906</td>
<td>0.135889</td>
<td>1.223</td>
<td>12.23</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.243934</td>
<td>0.277444</td>
<td>2.497</td>
<td>24.97</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.290134</td>
<td>0.510778</td>
<td>4.597</td>
<td>45.97</td>
</tr>
<tr>
<td>Nano drug flakes tablet</td>
<td>10</td>
<td>0.269036</td>
<td>0.404222</td>
<td>3.638</td>
<td>36.38</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.355298</td>
<td>0.839889</td>
<td>7.559</td>
<td>75.59</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.386472</td>
<td>0.997333</td>
<td>8.976</td>
<td>89.76</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

From UV Spectra of Simvastatin $\lambda_{\text{max}}$ was found to be 238 nm in Phosphate buffer pH 6.8. Simvastatin calibration curve was constructed in phosphate buffer pH 6.8 and scanned in UV-spectrophotometer at 238 nm (Table-3). The linearity of the calibration curve was found to be in the range of 10-50 µg/ml. A regression coefficient value of 0.9996 was noticed for Simvastatin (Fig-1). From all prepared formulations F1 to F9, the batches which contains surfactant ratio 1:1 was yields a good characterized products, whereas in case of the other formulations, when surfactant ratio was increased
from 1:1 to 1:2 and onwards, not producing physically acceptable product. Even during manufacturing process, the turbidity was also not disappeared, so those batches such as F2, F3, F5, F6, F8 and F9 was not considered for the further study. The evaluation test was carried out only for F1, F4 and F7 batches.

Nanoparticles of simvastatin were formulated by emulsification method followed by solvent evaporation. The surfactants viz soya lecithin, PVP and Tween 80 in the different ratio in Ethanol were tried in combination for optimization. From the Table 4 data it was clearly evident that the assayed drug content in the formulations was found to be within the range of ± 10% of the theoretical amount. Assay data also indicates that the method used for the formulation produced good yield and was suitable and reproducible in nature.

The solubility data of pure and flakes of simvastatin particles are shown in Table 5. In the case of commercial simvastatin particles, the equilibrium solubility (approximately 74.2µg/ml) was reached rapidly. In contrast, the maximum supersaturated concentrations from nanoparticles were about 275.3µg/ml for particles prepared with surfactants PVP and soya lecithin (1:1) at 0.2% concentration.

Pure simvastatin used for the study was characterized by relatively large particles with average value of about 28.3 µm as reported .The nanoparticles prepared after emulsification and solvent evaporation showed a drastic decrease in the particle size when compared to the pure drug particles. The results shown in Table 6 that the technique of Microemulsion method can be utilized as an effective tool in the reduction of particle size to the nano scale range. As per Noyes-Whitney equation, the decrease in the particle size will have a positive effect on the drug dissolution rate. Hence this decrease in the particle size achieved will have a significant effect in the drug solubility and dissolution characteristics.

XRD pattern of the pure drug and selected formulation are shown in fig 6 and fig 7. Characteristic diffraction peaks were observed for commercial simvastatin. On the other hand, the nano formulations particles prepared with surfactants PVP and soya lecithin (1:1) at 0.2% concentration i.e. (F7) was characterized by less intensity of the diffraction peak when compared to that of simvastatin. This clearly indicates the reduction in the crystallinity of the precipitated simvastatin nanoparticles.
The DSC curves of commercial simvastatin (fig 8) shows a broad endotherm ranging from 30 to 120°C indicating the loss of water and the sharp endotherm at 138.97°C might be due to the melting point of simvastatin. However no sharp endotherm was seen at 139°C for the DSC curves of the nanoparticles (fig 9) prepared of PVP: soybean lecithin (1:1) 0.2%. This shows the crystallinity of the drug has been reduced significantly in the nanoparticles. The DSC results were in support of the XRD analysis which also showed decrease in drug crystallinity.

The SEM images of pure drug particles and the nanoparticles are presented in fig 10, fig 11, and fig 12. While unprocessed simvastatin particles have appeared as irregular shaped crystals, a drastic change in the morphology and shape of drug was observed for the nanoparticles prepared of PVP: soybean lecithin (1:1).

The dissolution test of tablets containing the nanometric drug flakes revealed that, within 30 minutes, 89.76% (w/w) of the simvastatin in the tablet was dissolved. In comparison, the dissolution test of the conventional tablets revealed that under these testing conditions only 45.97% (w/w) simvastatin was dissolved (Table 7). This result demonstrates the significant advantage of simvastatin nanoparticles over the conventional particulate drug and the feasibility of the proposed method (Fig 13).

CONCLUSION

The prime objective of present work was accomplished by nanosuspension technology using Soya lecithin with PVP and Tween 80 as surfactants. The method used for the formulation was Emulsion-solvent evaporation followed by freeze drying. This method can be used as an effective tool for preparation of nanosized formulations. Simvastatin nanoparticles prepared by this method showed significant improvement in aqueous solubility as well as dissolution characteristics which may significantly improve its oral bioavailability. Further studies in animal models can be done to show the effectiveness of prepared nanoparticles in-vivo.

REFERENCES


For Correspondence:
Vinod Ramani
Department of Pharmacy,
Sumandeep Vidyapeeth University,
Baroda, India
Email: v.ramani007@gmail.com