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Development and Evaluation of Simvastatin Nanoparticles using Nanosuspension Technique

Kantilal Narkhede

Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India 333001.

ABSTRACT:

Most of poorly water soluble fails to solubilize because of their particle size, to overcome this particle size reduction upto nano level is most appropriate solution. Many times microsize have also its limitation therefore nano level size reduction is selected. Conversion to nano level involves many physical as well as chemical methods, but in physical method drug may be degraded hence chemical method is selected to achieve nanoparticles. Quasi emulsion solvent method is used to prepare nanoparticles of simvastatin by using Pluronic F-68 stabilizer. And suitable concentrations were selected by using 32 factorial designs.

KEY WORDS: Nanoparticles, Quasi emulsion solvent technique.

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INTRODUCTION:

The oral delivery route is commonly recognized as the most preferred and convenient route for the administration of drug formulations. In oral administration the drug must be dissolved in GI fluids, hydrophobic drugs belonging to Class II of the biopharmaceutical classification, the dissolution process of the drug acts as the rate controlling step and, therefore, it is necessary to improve the solubility and dissolution of the drug. Approximately, 30 % of formulations and 40 % of new chemical entities entering into market had too low aqueous solubility or poor oral bioavailability. Thus, one of the major current challenges facing the pharmaceutical industry involves the development of strategies to improve the aqueous solubility and dissolution rate of drugs. Drug solubility can be enhanced using traditional approaches such as designing Prodrug, reducing particle size of Micronization, co-solubilization by micellization and Complexation, solid dispersions, and use of solubilizing excipients. Recently, major research efforts have been focused on the development of nanotechnology-based drug delivery systems including biodegradable polymeric nanoparticles, smart polymeric micelles, Nanocrystals, Nanosuspension and Nanoemulsion to enhance the dissolution rate of poorly soluble drugs and improve oral bioavailability. The major advantages of Nanoparticle is to increase saturation solubility and consequently increase the dissolution rate of the drug.¹

Simvastatin is a cholesterol lowering agent; it is a white, non-hygroscopic, crystalline powder, having poor aqueous solubility and bioavailability. Simvastatin is a potent competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme a reeducates which is rate limiting in cholesterol biosynthesis. It

*For Correspondence:

Narkhede Kantilal

Research Scholar, Shri Jagdishprasad
Jhabarmal Tibrewala University,
Jhunjhunu,
Rajasthan, India 333001.

(www.jpsbr.org)

also increases break down of LDL cholesterol. Simvastatin is a hydrophobic drugs belonging to Class II of the biopharmaceutical classification, the dissolution process of the drug acts as the rate controlling step and, therefore, it is necessary to improve the solubility and dissolution of the drug. The drug is poorly absorbed from the gastrointestinal (GI) tract; it has a low solubility of 4.75 $\mu\text{g}/\text{ml}$ in water, so the bioavailability was less than 5%. Approximately 95% of an oral dose is not absorbed.² The plasma half-life of oral simvastatin is 3 hrs. it is excreted by kidney, and therefore it is important to enhance the aqueous solubility, dissolution rate, and improving bioavailability from its oral dosage forms.

The presented research work thus deals with the techniques on enhancement of solubility as well as dissolution and bioavailability of poorly aqueous soluble drug like Simvastatin.

METHODOLOGY

PREFORMULATION OF DRUG:³

The Preformulation study is mostly generate data useful to develop stable dosage forms that can be mass-produced for manufacturer.

Organoleptic Characteristics of Simvastatin

Physical examine was done to check Organoleptic Characteristics of Simvastatin like color and odor.

Determination of Melting Point of Simvastatin

Melting point of Simvastatin had been evaluated by the capillary method.

Solubility study of Simvastatin

The aqueous solubility of simvastatin in powder form was determined by a shake-flask method. Briefly, an excess amount of simvastatin was suspended in 10ml of water, and the suspensions were shaken at 37°C. Aliquots were withdrawn and filtered through a 0.22 μm Whatmen filter. The filtered solution was suitably diluted and the simvastatin concentration in the filtrate was analyzed by UV analysis method at 238nm (Systronic 2203, Japan).

Saturation solubility studies

Saturation solubility measurements was analyze by ultraviolet absorbance determination at 238 nm using a Shimadzu UV-Visible spectrophotometer. The saturation

solubility studies was carried out for both unprocessed pure drug. 10 mg of unprocessed pure drug Simvastatin was weighed and introduce into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks was seal and place in a rotary shaker for 24 hrs. at 37°C and equilibrate for 2 days. The samples was collect after the specific time interval, and it is filter and analyze. The dilute samples was analysed using UV spectrophotometer at 238 nm. The results were analyzed in triplicate and standard deviations are report.

Identification and Determination of Wavelength_{max} (λ_{max}) of Simvastatin

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 20 $\mu\text{g}/\text{ml}$ and λ_{max} of the solution was found out by scanning from 200 - 400 nm.

Preparation of Calibration Curve for Simvastatin

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 $\mu\text{g}/\text{ml}$ and λ_{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.

Identification of Drug- Simvastatin by FT-IR Spectroscopy

Fourier-transform infrared (FT-IR) spectra of moisture free powdered samples of SS, its lyophilized nanoparticles and PVPK-30 were obtained using a spectrophotometer (FTIR-8300, Shimadzu Co., Kyoto, Japan) by potassium bromide (KBr) pellet method. The scanning range was 750–4000 cm^{-1} and the resolution was 1 cm^{-1} .

Drug-Excipients Compatibility Studies by DSC

DSC scans of the prepared lyophilized powdered drug sample and pure drug samples were recorded using DSC-Shimadzu 60 with TDA trend line software. All samples

were weighed (8- 10 mg) and heated at a scanning rate of 10°C/min under dry nitrogen flow (100 ml/min) between 50 and 300° C. Aluminum pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

METHOD OF PREPARATION OF SIMVASTATIN NANOPARTICLE:⁴

Nanoparticles were prepared by Quasi- Emulsion Solvent Evaporation Method. Simvastatin was dissolved in an acetone at room temperature. This was poured into fixed amount of water containing fixed amount of different stabilizer Pluronic F-68 at a room temperature and subsequently stirred on magnetic stirrer to allow the volatile solvent to evaporate (Remi, magnetic stirrer, India.). Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the Nanosuspension at room temperature for 1 hour.

FORMULATION AND DEVELOPMENT OF SIMVASTATIN NANOPARTICLE USING DESIGN OF EXPERIMENT [DOE] APPROACH

CHARACTERIZATION OF SIMVASTATIN NANOSUSPENSION

Nanoparticles were prepared by Quasi- Emulsion Solvent Evaporation Method. Simvastatin was dissolved in an acetone at room temperature. This was poured into fixed amount of water containing fixed amount of different stabilizer Pluronic F-68 at a room temperature and subsequently stirred on magnetic stirrer to allow the volatile solvent to evaporate (Remi, magnetic stirrer, India.). Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the Nanosuspension at room temperature for 1 hour.

Particle size:

Particle size was determine by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This analysis yields the mean diameter (z-average, measuring range: 20–1000 nm). All the data presented are the mean values of three independent

samples produced under identical production conditions⁵.

Screening electron microscopy

Particle size and morphology was examined by SEM, The particle size distribution was determine by the IBAS I/II Image Analyzer System (Germany) via the obtained SEM photographs.^{5,6}

Drug content

The prepared Nanosuspensions was analyzed for drug content by UV spectroscopic method. Different batches of Nanosuspension equivalent to 20 mg of simvastatin weighed accurately and dissolve in 10 ml ethanol. The stock solutions was diluted with distilled water and analyze by UV spectroscopy at 238 nm.^{4,7}

Saturation solubility studies:

Saturation solubility measurements was analyze by ultraviolet absorbance determination at 238 nm using a Shimadzu UV-Visible spectrophotometer. The saturation solubility studies was carried out for both unprocessed pure drug and different batches of lyophilize Nanosuspension. 10 mg of unprocessed pure drug and Nanosuspension equivalent to 10 mg of Simvastatin was weighed and separately introduce into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks was seal and place in a rotary shaker for 24 hrs. at 37°C and equilibrate for 2 days. The samples was collect after the specific time interval, and it is filter and analyze. The dilute samples was analysed using UV spectrophotometer at 238 nm. The results were analyzed in triplicate and standard deviations are report.^{4,8}

In vitro Drug Release Study:⁵

Table 1 : In-vitro drug release study

| | |
|-----------------------------------|--|
| Instrument | : USP Dissolution apparatus |
| Type | : Paddle method |
| Medium | : 900 ml Phosphate buffer pH 6.8 |
| Temperature | : 37± 0.5°C |
| RPM | : 50 |
| Testing time | : 60 min |
| Amount withdrawn | : 5 ml |
| λ max | : 238 nm |
| Sample | : Tablets of Pure drug and Nanoparticles |
| Sample Withdrawal interval | : Every 5 Min |

Residual solvent Analysis

Solvent Residual Analysis was done to determine organic solvent residual traces present in nanoparticles formulation of used as internal phase.⁹

PROCEDURE FOR FREEZE DRYING OF NANOPARTICLES

The Nanosuspension was lyophilized using a Freeze dryer (Labconco™, Japan), for obtain free following Simvastatin NPs formulation. The freeze dried Nanosuspension is used to shelf life of Nanosuspension and to study the dissolution behaviour. 1% mannitol is added to each formulation as a Cryoprotectant at the time of Lyophilization. The sample is kept in deep freezer at -70° C overnight and then the sample is kept in Freeze dryer (Labconco™, Japan), for 2 days at -50° C at 2 millitorr.^{3,10,11.}

RESULTS AND DISCUSSION:

Organoleptic Characteristics

The colour of Simvastatin was visualized white with odourless having white powder.

Melting Point of Simvastatin

Melting point was carried out to determine the purity of sample. The Drug sample has melting point of 135-136°C which was in the range and indicate the purity of sample as Simvastatin.

Wavelength_{max} (λ_{max}) of Simvastatin

λ_{max} of sample was found to be 238.5 nm which is approximately equal to standard value of 238 nm.

Saturation solubility studies

Study reveals that simvastatin is very slightly soluble in water, acetate buffer, phosphate buffer, 0.1 N HCl but freely soluble in Acetone, alcohol such as methanol.

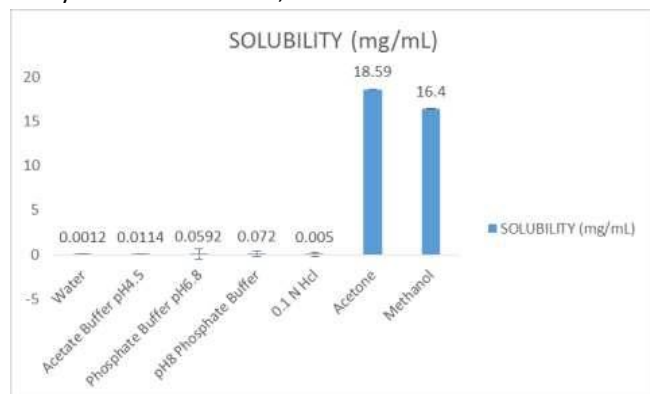


Fig 1. Saturation Solubility study
Calibration Curve for Simvastatin:

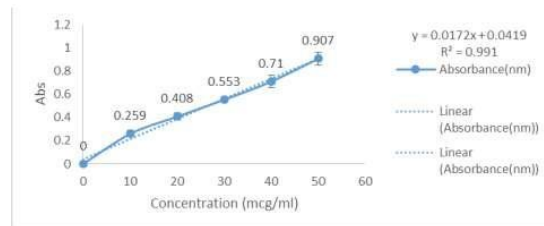


Fig. 2: Calibration Curve

Simvastatin by FT-IR Spectroscopy:

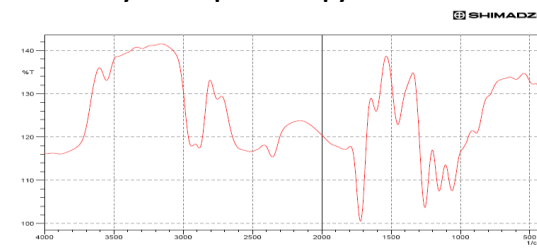


Fig.3: FT-IR Spectroscopy

Figure shows IR spectrum of Simvastatin. All major peaks of Simvastatin drug was observed at wave numbers 3549.14 (Free O – H stretching vibrations) and 3010.98 (Alkene C – H stretching vibrations), 1967.41 (C = O stretching, Ester and Lactone Carbonyl), 1460.16 (C- H bending), 1390.72 (-OH inplane bending) and 1269.27 (C- O- C asymmetric stretching) were observed which Confirms identity and purity of drug.

Drug-Excipients Compatibility Studies by DSC:

Drug-Excipients Compatibility Study was done by using DSC. DSC was carried out of drug Simvastatin and PLURONIC F68, and formulation. The thermogram of pure Drug (Fig.18) exhibits a sharp melting endotherm at 135.22°C which was compared with other DSC thermogram and shows indicate no alteration.

SIMVASTATIN NANOPARTICLES:

3² Factorial Design Approach:

| Independent Variables of Formulations | | | |
|--|---------|------------|----------|
| Independent Variables | Low(-1) | Medium (0) | High(+1) |
| Stabilizer Concentration (X ₁) | 1:1 | 2 : 1 | 3:1 |
| Speed (RPM) (X ₂) | 1500 | 2000 | 2500 |
| Dependent Variables | | | |
| Y1 = % Drug Content | | | |
| Y2 = % Entrapment efficiency | | | |
| Y3 = % Yield | | | |

Table 2: 3² Factorial Design

Compositions of Factorial Batches in Coded Form:

| SPVANS 3 ² = Batches | | |
|---------------------------------|-----------------------------------|-----------------|
| Batches | Variable level in coded form | |
| | Stabilizer Concentration- mg (X1) | Speed- RPM (X2) |
| SPVANS1 | -1 | -1 |
| SPVANS2 | -1 | 0 |
| SPVANS3 | -1 | +1 |
| SPVANS4 | 0 | -1 |
| SPVANS5 | 0 | 0 |
| SPVANS6 | 0 | +1 |
| SPVANS7 | +1 | -1 |
| SPVANS8 | +1 | 0 |
| SPVANS9 | +1 | +1 |

Table3: Compositions of Factorial Batches in Coded Form

Formulation Design by 3² Factorial Designs:

| SPVANS 3 ² = Batches | | |
|---------------------------------|-----------------------------------|-----------------|
| Batches | Variable level in De-coded form | |
| | Stabilizer Concentration- mg (X1) | Speed- RPM (X2) |
| SPVANS1 | 1:1 | 1500 |
| SPVANS2 | 1:1 | 2000 |
| SPVANS3 | 1:1 | 2500 |
| SPVANS4 | 2:1 | 1500 |
| SPVANS5 | 2:1 | 2000 |
| SPVANS6 | 2:1 | 2500 |
| SPVANS7 | 3:1 | 1500 |
| SPVANS8 | 3:1 | 2000 |
| SPVANS9 | 3:1 | 2500 |

Table 4: Formulation Design by 3² Factorial Design

Characterization of Batches SPVANS1-SPVANS9:

| Batch No | Drug Content (mg) | Entrapment Efficiency (%) | Yield (%) |
|----------|-------------------|---------------------------|-----------|
| SPVANS1 | 7.17 ± 0.25 | 41.66 ± 1.56 | 52.73 |
| SPVANS2 | 9.72 ± 0.21 | 45.21 ± 1.25 | 56.23 |
| SPVANS3 | 8.01 ± 0.41 | 43.79 ± 1.36 | 48.12 |
| SPVANS4 | 12.51±0.26 | 63.26±1.41 | 72.39 |
| SPVANS5 | 15.23±0.56 | 69.34±1.59 | 82.54 |
| SPVANS6 | 13.82±0.42 | 60.52±1.37 | 76.32 |
| SPVANS7 | 9.12±0.29 | 55.26±1.49 | 54.79 |
| SPVANS8 | 11.79±0.41 | 58.35±1.32 | 60.80 |
| SPVANS9 | 10.19±0.27 | 49.21±1.22 | 52.62 |

Table 5: Characterization of Batches SPVANS1-SPVANS9
From the results, all batches shows % yield greater than 50 %. By comparing them batch SPVANS5 shows higher results and selected as optimized batch and further evaluation is carried out,

Particle size Analysis of Optimized Batch:

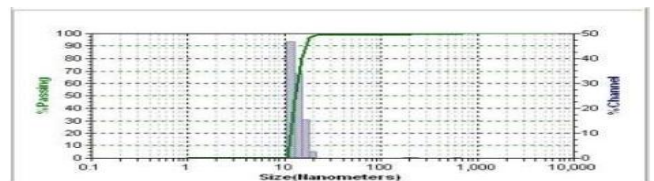


Fig. 4: Particle size Analysis

Scanning Electron Microscopy of Optimised Batch

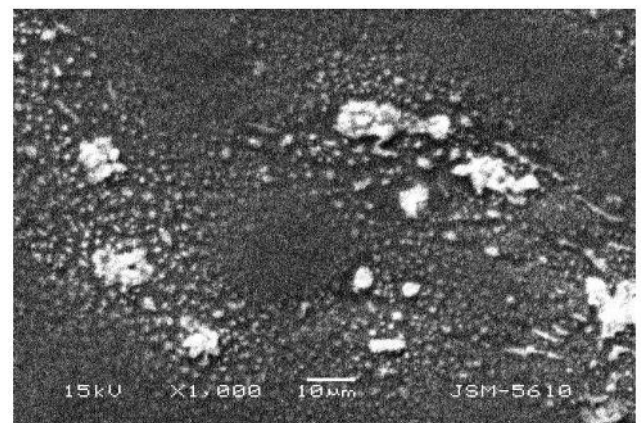
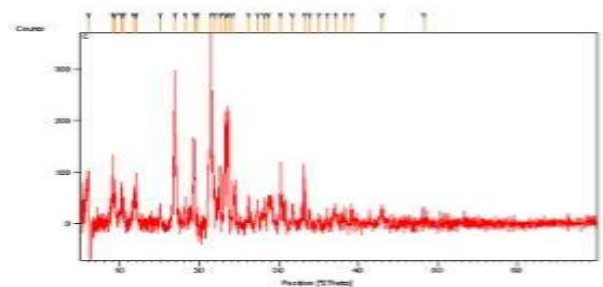


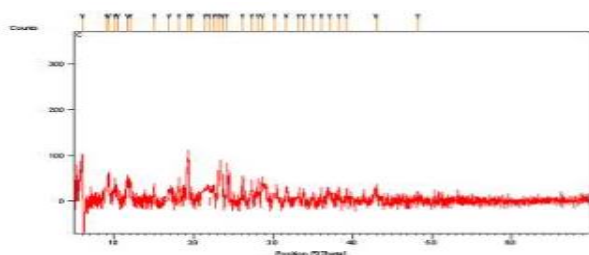
Fig. 5: SEM of optimised batch

X-Ray Diffraction of Optimised Batch:



Pattern of Pure Simvastatin

Fig.6: Pattern of pure simvastatin



Pattern of Simvastatin Nanoparticle

Fig.7: Pattern of Simvastatin Nanoparticles

SUMMARY AND CONCLUSION:

Nanoparticles containing Simvastatin was prepared by a quasi-emulsion Solvent Evaporation method using Pluronic F68 using DoE approach. Optimized Batches was subjected for % drug content, % Entrapment efficiency, Particle size Analysis, Scanning electron microscopy, XRD and *in vitro* drug release studies, the low SD and CV values indicate drug content was uniform and reproducible in all the formulations. The IR spectral analysis and DSC suggested compatibility between the drug and formulation additive. The drug exists in original form and available for the biological action. The dissolution parameters were studied by using dissolution software PCP DISSO V.3 for Nanoparticle loaded tablet which proved increase in Saturation Solubility and Dissolution rate. The Nanoparticles of SPVANS5 gave better physical, morphological and % encapsulation in either of the Stabilizers and Excipients were selected.

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