

PREPARATION AND SOLID STATE CHARACTERIZATION OF SIMVASTATIN NANOSUSPENSIONS FOR ENHANCED SOLUBILITY AND DISSOLUTION

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ABSTRACT

Objective: In this study, an attempt was made to improve the solubility and dissolution characteristics of poorly soluble drug [simvastatin].

Methods: The nanosuspensions of simvastatin were prepared using high pressure homogenization technique followed by its lyophilization using mannitol as a cryoprotectant. Nanoparticles characterized in terms of size and morphological characteristics. Characterization of the prepared nanosuspension was done with respect to particle size, zeta potential, saturation solubility, dissolution rate, morphology study [SEM], *in vitro* dissolution study. Evaluation of the crystalline state before and after particle size reduction was done by differential scanning calorimetry [DSC].

Results: The results indicated that the initial crystalline state of the drug reduced the following: particle size reduction and the dissolution rates of amorphous simvastatin nanoparticles were highly increased with comparison to pure drug by the enhancement of intrinsic dissolution rate and reduction of particle size resulting in a increased specific surface area.

Conclusion: Nanoparticles are seemed to promising approach for bioavailability enhancement because of the simple method of its preparation and its universal applicability.

Keywords: Nanosuspension, Simvastatin, Bioavailability enhancement and Lecithin.

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, 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 [1,2]. Approximately, 30 % of formulations and 40 % of new chemical entities entering into market had too low aqueous solubility or poor oral bioavailability [3]. 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 [4-6]. Drug solubility can be enhanced using traditional approaches such as designing prodrug [7], reducing particle size of micronization[8], co-solubilization by micellization and complexation [9], solid dispersions [10], and use of solubilizing excipients [11]. Recently, major research efforts have been focused on the development of nanotechnology-based drug delivery systems including biodegradable polymeric nanoparticles [12], smart polymeric micelles [13], nanocrystals [14], nanosuspension [15] and nanoemulsion[16] to enhance the dissolution rate of poorly soluble drugs and improve oral bioavailability. Pharmaceutical nanosuspension consists of dispersed solid drug particles in an aqueous vehicle with average particle sizes below 1 μ m. The major advantages of Nanosuspension technology is to increase saturation solubility and consequently increase the dissolution rate of the drug [3].

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 reductase which is rate limiting in cholesterol biosynthesis. It also increases break down of LDL cholesterol [1]. The drug is poorly absorbed from the gastrointestinal (GI) tract; it has a low solubility of 4.75 μ g/ml in water, so the bioavailability was less than 5 %. Approximately 95 % of an oral dose is not absorbed. 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.

MATERIALS AND METHODS

Simvastatin was obtained as a gift samples from micro labs limited, Hosur, India and Lecithin was gift samples from HI media laboratories, Mumbai. All other chemicals and solvents used are of analytical grade.

Preparation of Nanosuspension [17-19]

Nanosuspension of simvastatin was prepared by high pressure homogenization technique by using different concentration of the stabilizer like soya lecithin. The concentration of soya lecithin F1, F2, and F3 contains 0.5 %, 1 % and 1.5 % respectively, but in all the formulation drug concentration remain constant (5g). Simvastatin powder (5% w/v) was dispersed in aqueous surfactant solution using magnetic stirrer. After drug dispersion in the surfactant solution first size reduction step was carried out using an Ultra-Turrax T25 basic homogenizer at 9500 rpm for 10 minutes. The obtained mixtures were homogenized using Micron- LAB 40 high pressure homogenizer, the homogenization step includes first two cycles at 100 bar and next two cycles at 500 bars pressure as initial step. Finally the suspension was homogenized for 15 cycles at 1500 bar pressure.

Production of dry nanoparticles

The nanosuspension was lyophilized using a Vertis freeze dryer, USA. The freeze dried nanosuspension is used to shelf life of nanosuspension and to study the dissolution behavior. 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 Viritis freeze dryer for 2 days at -50°C at 2 millitorr.

Particle size analysis [20]

The particle size analysis was performed by microtac blue wave particle size analyzer (Germany). First, the lyophilized powder was diluted with de-ionized water to obtain a suitable concentration for measurement. The results obtained from particle size distribution were used to confirm the formation of Nano size particles.

In vitro drug release studies [21]

The *in-vitro* release of simvastatin and the nanosuspension was carried out in USP dissolution test apparatus using paddle method at a rotation speed of 50 rpm. The dissolution profile was carried out in freshly prepared acidic buffer (pH 1.2) and also in phosphate buffer (pH 7.0) containing 0.5 % sodium lauryl sulphate. 10 mg of pure drug and nanosuspension containing 10 mg of simvastatin equivalent was taken and placed in dissolution medium. The volume and temperature of dissolution medium were 900 ml and $37.0 \pm 0.2^\circ\text{C}$, respectively. Samples were withdrawn at fixed time intervals and were filtered. The filtered samples were analyzed at 238 nm using Shimadzu UV-Visible spectrophotometer. The results obtained for different batches of formulation were compared with the dissolution profile of unprocessed drug.

Saturation solubility studies [22]

Saturation solubility measurements were analyzed by ultraviolet absorbance determination at 238 nm using a Shimadzu UV-Visible spectrophotometer. The saturation solubility studies were carried out for both unprocessed pure drug and different batches of lyophilized Nanosuspension. 10 mg of unprocessed pure drug and Nanosuspension equivalent to 10 mg of Simvastatin were weighed and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in a rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. The samples were collected after the specified time interval, and it is filtered and analyzed. The diluted samples were analyzed using UV spectrophotometer at 238 nm. The results were analyzed in triplicate and standard deviations are reported.

Characterization of nanoparticles

Differential scanning calorimetry [20]

Thermal properties of powder samples were carried out by Differential scanning calorimeter (DSC) (DSCQ20 V24.4) Germany). The 5mg of sample was taken in the aluminum vial and kept in the instrument. The sample is then heated from 20°C to 200°C at heating rate of $10^\circ\text{C}/\text{m}$ under a stream of nitrogen at a flow rate of 50 mm/min. enthalpy changes (H) were calculated from peak areas of samples and study the polymeric changes of formulation.

Scanning electron microscopy

Scanning electron microscopy is a type of electron microscopy that images the surface of solid specimen by using focused beam of high-energy electrons. The scanning process and image formation in SEM depends on signal produced by elastic and inelastic interactions

between high energy electron beam and specimen surface. The particle size analysis of lyophilized Nanosuspension was carried out to confirm the Nano-size of formulation. The samples were lightly sprinkled on a double sided adhesive tape stuck to an aluminum stub and the stubs were coated with platinum. The stub containing sample was placed in scanning electron microscope chamber and analyzes the surface morphology.

Fourier Transform Infra- Red Spectroscopy [23]

FT- IR spectra nanosuspension were recorded on the sample prepared in KBr disks (2 mg sample in 200 mg KBr disks) using Shimadzu Fourier Transform Infra-Red spectrometer. The samples were scanned over a frequency range $4000\text{-}400\text{ cm}^{-1}$.

Re-Dispersibility and Percentage Drug Content Determination [21,24]

The prepared Nanosuspensions were analyzed for drug content by UV spectroscopic method. Different batches of nanosuspension equivalent to 10 mg of simvastatin weighed accurately and dissolved in 10 ml ethanol. The stock solutions were diluted with distilled water and analyzed by UV spectroscopy at 238 nm.

RESULTS

Characterization of nanoparticles

Drug content

Drug content of simvastatin nanosuspension was in the range of 98.83 % to 99.83 % which is acceptable according to the USP.

Particle size analysis

The particle size of the NS produced systems was analyzed by photon correlation spectroscopy. All samples were measured in suspension after particle preparation without further dilution. The particle size and poly- dispersibility index was in the range of F1 to F3 represented in Table 1. It could be inferred from the results there was significant impact of the drug to stabilizer

Particle size distribution of each formulation was carried out after homogenization and results indicates that all the formulation was found to be in the nanosized range.

Table 1: Particle size distribution

Formulation	Particle size (nm)	P.DI
F1	383.9	0.227
F2	453.1	0.026
F3	316.1	0.380

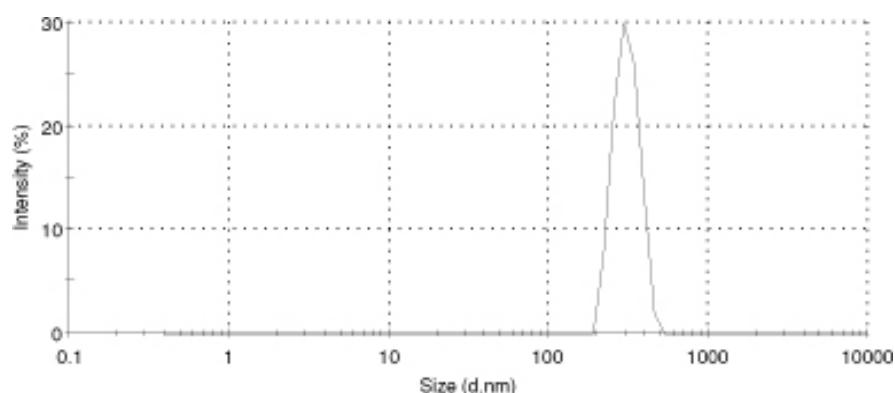


Fig. 1: Particle size distribution

Saturation solubility studies, p^H solubility profiles

The saturation solubility of the lyophilized nanoparticles and pure simvastatin was reported in Table 2. Simvastatin showed a solubility of $14.16\ \mu\text{g}/\text{ml}$ in distilled water, $20.49\ \mu\text{g}/\text{ml}$ in P^H1.2 buffer and $46.7\ \mu\text{g}/\text{ml}$ in P^H7.0 buffer. The saturation solubility increased with an increase in carrier proportion for carrier. This might be due to

better wetting ability associated with lecithin. So as the proportion of carrier increases, a large surface is presented for adsorption of the drug crystals. High pressure homogenization leads to an increase in interfacial area of contact between the drug particles and dissolution medium. The affinity between the hydrophilic inert carriers of the dissolution fluid facilitates rapid penetration into the particles, further enhances the dissolution process.

Table 2: Saturation solubility studies

Formulation	Solubility(µgm/ml)
F1	137.1
F2	142.94
F3	144.26

FTIR Spectroscopy

FTIR Spectroscopy was used to study the possible interaction between pure simvastatin and stabilizer in the nanosuspension. There was no significant difference in the FTIR spectra of pure drug, physical mixture and lyophilized nanoparticles. All major peaks of simvastatin was observed at wave numbers 3549.14 (Free O - H stretching vibrations) and 3010.98, 2968.55, 2955.04 and 2872.10 (Alkene C - H stretching vibrations), 1967.41 (C = O stretching, Ester and Lactone Carbonyl), 1556.61, 1541.18, 1519.96, 1508.38

combination, 1467.88, 1460.16 (C- H bending) 1390.72, 1369.50 (-OH inplane bending) 1269.27 (C- O- C asymmetric stretching) were retained in physical mixtures and Nanoparticles which clearly indicate that no interaction exists between pure drug and stabilized nanoparticles. The results of IR study are shown in Figure 2.

Differential scanning calorimetry

The DSC thermograms of SIM and SIM loaded lecithin nanoparticles are shown in Fig 3. Simvastatin was characterized by a single, sharp melting endothermic peak at 131.8 J /g and melting point range at 135.66° C. Suggesting that there was no physical or chemical interaction in SIM and HPH Nanosuspension.

Scanning Electron Microscopy (SEM)

SEM micrograph clearly showed great differences between pure simvastatin and lyophilized nanoparticles.

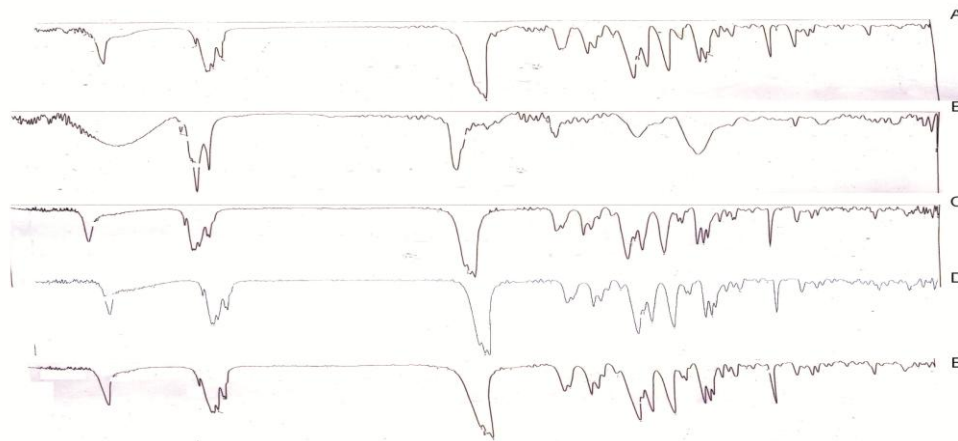


Fig. 2: FT-IR spectra of (A) Pure Simvastatin (B) Lecithin, (C) F3, (D) F2, (e) F1

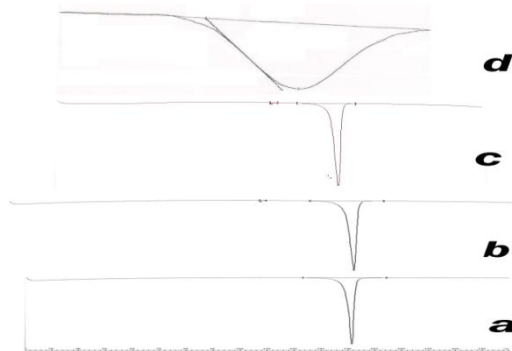


Fig. 3: DSC thermograms of (a) F1, (b) F2, (c) F3, (d) Pure simvastatin

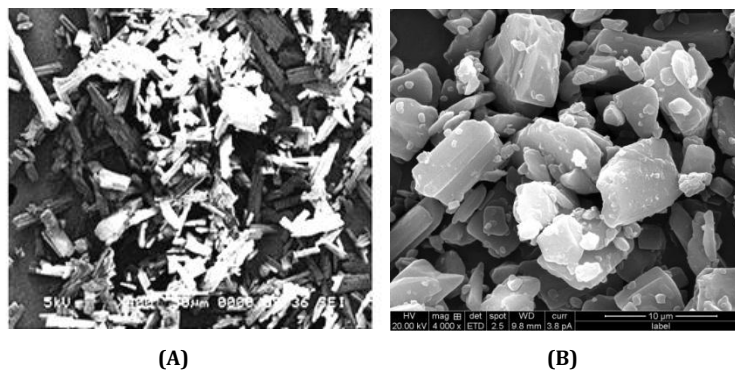


Fig. 4: SEM photograph of (A) Pure Simvastatin; (B) Lyophilized nanoparticles F3

In vitro dissolution studies

The dissolution profiles of the nanosuspension are shown in Figure 5. It is evident that the dissolution rate for SIM in PMs as well as nanosuspensions were higher as compared to the plain SIM for carrier. Pure drug substance showed poor dissolution profile i.e. only 29.5 % of drug was released at the end of 60 minutes, whereas PMs showed slight improvement due to effect of carrier present in the respective mixtures. The increase in dissolution rate was seen to

be proportionate with increase in carrier proportion. The lyophilized nanoparticles 1.5 % concentration F 3 formulation showed maximum drug release 74.68 % for 60 minutes

The improved dissolution could be attributed to a reduction in particle size of the drug, its deposition on the surface of the carrier and improved wettability. These results showed that lyophilized nanosuspension is a useful method for higher dissolution rate of simvastatin.

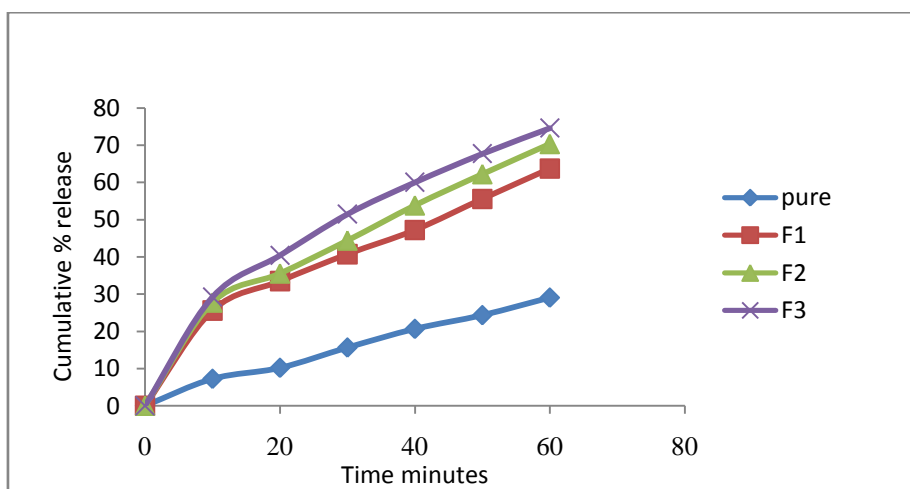


Fig. 5: Dissolution profile of simvastatin and lyophilized nanoparticles.

DISCUSSIONS

The FTIR results showed which clearly indicates that no interaction exist between pure drug and stabilized nanoparticles. Other hand in DSC curves of lyophilized simvastatin nanoparticles, a broad endotherm indicating the loss of water and a sharp endotherm at 135.66°C might be due to the melting point of simvastatin. However no sharp endotherms were observed in the DSC curves for the nanoparticles obtained by high pressure homogenization. These results indicates that the conversion of simvastatin crystalline nature to amorphous nature.

The saturation solubility results obtained in the study was in correlation with results already reported [25]. It is suggested that these dissolution behaviors of all processed particles might be due to the amorphous nature.

The dissolution profile obtained in the study clearly gives indication about the enhancement of solubility of the pure drug by using this technology. The HPH F3 cumulative percentage release about 74.68% at the end of 1 hour, where as the cumulative release of the pure drug was only about 29.5%. The increased dissolution rate can be explained by reduced particle size of nanoparticles.

CONCLUSION

In conclusion, Lyophilized nanoparticles of SIM with soya lecithin prepared by HPH method showed significantly higher dissolution in comparison with pure drug and PMs. FTIR and DSC studies showed no evidence of interaction between the drug and carrier, which were further SEM studies revealed by deposition of the drug in the polymeric matrix of lecithin. This is attributed to improved bioavailability due to enhancement in rate and extent of drug release when drug was administered as a lyophilized nanoparticles using soya lecithin carrier

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