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Der Pharmacia Lettre, 2014, 6 (2):145-155
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ISSN 0975-5071
USA CODEN: DPLEB4

Formulation, development and characterization of Simvastatin nanoparticles by solvent displacement method

Anilkumar J. Shinde* and Harinath N. More

Department of Pharmaceutics, Bharati Vidyapeeth, College of Pharmacy, Kolhapur

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ABSTRACT

The present study was to formulate nanoparticles (NPs) containing simvastatin (SV) prepared with Poly (D, L Lactide-co- Glycolide) by nano-precipitation-solvent displacement method to achieve a better release profile suitable for per oral administration with enhanced efficacy. The formulations were fabricated according to a 3^2 full factorial design, allowing the simultaneous evaluation of two formulation independent variables and their interaction. The dependent variables that were selected for study were particle size and % drug entrapment. The influence of various formulation factors (drug: polymer ratio and concentration of surfactants) on particle size, size distribution, zeta potential, drug loading and encapsulation efficiency were investigated. Encapsulation efficiency and drug loading capacity were found to be increased as drug concentration increases with respect to polymer. Addition of surfactants showed a promising result in decreasing particle size of NPs. Dissolution study revealed increased release of SV from NPs. Transmission electron microscopy (TEM) study revealed spherical morphology of the developed NPs. Differential scanning calorimetry (DSC) studies confirmed phase transition behavior of NPs. They also showed very significant change in saturation solubility in comparison with pure drug. The in vitro release data follows matrix and first order release kinetics mechanism, good correlation coefficients ($R^2 \geq 0.9915$) could be obtained.

Keywords: antilipidemic activity, nanoparticles, PLGA, solvent displacement, simvastatin

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INTRODUCTION

Recent days have seen tremendous strides in the solubility enhancement of poorly soluble drugs [1]. Solubility is an important criterion for drug efficacy, independent of route of administration. It also posses a major challenge for pharmaceutical industries, which are developing new pharmaceutical products, since 40% of the active substances being identified are either insoluble or poorly soluble in aqueous media. A limiting factor for *in vivo* performance of poorly water soluble drugs, following oral administration, is their resistance to being wetted and being dissolved into the fluid in the gastrointestinal tract. Increasing the dissolution rate of poorly water soluble drug, is thus important for optimizing bioavailability. The role of solubility enhancement is an attempt to shift the classification of a drug (II → I) in order to eliminate the problems associated with dissolution-limited compounds [2-4]. Poor solubility is in most cases associated with poor bioavailability [5]. There are two basic approaches to overcome the bioavailability problems of these drugs, increase of saturation solubility and dissolution velocity. However, many of the new compounds, show such a low solubility that micronisation does not lead to a sufficient increase in bioavailability after oral administration. Therefore the next step taken was nanonisation [6,7].

Over the last 10 years, nanoparticle (NP) engineering processes have been developed and reported for enhancement of solubility of poorly aqueous soluble drugs. In this approach, poorly water soluble compounds are formulated as nanometer sized drug particles. According to Muller, NPs are solid colloidal particles ranging in size from 1 to 1000

nm [8,9]. They have the advantage of having an even greater surface area, and being characterized, unlike micronized drugs, by an increase in saturation solubility [10,11].

Simvastatin (SV), which is a potent and effective lipid lowering agent from the family of statins with a good tolerability profile, has systemic bioavailability only 5% [12,13]. SV is used to control hypercholesterolemia (elevated cholesterol levels) and to prevent cardiovascular disease. SV is a powerful lipid lowering drug that can decrease low density lipoprotein (LDL) levels by up to 50%. From recent research it has become apparent that SV inhibit the progression of atherosclerosis beyond their effects on LDL.

Exhaustive research has been carried out on increasing dissolution and bioavailability of SV such as micronization, molecular dispersion, incorporation of surfactants, inclusion complexation with cyclodextrin, crystal modification, glass formation and co-precipitation these studies was explored earlier. Nanoparticles have drawn greater attention because of their solubilisation and transport properties [14-16].

In the present study, attempt has been made to investigate the utility of a 3^2 factorial design and optimization process to develop and improve formulation of PLGA nanoparticles containing SV using nano-precipitation-solvent displacement method to achieve a better release profile suitable for *per oral* administration with enhanced efficacy than previous SV delivery [17,18].

MATERIALS AND METHODS

Simvastatin (SV) was a obtained from gift sample from Aurobindo Pharmaceuticals Ltd., Hyderabad; Poly (D, L Lactide-co- Glycolide) (PLGA 50:50) was obtained as gift samples from Purac biochem Ltd. Netherland; Pluronic F 68 was purchased from sigma chemicals, Mumbai, dialysis bag (cellophane membrane, molecular weight cut off 10000-12000 Da, purchased from Hi-Media, Mumbai. India. All other reagents and chemicals used in this study were of analytical Grade.

Compatibility Studies

Compatibility of the simvastatin (SV) with PLGA used to formulate nanoparticles (NPs) was established from FTIR spectrum and DSC thermogram analysis. FTIR & DSC spectral analysis of SV and combination of SV and PLGA was carried out to investigate the changes in chemical composition of the drug after combining it with the excipients. Compatibility study was carried out on FTIR (Jasco V-530) and DSC (TA-60, Instruments SDT-2960, USA).

Experimental Design

The formulations were fabricated according to a 3^2 full factorial design, allowing the simultaneous evaluation of two formulation independent variables and their interaction. The experimental designs with corresponding formulations is shown in (Table 1& 2). The dependent variables that were selected for study were particle size (Y_1) and % drug entrapment (Y_2). The effect of the previously mentioned variables were investigated on the responses of the particle size and the encapsulation efficiency [19].

Table 1. Experimental design and Parameters for 3^2 Full Factorial design batches

Batch code	Variables level in coded Form	
	X_1	X_2
PS1	-1	-1
PS2	-1	0
PS3	-1	+1
PS4	0	-1
PS5	0	0
PS6	0	+1
PS7	+1	-1
PS8	+1	0
PS9	+1	+1

Table 2. Translation of coded levels to actual quantities

Coded Levels	+1	0	-1
Drug: Polymer ratios (X_1) (mg)	1:3 (150)	1:2 (100)	1:1 (50)
PluronicF68 (X_2) %	0.4	0.3	0.2

Preparation of PLGA Nanoparticles

PLGA nanoparticles were prepared by the nanoprecipitation-solvent displacement method [20]. Accurately measured simvastatin (SV) was dissolved in sufficient quantity of acetone. Hydrophilic stabilizer pluronic F-68 (0.2%, 0.3% and 0.4 %) dissolved in distilled water. PLGA was solubilized in acetone at various concentrations (1:1, 1:2 and 1:3. i.e 50mg, 100mg and 150mg). The organic phase was poured into the aqueous solution drop wise, at 1ml/min flow with syringe positioned with the needle directly into stabilizer containing water, which was stirred at 5000 rpm for 2h, thus forming a milky colloidal suspension. The organic solvent was evaporated by using a Rota evaporator. All experiments were performed in triplicates. Nanoparticles (NP's) were collected by centrifugation at 15,000 rpm for a period of 1h and supernants were discarded. The resultant dispersion was dried using a freeze-drying [21].

Characterization of Nanoparticles

Determination of particle size

The particle size and size distribution of the simvastatin (SV) loaded PLGA (50:50) nanoparticles was characterized by photon correlation spectroscopy using a Zetasizer 2000 Malvern Instruments, UK. Nanosuspension was diluted with filtered (0.22µm) ultra pure water and analysed using Zeta sizer. Polydispersity index is the ratio of weight of average molecular mass to the number of average molecular mass. Polydispersity was determined using data of particle size [22].

Determination of entrapment efficiency

The encapsulation efficiency of nanoparticles was determined by first separating the nanoparticles formed from the aqueous medium by centrifugation at 15000 rpm for 1 h. The amount of free SV in the supernant was measured by UV spectrophotometery at 238 nm (Shimadzu UV-1700) after suitable dilution. The SV entrapped in the nanoparticles was calculated using formula (2) [23].

$$\% \text{ Entrapment Efficiency} = \frac{(Tp - Tf)}{Tp} \times 100 \qquad (2)$$

Where, Tp is the total SV used to prepare the nanoparticles and Tf is the free SV in the supernant.

Determination of zeta potential

The zeta potential of the SV loaded PLGA nanoparticle was measured on a zetasizer (Malvern Instruments UK). All the samples were measured in water at 25°C in triplicate [24,25].

Percent process yield

Percent process yield was calculated as the weight of the lyophilized nanoparticle (NPs) from each batch in relation to the sum of starting material multiplied by hundred.

Percent drug content

The lyophilized nanoparticle (NP's) powder (10mg) was dissolved in 1 ml methanol and volume was made up to mark in 10ml volumetric flask with phosphate buffer pH 6.8. 0.1ml of above solution was further diluted to 10 ml and analyzed by spectrophotometrically at 238nm. The SV contents in nanoparticles (% w/w) were calculated.

***In vitro* drug release study**

In-vitro drug release studies were performed in USP Type II dissolution apparatus at rotation speed of 50 rpm. The SV-loaded PLGA nanoparticles, after separation by centrifugation, were re-dispersed in 5mL phosphate buffer solution pH 6.8, and immersed in 900ml of phosphate buffer solution in a vessel, and temperature was maintained at 37±0.20°C. The sample weight of formulations equivalent to 10mg of SV was used for dissolution study. Required quantity 5ml of the medium was withdrawn at specific time periods (5, 10, 20, 30, 60 min.) and the same volume of dissolution medium was replaced in the flask to maintain a constant volume. The withdrawn samples were filtered through a filter paper (0.22 µm, Whatman Inc., USA) and 5 ml filtrate was made up to volume with 100ml of Phosphate buffer pH 6.8. The samples were analyzed for drug release by measuring the absorbance at 238 nm using UV-visible spectrophotometer and calculated percent cumulative release of simvastatin (SV) [26].

Response Surface Analysis

The results from factorial design were evaluated using PCP Disso 2000 V3 software. Step wise backward linear regression analysis was used to develop polynomial equations for dependent variables particle size (Y_1) and % drug entrapment (Y_2) equation (1).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \qquad \text{Eq.. (1)}$$

Where, Y is the estimated response of dependent variable, β_0 arithmetic mean response of nine batches, and β_1 estimated coefficient for factor X_1. The main effects (X_1 and X_2) represent average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes, when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. The term ε is the random error.

Fourier Transform Infrared Spectroscopy analysis

Fourier Transform Infrared Spectroscopy analysis (FTIR) infrared spectrum of simvastatin (SV), nanoparticle formulation was determined by using Fourier Transform Infrared Spectrophotometer (FTIR-4100, Shimadzu) using KBr dispersion method. To evaluate the molecular states of nanoparticles and also for the drug interaction study.

Differential scanning calorimetry analysis

Differential scanning calorimetry analysis (DSC) measurements were carried out on a modulated DSC Instrument: SDT Q600 V20.9 Build 20 equipped with a thermal analysis data system (TA instrument). Thermal data analyses of the DSC thermograms were conducted using STARe software (version 5.21). [27].

X-ray Diffraction Study

X-ray diffraction analysis (PXRD) was employed to detect the crystallinity of the pure drug and the NPs formulation, which was performed using a Philips PW 3710 x-ray diffractometer (XRD) with a copper target and nickel filter (Philips Electronic Inst, Holland). XRD diffraction pattern of SV, Physical mixture and PS6 batch was obtained and peak intensity was calculated using 2-θ value by spekwin software 32 v 1.71.6.

Scanning electron microscopy study

The morphology of nanoparticles was examined by using scanning electron microscopy (SEM, JSM-6360LV scanning microscope Tokyo, Japan). SEM has been used to determine particle size distribution, surface topography, texture and examine the morphology of fractured or sectioned surface.

Transmission electron microscopy study

The morphology of nanoparticles was observed by Transmission electron microscopy instrument (TEM, Tecknai G^2, 20 U- Twin, FEI, Netherland).

RESULTS AND DISCUSSION

Compatibility Studies

FTIR studies that the fundamental peaks of SV are retained. Comply with peak of PLGA and SV, indicate that SVcompatible with the PLGA and pluronic F68. DSC curve of simvastatin (SV) /PLGA physical mixture showed a glass transition peak at 33.83°C corresponding to the PLGA, followed by the endothermal melting peak at 138.98°C indicating its crystalline nature (the endothermic value was 25.73 j/g). Results from FTIR and DSC spectras indicate that there was no chemical interaction between simvastatin (SV) and excipients used in the formulation hence, can be used in the formulation of nanoparticles (NPs). The solubility of SV was 16.82±0.73µg/ml. Simvastatin-loaded nanoparticles resulted in maximum supersaturated concentrations from nanoparticles, hence increase in solubility after 48h (81.58 ±1.60µg/ml) in comparison with simvastatin pure drug, i.e increase in solubility approximately 5 fold.

Particle size and Polydispersity index

The results of mean particle size and Polydispersity index (PI) of prepared nanoparticles batches are shown in (Table 3). Analysis of results indicates that particle size range was 100- 300 nm. As the concentration of PLGA was increased, particle size also increased but Pluronic F68 surfactant concentration played important role in maintaining particle size in submicron range, which is evident from particle size of batch PS6, which was 122± 1.52 nm with 0.4 % surfactant concentration and 100 mg of PLGA with (1:2) proportion of SV and PLGA. Particle size of nanoparticles was not only dependent on the PLGA amount used in formulation but also dependent on the Pluronic F68 surfactant concentration (i.e increase concentration of surfactant, decease particle size of nanoparticles, which when optimum, submicron particle size was achieved with low particle size distribution. Particle size of optimized batch of PS6 shown in Fig.1. Polydispersity index (PI) of prepared nanoparticles batches were found in the range 0.4508 to 0.9669, which was near to 1 for all nanoparticles batches.

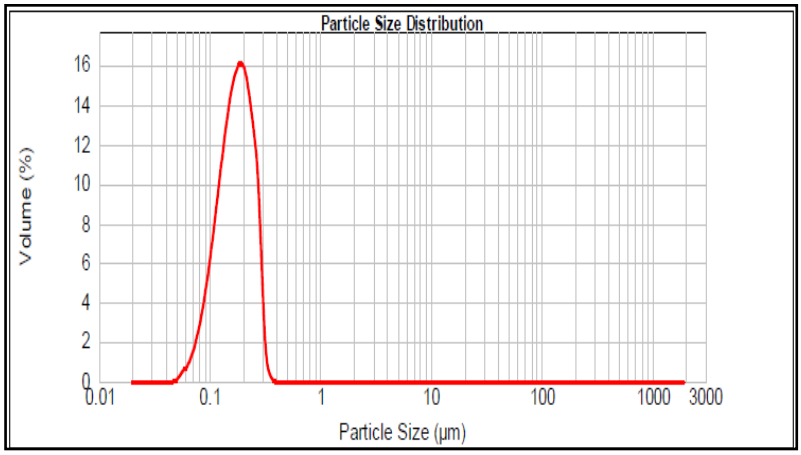

Fig.1: Particle size of nanoparticles of PS6 batch

Percentage entrapment efficiency (EE)

The percentage entrapment efficiency of batches under investigation was in the range of 70.0- 85.43%, batch PS6 showed entrapment efficiency 85.43 ± 0.49% shown in (Table 3). The entrapment efficiency not only depends on the PLGA concentration but also depends on the concentration of surfactant used. Prepared nanoparticles batches, PS6 batch zeta potential was (-23.32 ± 0.01), means near to range, which indicates good physical stability of nanoparticles, shown in (Table 3) and figure 2. The % process yield of all nanoparticles batches were found to be from 81.56 ± 0.59% to 89.62± 0.93%. The result reveals that % loss of all batches is very negligible during processing of freeze-drying, shown in (Table 3).

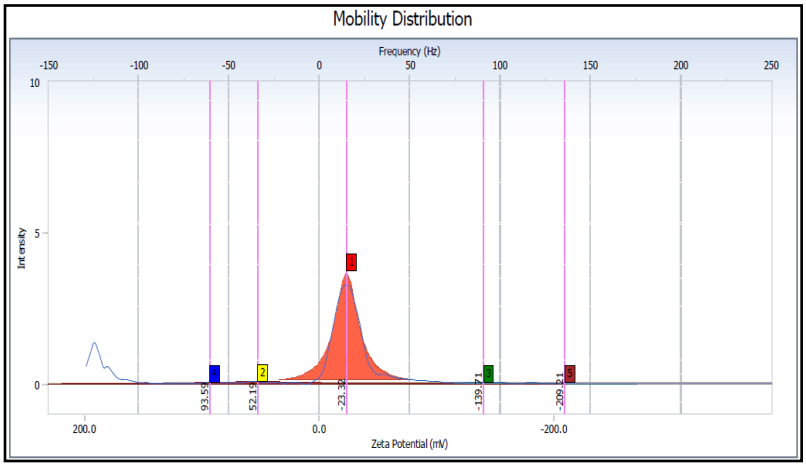

Fig. 2: Zeta potential of nanoparticles of PS6 batch

Table 3: Average particle size, % drug Entrapment, PI, Zeta potential, % Process yield and % Drug content of nanoparticles

Batch code	Particle size (nm) *	Polydispersity Index (PI)	% drug Entrapment*	Zeta potential (Mv) *	% Process yield*	% Drug content*
PS1	212 ± 3.51	0.9669	80.23 ± 0.31	-13.93 ± 0.02	81.56 ± 0.59	91.27 ± 0.56
PS2	189 ± 2.08	0.9259	77.25 ± 0.76	-17.65 ± 0.02	84.23 ± 0.45	89.70 ± 0.33
PS3	175 ± 1.01	0.4860	70.59 ± 0.62	-19.56 ± 0.03	86.65 ± 0.60	86.07 ± 0.93
PS4	209 ± 1.52	0.8133	79.40 ± 0.90	-14.26 ± 0.02	85.69 ± 0.49	96.08 ± 0.37
PS5	140 ± 2.08	0.7928	76.57 ± 0.92	-21.53 ± 0.02	87.95 ± 0.75	95.26 ± 0.77
PS6	122 ± 1.52	0.4508	85.43 ± 0.49	-23.32 ± 0.01	89.62 ± 0.93	98.48 ± 0.44
PS7	293 ± 2.64	0.9522	84.18 ± 0.22	-15.95 ± 0.01	88.25 ± 0.43	93.96 ± 0.42
PS8	272 ± 3.05	0.9264	81.25 ± 0.27	-16.58 ± 0.01	87.67 ± 0.66	92.85 ± 0.27
PS9	205 ± 2.51	0.8146	79.03 ± 0.94	-12.04 ± 0.02	85.54 ± 0.87	90.68 ± 0.35

** Indicates average ±SD (n=3)*

Drug content determination

The drug content of the freeze dried nanoparticles batches were determined by UV-visible spectroscopic method at 238 nm. Drug content of optimized PS6 batch was found to be 94.48 ± 0.44 % respectively, shown in (Table 3). Low loss of drug content of optimized PS6 batch during freeze drying resulted in good recovery of nanoparticles.

***In vitro* drug release study**

In vitro drug release studies were carried out using USP Type II dissolution apparatus, at rotation speed of 50 rpm. The cumulative percentage drug release of SV in Phosphate buffer pH 6.8 medium of PS1-PS9 batches were shown in fig. 2. The release rate of nanoparticles by diffusion and biodegradation process. It is generally anticipated from a bulk eroding polymer such as 50:50 PLGA to give an initial burst release, which may be probably due to the drug that was close to the surface of the nanoparticles. Cumulative drug release for all formulations batches PS1–PS9 were found to be 77.24± 0.317% to 96.53 ± 0.501% respectively, after 60min. Cumulative drug release for PS6 was found to be 96.53 ± 0.501% respectively, after 60min. Finally, it can be concluded that smaller the particle size of nanoparticle their surface area will be more and the drug release is faster. The in vitro release data of the optimized formulation was compared with different kinetic models to select the best fitting model. Good correlation coefficients ($R^2 \geq 0.9915$) could be obtained. The drug release follows matrix and first order release kinetics mechanism.

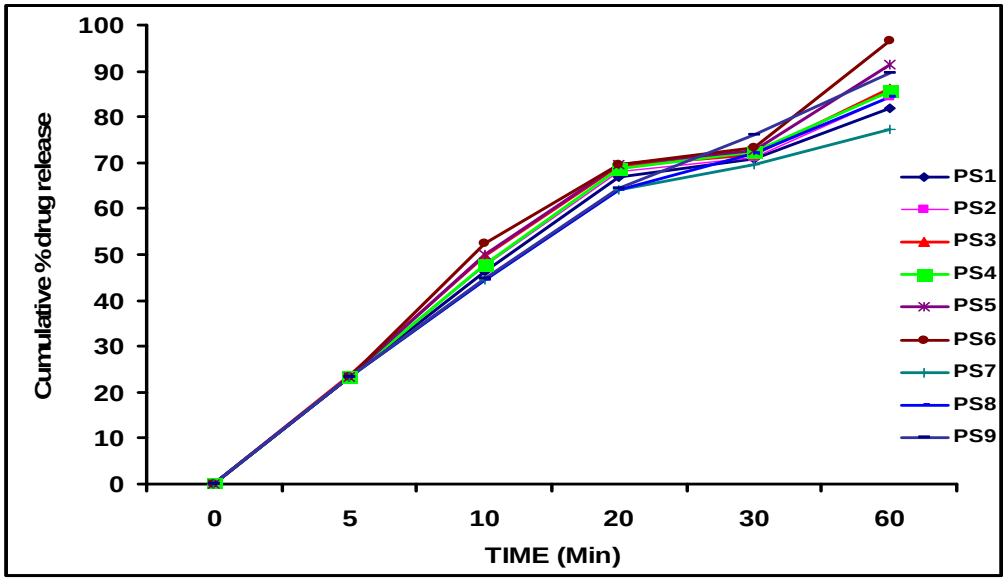

Fig. 3: In vitro drug release of PS1-PS9 batch

Development of Polynomial Equations

The experimental design and Parameters in (Table 1 & 2) for factorial formulations PS1 to PS9, polynomial equations for two dependent variables, particle size and % drug entrapment have been derived using PCP Disso 2000 V.3 software.

The equation derived for particle size is:

$$Y1 = 157.15 + 33.6125\ X1 - 35.333\ X2 + 68.6125\ X1^2 - 0.2250\ X2^2 - 7.6750\ X1\ X2 \quad \text{Eq. (2)}$$

The equation derived for % drug entrapment is:

$$Y2 = 79.2367 + 2.5358X1 - 1.460X2 - 1.9075\ X1^2 + 1.8450\ X2^2 + 1.1750X1X2 \quad \text{Eq. (3)}$$

In equation no. (2) negative sign for coefficient of X2 indicates that the particle size of nanoparticles increases, when concentration of stabilizer Pluronic F68 is decreased and positive sign for coefficient of X1 indicate positive effect of concentration PLGA on particle size. In equation no. (3), positive sign for coefficient of X1 indicates that the % drug entrapment increases, when concentration of PLGA increases and negative sign for coefficient of X2 indicates that % drug entrapment of nanoparticles increases, when concentration of stabilizer Pluronic F 68 decreases**.** The closeness of predicted and observed values for particle size and % drug entrapment indicates validity of derived equations for dependent variables. The data clearly indicates that the particle size and entrapment

efficiency are strongly dependent on the selected independent variables i.e PLGA and pluronic F 68 concentration. The values of the correlation coefficient indicate good fit.

Response Surface Plots

The response surface plots of particle size and % drug entrapment are shown in fig. 4 & 5 respectively. The response surface plots illustrate that as concentration of PLGA increases, the value of dependent variable, particle size increases and as concentration of Pluronic F 68 increases the value of dependent variable, particle size decreases. Similarly the response surface plots for % drug entrapment shows positive effects of independent variable, PLGA concentration and negative effect of other independent variable, concentration of Pluronic F68.

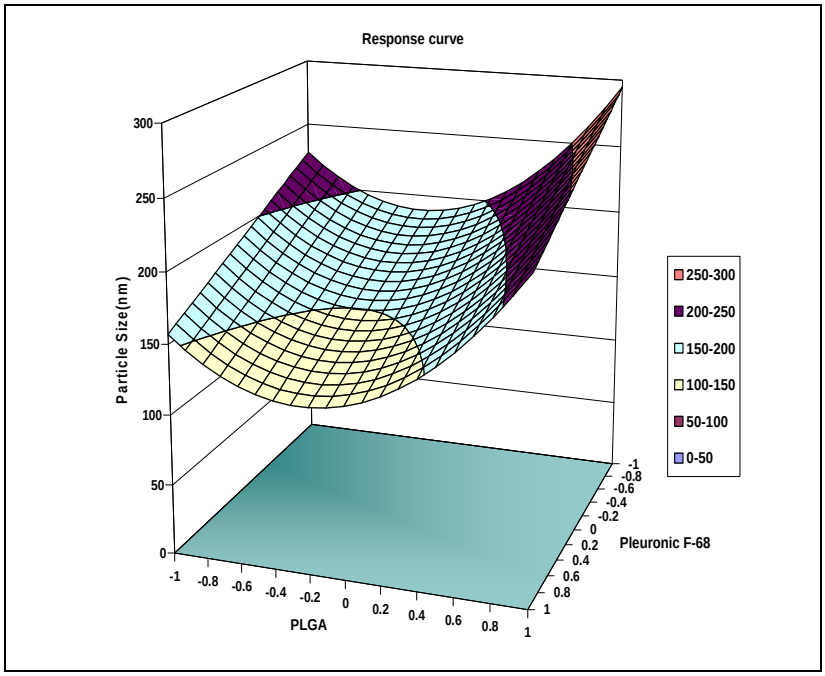

Figure 4. Response surface plot showing effect of factorial variables on particle size

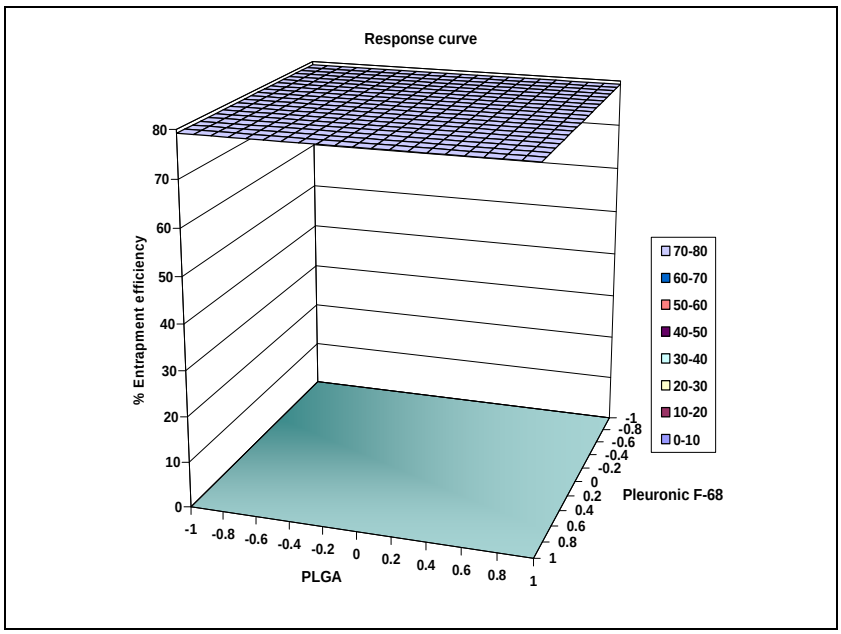

Figure 5. Response surface plot showing effect of factorial variables on % drug entrapment

Fourier Transform Infrared Spectroscopy study

The results of FTIR spectrum reveal few minor shifting of peaks but no major changes as well as no loss of functional peaks. This indicates absence of chemical interaction or any changes in chemical composition of SV

during freeze drying of nanoparticle dispersion. The compliance of FTIR spectrum of SV in freeze dried formulation with standard SV reveals the stability of SV. The overlain spectra of pure drug simvastatin, PLGA, physical mixture and freeze dried optimized batch PS6 shown in fig. 6.

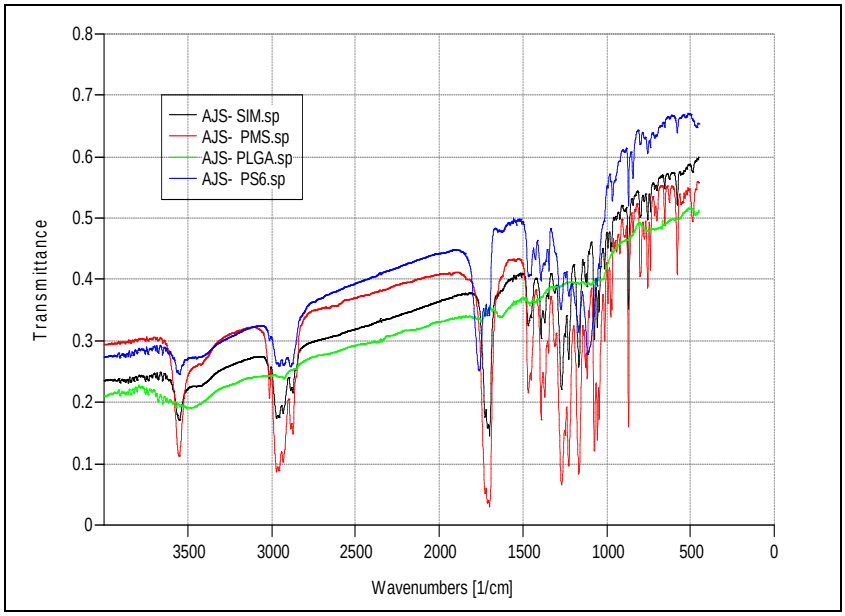

Fig. 6: FTIR spectrum of SV, PLGA, Physical mixture and optimized nanoparticles

Differential Scanning Calorimetry study

The DSC curves of commercial SV shows a broad endotherm ranging from 135 to 160°C indicating the loss of water and by a single, sharp melting endothermic peak at 139.53ºC, which corresponded to its intrinsic melting point indicating its crystalline nature (the endothermic value was 40.65 j/g). PLGA exhibited a glass transition peak at 34.91°C and no melting endothermic peak was observed, because PLGA appears less crystalline in nature. The DSC curve of SV /PLGA physical mixture showed a glass transition peak at 33.83°C corresponding to the PLGA, followed by the endothermal melting peak at 138.98°C indicating its crystalline nature (the endothermic value was 25.73 j/g). However, no sharp endotherm was seen at 130.19°C for the simvastatin nanoparticles and endothermic value was 3.122 j/g), suggesting that SV in nanoparticles was molecularly dispersed as a less crystalline form. This SV as amorphous after being precipitated as nanoparticles; its melting point was decreased indicating reduced crystallinity.

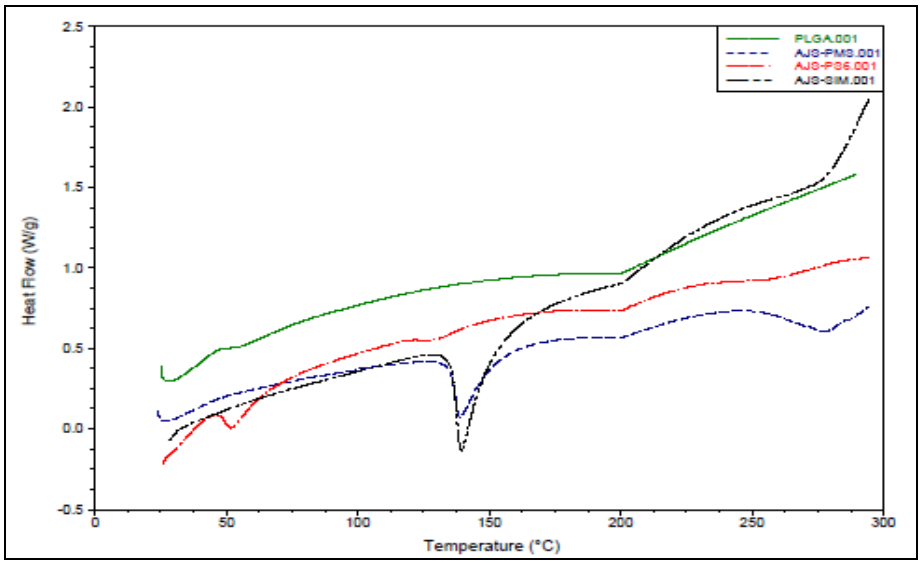

Fig.7: DSC thermogram of SV, PLGA, Physical mixture and optimized nanoparticles

The degree of crystallinity of lyophilized nanoparticles was calculated by comparing the enthalpy of nanoparticles with the enthalpy of SV pure drug. The crystallinity of SV pure, physical mixture (1:1 SV & PLGA) and lyophilized PS6 batch was 100%, 63.29 % and 07.68 % respectively. The melting points of SV, physical mixture (1:1) and lyophilised PS6 batch was 139.53 ºC, 138.98 ºC and 130.19 ºC respectively, results are depicted in (Table 4). Overlain Differential Scanning Calorimetry thermogram is shown in fig. 7. Hence it could be concluded that in both the prepared PLGA nanoparticles loaded SV was present in the less crystalline phase and may have been homogeneously dispersed in the PLGA matrix.

X-ray Diffraction Study

The nanoparticles prepared with PLGA of PS6 batch was characterized by less intensity of the diffraction peak, when compared to that of SV, which demonstrates that the chemical structure of the drug was not changed before and after the precipitation process. This clearly indicates the significant reduction in the crystallanity of the precipitated SV nanoparticles and the less ordered crystals were in majority and the amorphous state would contribute to the higher drug loading capacity. It was confirmed that simvastatin existed in amorphous state in the SV nanoparticles because of the disappeared sharp peak of SV in the diffraction pattern. The overlain spectra of SV, PLGA, physical mixture and PS6 batch were shown in fig. 8.

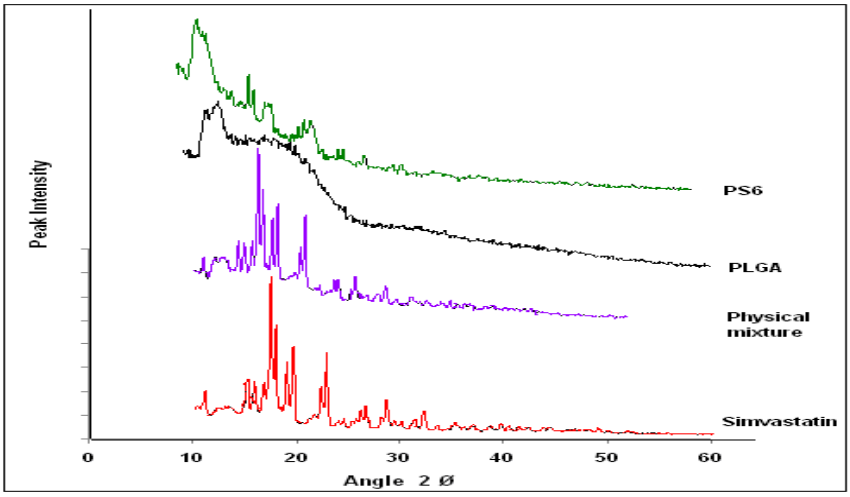

Fig. 8: PXRD peak of SV, PLGA, Physical mixture and optimized nanoparticles

Scanning electron microscopy study

It can be revealed from the SEM of SV pure drug that consisted of a mixture of needle-shaped large crystals, indicating its crystalline nature. However, the prepared SV-loaded PLGA nanoparticles of batch PS6 had a drastic change in the morphology and shape of drug, nearly spherical shape with a relatively uniform size of about 122 nm in diameter and no drug crystals were present in nanoparticles. The SEM of SV pure drug (A), PS6 (B) batches were nearly spherical in shape shown in fig. 9.

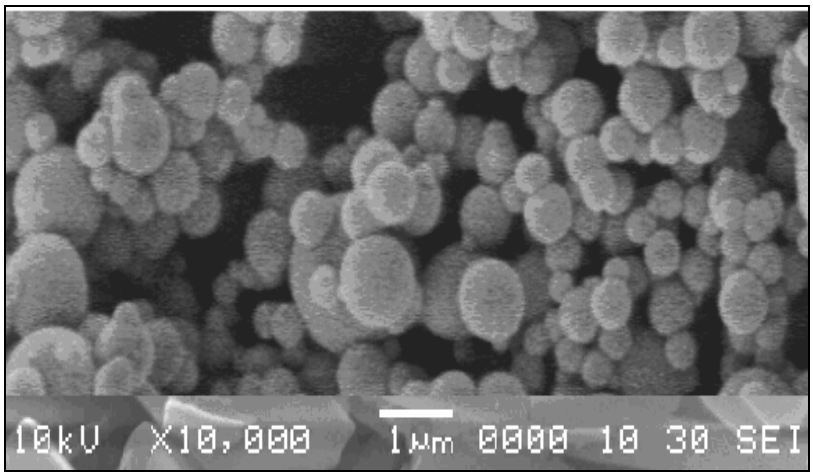

Fig. 9: SEM of simvastatin nanoparticles

Transmission electron microscopy study

The morphology of nanoparticles was observed by Transmission electron microscopy (TEM)of optimized batch PS6, spherical shape and uniform size, shown in fig. 10.

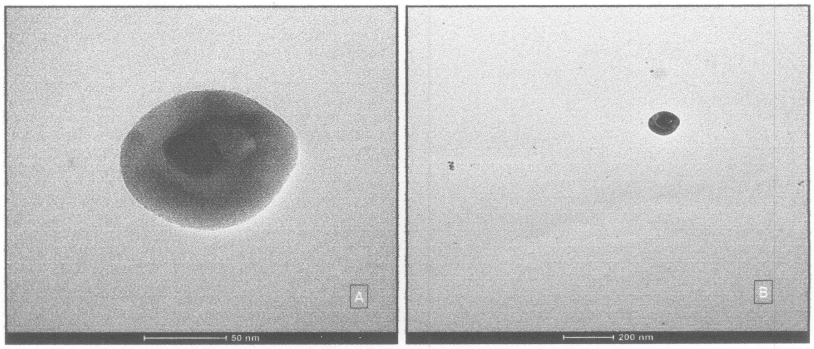

Fig. 10: TEM of simvastatin nanoparticles

Stability studies

Stability studies results indicate that after the 1, 2 and 3 months accelerated stability studies reveals no morphological changes but particle size increased, drug content and % cumulative release decreased in nanoparticles dispersion,127±0.2, 95.22±0.2 and 95.48± 0.2 respectively. There was no morphological changes but particle size increased, drug content and % cumulative release decreased in freeze dried nanoparticles, 143 ± 2.5, 93.35±0.6 and 95.54± 0.1 respectively. After the 1, 2 and 3 months accelerated stability studies reveals that % cumulative release decreased in pure tablet, marketed tablet and freeze dried nanoparticles tablets results shown in table 4. Thus we may conclude that the drug does not undergo degradation on storage (Lourenco C et al. 1996).

Table 4: Changes in particle size, % drug content and % cumulative release of freeze dried optimized batch (PS6) during stability

Time of sampling (month)	Freeze dried optimized batch (PS6)					
	Particle size (nm) *		% drug content*		% Cumulative release	
	Zero timing	After sampling	Zero timing	After sampling	Zero timing	After sampling
1	135 ± 3.2	139 ± 4.5	94.48 ± 0.4	94.1 ± 0.5	96.53±0.5	96.13± 0.7
2	135 ± 3.2	141 ± 2.0	94.48 ± 0.4	93.82±0.3	96.53± 0.5	95.78± 0.1
3	135 ± 3.2	143 ± 2.5	94.48 ± 0.4	93.35±0.6	96.53± 0.5	95.54± 0.1

** Indicates average ±SD (n=3)*

CONCLUSION

The present study was carried out to develop nanoparticles of SV in order to enhance solubility, dissolution and bioavailability by decreasing the particle size of the drug. Successful incorporation of SV drug was carried out in to nanoparticles by precipitation-solvent displacement method. DSC studies result reveals that the prepared nanoparticles were present in the amorphous phase and may have been homogeneously dispersed in the polymer matrix. SEM and TEM studies prepared nanoparticles were spherical in shape and no drug crystals were present. Cumulative drug release for all formulations batches PS1–PS9 were found to be 77.24± 0.317% to 96.53 ± 0.501% respectively, after 60min. The in vitro release data of the optimized formulation was compared with different kinetic models to select the best fitting model. Good correlation coefficients ($R^2 \geq 0.9915$) could be obtained. The drug release follows matrix and first order release kinetics mechanism.

Thus resulting in improved therapeutic outcome, thereby minimizing the dose-dependent adverse effects and maximizing the patients compliance.

Acknowledgements

Authors are wish to acknowledge Aurobindo Pharmaceutical Ltd. (Hyderabad, India), for providing simvastatin as gift sample. We also grateful to Bharati vidyapeeth, Poona college of Pharmacy, Pune for providing Malvern Mastersizer facility, Shivaji university Kolhapur for SEM, DSC & XRD facilities and Purac biochem, USA for providing, Polylactic co- glycolic acid as gift samples.

Ethical Approval

All authors hereby declare that "Guidelines for care and use of laboratory animals" were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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