Review Article

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Design fabrication and characterization of lovastatin nanoparticles by nanoprecipitation-solvent displacement method

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ABSTRACT

Nanoparticles are particles between 1 and 100 nanometers in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter. The present study was to formulate nanoparticles containing Lovastatin 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 ($R2 \geq 0.9915$) could be obtained.

Keywords: Lovastatin, Factorial Design, Transmission electron microscopy, Differential scanning calorimetry.

INTRODUCTION

Nanoparticles are particles between 1 and 100 nanometres (nm) in size with a surrounding interfacial layer. The interfacial layer is an integral part of nanoscale matter, fundamentally affecting all of its properties. The interfacial layer typically consists of ions, inorganic and organic molecules. Organic molecules coating inorganic nanoparticles are known as stabilizers, capping and surface ligands, or passivating agents. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to

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its transport and properties. Particles are further classified according to diameter [1-3].

MATERIALS AND METHODS

Lovastatin (SV) was a obtained from gift sample from Aurobindo Pharmaceuticals Ltd., Hyderabad; Poly (D, L Lactide-co- Glycolide) (PLGA 50:50

Compatibility Studies

Compatibility of the Lovastatin (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 [4-6].

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. 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

Preparation of PLGA Nanoparticles

PLGA nanoparticles were prepared by the nanoprecipitation-solvent displacement method . Accurately measured Lovastatin (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.

CHARACTERIZATION OF NANOPARTICLES [7-10]

Determination of particle size

The particle size and size distribution of the Lovastatin (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.

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.

Determination of zeta potential

The zeta potential of the SV loaded PLGA nanoparticle was measured on a zetasizer.
All the samples were measured in water at 25°C.

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 equiva lent 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 Lovastatin (SV).

Fourier Transform Infrared Spectroscopy analysis

Fourier Transform Infrared Spectroscopy analysis (FTIR) infrared spectrum of Lovastatin (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.

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 XRD diffraction pattern of SV, Physical mixture and PS6 batch was obtained .

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.

RESULTS AND DISCUSSION

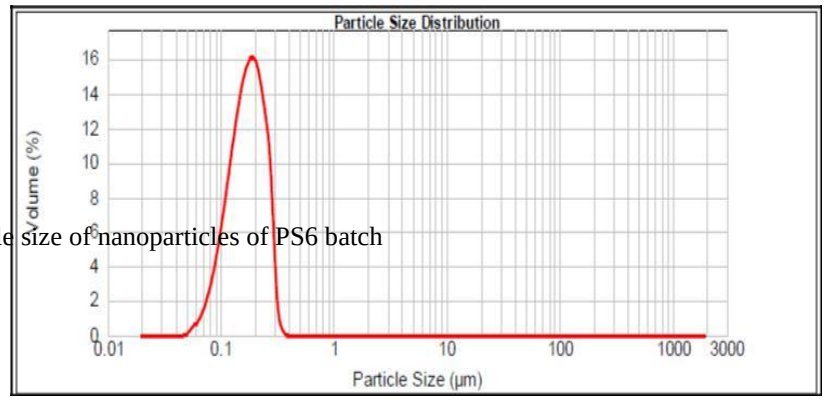
Compatibility Studies

FTIR studies that the fundamental peaks of SV are retained. Comply with peak of PLGA and SV, indicate that SV compatible with the PLGA and pluronic F68. DSC curve of Lovastatin (SV) /PLGA physical mixture showed a glass transition peak at 31.83°C corresponding to t he PLGA, followed by the endothermal melting peak at 132.98°C indicating its crystalline nature (the endothermic value was 22.73 j/g). Results from FTIR and DSC spectras indicate that there was no chemical interaction between Lovastatin (SV) and excipients used in the formulation hence, can be used in the formulation of nanoparticles (NPs). The solubility of SV was 19.82±0.73µg/ml. Lovastatin-loaded nanoparticles resulted in maximum supersaturated concentrations from nanoparticles, hence increase in solubility after 48h (81.58 ±1.60µg/ml) in comparison with Lovastatin pure drug.

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- 400 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 120± 2.52 nm with 0.1% 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. Polydispersity index (PI) of prepared nanoparticles batches were found in the range 0.5 to 0.7669, which was near to 1 for all nanoparticles batches.

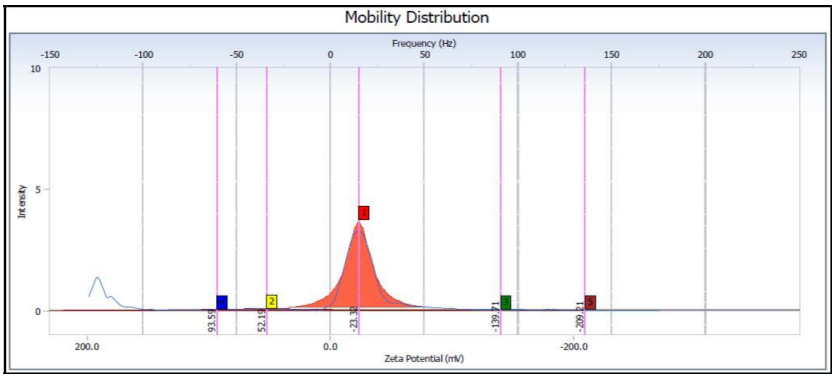


Particle size of nanoparticles of PS6 batch

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 reve als that % loss of all batches is very negligible during processing of freeze-drying,



Zeta potential of nanoparticles of PS6 batch

Batchcode	Particlesize(nm)	*Polydispersity Index (PI)	% drugEntrapment*	% Processyield*	% Drug content*
PS1	212 ± 3.51	0.9669	80.23 ± 0.31	81.56 ± 0.59	91.27 ± 0.56
PS2	189 ± 2.08	0.9259	77.25 ± 0.76	84.23 ± 0.45	89.70 ± 0.33
PS3	175 ± 1.01	0.4860	70.59 ± 0.62	86.65 ± 0.60	86.07 ± 0.93
PS4	209 ± 1.52	0.8133	79.40 ± 0.90	85.69 ± 0.49	96.08 ± 0.37
PS5	140 ± 2.08	0.7928	76.57 ± 0.92	87.95 ± 0.75	95.26 ± 0.77
PS6	122 ± 1.52	0.4508	85.43 ± 0.49	89.62 ± 0.93	98.48 ± 0.44

PS7	293 ± 2.64	0.9522	84.18 ± 0.22	88.25 ± 0.43	93.96 ± 0.42
PS8	272 ± 3.05	0.9264	81.25 ± 0.27	87.67 ± 0.66	92.85 ± 0.27
PS9	205 ± 2.51	0.8146	79.03 ± 0.94	85.54 ± 0.87	90.68 ± 0.35

Average particle size, % drug Entrapment, PI % Process yield and % Drug content of nanoparticles

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. 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% re spectively, 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 ($R2 \geq 0.9915$) could be obtained. The drug release follows matrix and first order release kinetics mechanism.

CONCLUSION

The present study was carried out to develop nanoparticles of SV in order to enhance solubility, dissolution 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 75.24± 0.317% to 97.53 ± 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 ($R2 \geq 0.9905$) could be obtained. The drug release follows matrix and first order release kinetics mechanism.

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