Research and Reviews: Drug Delivery

Formulation, Development and Characterization of Simvastatin Nanoparticles by Ionotropic Gelation Method

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Research Article

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ABSTRACT

The aim of this study is to formulate and evaluate nanoparticles containing Simvastatin using chitosan as a biodegradable polymer. The Simvastatin loaded nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions. Polymeric Nanoparticles of various core: coat ratio were prepared and evaluate%products yield, entrapment efficiency, particle size, and zeta potential, *in vitro* drug release and stability studies. The prepared simvastatin nanoparticles were white, free-flowing and in a spherical shape. The IR Spectra showed the stable character of Simvastatin within the drug-loaded nanoparticles and displayed the absence of drug-polymer interactions. The chitosan nanoparticles have a particle diameter ranging between 132.1 ± 5.60 to 774.8 ± 2.60 nm and a zeta potential +11.93 to+43.23 mV. The in vitro release profile from the entire drug loaded formulation batches were found to follow first order and provided sustained release over a period of 10 h. No considerable difference was observed within the extent of degradation of the product during 90 days during which nanoparticles were stored at different temperatures. The discharge of Simvastatin was influenced by the drug to polymer ratio and particle size. These results indicate that Simvastatin nanoparticles might be effective in sustaining drug release for a prolonged period of time.

INTRODUCTION

Simvastatin (SV) is a cholesterol-lowering agent that's derived synthetically from a fermentation product of Aspergillus terreus and widely accustomed treat hypercholesterolemia[1].When given orally, SV (a lactone) is quickly hydrolyzed in vivo to the corresponding β, δ-dihydroxy acid form, a potent competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) the enzyme that catalyzes the conversion of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) to mevalonate, which is an early and rate-limiting step within the biosynthesis of cholesterol [2]. However, it's a short half-life and is practically insoluble in water. Half-life is also commonly considered that compounds with less water solubility will show dissolution rate-limited absorption in vivo and hence poor absorption, distribution, and site-specific delivery. Conventional drugs delivery system has been characterized by instant release and frequent dosing of the drug which could result in the danger of dose fluctuation. The main objectives of designing nanoparticles as a drug delivery system are very important particle size determination, surface properties and to deliver pharmacologically active agents at the right place, at the rational rate and dose. Therefore it's important to introduce effective methods to boost the solubility and dissolution rate of drug, substantially resulting in its improved oral bioavailability [3]. Sustained-release formulations, nanoparticles, are reported to resolve these problems because of the alteration of its tissue delivery, civilizing the drug efficacy, decreasing the drug toxicity, and prolonging the half-lives in blood. Chitosan (CS) may be a natural cationic polysaccharide obtained by the N-deacetylation of chitin; a product found within the shells of crustaceans the first amine groups provide special properties that make CS very useful in pharmaceutical applications. The produced nanoparticles are biocompatible, biodegradable, non-toxic and competent to sustain the release of encapsulated materials more competently than either alginate or chitosan alone. This necessitated the development of novel chitosan nanoparticles as novel drug delivery system for Simvastatin order to provide pH dependent, sustained drug release and increase oral bioavailability [4].

MATERIALS AND METHODS

Materials

Simvastatin was procured as gift sample from Aurobindo Pharma ltd. Hyderabad. Sodium tripolyphosphate was purchased from sigma-Aldrich, Mumbai; Chitosan (high viscosity) was purchased from Central Institute of Fisheries Cochin and all Reagents and chemical used were of analytical grade.

Preparation of chitosan nanoparticles

Chitosan nanoparticles containing Simvastatin were prepared by ionotropic gelation method. Chitosan was dissolved in 1% acetic acid solutions at various concentrations to obtain (0.1%, 0.2% & 0.3% i.e. 35 mg, 65 mg & 90 mg) and adjusted the pH 5-6 with 0.1N sodium hydroxide solution, while STPP was dissolved in deionized water at various concentrations to obtain 0.1%, 0.15% and 0.20% while stirring at 750 rpm. Simvastatin 50 mg was dissolved in ethanol/ water mixture (1:1) (1% tween 80) to obtain clear solution. Simvastatin solution was added dropwise during probe sonication with syringe needle size 0.45 mm to 40 ml chitosan solution. Repeat the sonication cycles for 1 h .The 20 ml of STTP solution was added dropwise 0.75 ml/min. under stirring (1000 rpm) at ambient temperature [5]. The formulation was stirred for 30 minutes so as to remove ethanol content. All the formulation was sonicated at fixed time for 30 minutes. Nanoparticles were collected by centrifugation at 9000 rpm for a period of 1 h and supernant was analyzed using UV-Visible spectro photo meterically to determine encapsulation efficiency. Pellet was redissolved for sonicated for 15 min. The sample was freeze dried at -40°C and lyophilized to get dry powder using 2% Mannitol as cryoprotectant.

Freeze drying of nanoparticles

Briefly, by taking 5 ml of nanoparticles dispersion was filled in 10 ml glass vials, covered with special stoppers for lyophilization and placed in a freeze dryer (Southern scientific lab Instrument, India) After freeze drying all sample vials were stored at 2°C-8° C.

Experimental design

The formulations batches were design according to a 3^2 full factorial, allowing the simultaneous evaluation of two formulation variables and their interaction (Tables 1 and 2).

Table 1: Parameters for 3^2 full factorial design batches code and experimental design.

Batch code	Drug: polymer ratios	STTP
	(X1)	(X2)
F1	2	2
F2	3	1
F3	3	2
F4	1	2
F5	1	1
F6	1	3
F7	2	3
F8	2	1
F9	3	3

Table 2: Coded levels to actual quantities translation.

Coded Levels	1	0	-1
Drug: Polymer ratios (X1) in mg	1:3 (90)	1:2 (65)	1:1 (35)
STTP (X2) in%	0.2	0.15	0.1

Evaluation of simvastatin chitosan nanoparticles

Determination of particle size and Poly dispersity index: The size distribution and Polydispersity Index (PDI) of the formulations was considered by Dynamic Light Scattering zeta Analyzer (Nanoplus 3, Micromeritics, USA). The average diameter and a determinaion of the distribution width (polydispersity) were measured from the particle size distribution data. Polydispersity Index (PDI) varies from 0.0 to 1.0. The usual range of Polydispersity index values: 0-0.05 (monodisperse standard) [5-7].

X-Ray diffraction (XRD) analysis: XRD patterns were obtained at room temperature using a very high-resolution Cu-Kα radiation diffraction system (Bruker D8 Advance) operating at a voltage of 40 kV and current of 30 mA. This were analyzed in the 2θ angle range of 0–80°.0.05-0.08 (nearly-monodisperse), 0.08-0.7 (midrange polydispersity),>0.7 (very polydisperse).

Fourier transforms infrared spectroscopy (FTIR): Infrared spectroscopy analysis was carried out to see the chemical composition of the prepared microspheres using FTIR (Nicolet, USA) operating within the frequency range of 400 cm^{-1}–4000 cm^{-1} at the absorption mode [8].

Scanning electron microscopy (SEM): The prepared microspheres were coated with a thin layer of gold by sputtering (Hitachi High E-1010, Japan) and so the microstructure were observed in a scanning microscope (SEM; Hitachi High S-4800,Japan) that operated at an acceleration voltage of 20 kV.

Determinations of drug content: A quantity of simvastatin drug loaded nanoparticles equivalent to 1 mg was added to 10 ml methanol and phosphate buffer pH 6.8 (1:10) mixtures and stirred continuously for 2 hr and so the ultimate colloidal suspensions were ultracentrifuged at 10000 rpm for half an hour [9]. The supernatant was analyzed for drug content by measuring the absorbance at 238 nm using UV spectrophotometer.

Entrapment efficiency: The Entrapment efficiency of nanoparticles was determined by the separation of drug-loaded Nanoparticles from the aqueous medium containing non-associated Simvastatin by ultracentrifugation at 12,000 rpm at 4°C for 1hr. the quantity of Simvastatin loaded into the nanoparticles was calculated as the difference between the total amount used to prepare the nanoparticles and also the amount that was found within the supernatant. The quantity of free Simvastatin within the supernatant was measured by UV Spectrophotometer. Entrapment efficiency was then calculated as follows: Entrapment efficiency was calculated by Equation 1.

$$\%\ EE = \frac{\text{Total amount of drug-non bound drug}}{\text{Total amount of drug added}} \times 100 \quad \ldots Eq\ 1$$

Percentage yield

Fixed volumes of Simvastatin nanosuspension were centrifuged at 9000 rpm for 30 min at 15°C. The obtained sediment was dried and weighed. The percentage yield was calculated by Equation 2.

$$\text{Percentage yield} = \frac{\text{Weight of nanoparticles obtained}}{\text{Weight of drugs and excipient}} \times 100 \quad ..Eq\ ..2$$

Zeta potential: The zeta potential value of optimized Simvastatin loaded chitosan nanoparticle formulation was measured with the Zetasizer. To determine the zeta potential, optimized formulation was diluted with double-distilled water and placed in an electrophoretic cell.

In vitro drug release: The release of Simvastatin from nanoparticles was evaluated using USP type II paddle apparatus over 24 hr, dialysis membrane was loaded with nanoparticle formulation containing 10 mg equivalent of drug, which was suspended initially for 2 hrs in 900 ml of 0.1 N HCl buffer of pH 1.2 and so in pH 6.8 phosphate buffer upto 24 hr maintained at 37° C ± 0.5°C and 50 rpm. At standard intervals aliquots of 1 ml of the sample

were withdrawn and replaced with the identical volume of the respected fresh buffer solution. The amount of released drug was assessed by UV-1700 analysis at 238 nm (Shimadzu UV-1700, Japan) after dilution [10].

Stability studies: Optimized formulation was chosen to perform short term stability studies. Samples were stored in glass vials for 3 months at 5ºC ± 3°C in freeze and at 30 ºC ± 2°C/65 ± 5%RH. After 30, 60 and 90 days samples were observed for particle size, %entrapment efficiency and percent drug release were carried out for optimized formulation at each one month interval.

RESULTS AND DISCUSSION

Particle size and size distribution

The mean particle size for formulations F1 to F9 varied in range of 132.1 ±. 5.60 to 774 ± 2.60. It was observed that mean particle size increases with the increase in the polymer concentration upto a level. The mean polydispersity index values for the Simvastatin loaded chitosan nanoparticle formulations F1 to F9 are in the range of 0.270 - 0.628 as shown in (Table 3).

Table 3: Average particle size, pdi, %yield, drug content and %ee of nanoparticles.

Formulation batches	Particle Size (nm)*	%Drug Content	Product Yield (%)	Polydispersity index
F1	401.9 ± 4.51	68.46 ± 0.12	54.7 ± 0.414	0.27
F2	570.5 ± 6.03	68.00 ± 0.24	48.5 ± 0.320	0.373
F3	774.8 ± 2.60	71.17 ± 0.32	45.1 ± 0. 212	0.477
F4	330.4 ± 4.05	82.26 ± 0.35	55.1 ± 0.456	0.386
F5	612.8 ± 6.05	73.12 ± 0.12	46.3 ± 0.876	0.392
F6	414.6 ± 2.51	80.90 ± 0.25	53.4 ± 0.468	0.628
F7	317.9 ± 4.21	65.06 ± 0.10	53.4 ± 0.524	0.284
F8	132.1 ± 5.60	70.27 ± 0.291	56.4 ± 0.281	0.395
* Indicates average ± SD (n=3).				

Powder x- ray diffraction (PXRD) studies

XRD pattern of the Simvastatin and selected nanoparticle formulation. The nanoparticle fSimvastatin. Simvastatin the characteristic peaks at 9.3°, 10.98°, 14.84°,15.42º,16.39º,17.11º,17.62º,18.64º,19.16º,22.43º,26.14º,28.08º,31.68º 2θ while diffractogram of Simvastatin NPs showed the characteristic peaks at 9.6°, 10.77°, 13.72°,14.74°,17.32°,18.86°,20.49°,21.41°,23.57°,28.39°,29.62°,33.74°,36.19º,38.75º,44.08º 2θ. This means that reduction in the crystallanity of the precipitated Simvastatin nanoparticles (Figure 1).

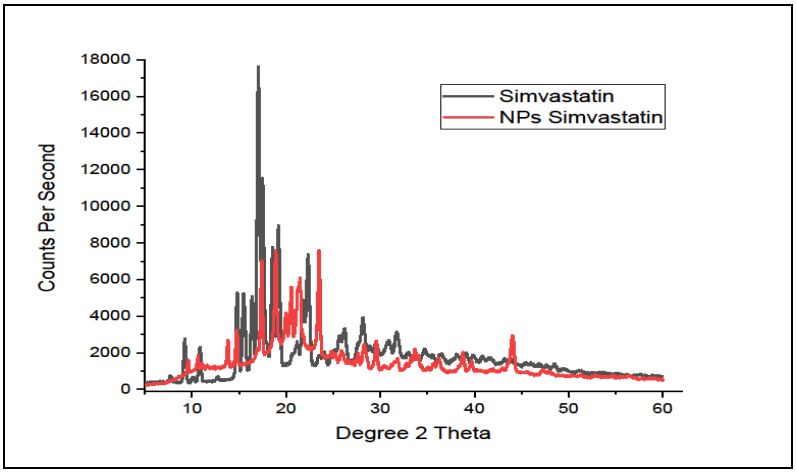

Figure 1: XRD pattern of pure drugs & NPs Simvastatin (F4).

Compatibility studies

FTIR spectra of Simvastatin and chitosan physical mixture and optimized nanoparticle batch. From FTIR studies it can be seen that the fundamental peaks of Simvastatin are retained. From FTIR indicated that there was no chemical interaction between Simvastatin and chitosan used in the formulation hence, can be used in the formulation of nanoparticles (Figure 2).

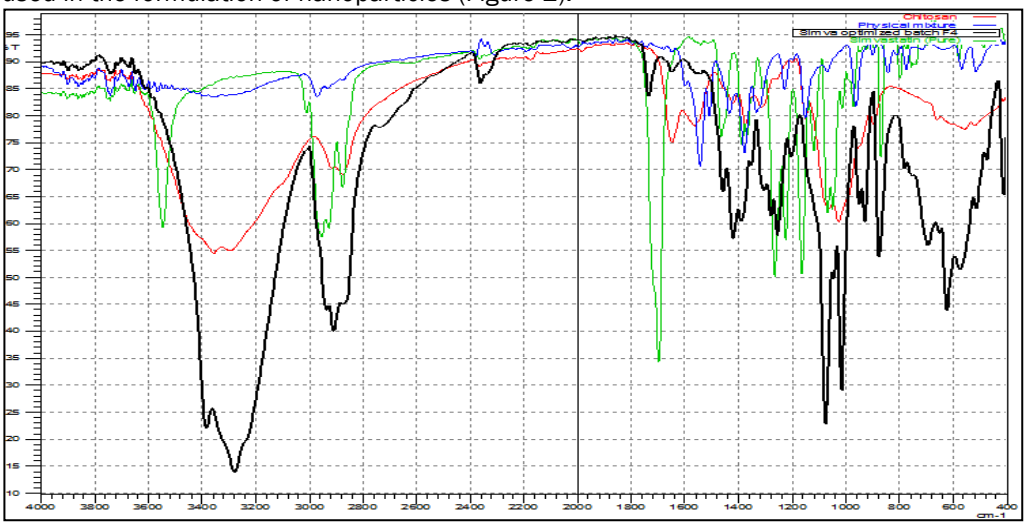

Figure 2: FTIR spectra of Simvastatin and chitosan physical mixture (1:1).

Scanning microscopy study

The revealed from the SEM of Simvastatin consisted of a mix of enormous crystals, indicating its crystalline nature. However, the prepared Simvastatin - loaded CS NP's of batch F4 had a spherical shape with a comparatively uniform size of about 330.4 nm in diameter and no drug crystals were present which was shown in SEM of pure Simvastatin. F4 batches were nearly spherical in shape depicted in (Figure 3).

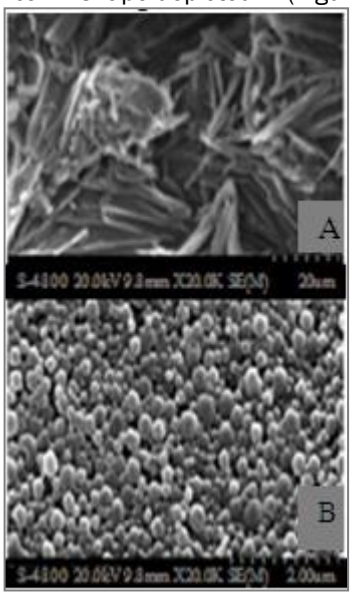

Figure 3: SEM of SIM (A) &optimized batch of F4 (B).

Percent drug content study

Drug content varies in the range of 65.06 ± 0.10 to 82.26 ± 0.35 and was determined using the UV spectroscopic analysis at 238 nm.

Entrapment efficiency

Encapsulation efficiency of the nanoparticles was found to vary between 87.0 ± 0.28 to 97.9 ± 0.12 suggested that at intermediate concentration of chitosan and STTP the encapsulation of drug was maximum. Formulation F4 (97.9 ± 0.12) show maximum entrapment efficiency Based on entrapment efficiency and drug content formulation F4 was taken as optimized formulation.

Percentage yield

Percentage yield was found to be 42.7 ± 0.358% to 55.1 ± 0.456% for formulation F1 to F9. Percentage practical yield depends on the concentration of polymer added, as the concentration of polymer increases there is increases in the % yield. Maximum yield obtained is 55.1 ± 0.456% for formulation F4.

Zeta potential

Zeta potential is a key factor to analyze the long-term stability of the nanoparticles. Zeta potential of formulation batches F1-F9 was found to be in range of 11.93 to 43.23. Zeta potential of the optimized formulation F4 was found to be 31.9 Mv ± 0.072 Mv.

In vitro drug release

In vitro drug release studies were carried out using USP Type II dissolution apparatus (EDT.08LX, 1292092, Elestro Lab, India), at rotation speed of 50 rpm. The cumulative% drug release of Simvastatin in Phosphate buffer pH 6.8 medium of F1 to F9 batches. Cumulative percentage drug released for F1,F2,F3,F4,F5,F6,F7,F8,and F9 after 10 h were found to be 82.33%, 66.57% 84.43%, 98.60%,89.00%, 93.24%,86.12%,79.74%and 78.58% respectively. It was showed that in vitro release of Simvastatin showed a very rapid initial burst, and then followed by a very slow drug release (Figure 4).

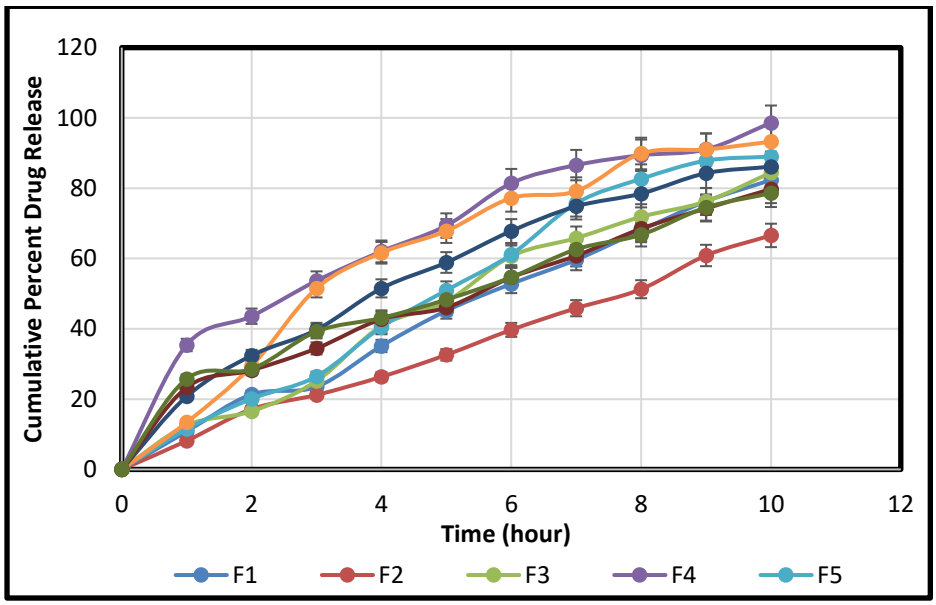

Figure 4: Comparative in vitro drug release profile of F1 to F9.

CONCLUSION

This paper report, the possibility to entrap hydrophobic Simvastatin within CS-STPP nanoparticles using a modified ionotropic gelation technique, strong electrostatic interactions are present in the polymeric nanoparticles. Chitosan nanoparticles indication a novel controlled drug delivery, which offer several potential benefits. The present study was aim to develop Simvastatin loaded chitosan Nanoparticles. Chitosan concentrations and drug: Polymer ratios in the nanoparticles influence the physiochemical parameter such as zeta potential polydispersity index, and average nanosize diameter or percentage encapsulation efficiency of Simvastatin. Average Nanosize diameter, Polydispersity index, zeta potential, percentage encapsulation efficiency, stability study was found to be good for optimum formulation (F4, The concentration of polymer and cross linking agent are the important factors in the development of Simvastatin nanoparticles. 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 ≥ 0.99) for Simvastatin NPs tablet could be obtained. Thus resulting in improved therapeutic outcome, thereby minimizing the dose-dependent adverse effects and maximizing the patient's compliance.

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