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Development and characterization of atorvastatin calcium loaded chitosan nanoparticles for sustain drug delivery

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ABSTRACT

The aim of this study is to formulate and characterize atorvastatin loaded chitosan loaded nanoparticles prepared by solvent evaporation method for sustained release. Low oral bioavailability of Atorvastatin calcium (14%) due to an extensive high first-pass effect makes it as prime target for oral sustained drug delivery. Weighed amount of drug and polymer were dissolved in suitable organic solvent DMSO and 2% acetic acid as an organic phase. This solution is added drop wise to aqueous solution of Lutrol F68 and homogenized at 25000rpm followed by magnetic stirring for 4hrs. Nanoparticles were evaluated for its particle size, scanning electron microscopy (SEM), Fourier-Transform infrared spectroscopy (FTIR), percentage yield, drug entrapment and for in vitro release kinetics. Among the four different ratios, 1:4 ratio showed high drug loading and encapsulation efficiency. SEM studies shows that prepared nanoparticles were spherical in shape with a smooth surface. Particle size of prepared nanoparticles was found to be in range between 142 nm to 221 nm. FTIR and DSC shows drug to polymer compatibility ruling out any interactions. *In vitro* release study showed that the drug release was sustained up to 7 days. Hence, prepared nanoparticles proved to be promising dosage form for sustained drug delivery of atorvastatin reducing dosing frequency, thus increasing the patient compliance. Copyright © 2012 VBRI press.

Keywords: Atorvastatin calcium; nanoparticles; chitosan; solvent evaporation.

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Introduction

One of the main dilemma associated with the development of new molecular entities as drug formulation is poor solubility and poor permeability of lead compounds [**1**]. Dissolution rate limited bioavailability is the major obstacle allied to the profitable use of many poorly soluble drugs. Many approaches have been developed to improve solubility and to enhance the dissolution rate and oral bioavailability of poorly soluble drugs e.g. salt formation [**2**], solid dispersion [**3, 4**], inclusion complex [**5**], micronization [**6**] etc., which often aims to increase the surface area, solubility, wettability of the particles.

Over the last few years, polymeric nanoparticles have been used as potential drug delivery systems because of several advantages such as controlling particle size, surface properties and sustained release of pharmacologically active ingredient in order to achieve site specific action of the drug at therapeutically optimal rate [**7**]. Oral route is the most accepted route of administration used for sustained delivery of drugs. It offers ease of administration, greater flexibility in dosage form design, low cost.

Nanoparticles are colloidal particles with diameter in the range of 1- 1000 nm. They are the drug carriers in which the active ingredient is dissolved, dispersed,

entrapped, encapsulated, adsorbed or chemically attached [**8**].

Many natural polymers had gained importance in recent years for their use in new drug delivery applications. The dissolution rate of drugs from the formulations containing viscous carriers is generally low due to the formation of gel layer on the hydrated surfaces, however, it is reported that the viscous ability of the carrier retards dissolution rate of highly water soluble drug.

Natural chitosan materials gained great interest in pharmaceutical sector because of its advantages like biodegradability, biocompatibility, non-toxicity, non-immunogenicity & low cost. Chitosan is a natural hydrophilic cationic polysaccharide derived by deacetylation of chitin [**9**]. The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino) glucose. These units combined by (1-4) glycosidic linkages, forming a long chain linear polymer. It is soluble in most of the organic acidic solutions at pH less than 6.5 such as acetic acid, tartaric acid etc [**10, 11**].

Atorvastatin calcium (AC), [R-(R*,R*)]-2-(4-fluorophenyl)- b,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, is a BCS class II drug used in the treatment of hypercholesterolemia. It acts by competitive inhibition of HMG-CoA reductase. Hence it prevents the conversion of HMG-CoA to mevalonate, an early rate-limiting step in the biosynthesis of cholesterol [**12, 13**]. It is insoluble in aqueous solutions at pH 4 and below. It exhibits low oral bioavailability of 12% which is ascribed to the presystemic clearance in the gastrointestinal mucosa and extensive hepatic first pass metabolism. The present study aimed to increase the bioavailability of the drug by developing sustained release nanoparticles of AC by solvent evaporation method [**14, 15**].

Experimental

Materials

Atorvastatin calcium was purchased from Microlabs, Bangalore. Chitosan (CN) was obtained as a gift sample from Sigma Aldrich Chemicals, Bangalore. Lutrol F68 was obtained from BASF Pvt. Ltd, Mumbai. DMSO was procured from Loba Chemie. All other chemicals are of analytical grade.

Preparation of chitosan nanoparticles

Nanoparticles are prepared by solvent evaporation method using chitosan as a coating material and AC as a core material. Drug and polymer in different ratios were weighed and were dissolved in a suitable organic solvent, DMSO & 2% acetic acid forming an organic phase. This organic solution was then added dropwise to aqueous solution of Lutrol F68 and homogenised at 25000 rpm using Polytron PT 1600 E, Mumbai followed by magnetic stirring for 4hrs. The formed nanoparticles were then recovered by centrifugation (REMI cooling centrifuge, Vasai) at 28000 rpm for 20 min followed by washing with petroleum ether and lyophilized.

Nanoparticle yield

The nanoparticle yield was calculated according to the equation given below.

$$\text{nanoparticle yield} = \left\{ \frac{\text{mass of nanoparticles recovered}}{\text{Mass of polymeric nanoparticles, drug \& other excipients used in formulation}} \right\} \text{X } 100$$

Determination of drug content and entrapment efficiency

Freeze-dried nanoparticles were dissolved in suitable solvent and the amount of drug was measured by UV spectroscopy at 245nm (Shimadzu UV 1800).

$$\text{Drug Content (\%)} = \left\{ \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles recovered}} \right\} \text{X } 100$$

$$\text{Entrapment Efficiency} = \left\{ \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug used in formulation}} \right\} \text{X } 100$$

Measurement of particle size and zeta potential of prepared nanoparticles

Particle size and zeta potential of AC nanoparticles were measured by photon correlation spectroscopy (PCS) using Malvern Zetasizer (Malvern Instruments, UK). The particle size analysis was performed at a scattering angle of 90°C at room temperature. The diameter was averaged from three parallel measurements and expressed as mean ± standard deviation.

Scanning electron microscopic (SEM)

SEM photographs were taken with a scanning electron microscope Model Joel- LV-5600, USA, at the required magnification at room temperature. The Nanoparticles were deposited on a glass disc applied on a metallic stub and evaporated under a vacuum overnight. Before the SEM analysis, the samples were metallized under an argon atmosphere with a 10-nm gold palladium thickness (EMITECH- K550 Sputter Coater, Houston, TX).

Transmission electron microscopic (TEM) study

TEM was used to observe the morphology of AC loaded chitosan nanoparticles. Sample was suitably diluted and a drop was placed on a carbon coated 400 mesh copper grid and dried in an oven at 40^0C for 15 min. The images were taken using a Hitachi Ultra-thin film evaluation system (HD-2300A) in Phase contrast, Z contrast, Secondary Electron (SE) modes.

Differential scanning calorimetry (DSC)

All dynamic DSC studies were carried out on Du Pont thermal analyzer with 2010 DSC module. The instrument was calibrated using high purity indium metal as standard.

The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10 °C/min. The runs were made in triplicate.

Fourier Transform Infrared Radiation Measurements (FT-IR)

FT-IR analysis was carried out for pure drug and for nanoparticles obtained using KBr pellet method on FTIR spectrophotometer type Shimadzu model 8033, USA.

Powder X-ray diffraction (PXRD)

PXRD diffractograms of each pure atorvastatin, chitosan and atorvastatin formulations were recorded using a Siemens Diffractometer D5000 (Siemens, Germany) with Ni-filtered Cu Kα radiation.

In vitro release study

The in vitro release study of AC loaded CN nanoparticles were carried out using USP dissolution apparatus type II (paddle method) at a rotating speed of 50rpm for 7days. Samples were collected at specific time intervals. The amount of drug release was measured by taking absorbance at 245nm using single beam UV spectrophotometer (Shimadzu UV, 1801). The mechanism of drug release was analysed by fitting the drug release data into various release models like first order, zero order, higuchi, korsemeyer-peppas and hixon-crowell cube root law.

Stability studies

The stability of different formulations was evaluated after storing the formulations at room temperature (20^{o}C) or at 4^{o}C in a freeze for a period of 6months. Samples were collected at every 1 month interval and are viewed for sedimentation. Any changes in the particle size and zeta-potential were assessed. Results are given as mean ± S.D. of at least three measurements.

Results and discussion

Formation of polymeric nanoparticles

The atorvastatin loaded chitosan nanoparticles were prepared by solvent evaporation method in four different ratios (1:1, 1:2, 1:3, 1:4). A suspension of chitosan and atorvastatin in DMSO and 2% acetic acid is prepared to form an organic phase. This suspension is then added dropwise to aqueous solution of Lutrol F68. The partition of organic phase into aqueous phase takes place leading to precipitation of polymer on the drug. The subsequent evaporation of organic solvent lead to the formation of AC loaded chitosan nanoparticles.

Determination of nanoparticle yield, drug content and entrapment efficiency

Drug content and entrapment efficiency is mainly affected by drug to polymer ratio (**Table 1**). It was found that increase in the amount of polymer as compared to amount of drug, more was the efficiency of entrapment. Particles with larger size were able to entrap more amount of drug. According to efficiency of yield and entrapment, 1:4 ratio showed better yield compared to other 3 ratios. Increased drug entrapment causes increased yield. It was assumed that the high entrapment of AC was due to its poor aqueous solubility, high binding of drug and polymer in organic solvent and increased polymer ratio. Compared to all the four ratios, 1:1 ratio showed high drug content with low entrapment efficiency leading to enhanced drug leakage and increased initial burst. High drug content shows increase amount of drug on the surface of nanoparticles and hence initial burst release of drug. The increase amount of drug in core of nanoparticles is responsible for prolonged release of drug.

Tabe 1. Yield, drug content and entrapment efficiency of different batches of nanoparticles prepared.

Drug: polymer	Formulation code	Nanoparticles yield	Nanoparticles yield	Drug content	Entrapment efficiency
1:1	F1	53.45±0.324	53.45±0.324	11.76±0.09	61.2±0.578
1:2	F2	65.89±0.289	65.89±0.289	12.05±0.12	75.3±0.325
1:3	F3	74.32±0.452	74.32±0.452	12.46±0.32	87.1±0.11
1:4	F4	88.98±0.521	88.98±0.521	13.25±0.24	98.2±0.04

Particle size and zeta potential of nanoparticles

Particle size is usually used to characterize the nanoparticles, because it facilitates understanding of dispersion and aggregation. Larger surface area and attractive forces between particles accompanies more aggregation of particles. The result showed that the average size of prepared nanoparticles varies from 121.5±1.18 to 150.5±1.24 with a polydispersity index in the range of 0.232±0.012 to 0.364±0.013 as shown in **Table 2**. As the amount of polymer increased, size of the nanoparticles also increased. It was reported that polydispersity index more than 0.5 is indicative to aggregation of particles. The addition of Lutrol F68 aids to reduce aggregation of nanoparticles, which in turn was confirmed by low polydispersity index of particles.

Table 2. Particle size and zeta potential of prepared nanoparticles.

Formulation code	Polydispersity index	Average size (nm)	Zeta potential (mV)
F1	0.324 ± 0.017	121.5 ± 1.18	+16.3 ± 1.6
F2	0.286 ± 0.02	128.5 ± 2.27	+17.6 ± 1.8
F3	0.241 ± 0.016	139.6 ± 1.13	+19.8 ± 2.2
F4	0.232 ± 0.012	150.5 ± 1.24	+20.5 ± 1.6

Zeta potential of prepared nanoparticles was found to range between +16.3+1.6 to +20.5+1.6mV. It was found that higher the zeta potential, less will be the particle aggregation, due to electric repulsion and hence more will be the stability of nanoparticles. It was observed that positive charge appear on nanoparticles surface which is attributed to the presence of the quarternary ammonium groups of chitosan.

Fourier transforms infrared spectroscopy

The IR spectrum showing percentage transmission (%T) versus wave number (cm^{-1}) of Atorvastatin calcium (AC) is

shown in **Fig. 1** with characteristic peaks of aromatic N-H stretching and C=O stretching at 3364.21 cm^{-1} and 1649.81 cm^{-1}, respectively. However formulation exhibited similar peaks but with a neglible shift for aromatic N-H stretching and C=O stretching at 3363.17 cm^{-1} and 1647.67 cm^{-1}. It is evident from the figure that AC in nanoparticles doesn't undergo any chemical reaction with any of the excipients used in the preparation.

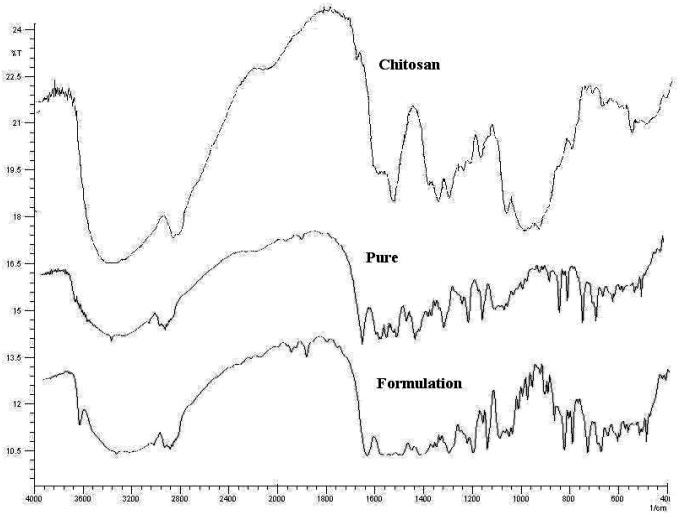

Fig. 1. FTIR of pure AC and AC loaded chitosan nanoparticles formulation.

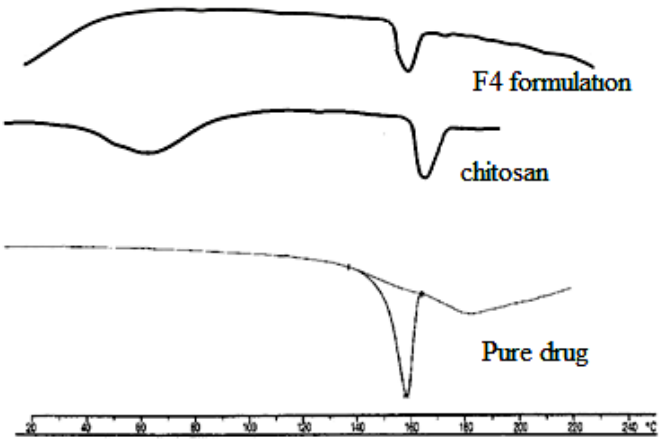

Fig. 2. DSC of pure drug AC, chitosan & F4 formulation.

Differential scanning colorimetry (DSC)

DSC studies of a pure AC clearly showed a sharp endothermic peak at 159^0Cshowing crystalline nature and nanoparticulate formulation showed a peak at 158.2^0C. The slight shift in the peak of formulation is due to incorporation of polymer. Result of DSC studies completely indicates compatibility between drug and polymer used thus ruling out any interaction as shown in **Fig. 2**.

X-ray diffraction study

Characteristic diffraction peaks were observed for Pure atorvastatin as shown in **Fig. 3**. In case of formulation no sharp peaks were observed indicating the absence of crystalline atorvastatin calcium.

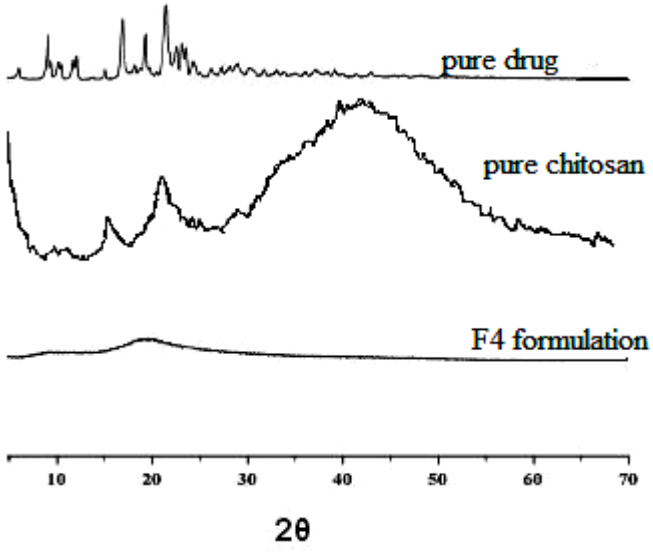

Fig. 3. XRD patterns of pure drug, chitosan and F4 formulation.

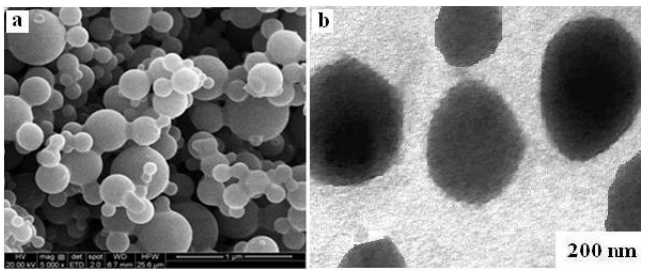

Fig. 4. SEM (a) & TEM (b) of AC loaded CN nanoparticles.

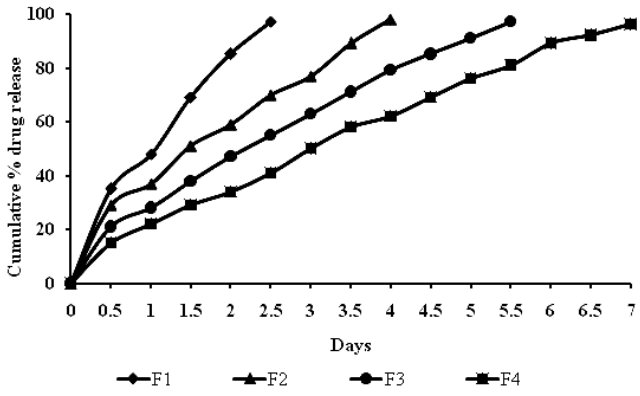

Fig. 5. In-vitro release study of different nanoparticles formulations.

Morphological characterization of prepared nanoparticles

SEM microphotographs are shown in the **Fig. 4(a)** which show surface morphology of nanoparticles which have discrete, smooth surface spherical shape nanoparticles. TEM images shown in **Fig. 4(b)** were in conformity with the SEM studies.

In-vitro release study

In-vitro drug release data for different formulations was shown in **Fig. 5**. It was found that as the amount of polymer

increased, drug release was sustained. It is clearly shown from **Fig. 5**. that drug from the NPs batches were released in controlled manner which is due to the hydration ability of chitosan, which on coming in contact with dissolution media leads to the formation of gelatinous mass which act as retardant material for the drug to diffuse out. Thus a prolonged release of drug is attained. At the end of second day, drug release from F1 was found to be 85.26±1.24, F2 showed 59.32±2.21, F3 showed 47.14±1.25 and F4 showed 34.18±1.26 respectively. It was found that F4 formulation was able to sustain the drug release for more period of time compared to other prepared formulations.

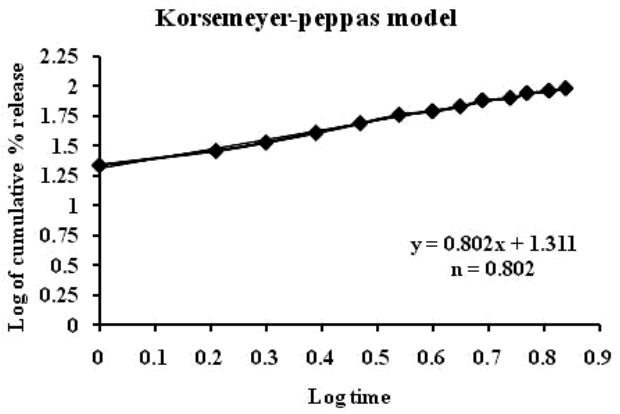

Fig. 6. Korsemeyer-peppas equation data for F4 formulation**.**

In-vitro release kinetics study

The data obtained from dissolution study of F4 formulation was fitted into various release kinetics models and the value of resulting regression coefficient is calculated (**Fig. 6**). The data was also fitted into korsemeyer-peppas model in order to obtain the 'n' value to describe the mechanism of drug release. The 'n' value of 0.802 indicates that the drug release follows anolamous (non-fickian) diffusion mechanism which signifies that the drug release is both diffusion-controlled and swelling-controlled. From these results, we concluded that the release of AC from AC-CN nanoparticles follows first order and diffusion and swelling mechanism.

Stability studies

Stability studies were carried out for F4 formulation in order to check any changes in particle size, zeta potential and polydispersity index. No significant changes were shown in the particle size, zeta potential and polydispersity index of the formulation after storage for 6 months.

Conclusion

Atorvastatin loaded chitosan nanoparticles were successfully prepared by solvent evaporation method in four different ratios 1:1, 1:2, 1:3 & 1:4. According to efficiency of yield and entrapment, 1:4 ratio showed better yield compared to other 3 ratios. The entrapment efficiency was found in the range of 61.2±0.578% to 98.2±0.04%. Average size of prepared nanoparticles varies from 121.5±1.18 to 150.5±1.24 with a polydispersity index in the range of 0.232±0.012 to 0.364±0.013. As the amount of polymer increased, size of the nanoparticles also increased. It was found that higher the zeta potential, less will be the particle aggregation and hence more will be the stability of nanoparticles. DSC and FTIR completely suggest the drug to polymer compatibility. In-vitro release study showed sustained release of drug from F4 formulation upto 7days following diffusion and swelling mechanism. From the present study, it is concluded that atorvastatin calcium loaded chitosan nanoparticles is an effective carrier for the design of controlled drug delivery of poor water soluble drug like atorvastatin calcium.

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