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Original Article

ROSUVASTATIN CALCIUM LOADED CHITOSAN NANOPARTICLES: PREPARATION EVALUATION AND *IN VITRO* RELEASE STUDIES

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ABSTRACT

Objective: The objective of the present study was to develop sustained release biodegradable polymeric nanoparticles of rosuvastatin calcium.

Methods: Nanoparticles were prepared by modified ionotropic gelation method using 3^2 full factorial designs. From the preliminary trials, the constraints for independent variables X1 (concentration. of chitosan) and X2 (concentration. of sodium tripolyphosphate) have been fixed. Factors included concentration of chitosan and sodium tripolyphosphate, have been examined to investigate effect on particle size, encapsulation efficiency, zeta potential, % release, scanning electron microscopy, Fourier transfer infrared study and X-ray diffraction and release study of rosuvastatin calcium nanoparticles. 0

Results: The prepared nanoparticles were white, free-flowing and spherical in shape. The infrared spectra showed stable character of rosuvastatin calcium in the drug-loaded nanoparticles and revealed the absence of drug polymer interactions. The chitosan nanoparticles have a particle diameter ranging approximately 114.5±3.61 to 724±.2.51 nm and a zeta potential-13.12 to-52.63 mV. The *in vitro* release behavior from all the drug loaded batches were found to follow first order and provided sustained release over a period of 10 h. The Zeta potential of all the batches were in the range of-13.12 to-52.63 mv. The release profiles of all batches were very well fitted by Korsmeyer Peppas model.

Conclusion: The best-fit release kinetics was achieved with Korsmeyer peppas model. The release of rosuvastatin calcium was influenced by the drug to polymer ratio and particle size. These results indicate that rosuvastatin calcium nanoparticles could be effective in sustaining drug release for a prolonged period.

Keywords: Rosuvastatin calcium, Biodegradable, Particle size analysis, Ionotropic gelation

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INTRODUCTION

One of the most attractive areas of research in drug delivery today is the design of nanoparticulate systems that are able to deliver drugs to the right place, at appropriate times and at the right dosage. Nanoparticulate delivery systems, are unit the reduction of drug particle to the nano-scale, increases dissolution velocity and saturation solubility, have the potential power to improve drug stability, increase the duration of the therapeutic effect and permit administration through enteral or parenteral administration, which may prevent or reduces the drug degradation and metabolism as well as cellular efflux. Therapeutic efficacy of a drug mainly depends upon bioavailability and, ultimately upon the solubility of drug molecules. Solubility is one of the factors to attain the desired concentration of drug in systemic circulation for pharmacological response to be shown.

Rosuvastatin calcium is the one of the most effective antihyperlipidemic drug and is termed "superstatin". It reduces the low-density lipoprotein-C (LDL-C) by 63% after an administration of 40 mg dose [1, 2]. Rosuvastatin calcium is a poorly water-soluble drug with a low oral bioavailability i.e. 20%. It is classified by biopharmaceutical class II drug. It is a poorly water-soluble 3-hydroxy-3-methyl glutaryl CoA reductase inhibitor. Due to this enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in cholesterol biosynthesis, a potent lipid lowering ingredient and used as a hyperlipidemic agent [3]. The poor solubility of Rosuvastatin calcium affects its dissolution rate and, in turn, its bioavailability. Thus, enhancing the dissolution of Rosuvastatin calcium can lead to improve its oral bioavailability. Accordingly, several nanosization approaches were adopted to improve Rosuvastatin calcium dissolution and bioavailability [4].

The conventional drug delivery system has been characterized by immediate drug release and repeated doses of the drug, which could result in the sudden of dose fluctuation [5]. The main aim of designing nanoparticles as a drug delivery system is very important particle size, surface properties and to deliver pharmacologically active agents at the right place, at the rational rate and dose [6, 7]. Therefore, it's important to introduce effective methods to boost the solubility and dissolution rate of the drug, substantially resulting in its enhanced oral bioavailability. Sustained-release formulations nanoparticles are reported to resolve these problems because of the alteration of its tissue distribution, improving the drug efficacy, reducing the drug toxicity, and prolonging the half-lives in blood [8, 9].

Chitosan (CH) is a natural cationic polysaccharide consisting of (1-4)-2-amino-2-deoxy-D-glucopyranosyl units. It breaks down are slowing the harmless products (amino sugars), which are completely absorbed by the human body [11, 12]. The degree of distillation and molecular weight are the two fundamental parameters that can affect the properties and functionality of chitosan. These properties include solubility, viscosity, reactivity of proteinaceous material coagulation, and heavy metal ion chelation and physical properties of films formulated using chitosan such as tensile strength, elasticity, elongation, and moisture absorption [10]. Chitosan has been used as a nanoparticle material owing to its versatile biodegradability, biocompatibility, a natural origin. Biodegradable nanoparticulate systems have received considerable attention as potential drug delivery vehicles [13].

The objective of the present study was to evaluate the possibility of chitosan nanoparticles as carriers for Rosuvastatin calcium. The challenge was to entrap a hydrophobic molecule into hydrophilic nanoparticle formed by the process of ionotropic gelation technique based on the interaction between the negatively charged of the sodium tripolyphosphate (STPP) and the positively charged amino groups of chitosan. This ionotropic gelation process is due to the formation of inter and intra cross-linkages between/within polymer chains, mediated by the polyanions. The cationic nature of chitosan

has been conveniently exploited for the development of particulate drug delivery systems. More recently, chitosan NPs have been developed based on the ionotropic gelation of chitosan with sodium tripolyphosphate, for drug encapsulation [14].

MATERIALS AND METHODS

Materials

Rosuvastatin calcium was procured as gift sample from MSN Laboratories Pvt. Hyderabad. Sodium tripolyphosphate was purchased from Sigma-Aldrich, Mumbai; Chitosan (high viscosity) was purchased from Central Institute of Fisheries Cochin. All other reagents and chemicals used were of analytical grade.

Experimental methods

Preparation of chitosan nanoparticles

Chitosan nanoparticles containing Rosuvastatin calcium were prepared by ionotropic gelation method. Chitosan was dissolved in 1 % acetic acid solutions at various concentrations to obtain (0.1 %, 0.2 % and 0.3 % i.e. 35 mg, 65 mg and 90 mg) and adjusted the pH 5-6 with 0.1 N sodium hydroxide solutions, while sodium tripolyphosphate was dissolved in deionized water at various concentrations to obtain 0.1 %, 0.15 % and 0.20 % while stirring at 750 rpm. Rosuvastatin calcium 50 mg was dissolved in ethanol/water mixture (1:1 v/v) (1 wt. % tween 80) to obtain clear solution. Rosuvastatin calcium 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 sodium tripolyphosphate solution was added dropwise 0.75 ml/min. under stirring (1000 rpm) at ambient temperature. The formulation was stirred for 30 min so as to remove ethanol content. All the formulations were sonicated at fixed time for 30 min. All experiments were performed in triplicates. Nanoparticles were collected by centrifugation at 9000 rpm for a period of 1 h and supernatant was analyzed using UV-Visible spectrophotometerically 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 [15-17].

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 2-8 °C.

Experimental design

Full factorial design Optimization of rosuvastatin calcium loaded nanoparticles was done by using 3^2 factorial designs. In this design, amount of chitosan (CH) (X1) and amount of STPP (X2) were evaluated as independent variables. Formulated 9 possible combinations using 32 factorial design by taking each independent variable at 3 different levels as shown in table 1. Evaluated fixed responses particles size and % cumulative drug release as Y1 and Y2, respectively.

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

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

Batches	Variable level in coded form		Chitosan (mg)	STPP (mg)	Drug (mg)	Stirring rate (rpm)	Tween 80 (ml)
	Drug: polymer ratios (X2)	STPP (X2)					
RF1	2	2	65	30	200	750	1.5
RF2	3	1	90	20	200	750	1.5
RF3	3	2	90	30	200	750	1.5
RF4	1	2	35	30	200	750	1.5
RF5	1	1	35	20	200	750	1.5
RF6	1	3	35	40	200	750	1.5
RF7	2	3	65	40	200	750	1.5
RF8	2	1	65	20	200	750	1.5
RF9	3	3	90	40	200	750	1.5

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)**
Sodium tripolyphosphate (X2) in %	0.2	0.15	0.1

Characterization of rosuvastatin calcium nanoparticles

Determination of particle size and polydispersity index

The size distribution and polydispersity index (PDI) of the formulations were measured by Dynamic Light Scattering Particle Size Analyzer (Nanoplus 3, Micromeritics USA). The average diameter and a measure of the distribution width (polydispersity) were determined from the particle size distribution data. Polydispersity index varies from 0.0 to 1.0. The usual range of PDI values: 0-0.05 (monodisperse standard).

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 diffractometer). The instrument was equipped with high-speed dispersive LYNXEYE XE-T detector and monochromatic Kβ radiation the sample was scanned over 2θ of 3-50° [18].

Fourier transforms infrared spectroscopy (FTIR)

Infrared spectroscopy analysis was carried out to see the chemical composition of the prepared nanoparticles using FTIR (Nicolet, USA) operating within the frequency range of 400–4000 cm-1 at the absorption mode.

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 was 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 rosuvastatin calcium drug-loaded nanoparticles equivalent to 1 mg was added to 10 ml methanol and phosphate buffer pH 7.4 (1:10) mixtures and stirred continuously for 2 h and so the ultimate colloidal suspensions were ultracentrifuged at 10000 rpm for half an hour. The supernatant was analyzed for drug content by measuring the absorbance at 240 nm using UV spectrophotometer [19].

Entrapment efficiency

The Entrapment efficiency of nanoparticles was determined by the separation of drug-loaded Nanoparticles from the aqueous medium containing non-associated rosuvastatin calcium by

ultracentrifugation at 12,000 rpm at 4 °C for 1 hr. the quantity of rosuvastatin calcium 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 rosuvastatin calcium within the supernatant was measured by UV Spectrophotometer [20, 21]. Entrapment efficiency was then calculated as follows: Entrapment efficiency was calculated by Eq.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 Rosuvastatin calcium nanosuspension were centrifuged at 9000 rpm for 30 min at 15 °C. The obtained sediment was dried and weighed [20]. The percentage yield was calculated by Eq.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 Rosuvastatin calcium 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 [22].

***In vitro* drug release**

The release of Rosuvastatin calcium 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 the drug, which was suspended initially for 2 h in 900 ml of 0.1N HCl buffer of pH 7.4 phosphate buffer upto 10 hr maintained at 37±0.5 °C and 50 rpm. At regular 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 240 nm (Shimadzu UV-1700, Japan) after dilution.

Kinetic modelling

To study the release kinetics from NPs loaded rosuvastatin calcium, the release data of the optimized formulation RF4 nanoparticle batch were fitted to Zero order (Eq. (1), First-order (Eq. (2), Higuchi (Eq. (3) and Korsemeyer-Peppas (Eq. (4). These kinetic modelling were analyzed by using Microsoft Office Excel (2013) to obtain the best fit model for the *in vitro* release [21].

$$Qt = Q_0 + K_0 t \quad \ldots\ldots\ldots (1)$$

$$Log\ C = log\ C_0 - Kt/2.303 \quad \ldots\ldots (2)$$

$$f = t^{1/4}\ K_H\ t^{1/2} \quad \ldots\ldots (3)$$

$$f_t = at^n \quad \ldots\ldots (4)$$

Statistical analysis

Statistical analysis was performed for different formulae by applying factorial design using DOE by Minitab 17. The effect of the polymer and particle size of different formulations on the percent cumulative drug release was found out to optimize the best formulation for further studies. 3D surface plots and contour plots were drawn for supporting the selected ratios and selected formulations.

Stability studies

Stability studies were performed for polymeric nanoparticles to investigate the loss of drug from nanoparticle and change in nanoparticle structure during storage condition. Optimized formulation RF4 nanoparticles were subjected to accelerated stability studies as per ICH guidelines (at 5±3 °C in freeze and at 30±2 °C/65±5% RH) for a period of 3 mo. Further, the samples were observed for particle size, % entrapment efficiency and drug release were carried out at every one-month interval [22].

RESULTS AND DISCUSSION

Particle size and Size distribution

The mean particle size for formulations RF1 to RF9 varied in range of 114±0.16 to 724±2.51 (table 3). 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 rosuvastatin calcium loaded chitosan nanoparticle formulations RF1 to RF9 are in the range of 0.341-0.732 as shown in (table 3). The results of PDI can be simultaneously checked with particle size analysis. A monodisperse sample indicates PI value nearer to 0. However, PDI<1 indicates polydisperse samples. Therefore, PI determination was essential to confirm the size distribution of the particles [14].

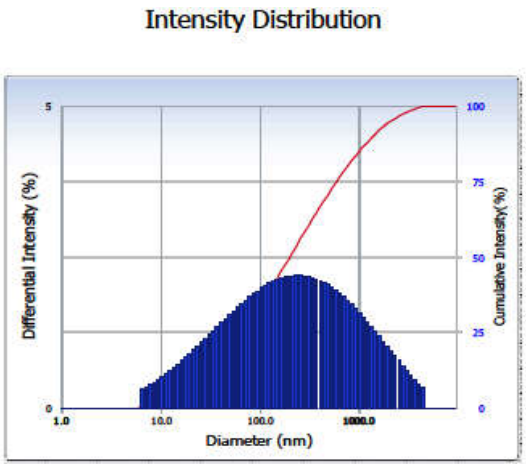

Fig. 1: Particle size analysis of formulation RF4 batch

Table 3: Average particle size, PDI, % product yield and % drug content of nanoparticles

Formulation batches	Particle size (nm)*	% Drug content	Product yield (%)	Poly dispersity index (PDI)
RF1	81.2±3.51	86.05±0.575	54.1±0.30	0.673
RF2	268.3±5.03	66.75±0.492	48.8±0.15	0.448
RF3	581.5±3.60	61.49±0.342	52.7±0.25	0.372
RF4	381.9±3.05	65.87±0.356	53.8±0.80	0.511
RF5	166.9±3.05	68.50±0.132	54.0±0.40	0.732
RF6	724.0±2.51	65.10±0.325	39.0±0.25	0.346
RF7	624.3±3.21	62.36±0.120	42.1±0.65	0.341
RF8	114.5±.3.61	64.12±0.491	47.2±0.71	0.421
RF9	652.0±2.51	65.20±0.545	46.0±0.55	0.408

*Data expressed as (mean±SD (N=3))

Powder X-ray diffraction (PXRD) studies

Drug crystallanity peaks were also detectable in the formulation. Compared with the PXRD patterns of pure rosuvastatin calcium and other formulation, the PXRD patterns of pure drug rosuvastatin calcium has the highest peak (2946) at 2θ range of 24.7, other peaks were (2385) at 2θ range of 20.1, (2294) at 2θ range of 1808, (2094) at 2θ range of 17.9, (1869) at 2θ range of 16.9 and (1599) at 2θ range of 15.9. While RF4 batch formulation has the highest peak (2825) at 2θ range of 9.47, other peaks were (7510) at 2θ range of 13.69, (21329) at 2θ range of 17.28, (10610) at 2θ range of 20.41, (14607) at 2θ range of 21.37,(4157) at 2θ range of 23.42, (3470) at 2θ range of 27.5, (3216) at 2θ range of 34.0, and (2006) at 2θ range of 43.8 indicating the amorphous nature of the drug in the formulation. These results confirm that rosuvastatin calcium is present as a crystalline material. The overlain spectra of rosuvastatin calcium and RF4 batch were shown in fig. 2.

FTIR analysis

The samples that were analyzed by FTIR were plain drug samples, physical mixture of drug and chitosan (1:1) and nanoparticulate formulation (Batch RF4). The IR spectrum of RC exhibited characteristic peaks at 3367.71 cm-1 (carboxylic OH stretch), 2968.45 cm-1 (N-H stretch), 1543.05 cm-1 (C=C stretch), 1435.04 cm-1 and 1377.17 cm-1 (asymmetric and symmetric vibrations of CH3), 1149.57 cm-1 (C-F stretch) and 514.99 cm-1, 596.0 cm-1, (absorption bands of out of plane C=C of benzene ring). These peaks were retained in the overlain IR spectra of both the physical mixture of RC and polymer and in the IR spectrum of optimized formulation (fig. 3). These observations confirm the compatibility between drug and excipients and lack of any chemical interactions.

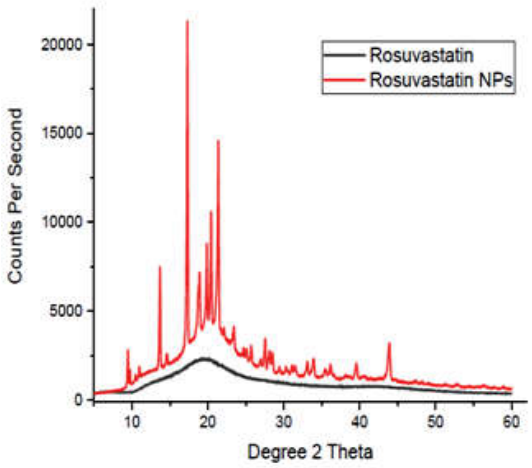

Fig. 2: Overlay XRD pattern of pure drugs and rosuvastatin calcium NPs

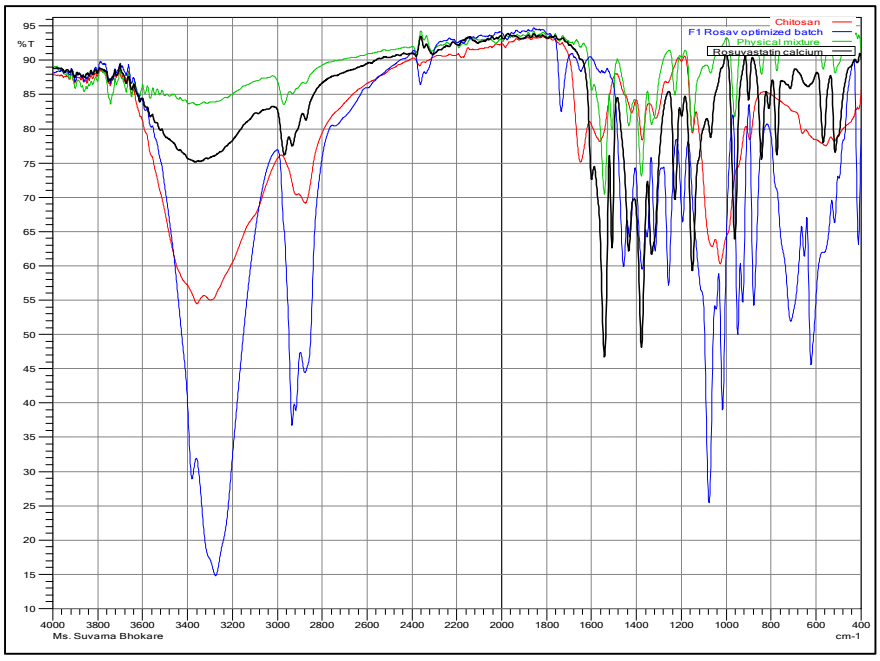

Fig. 3: Overlain FTIR spectra of chitosan (red), rosuvastatin (black), physical mixture (green) and NPs batch (blue)

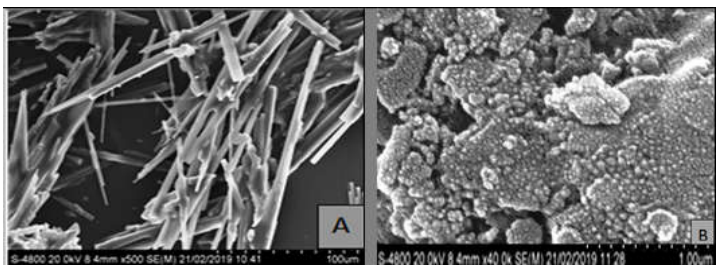

Fig. 4: Scanning electron microscopy of rosuvastatin calcium (A), and optimised batch of RF4 (B)

Scanning electron microscopy study

The exhibited from the SEM of Rosuvastatin calcium pure drug consisted of a mixture of large crystals, indicating its crystalline nature. However, the prepared Rosuvastatin calcium-loaded chitosan NP's of batch RF4 had a spherical shape with relatively uniform size and no drug crystals were present, which was shown in SEM of pure Rosuvastatin calcium. The SEM of pure Rosuvastatin calcium (A), RF4 batches (B) were nearly spherical in shape depicted in fig. 4.

Drug content study

Drug content varies in the range of 61.49±0.28 to 86.05±0.85 % and was determined using the UV spectroscopic analysis at 240 nm. Drug content of optimized RF4 batch was found to be 65.87±0.356 %. Low loss of drug content of all formulation batches during freeze-drying resulted in good recovery.

Entrapment efficiency

The encapsulation efficiency of the nanoparticles was found to vary between 78.6±0.21-98.5±0.30, which shown in table 4. Formulation RF4 shows 98.5±0.035 maximum entrapment efficiency. Based on entrapment efficiency and drug content formulation RF4 was taken as an optimized formulation [19, 20]. The entrapment efficiency not only depends on the chitosan concentration but also depends on the concentration of the surfactant used.

Table 4: %drug entrapment efficacy and zeta potential of nanoparticles (mean±SD; n=3)

Formulation batches	% drug entrapment ±SD*	Zeta potential±SD*
RF1	98.0±0.25	-52.63±0.30
RF2	94.3±0.10	-35.53±0.36
RF3	78.6±0.21	-42.6±0.31
RF4	98.5±0.30	-45.11±0.15
RF5	96.7±0.15	-44.56±0.56
RF6	84.3±0.41	-49.64±0.10
RF7	81.4±0.20	-29.14±0.20
RF8	94.2±0.34	-24.22±0.25
RF9	93.6±0.38	-13.12±0.10

*Data expressed as (mean±SD (N=3))

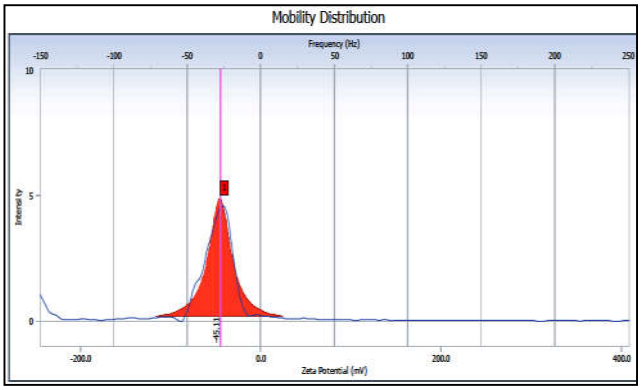

Fig. 5: Zeta potential of optimized formulation (RF4 batch)

Percentage yield

Percentage yield was found to be 39±0.605 % to 54±0.860 % for formulation RF1 to RF9 (table 3). Percentage practical yield depends on the concentration of polymer added, as the concentration of polymer increases, there is increases in the % yield. % yield obtained is 53.8±0.734% for formulation RF4 batch.

Zeta potential

Zeta Potential of the formulations was found, ranging from-13.12±0.010to-52.63±0.030 (table 4). Zeta potential indicates the stability of the dispersed particles in the dispersion medium. High zeta potential shows high repulsion between the particles and particle aggregation is less likely to occur. A negative value of the zeta potential shows that formulations have good stability and dispersion quality [20].

***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 percentage drug release of Rosuvastatin calcium in Phosphate buffer pH 7.4 medium of RF1 to RF9 batches were shown in fig. 6. Cumulative percentage drug released for RF1, RF2, RF3, RF4, RF5, RF6, RF7, RF8, and RF9 after 10 h were found to be 99.50%, 80.55% 79.97%, 88.49%,80.39%, 89.41%,86.12%,90.02% and 90.73% respectively. It was apparent that *in vitro* release of Rosuvastatin calcium showed a very rapid initial burst and then followed by a very slow drug release. Evaluation of the release profiles of pure drug showed that almost all the Rosuvastatin calcium was released immediately during first 3 h it has reached 36.51±0.02%, suggesting that the developed nanoparticles can be used as an important platform for sustained drug release which is shown in fig. 7, which would contribute to lower dosing frequency. Finally, it can be concluded that the different drug release rates may be attributed to different sizes of the nanoparticles. It is expected as the particle size of chitosan nanoparticle is smaller, their surface area will be more and the drug release is faster [24].

Release kinetics

It was found that the *in vitro* drug release of RF4 was best explained by Zero-order, as the plots showed the highest linearity (R2 = 0.982), followed by Higuchi's equation (R2 = 0.981), and First-order (R2 = 0.843). The corresponding plot (log % cumulative drug release vs. log time) for the Korsmeyer-Peppas equation (R2 = 0.960) indicated good linearity. The release exponent 'n' was found to be 0.631 [27]. Upon fitting the *in vitro* release data into different equations, the optimized formulation showed Zero-order release as it has high linearity, followed by Higuchi's equation and First-order as shown in table 5. The value of release component 'n' obtained using the Korsmeyer-Peppas equation is 0.621 which appears to

indicate the anomalous, non-Fickian diffusion suggesting that the drug release is controlled by more than one process i.e. superposition of both phenomena, the diffusion-controlled and swelling-controlled release [25].

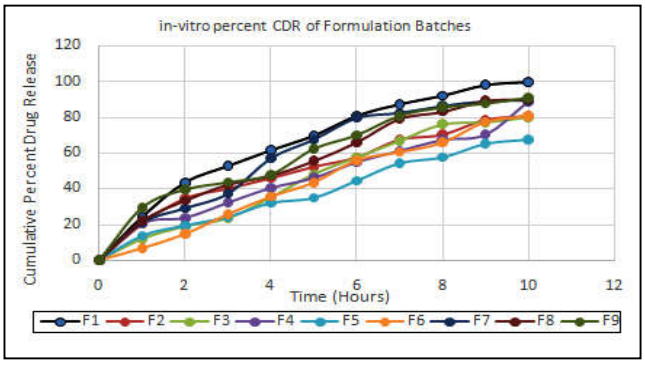

Fig. 6: Comparative *in vitro* drug release profile of RF1 to RF9

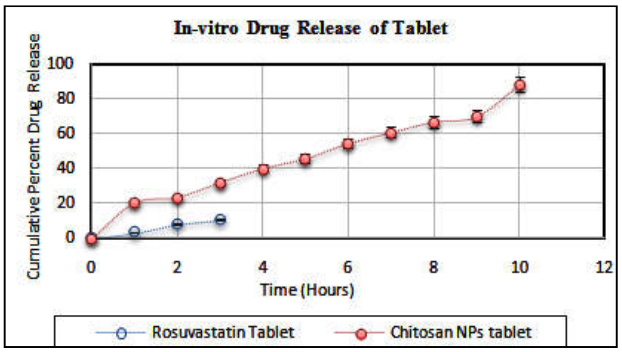

Fig. 7: Comparative *in vitro* drug release profile of rosuvastatin calcium-chitosan NPs tablet and pure drug tablet

Full factorial design

The effect of independent variables chitosan and STPP was analyzed by response surface plots using Minitab software 17. Fig. 8 and 9 shows responses for particles size (Y1) and % cumulative drug release (Y2) by the effects of independent variables. Observed coefficient values for the drug-loaded nanoparticles are represented in Eq. (5) and Eq. (6) [24].

$$Y1=403.6+20.7\ X_1+42.87\ X_2+37.03X_1X_2-36.53X_1^2-11.97\ X_2^2 \text{ ....} (5)$$

$$Y2 =85.20+7.787\ X_1-5.782\ X_2-5.518\ X_1X_2 \text{ ....} (6)$$

The effect of the particle size and chitosan was thoroughly proven by using full factorial design. From the above-generated surface response plots illustrated that as the concentration of chitosan increases, the value of the dependent variable, particle size increases and as the concentration of sodium tripolyphosphate increases the value of the dependent variable, particle size also increases. Similarly the response surface plots for % drug release shows negative effects of independent variable, chitosan concentration increases the % drug release was decreased and as the concentration of sodium tripolyphosphate increase the % drug release was also decreased. The best was selected based on the statistical data supportive evaluation. In the graph, it can be seen that the polymer ratio of 1 has correlated with the highest percent cumulative drug release and also the lowest particle size. Contour plot further strengthens the evidence that indicates the various colored regions with percent cumulative drug release [23].

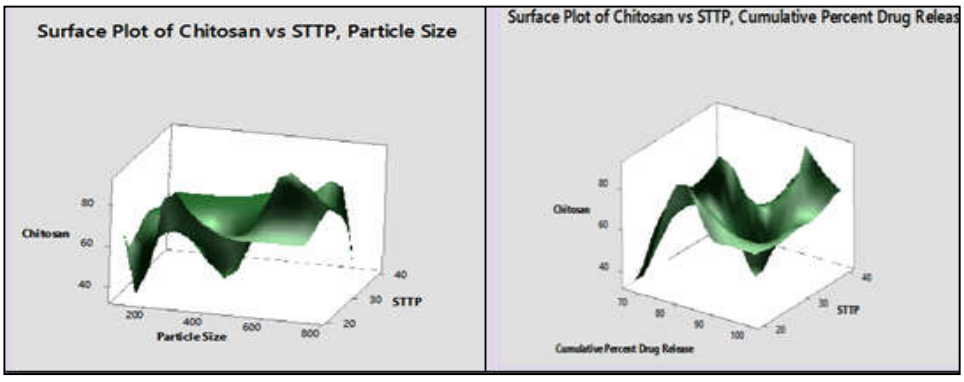

Fig. 8: Response surface plot showing effect of factorial variables on particle size and % cumulative drug release

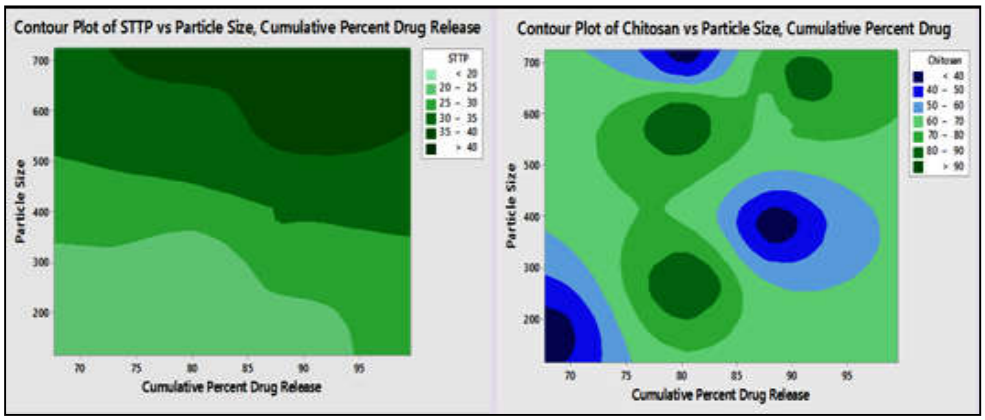

Fig. 9: Contour plot of chitosan and sodium tripolyphosphate cumulative drug release and particle size

Stability studies

Stability studies results indicate that after the 1, 2 and 3 mo accelerated stability studies reveals no morphological changes but particle size increased, percentage entrapment efficiency and % cumulative release decreased in freeze-dried nanoparticles. Stability studies were carried out on the optimized formulation RF4 as per ICH guidelines for 90 d.

By comparing this data with initial data it was observed that there was a slight decrease in the percentage entrapment efficiency and increase in particle size due to degradation of polymer and aggregation of particles (table 5). There was not much change in the cumulative percent drug release. Formulation stored at (5±3 °C) showed better stability as compared to the formulation stored at 30±2 °C/65±5% RH. Thus we may conclude that the drug does not undergo degradation on storage. Rosuvastatin calcium-chitosan nanoparticles can be successfully prepared by the ionotropic technique. *In vitro* release study showed that chitosan nanoparticles showed sustained release of drugs for a prolong period of time [26].

Table 5: Stability data

Freeze dried optimized batch (RF4)							
			Final at 5±3 °C		**Final at 30±2 °C/65±5%RH**		
	Initial	**30 d**	**60 d**	**90 d**	**30 d**	**60 d**	**90 d**
Particle size (nm)	381.9±2.10	384.5±3.81	388.2±9.12	394.3±5.24	393.4±5.21	398.9±0.93	402.7±3.81
% Entrapment efficiency	98.5±0.30	95.6±0.10	93.6±0.21	89.8±0.43	93.7±0.34	91.4±0.21	84.8±0.52
% Drug release	88.4±0.15	86.7±0.14	85.5±0.28	81.3±0.30	82.4±0.22	81.7±0.25	79.2±0.31

*Data expressed as (mean±SD (N=3))

CONCLUSION

A total of nine formulations (RF1-RF9) were formulated by varying the concentration of chitosan (0.1%, 0.2% and 0.3%) and sodium tripolyphosphate (0.1%, 0.15% and 0.20% using ionotropic technique and the various parameters were evaluated. The sizes of nanoparticles were in nanosize range, spherical and discrete, particle size increase with an increase in polymer concentration. Entrapment efficiency increased with increasing the polymer concentration to a certain level and then decreased. RF4 formulation was considered as optimized formulation based on drug release higher entrapment efficiency (98.5±0.30 %), drug content and good Zeta potential of-45.11 mV. *In vitro* release study showed initial burst effect, and then followed by a very slow drug release. Evaluation of the release profiles of pure drugs showed that almost all the Rosuvastatin calcium was released immediately during first 4 h, suggesting that the developed nanoparticles can be used as an important platform for sustained drug release up to 10 h which would contribute to lower dosing frequency. The oral bioavailability of Rosuvastatin calcium–chitosan nanoparticles was improved by than that of pure drug. From the stability studies, it can be found that 5±30 °C is the ideal temperature for the storage of nanoparticles. The concentrations of polymer and crosslinking agent are the important factors in the development of rosuvastatin calcium nanoparticles. In conclusion, this work confirmed that the modified ionic gelation method is offers an interesting potential for the delivery of hydrophobic drugs with chitosan nanoparticle.

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ABBREVIATION

SEM: Scanning electron microscopy; XRD: X-Ray Diffraction; PDI: Polydispersity index; FTIR: Fourier transforms infrared spectroscopy.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

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