ORIGINAL ARTICLE

Application of quality by design approach to optimize process and formulation parameters of rizatriptan loaded chitosan nanoparticles

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ABSTRACT

The purpose of present study was to optimize rizatriptan (RZT) chitosan (CS) nanoparticles using ionic gelation method by application of quality by design (QbD) approach. Based on risk assessment, effect of three variables, that is CS %, tripolyphosphate % and stirring speed were studied on critical quality attributes (CQAs); particle size and entrapment efficiency. Central composite design (CCD) was implemented for design of experimentation with 20 runs. RZT CS nanoparticles were characterized for particle size, polydispersity index, entrapment efficiency, *in-vitro* release study, differential scanning calorimetric, X-ray diffraction, scanning electron microscopy (SEM). Based on QbD approach, design space (DS) was optimized with a combination of selected variables with entrapment efficiency > 50% w/w and a particle size between 400 and 600 nm. Validation of model was performed with 3 representative formulations from DS for which standard error of − 0.70–3.29 was observed between experimental and predicted values. *In-vitro* drug release followed initial burst release 20.26 ± 2.34% in 3–4 h with sustained drug release of 98.43 ± 2.45% in 60 h. Lower magnitude of standard error for CQAs confirms the validation of selected CCD model for optimization of RZT CS nanoparticles. *In-vitro* drug release followed dual mechanism via, diffusion and polymer erosion. RZT CS nanoparticles were prepared successfully using QbD approach with the understanding of the high risk process and formulation parameters involved and optimized DS with a multifactorial combination of critical parameters to obtain predetermined RZT loaded CS nanoparticle specifications.

Key words: Central composite design, chitosan, design space, ionic gelation, quality by design, rizatriptan

INTRODUCTION

Rizatriptan (RZT) is a potent and selective 5-hydroxytryptamine 1B/1D receptor agonist for the treatment of acute migraine headaches in adults and it is considered better than the traditional triptans for the treatment of an acute migraine attack.[1] The bioavailability of RZT is about 45% due to first-pass metabolism and half-life is of 2–3 h.[2] Nasal drug delivery used to achieve longer retention of drug and brain targeting through the olfactory mucosa of the nasal cavity. Different approaches implemented includes bioadhesive gel, *in-situ* gel,[3] dry powder,[4] microparticles,[5] nanoparticles[6,7] using biodegradable and/or nonbiodegradable-natural (alginate, chitosan [CS], starch, dextran)[4] and/or synthetic (carbopol, poloxamer, PLGA, PLA, poly-caprolactone)[4] polymers. Nanoparticles have better advantages as it might cross the nasal mucosal epithelium intact, overlaying the mucosal associated lymphoid tissue, which can provide sustained release for drug for long period of time better than that of microspheres and other dosage forms.[8,9]

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Chitosan is nontoxic, biodegradable, biocompatible, hydrophilic, and it has antibacterial activity, protein affinity, positive polyanions and also approved as GRAS by the USFDA. Furthermore, it has been reported that the bioavailability of drug, proteins, and vaccines was raised by opening the tight junctions of epithelial cell layers and increasing the retention time of drug delivery locally using CS as polymer.[7,10-12] Ionic gelation method involves ionic cross-linking of amino groups of CS and phosphate groups of tripolyphosphate (TPP), to form spontaneous gelation in aqueous solution.[13,14] Many properties of CS nanoparticles such as surface morphology, entrapment, and release characteristics are highly related to formulation and process parameters, such as concentration and molecular weight of CS, pH and concentration of cross-linker agent, curing time, stirring time, and speed.[7,15] Quality by design (QbD) approach can be applied for better understanding of the process and formulation variables, which can lead to better and robust quality into the product assuring the target quality product profile. Based on risk assessment of process and formulation variables, design of experimentation (DoE) study need to conduct on critical parameters to establish certain ranges for critical parameters within certain range to obtain design space (DS).[16-20]

In present study, we aimed to develop RZT CS nanoparticles formulation using QbD approach to understand the effect of process and formulation variables on critical quality attributes (CQAs) of RZT CS nanoparticles and to establish DS with accepted Quality Target Product Profile (QTPP).

MATERIALS AND METHODS

Rizatriptan, CS was supplied as gift sample from Cipla Ltd. (Mumbai, India) and Central Institute of Fisheries Technology, Cochin, India (medium molecular weight, 95% deacetylated) respectively. All other excipients, solvents were of pharmaceutical and analytical grade.

Formulation of rizatriptan chitosan nanoparticles

Nanoparticles were prepared using modified ionic gelation method,[21,22] where CS was dissolved in 1% acetic acid solution to a various concentration and TPP was dissolved in distilled water with various concentrations, based on the results of preliminary study. RZT was uniformly dispersed in TPP solution and this solution was added drop-wise to CS solution under continuous stirring at room temperature. RZT CS nanoparticles formed based on the principle of electrostatic attraction between positively charged primary amino groups on CS chains and charged polyanions (TPP). RZT CS nanoparticles were centrifuged at 6000 rpm for 30 min (Remi R-88). The supernatant liquid was separated and nanoparticles were redispersed in PBS at pH 6.8 and ultrasonicated for 5 min to disaggregate the CS nanoparticles. Three nanoparticles optimized batches, were redispersed in deionized water containing 1% w/v mannitol as cryoprotectant, and lyophilized primarily for 12 h at −20°C and secondary for 36 h at − 54°C with vacuum pressure of 0.001 mbar using Christ freeze-dryer (Christ Alpha 1-2 LD). Nanoparticles were collected, kept in glass vials and stored in dessicator.

Optimization of rizatriptan chitosan nanoparticles

The QTPP is an essential element of a QbD approach and forms the basis of design of the product. QTPP for RZT CS nanoparticles were presented in Table 1 considering the formulation and process to develop nanoparticles.

Risk assessment

Initial risk assessment of process parameters and formulation components of RZT CS nanoparticles was performed to identify critical parameters and components having a high-risk of impacting the drug product CQAs. High-risk parameters to the CQAs of RZT CS nanoparticles were further evaluated by performing experiments as per the DoE to reduce the risk.

Optimization using central composite design

Based on risk assessment and preliminary studies, optimization of three high-risk parameters at more than 3 levels needed to identify main and interaction effect of selected parameters on responses with minimum number of runs. Central composite design (CCD) was selected for RZT CS nanoparticles with % CS (X1), % TPP (X2), and stirring speed (X3) at 3 levels and 2 more levels as star points ($-\alpha$, $+\alpha$) was selected as shown in Table 2. The obtained RZT CS nanoparticles suspensions were further evaluated for particle size, entrapment efficiency.

Optimization of design space and validation of model

Design space was generated by setting acceptance criteria to CQAs. The 3 optimization formulations were prepared within DS and compared with predicted results of the responses and percentage error was calculated to validate the selected model.

Table 1: QTPP for RZT CS nanoparticles

Profile component	Target	Justification
Dosage form	Nanoparticles	Novel dosage form for targeted drug delivery
Dosage design	Sustained release nanoparticles	For long-term treatment of RZT
Particle size (nm)	350-650	Narrow distribution
Entrapment efficiency (%)	>50	Higher entrapment is better for the nanoparticulate dosage form
Drug release (h)	>48	To achieve sustained drug release for long period of time

RZT: Rizatriptan, QTPP: Quality target product profile, CS: Chitosan

Entrapment efficiency

The entrapment efficiency was determined by measuring the amount of unentrapped drug in the supernatant recovered after centrifugation (Remi R-88) of prepared RZT loaded CS nanoparticles by UV spectrophotometer at 227 nm wavelength. Results obtained were reported in triplicates.

$$\text{Entrapment efficiency}(\%) = \frac{\text{Total amount of drug} - \text{Total amount of unentrapped drug}}{\text{Total amount of drug}} \times 100$$

Characterization of rizatriptan chitosan nanoparticles

Nanoparticles were analyzed for particle size, polydispersity index (PDI) using particle size analyzer (Malvern ZS 90); each time with fresh polystyrene cuvette and sample (n) = 3 as per the SOP.

Morphology of RZT CS nanoparticle was observed and photographed using SEM (JFC-1100, JEOL, University of Pune). Nanoparticles were coated with gold (<20 nm thick) using sputter for 5 min at 20 mA, an accelerating voltage of 5 kV, a working distance of 10 mm, at argon atmosphere in a high-vacuum evaporator at × 20,000.

Differential scanning calorimetric (DSC) carried out with a thermal analysis data system (DSC 2920, TA Instruments, Alzenau, Germany). The endothermic melting temperature for RZT, CS, physical mixture of RZT/CS, and RZT CS nanoparticles was determined. 10 mg of samples were scanned from 20°C to 270°C at a rate of 10°C/min and thermograms were recorded.

Powder X-ray diffraction (XRD) patterns were performed using X-ray diffractometer (a Philips 171) with a copper target and nickel filter was used to obtain XRD result for the

Table 2: Central composite design matrix for RZT CS nanoparticles

Experiments number	X1: CS (% w/v) (mg)	X2: TPP (% w/v) (mg)	X3: Stirring speed (rpm)	Y1: Particle size (nm)	Y2: Entrapment efficiency (%)
1	2.00 (800)	1.00 (200)	600.00	385.50	27.16
2	4.00 (1600)	1.00 (400)	600.00	330.50	33.58
3	2.00 (800)	3.00 (200)	600.00	480.10	52.94
4	4.00 (1600)	3.00 (400)	600.00	371.60	61.54
5	2.00 (800)	1.00 (200)	1200.00	315.60	24.82
6	4.00 (1600)	1.00 (400)	1200.00	282.30	32.61
7	2.00 (800)	3.00 (200)	1200.00	404.60	44.28
8	4.00 (1600)	3.00 (400)	1200.00	348.10	55.81
9	1.32 (528)	2.00 (132)	900.00	380.90	43.58
10	4.68 (1872)	2.00 (468)	900.00	304.80	50.46
11	3.00 (1200)	0.32 (300)	900.00	423.40	20.45
12	3.00 (1200)	3.68 (300)	900.00	621.30	67.15
13	3.00 (1200)	2.00 (300)	395.46	588.40	51.62
14	3.00 (1200)	2.00 (300)	1404.54	297.80	39.58
15	3.00 (1200)	2.00 (300)	900.00	378.40	55.67
16	3.00 (1200)	2.00 (300)	900.00	384.90	56.41
17	3.00 (1200)	2.00 (300)	900.00	356.20	58.26
18	3.00 (1200)	2.00 (300)	900.00	364.75	57.66
19	3.00 (1200)	2.00 (300)	900.00	360.91	58.1
20	3.00 (1200)	2.00 (300)	900.00	367.30	51.26

For all batches rate of addition of TPP and RZT solution to chitosan in acetic acid solution - 5 mL/min; CS: TPP ratio - 4:1; Volume of batch varied to maintain CS % and TPP % and their ratio; Temperature – 25±20°C (Cryostat bath). RZT: Rizatriptan, CS: Chitosan, TPP: Tripolyphosphate

Table 3: Initial risk assessment for RZT CS nanoparticles

CMAs/CPPs	CQAs				
	Acetic acid (% v/v)	CS (% w/v)	TPP (% w/v)	Stirring speed (rpm)	Rate of addition of TPP solution (mL/min)
Particle size	Low	High	High	High	Low
Entrapment efficiency	Medium	High	High	Low	Low
Drug release %	Low	High	Low	Medium	Medium

RZT: Rizatriptan, CS: Chitosan, CMAs: Critical material attributes, CPPs: Critical process parameters, TPP: Tripolyphosphate, CQAs: Critical quality attributes

samples. Powder were mounted on aluminum stages with glass bottoms and smoothed to a level surface. XRD pattern was measured from 10 to 500 at 2θ using a step increment of 0.1^0 (2θ) and a dwell time of 1 s at each step and XRD patterns were recorded.

In vitro drug release

The *in-vitro* drug release studies were performed using following method.[22,23] RZT CS nanoparticles were suspended in PBS at pH 7.4 and free RZT centrifuged to collect nanoparticles and resuspended in PBS. The nanoparticle was poured in dialysis tube and tied at both end (HiMedia, Mumbai) with cut-off of 12 kDa and kept in 50 ml PBS at pH 7.4 and placed in bath shaker at 37°C for 60 h. An aliquot of release medium withdrawn using syringe at different time interval including 1, 3, 6, 9, 12, 24, 36, 48, 60 h and replaced with equal amount of fresh release medium. The concentration of RZT was quantified using UV/VIS spectrophotometer at 227 nm.

Stability studies

Optimized formulations from DS were subjected to accelerated stability testing as per ICH guidelines at a temperature 40 ± 2°C and RH 25 ± 5% for a period of 3 Months. Nanoparticles were filled in sealed glass vials and kept in stability chamber (specifications - capacity 200 L, temperature 10–60°C, humidity 40–95%) and were analyzed for particles size, zeta potential, and entrapment efficiency as per SOP.

Statistical evaluation

All obtained data were analyzed by the Student's *t*-test (α = 0.05) and calculated values were expressed as their mean ± standard deviation for statistical significance.

RESULT AND DISCUSSION

Rizatriptan CS nanoparticles were prepared successfully using ionic gelation method with certain advantages as compared to other solvent based preparation methods due to the absence of nontoxic solvent, higher yield, better entrapment, and easy method. Risk assessment, an element of QbD approach was implemented to get a detailed insight of critical material attributes and critical process parameters on the CQAs based on the few preliminary experiments and knowledge space as shown in Table 3. Based on different criteria's such as type of study, that is, optimization, nature (process and formulation parameters), and number (3) of critical parameters and their levels (>2 levels), type of effect to know (main and interaction effect), feasibility of time and cost involvement, CCD (rotatable α ± 1.4142) was selected as compared to factorial design, Taguchi, placket Burman designs.[16-20] 20 experiments were conducted at 5 levels for each factor (−α, −1, 0, +1, +α) which provide excellent predictability, shield to missing data and giving main and interaction effects of critical parameters on response.

From preliminary screening, experimental levels of critical parameters were established between 2 and 4% of CS, 1–3% of TPP and 600–1000 rpm, stirring speed. 100% fraction of design space indicates that the design will provide a fitted response surface that is precise throughout the region of interest at 99% TI [Figure 1].

Response surface analysis for particle size and entrapment efficiency

Particle size histogram shows single peak with good intensity and narrow PDI of 0.377 [Figure 2]. Particle size was significantly affected by % TPP and stirring speed as compared to % CS, which can be depicted from "$P > F$" value from ANNOVA Table 4. All batches showed higher particle size values; with increase in % TPP and decrease in particle size with increase in stirring speed [Figure 3a] whereas model follow quadratic polynomial equation [Table 5] (Model $P > F < 0.05$). The effect of % TPP on nanoparticles size can be justified as, at augmented level of % TPP and CS, more electrostatic attraction between negatively charged TPP and the positively charged amino groups of CS, this could make CS chains too close together and forming multilayer around itself resulting into increase in particle size of matrix nanoparticles.[23] Further, the effect of stirring speed on particle size observed was prominent might be due to breaking of CS-TPP complex to smaller particle at higher stirring speed.

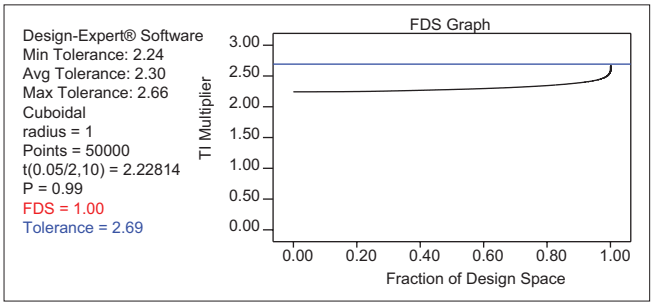

Figure 1: Fraction of design space plot from central composite design of rizatriptan loaded chitosan nanoparticles

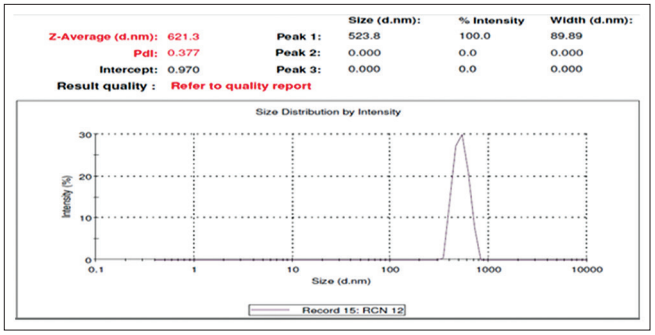

Figure 2: Particle size histogram of rizatriptan chitosan nanoparticles (RCN 12)

Entrapment efficiency of RZT loaded CS nanoparticles was mainly augmented by % TPP and % CS in the formulation (67.15% at 3% CS, 3.68% TPP at 900 rpm) ($P > F < 0.05$). Higher concentration of TPP leads to formation of strong poly-electrolytic matrix with positively charged amino group of CS enhancing drug entrapment efficiency as shown in three-dimensional (3D) surface plot [Figure 3b], the same results were reported by Luo *et al*.[24] From the polynomial equation, it can be concluded that, the interactive effect of variables was relatively low as compared to main effect of variables on entrapment efficiency. Whereas stirring speed had significant effect on entrapment efficiency as it was 52.94% at low level of stirring speed and reduced to 44.28% at higher level of stirring speed but less as compared to other variables presented in 3D surface plot [Figure 3c].

In-vitro drug release

The effect of % CS and % TPP on drug release from nanoparticles is shown in Figure 4. RZT release was decreased with increase in both % CS and % TPP, because of strong poly-electrostatic attraction between polymer and polyanions which leads to the formation of strong matrix. Nanoparticles exhibited almost 100% drug release

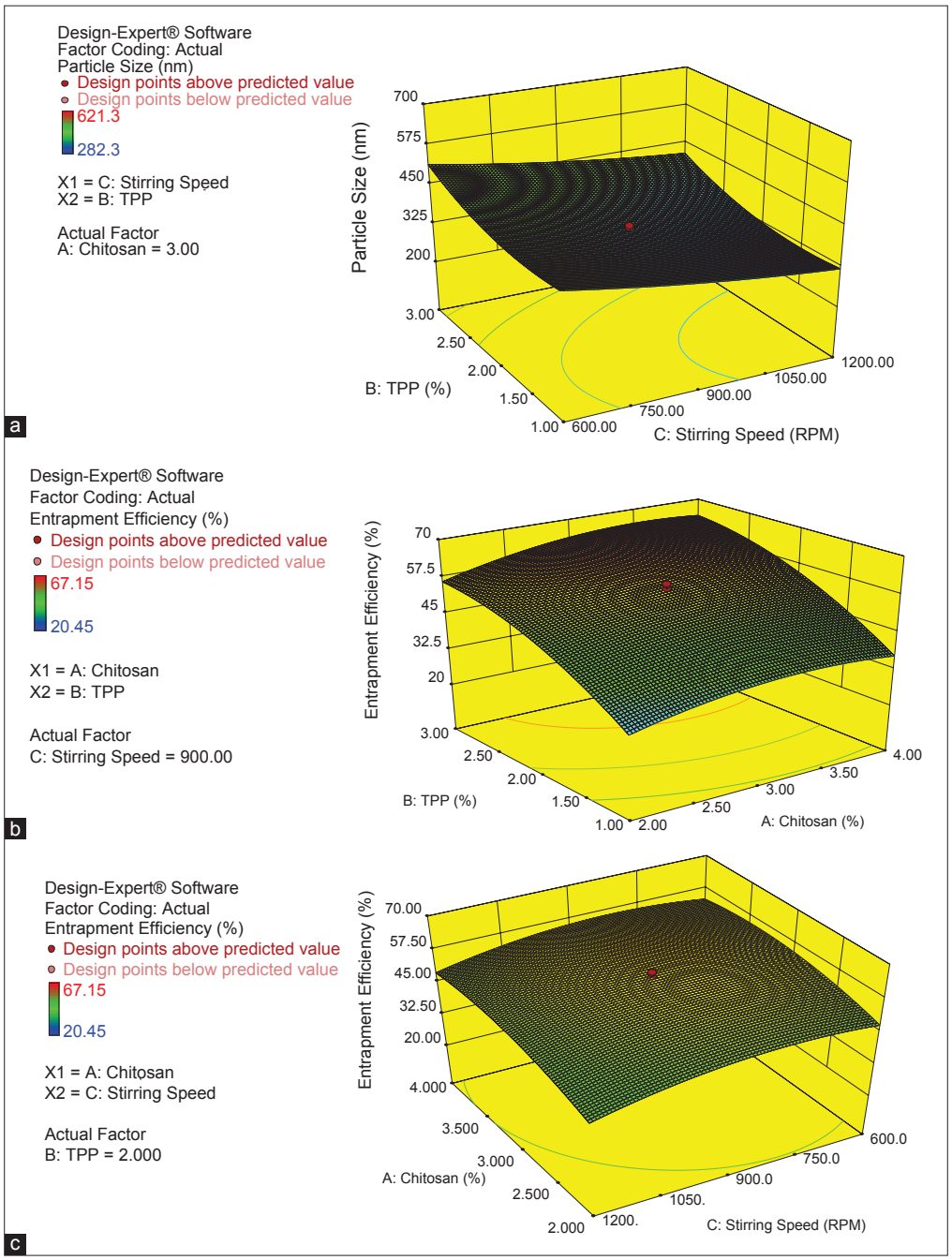

Figure 3: Three-dimensional surface plot of (a) particle size, (b and c) entrapment efficiency of rizatriptan chitosan nanoparticles

in 9–12 h at low level of % CS and % TPP. Whereas at 3.68% (+α) TPP and 3% CS drug was retarded maximum up to 60 h as compared to other combination. The drug release profile exhibit the biphasic patterns, initial 20–25% burst release of drug in 3–4 h might be attributed to that of surface bound and superficial embedded drug in CS nanoparticles, and later sustained drug release up to 60 h was observed due to strong cross-linking complex of CS and TPP resulting into denser particle which retards RZT release due to swelling of CS which lowers the membrane permeability for 4RZT. The similar pattern was observed in previous studies.[23,24]

Table 4: ANNOVA for responses of RZT loaded CS nanoparticles

Source	Sum of squares	df	Mean square	*F*	*P* *P>F*
Particle size - quadratic model					
Model	1.117E+005	9	12406.07	3.82	0.0241
X1	10645.04	1	10645.04	3.28	0.1002
X2	28449.91	1	28449.91	8.77	0.0143
X3	36479.45	1	36479.45	11.24	0.0073
X1X2	735.36	1	735.36	0.23	0.6443
X1X3	678.96	1	678.96	0.21	0.6571
X2X3	45.60	1	45.60	0.014	0.9080
X12	9054.35	1	9054.35	2.79	0.1258
X22	21247.31	1	21247.31	6.55	0.0284
X32	1552.19	1	1552.19	0.48	0.5049
Residual	32449.89	10	3244.99		
Lack of fit	31858.88	5	6371.78	53.91	0.0002
Pure error	591.02	5	118.20		
Cor total	1.441E+005	19			
Entrapment efficiency - quadratic model					
Model	3258.14	9	362.02	35.36	<0.0001
X1	154.34	1	154.34	15.08	0.0030
X2	2240.92	1	2240.92	218.90	<0.0001
X3	105.45	1	105.45	10.30	0.0093
X1X2	4.38	1	4.38	0.43	0.5278
X1X3	2.31	1	2.31	0.23	0.6449
X2X3	15.35	1	15.35	1.50	0.2489
X12	223.30	1	223.30	21.81	0.0009
X22	371.14	1	371.14	36.25	0.0001
X32	283.89	1	283.89	27.73	0.0004
Residual	102.37	10	10.24		
Lack of fit	67.66	5	13.53	1.95	0.2407
Pure error	34.71	5	6.94		
Cor total	3360.52	19			

RZT: Rizatriptan, CS: Chitosan, df: Degree of freedom

X-ray diffraction

X-ray diffraction graph for RZT, CS, TPP and RZT loaded CS nanoparticles is shown in Figure 5. XRD of RZT at 2θ shown crystalline nature with sharp peaks at 17°, 19°, 21°, 23°, and 25.5°. CS shows two peaks at 2θ of 10°, 21° exhibit the amorphous nature, whereas TPP shows some sharp characteristic peaks at 2θ of 19°, 25°, 34°, and 35° and nanoparticles formulation shows peaks at same degree as of pure drug RZT but with almost half

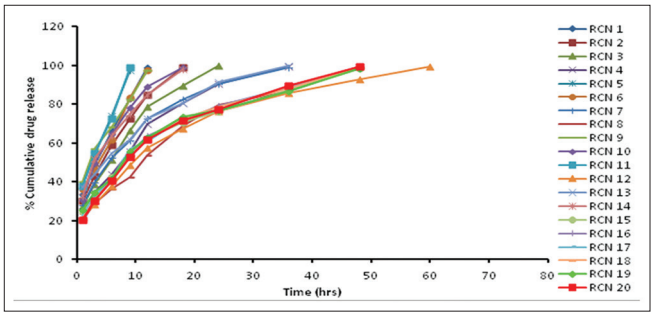

Figure 4: *In-vitro* drug release of rizatriptan chitosan nanoparticles (*RCN – RZT CS nanoparticles)

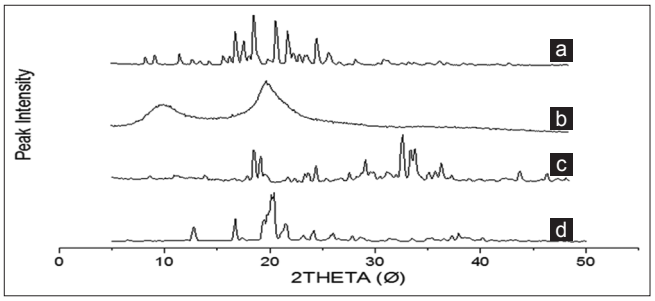

Figure 5: X-ray diffraction pattern (a) rizatriptan (RZT), (b) chitosan (CS), (c) tripolyphosphate, and (d) RZT CS nanoparticles

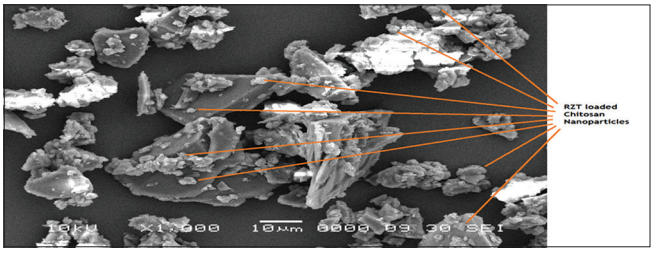

Figure 6: Scanning electron microscopy of rizatriptan chitosan nanoparticles

Table 5: Polynomial equations for CQAs of RZT loaded CS nanoparticles

Response	Polynomial equation
Particle size	$371.18-27.92X_1+45.64X_2-51.68X_3-9.59X_1X_2+9.21X_1X_3+2.39X_2X_3-25.07X_1^2+38.40X_2^2+10.38X_3^2$
Entrapment efficiency	$56.33+3.36X_1+12.81X_2-2.78X_3+0.74X_1X_2+0.54X_1X_3-1.38X_2X_3-3.94X_1^2-5.07\ X_2^2-4.44X_3^2$

CQAs: Critical quality attributes, RZT: Rizatriptan, CS: Chitosan

intensity as compared to pure drug might be due to matrix formation with polymer. This indicates that there was no any interaction of drug and polymer in RZT CS nanoparticles.

Scanning electron microscopy

Freeze – dried nanoparticles with mannitol as a cryoprotectant in powder formulation appears to be slightly spherical and rough [Figure 6] which could be due to CS as a natural polymer with less elasticity compared to synthetic polymer. SEM photographs showed CS nanoparticles adhered to mannitol particles, further optimization of freeze drying process may lead to free flowing nanoparticles.

Differential scanning calorimetry

Differential scanning calorimetric thermogram of pure drug RZT [Figure 7] showed a sharp endothermic peak at its melting point at 178–180°C revealing the crystalline nature. CS thermogram reveals the amorphous nature with a peak around 75–80°C. Physical mixture of RZT, CS, and TPP showed less intense peak at the melting point of drug,

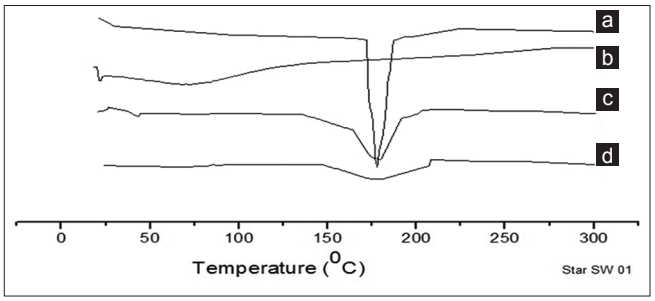

Figure 7: Differential scanning calorimetric thermograms for (a) rizatriptan (RZT), (b) chitosan (CS), (c) RZT + CS physical mixture, and (d) RZT CS nanoparticles

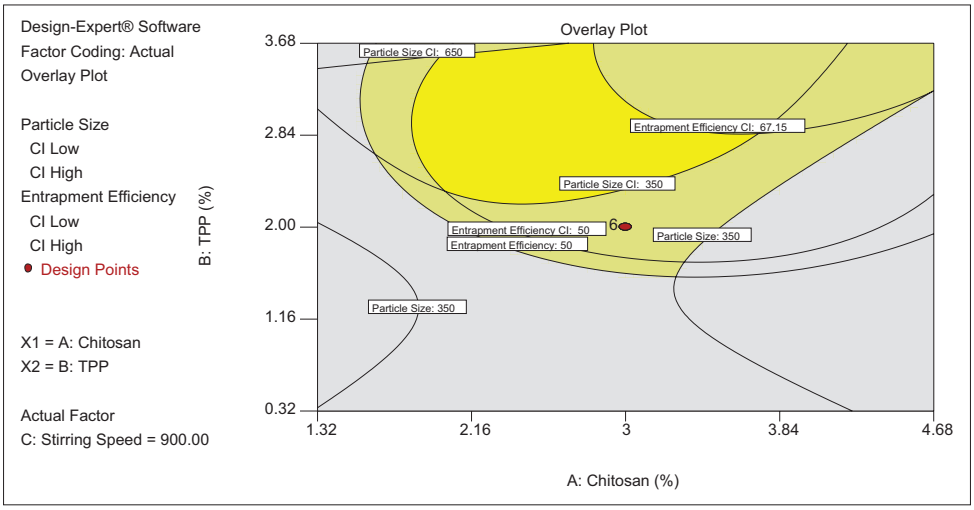

Figure 8: Design space for rizatriptan loaded chitosan nanoparticles

Table 6: Validation of design space of RZT CS nanoparticles

Formulation code	Composition (% w/v)		Response	Predicted value	Experimental value	SE
	X_1 CS	X_2 TPP				
21	2.74	3.68	Y_1 - Particle size	565.58	570.24	1.33
			Y_2 - Entrapment efficiency	62.08	63.14	1.03
22	3.30	2.83	Y_1 - Particle size	422.42	437.21	2.62
			Y_2 - Entrapment efficiency	64.28	63.78	−0.75
23	2.96	3.17	Y_1 - Particle size	479.16	491.24	3.29
			Y_2 - Entrapment efficiency	64.21	65.8	0.70

RZT: Rizatriptan, CS: Chitosan, TPP: Tripolyphosphate, SE: Standard error

Table 7: Accelerated stability results for RZT CS nanoparticles

Batch number	Entrapment efficiency* (% w/w)		Particle size (nm)		Zeta potential (mV)		PDI	
	Initial	3 months	Initial	3 months	Initial	3 months	Initial	3 months
21	99.57±2.4	96.54±1.59	570.24	584.14	+33.56	+32.68	0.247	0.226
22	101.52±2.3	98.14±2.45	437.21	448.75	+35.6	+34.81	0.256	0.216
23	99.75±2.61	96.57±2.74	491.24	496.97	+32.43	+31.98	0.128	0.142

*For entrapment efficiency of RZT loaded CS nanoparticles were weighed equivalent to RZT 25 mg. RZT: Rizatriptan, CS: Chitosan, PDI: Polydispersity index

whereas RZT CS nanoparticles exhibit peak with change in intensity.

Optimization of design space and validation of model

Design space was generated [Figure 8] with the acceptance criteria particle size in narrow range of 350–650 nm; % entrapment efficiency >50%. Checkpoint formulations were evaluated for particle size and entrapment efficiency and compared with predicted values yielding the percentage error between −0.75 and 3.29. Thus, the low magnitudes of error in the current study indicated a high prognostic ability of CCD model in the optimization of RZT CS nanoparticles [Table 6].

Stability results

Particle size were slightly increased on stability might be due to attraction of small nanoparticles together leading to increased nanoparticles. Whereas PDI obtained within 0.142–0.226 for three batches indicated good polydispersity after stability. Zeta potential data for initial period was showing range from + 32 to + 35 considered as stabile, whereas on 3M stability zeta values remains unchanged as shown in Table 7 revealing the stable formulation. Entrapment efficiency of RZT was slightly decreased after 3 M of stability study but it was within accepted specification for RZT.

CONCLUSION

In this study, QbD approach was successfully implemented to gain understanding the effect of process and formulation parameters in the development of RZT CS nanoparticles. Checkpoint formulations for RZT CS nanoparticles prepared within DS showed acceptable results with the assurance of product quality. CCD RSM design provided the effect of critical parameters within and outside its levels of variables on the CQAs with certain advantages such as no data missing and obtaining two-factor interactions. Moreover, QbD approach improved the elucidation of critical parameters as compared to routine one factor at a time study. Accelerated stability data of optimized nanoparticles showed stability of RZT loaded CS nanoparticles, which lead to better drug product formulation. In further research, *in-vivo* animal study can be carried out on RZT CS nanoparticles to obtain bioavailability data to provide more detailed insights of CS-based nasal drug delivery.

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