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Formulation and *In Vitro* Characterization of Anastrozole Loaded Nanoparticles with Factorial design Based Studies

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ABSTRACT

The purpose of this study was to develop chitosan based anastrozole nanoparticles for treatment of breast cancer. An ionic gelation method was used to prepare anastrozole controlled-release nanoparticles. A 3^2 full factorial design was employed. Experimental variables such as concentration of CS and cross-linking agent sodium TPP were varied to study their effect on drug entrapment efficiency and release rates of drug from nanoparticles. Fourier transform infrared spectroscopic (FTIR) analysis and differential scanning calorimetry (DSC) were employed to determine any interactions between drug and polymer. The FTIR studies revealed no chemical interaction between the drug and the polymer. Entrapment efficiency of nanoparticles ranged between 51.51 ± 0.81 % to 84.35 ± 1.06 %.In-vitro release studies were performed in phosphate buffer saline of pH 7.4. A slow release of anastrozole up to 72 h was observed. Mean particle size of nanoparticles ranged between 1635 nm to 72.30 nm with mean particle size of 273.6 nm, while zeta potential 0.52 mV. DSC results indicated that the anastrozole entrapped in the nanoparticles existed in an amorphous or disordered-crystalline status in the polymer matrix. Scanning electron microscopy was done to study the surface morphology. Results revealed that more spherical shaped particles with possible aggregation. The highest correlation coefficients were obtained for the Higuchi model, suggesting a diffusion mechanism for the drug release. The results demonstrated that anastrozole nanoparticles with chitosan could be an alternative delivery method for the long-term treatment of breast cancer.

Keywords: Anastrozole; Nanoparticles; Factorial design; Ionic gelation; Controlled release.

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INTRODUCTION

Breast cancer is the most well-known reason for death in women. Estrogens are involved in various physiological processes including the development and maintenance of the female sexual organs, the reproductive cycle, proliferation, and different neuroendocrine functions. In addition, estrogens are also involves ingrowth and development of certain objective cells, for example, breast epithelial cells and estrogen-subordinate mammary carcinoma cells[1]. Most cases (around 80%) of breast cancer occur in postmenopausal women, and the majority of the tumor is found to be hormone-dependent, where estrone (E1) and estradiol (E2) play an important role in the development and evolution of the disease[2–5]. The conversion of E1 and E2 from androst-4-ene-3,17-dione (and rostenedione, AD) and testosterone is catalyzed by aromatase. Compounds that inhibit enzyme aromatase have applications in the treatment of advanced estrogen-dependent breast cancer[6,7]. Anastrozole is a nonsteroidal aromatase inhibitor. It is chemically described as 1,3-benzene diacetonitrile, a, a, a', a'-tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl). It is a potent and selective nonsteroidal aromatase inhibitor. The dose of anastrozole (Arimidex tablet) is 1 mg orally once a day. For patients with advanced breast cancer, the drug should be continued until tumor progression ends. For adjuvant treatment of early breast cancer in postmenopausal women, the median duration of therapy is 31 months. To increase patient compliance, a sustained delivery system of anastrozole could be used[8]. One of the technological resources used to improve the permanence of drugs at the site of action is the use of therapeutic systems prepared using biodegradable polymers. Erodible matrices offer the advantage of biodegrading, disappearing gradually while releasing the drug from the site of action[9]. One of the suitable methods to achieve these objectives could be association with biodegradable polymeric carriers such as nanoparticles. The nanometric size of these carrier systems allows efficient crossing of biological barriers, amelioration in tissue tolerance, improved cellular uptake and transport, thus enabling efficient delivery of the therapeutic agents to the target sites like liver, brain and solidtumor[10-12]. Nanoparticles may become one of the successful carriers by overcoming problems caused by infections that are refractory to conventional treatment. Chitosan possesses some ideal properties of a polymeric carrier for nanoparticles such as biocompatibility, biodegradability, non-toxicity, and low cost. It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan nanoparticles[13]. Hence, these nano systems are being used to target drugs to a specific site only in the body, to improve oral bioavailability, to sustain drug effect in the target tissue, to solubilize drugs for intravascular

delivery, and to improve the stability of drugs against enzymatic degradation. The objective of the work was to formulate chitosan nanoparticles containing anastrozole by ionic gelation method, evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and in vitro release characteristics.

MATERIALS AND METHOD

Anastrozole was a gift sample from Natco pharma Pvt. Ltd., Hyderabad, Chitosan, sodium tripolyphosphate (TPP), Glacial acetic acid and other reagents were made available at department through distributor. All chemicals used were of analytical grade.

Preparation of chitosan nanoparticles

Chitosan (CS) nanoparticles were prepared by the ionic gelation method. The preparation of CS nanoparticles is based on an ionic interaction between positively charged CS solution and negatively charged sodium TPP solution[14]. The accurately weighed amount of anastrozole was dissolved in polymeric solution of chitosan in 1% aqueous acetic acid solution and TPP was dissolved in distilled water. Then, 12 mL of sodium TPP solution was dropped into 30 mL CS solution under magnetic stirring (1000 rpm) at room temperature. CS nanoparticles were formed instantaneously. CS nanoparticle suspension was kept stirring for 30 min for further cross-linking of nanoparticles. Finally, CS nanoparticles were collected by centrifugation at 15,000 rpm and freeze-drying at – 20 ^{o}C for 3 h.

Experimental Design

The formulations were fabricated according to a 3^2 full factorial design, allowing the simultaneous evaluation of two formulation variables and their interaction. At 3^2 full factorial design requires 9 experimental runs to determine the experimental error and the precision of the design. The experimental designs with corresponding formulations are outlined in table 1. The dependent variable that was selected for study was % drug entrapment (Y1).

Table 1: Experimental run of chitosan nanoparticles as per 3^2 full factorial design and percentage entrapment efficiency

BATCH	X1	X2	% EE
ANS-1	-1	-1	64.56 ± 1.05
ANS-2	-1	0	71.90 ± 1.45
ANS-3	-1	+1	67.31 ± 0.7
ANS-4	0	-1	60.26 ± 1.06
ANS-5	0	0	74.70 ± 1.22
ANS-6	0	+1	84.35 ± 1.06
ANS-7	+1	-1	45.82 ± 0.96
ANS-8	+1	0	51.51 ± 0.81

ANS-9	+1	+1	62.67 ± 0.77

X1 = Chitosan concentration (mg/ml), X2 = Sodium TPP concentration

Characterization of prepared nanoparticles [15,16]

Drug polymer interaction study

The FTIR spectra of pure anastrozole, chitosan, sodium TPP and mixture of all these three ingredients were recorded to check drug polymer interaction and stability of drug.

Drug entrapment efficiency

Drug entrapment efficiency was determined by centrifugation method. The redispersed nanoparticles suspension was centrifuged at 15,000 rpm for 30 min at 8 °C to separate the free drug in the supernatant. Concentration of anastrozole in the supernatant was determined by using UV-Visible spectrophotometer at 263 nm after suitable dilution. The drug entrapment efficiency was determined using the relationship given in following equation 1.

$$\% \text{ EE} = \frac{W_{\text{total drug}} - W_{\text{free drug}}}{W_{\text{total drug}}} \text{ X } 100 \text{.....(1)}$$

In vitro release studies [17]

In vitro release studies were carried out by using dialysis tubes with an artificial membrane. The prepared chitosan nanoparticles were redispersed in 5 ml of phosphate buffer pH 7.4 and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 50 ml of phosphate buffer pH 7.4. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ± 0.5 °C. 5 ml sample of receptor compartment was taken at various intervals of time over a period of 72 h and each time 5 ml fresh buffer was replaced. The amount of drug released was determined spectrometrically at 263 nm.

Statistical analysis

Various computations for optimization of current study using RSM were carried out by employing Design-Expert software (Version: 9.0.3). Statistical model including interaction and polynomial equations (Eq. 2) were generated for response variable i.e. percentage entrapment efficiency.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X^2_1 + \beta_4 X^2_2 + \beta_5 X_1 X_2 + \beta_6 X^2_1 X_2 + \beta_7 X_1 X^2_2 + \beta_8 X^2_1 X^2_2 \text{.....(2)}$$

Where Y is dependent variable, Where, Y is the measured response, β_0 to β_8 are regression coefficients and X_1, X_2 are independent factors. The main effects (X1 and X2) represent average result of changing one factor at a time from its low to high value. The interaction term (X1X2) shows how the response changes when two factors are simultaneously changed. The polynomial terms X^2_1 and X^2_2 are included to investigate non-linearity. The validity of the developed polynomial equations was verified by preparing check point formulation.

Construction of contour plots:

Two dimensional and three dimensional contour plots were established using reduced polynomial equation and percentage entrapment efficiency was taken as response using Design-Expert software version 9.0.3.

Optimization by desirability function

In the present study, only one response was optimized by a desirability function in the Design-Expert software. Each response is associated with its partial desirability function (i), where an unacceptable response is assigned the value 0 and an acceptable response a value between 0 and 1, depending on the closeness of the response to its target value, that is, the least to the most desirable. Any response that falls outside the desired limit is considered completely unacceptable.

For the response to be maximized, the desirability function can be defined as mention in equation 3:

$$d_{i,max} = \frac{Y_i - Y_{min}}{Y_{max} - Y_{min}} \text{.....(3)}$$

Where $d_{i,max}$is the individual desirability of the response to be maximized, Y_i is the experimental result, and Y_{min} and Y_{max} represents the minimum and maximum possible values. If Y_i is equal to or less than $Y_{min,}$ then di,max = 0; and if Y_i is higher or equal to Y_{max}, then $d_{i,max}$= 1.

After obtaining the individual desirability values for each response, the results are usually combined as a geometric mean to give a global desirable value (D), which is explained by equation as given below in equation 4;

$$D = (d_1 \times d_2 \times d_3 \times d_4 \times \ldots\ldots\ldots \times d_n)^{1/n} = \left(\prod_{i=1}^{n} d_i\right)^{1/n} \text{.....(4)}$$

Where, n specifies the number of responses being optimized.

According to the simultaneously assigned goals for all responses, the Design-Expert software determines the maximum desirability value by an extensive grid search over the domain.

Response analysis for optimization

To find the compositions of optimized formulation over the whole experimental region, validation of RSM results were conducted. Three optimum checkpoint formulations were selected to validate the chosen experimental domain and polynomial equation. The optimized checkpoint formulations were prepared and evaluated for response properties. The consequent experimental value of the response was quantitatively compared with that of the predicted values.

Kinetics of drug release

In order to investigate the mechanism of drug release from optimized chitosan nanoparticles formulation (OANS 1), the release data obtained from in-vitro release studies were fitted to various kinetics equations. The kinetics models used were a zero order equation (Qt = Q0 – K0t), first order equation (lnQt = lnQ0 – Kt), Higuchi's equation (Qt = Kht1/2). Where Qt is the percent of drug released at time t, Q0 is the initial amount of drug present in chitosan nanoparticles and K0, K and Kh were constant of the equation of zero order, first order and Higuchi model respectively.

Particle size distribution

The particle size distribution of the nanoparticles was determined by laser particle size analyzer (Zetatrac 10.6.2, Microtrac Inc.) using distilled water as dispersant. The nanoparticle dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the nanoparticles. The average volume-mean particle size was measured after performing the experiment in triplicate.

Zeta potential

The zeta potential of drug loaded nanoparticles was measured by Zeta sizer (Zetatrac 10.6.2, Microtrac Inc.). To determine the zeta potential, nanoparticles samples were diluted with KCL (0.1 Mm) and placed in electrophoretic cell where an electrical field of 15.2 Vcm^{-1} was applied. Each sample was analyzed in triplicate.

Differential scanning colorimetry (DSC)

The DSC analysis of pure drug and drug loaded nanoparticles were carried out using a DSC (PerkinElmer, USA) to evaluate any possible drug-polymer interaction. The analysis was performed at a rate 5.00 $Cmin^{-1}$ from 10 ^{o}C to 300 ^{o}C temperature range under nitrogen flow of 25 $mlmin^{-1}$.

Surface morphology study (SEM)

Scanning electron microscopy (Zeiss, TIFR, Mumbai) of the chitosan nanoparticle was performed to examine the particle size and surface morphology. The photographs were taken using a scanning electron microscope under magnification of 10 KX – 12 KX. The nanoparticles were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument.

Stability study

The stability study was carried out using the optimized batch OANS1. Formulation OANS1 was divided into 2 sets of samples and stored at room temperature i.e 25 ± 2 ^{o}C/60 ± 5 %RH and at 40

± 2 °C/75 ± 5 %RH in humidity and temperature control cabinet (MSW 125, MAC). After 60 days, physical appearance, drug content and in-vitro drug release of all samples were determined[21].

RESULTS AND DISCUSSION

Drug polymer interaction

The interaction study between the drug and polymer was evaluated using FT-IR spectrophotometer. There was no significant difference in the IR spectra of pure drug loaded nanoparticles as shown in figure 1.

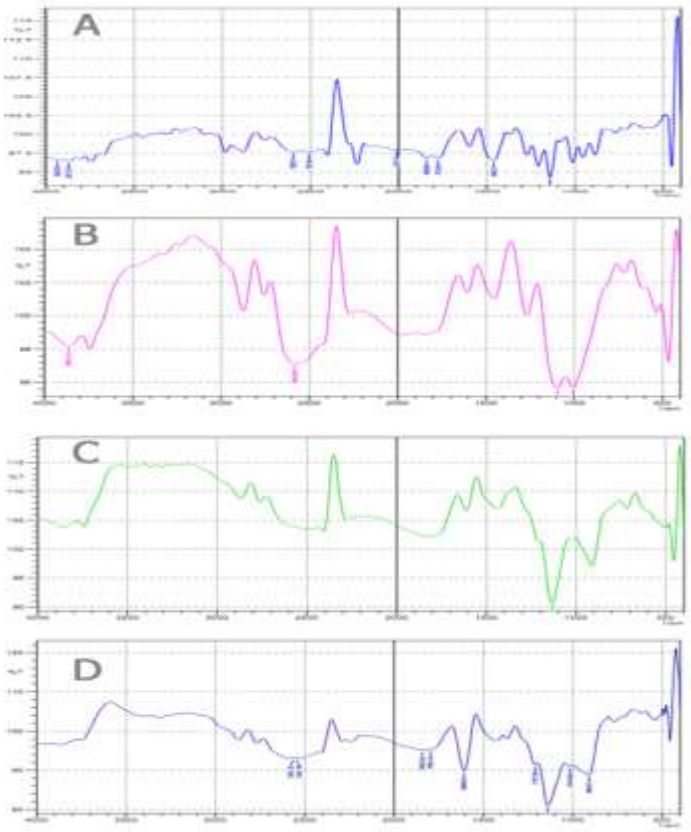

Figure 1: FTIR spectrum of A: Anastrozole, B: Chitosan, C: Sodium TPP, D: Mixture of Anastrozole, Chitosan and sodium TPP

Method of preparation

Nanoparticles prepared by ionic gelation technique. The ionic interactions between the positively charged amino groups and negatively charged counter-ion tripolyphosphate were used to prepare chitosan nanoparticles. The anionic counter-ion TPP can form either intermolecular or intra-molecular linkages: this is responsible for the successful formation of the nanoparticles.

Entrapment Efficiency

Table 1 shows the percentage entrapment efficiency of formulations. The entrapment efficiencies of chitosan–TPP nanoparticles ranged from 51.51 ± 0.81 % to 84.35 ± 1.06 %. The results of the

present study demonstrated that the encapsulation efficiency of chitosan–TPP nanoparticles was affected by the concentration of chitosan and sodium TPP. The maximum percentage drug entrapment was obtained for the formulation ANS-6. It was observed that increase in concentration of chitosan and sodium TPP significantly increases percentage entrapment. However, further increase in chitosan concentration from 6 mg/ml to 8 mg/ml and sodium TPP concentration from 4 mg/ml to 6 mg/ml, cause decrease in percentage entrapment. It may be due to the slower reaction rate at higher concentration.

In-vitro release study

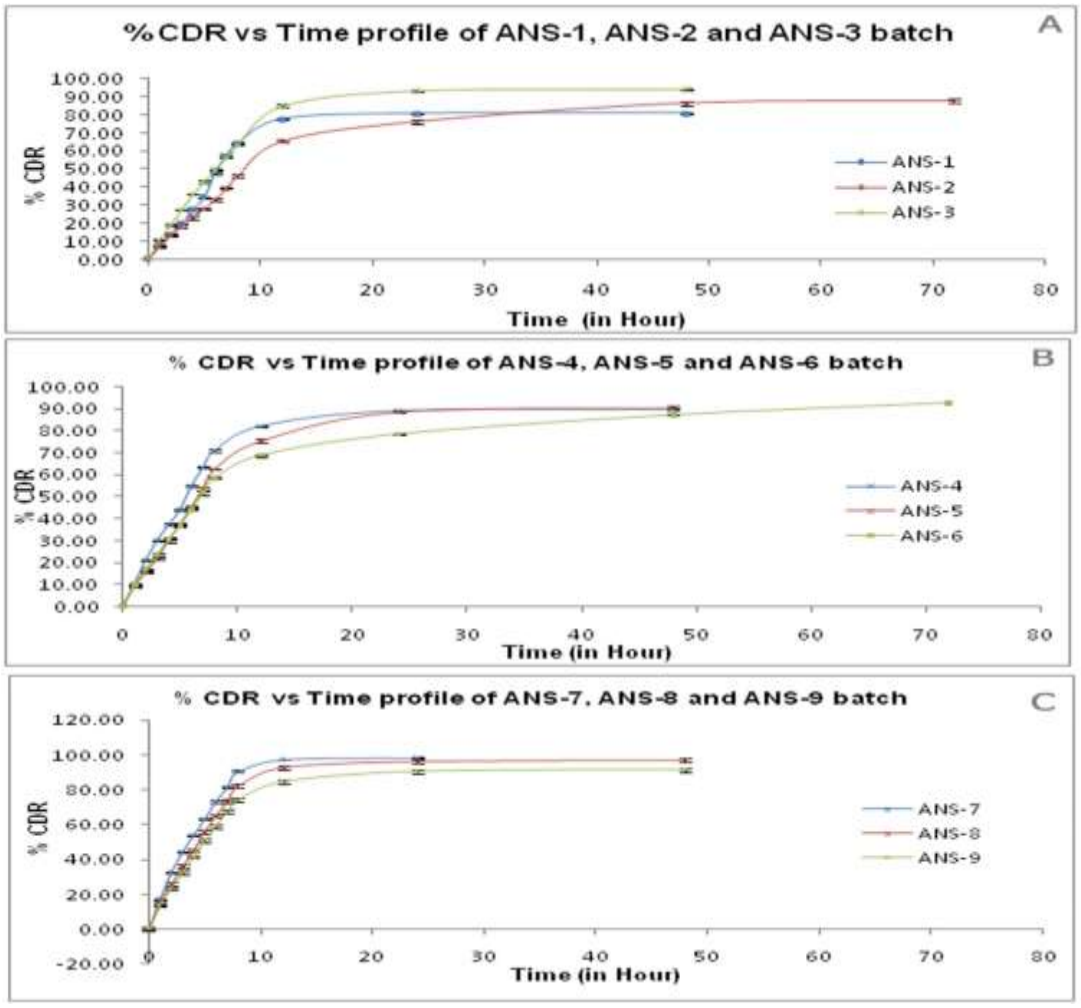

Figure 2: In – vitro release profile of chitosan nanoparticles A: ANS1 – ANS3, B: ANS4 – ANS6, C: ANS7 – ANS9 formulations

Figure 2 shows percentage cumulative drug released from formulations prepared by using 3^2 factorial design. In-vitro drug release study was performed for each formulation by using dialysis sac method. The maximum release was observed for 72 h for the formulation ANS-2 and ANS-6. The formulation ANS-1, ANS-3, ANS-4, ANS-5, ANS-8 and ANS-9 were show release of drug

within 48 h, whereas ANS-7 shows the release up to 24 h. It was also found that similar concentration of chitosan and sodium TPP shows prolonged release of drug from formulation. The formulations show initial burst release followed by a constant and continuous release. The initial burst drug release was may be due to desorption and diffusion of drugs from the surface of the drug loaded nanoparticles. Afterwards, the chitosan nanoparticles may undergo gradual swelling, leading to the constant and slow release of drug.

Optimization of formulation

On the basis of obtained result for characterization of nanoparticle, it was found that change in the concentration of chitosan and sodium TPP has higher influence on entrapment efficiency. Therefore entrapment efficiency was chosen as a response variable to optimize the formulation. The 3^2 full factorial design was used to optimized the formulation.

The popular method in the development and optimization of the drug delivery system is response surface methodology (RSM). Depending on the principles of design of experiments, the methodology involves the use of various types of experimental designs, generation of polynomial mathematical equations and plotting the response over the experimental domain to choose the optimum formulation. A full factorial statistical design is one type of RSM. It specifies the required experimental runs and consumes less time and thus provides a far more efficient and cost-effective technique than the conventional techniques of formulation and optimization of dosage forms.

Fitting the model to the data

All the responses observed for 9 formulations prepared were simultaneously fitted to linear, 2FI, quadratic and cubic models using Design-Expert 9.0.3. It was observed that the best-fitted model was reduced quadratic and the comparative values of R^2, SD and % CV were found to be 0.8632, 5.48 and 8.46 respectively.

The full model regression equation was generated having the model F – value of 6.45 implies there is a 7.80 % chance that an F – value this large could occur due to noise. Values of P > F is less than 0.05, i.e. 0.0134 indicate that model terms are significant. In this case X1 and X2 i.e. chitosan concentration (mg/ml) and sodium TPP concentration (mg/ml) are significant model terms. Values greater than 0.1 indicate the model terms are not significant and hence, by removing any insignificant model terms resulted into improved and reduced the model.

For the reduced quadratic model F – value was obtained 10.52 implies the model is significant. There is only a 1.34 % chance that an F – value this large could occur due to noise.

The adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. It was found to be 9.393 indicates an adequate signal. Thus, this model can be used to navigate the design space.

$$\% EE = 74.35 - 7.30X_1 + 7.28X_2 + 3.53X_1X_2 - 12.47X^2_1 - 1.87X^2_2 \ldots\ldots\ldots(5)$$

As shown in polynomial equation (equation 5), positive value indicates an effect that favors the optimization, whereas a negative value represents an effect that inverse the relationship between the factor and response. A response is considered to be significantly affected by the factors if the effect is different from zero and has p value less than 0.05. It can be seen that % EE was significantly affected by the antagonistic effect of chitosan concentration and synergistic effect of sodium TPP concentration as they have p value 0.0225 and 0.0226 respectively. Whereas quadratic term of chitosan concentration (X^2_1) with p value 0.0235 also shows the antagonistic effect on % EE.

Contour plots and response surface analysis

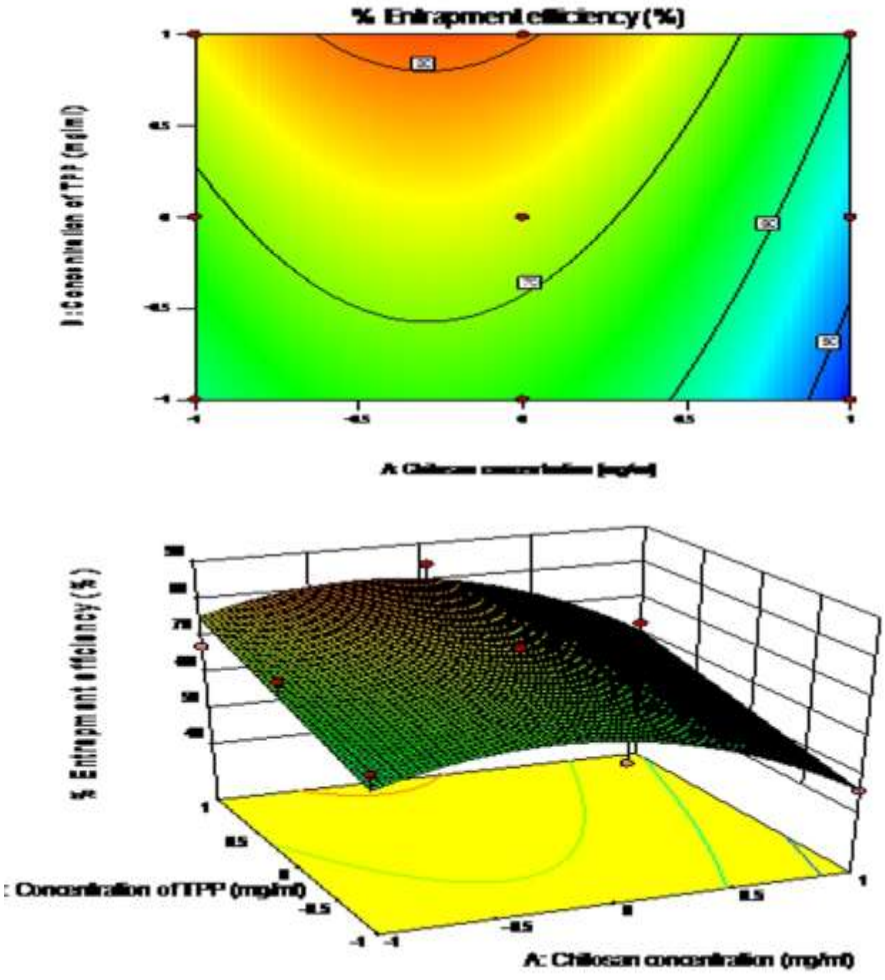

Figure 3: 2D and 3D images of contour plots

Two dimensional and three dimensional contour plots were prepared for the response percentage entrapment efficiency. Entrapment efficiency is considered to be one of the most crucial factors for assessing the quality of chitosan nanoparticles. The contour plot as shown in figure 3 indicates that at the low level of X_1 and X_2, % EE was also low. In addition, increase in X1 andX2 at certain limit increases % EE. On the other hand, it was observe that further increase in X1 decreases the % EE at low level of X2.

Desirability function

The search for the optimized formulation composition was carried out using the desirability function approach with Design expert software, criterion being one having the maximum desirability value. The optimization process was performed by setting the entrapment efficiency at maximum while concentration of chitosan and TPP within the range obtained as shown in figure 4. The optimized formulation was achieved at chitosan concentration 5.42 mg/ml and TPP concentration at 6 mg/ml with the corresponding desirability *(D)* value of 0.925 as given in table 2. This factor level combination predicted the entrapment efficiency 81.45%.

Table 2: Response surface analysis and desirability for optimized formulation

Sr. No.	Chitosan (mg/ml)	Sodium TPP (mg/ml)	% Entrapment efficiency	Desirability
1	5.42	6	81.45	0.925
2	5.40	6	81.45	0.925
3	5.36	6	81.44	0.925
4	5.52	6	81.41	0.924
5	5.26	6	81.37	0.923

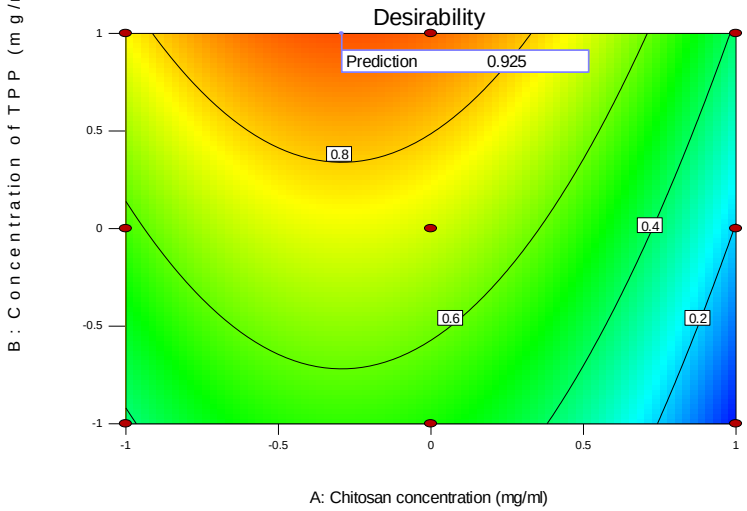

Figure 4: Desirability plot for chitosan nanoparticles

Finally, to confirm the validity of the optimal parameters and predicted responses calculated, three batches of the optimized formulations were prepared. All of the responses were evaluated for each

optimized formulation. The comparisons of predicted and experimental results shows very close agreement as mentioned in table 3, indicating the success of the design combined with a desirability function for the evaluation and optimization of chitosan nanoparticulate formulations.

Table 3: Response surface analysis for optimized formulation

Test batches	Response	Factors		Predicted value	Experimental value	% Error
		X1 (mg/ml)	X2 (mg/ml)			
OANS 1	% EE	5.42	6	81.45	80.78	0.83
OANS 2	% EE	5.40	6	81.45	80.86	0.73
OANS 3	% EE	5.52	6	81.41	80.67	0.92

Kinetics of drug release

The in-vitro release profile data of an optimized formulation were fitted in various kinetic dissolution models like zero order, first order and Higuchi release kinetics. The R^2 value obtained were 0.648, 0.905 and 0.858 for zero order, first order and Higuchi model respectively. As indicated by higher R^2 values, the drug release from optimized formulation follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled.

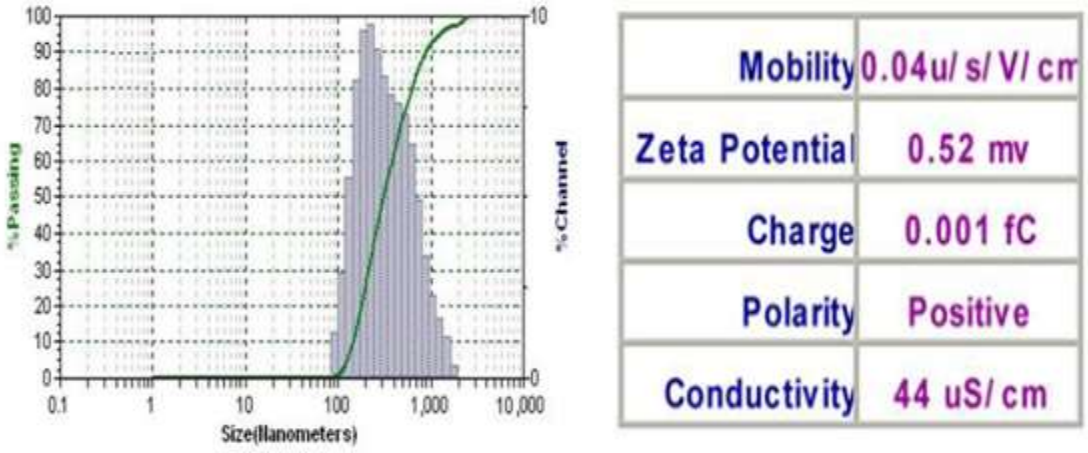

Figure 5: Particle size distribution and zeta potential of chitosan nanoparticles

Particle Size and Zeta Potential

The average diameter and zeta potential of optimized formulation was determined by photon correlation spectroscopy (PCS) at room temperature. The particle size of optimized formulation was found to be mean average of 273.6 nm with maximum intensity of 536.0 nm particles. It shows the wider range of particle size distribution from 1635 nm to 72.30 nm.

As Zeta Potential is an important tool for prediction of long term stability and understanding the state of the nanoparticle surface. The value greater than + 25 mV or less than – 25 mV have high degree of stability. Zeta potential was found to be 0.52 mV for optimized formulation, thus it

suggests that possible aggregation of particles as shown in figure 5.

Differential scanning calorimetry (DSC)

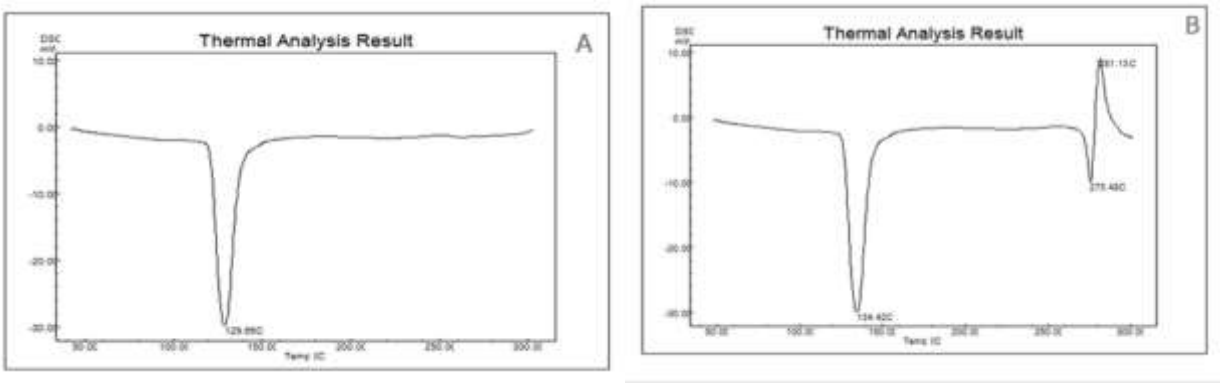

Figure 6: DSC image of A: anastrozole and, B: Optimized chitosan nanoparticles (OANS1)

Differential scanning calorimetry is widely used in thermal analysis to monitor endothermic processes (melting, solid-solid phase transitions and chemical degradation) as well as exothermic processes (crystallization and oxidative decomposition). It could be extremely useful since it indicates the existence of possible drug-excipients or excipient-excipient interactions in formulation. As shown in figure 6, thermograms of pure drug anastrozole shows an endothermic peak at 129.66 ºC which indicates the purity of the drug as the reported melting point of the drug was 130.14 ºC. The thermogram of an optimized formulation were shows two endothermic peak at 134.42 ºC, 275.48 ºC and one exothermic peak at 281.13 ºC. In this, endothermic peak at 134.42 ºC indicates the presence of drug and its stability into the formulation while peak at 275.48 ºC and 281.13 ºC indicates melting point of sodium TPP and chitosan respectively.

Scanning Electron Microscopy (SEM)

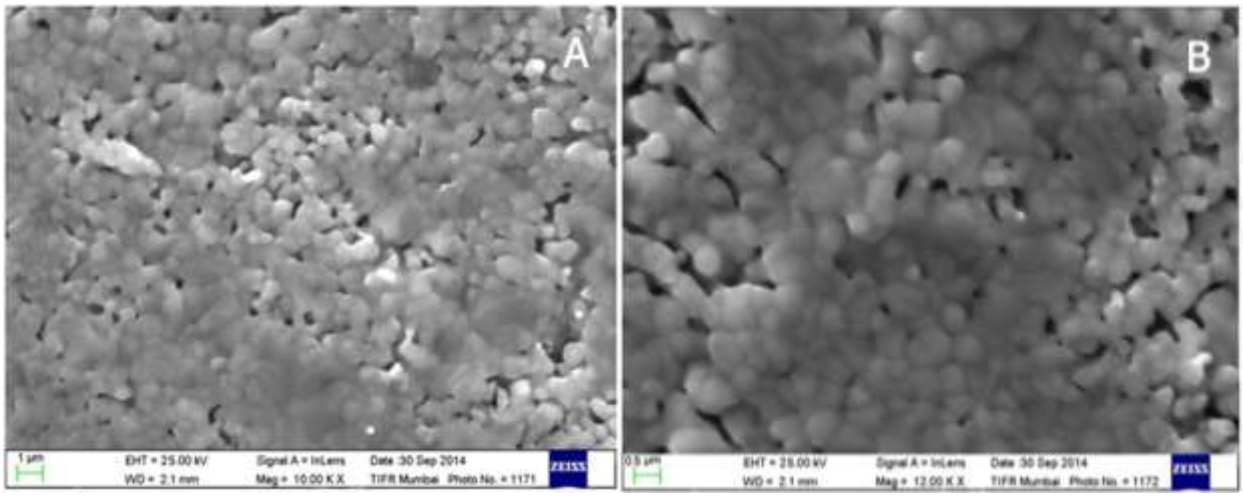

Figure 7: SEM images of optimized chitosan nanoparticles (OANS1) at A: 10.00KX and B: 12.00KX

The surface morphology of optimized formulation was studied using scanning electron microscopy at two different magnifications i.e. at 10.00 KX and 12.00 KX. SEM is an instrument that produces largely magnified image by using electrons instead of light. Electron gun produces a beam of electrons, which follows the vertical path through the microscope between electromagnetic fields and lenses towards the sample due to which electrons, and X-rays are ejected from sample. The particle shape was found to be fairly spherical structure with possibility of aggregation as few large particles were observed as shown in figure 7.

Stability study

The optimized formulation was subjected to stability studies at various ICH storage conditions i.e. 25 ºC ± 2 ^{o}C/60 ± 5 % RH and 40 ºC ± 2^{o}C/75 ± 5 % RH for a period of 60 days. The formulation was evaluated for physical appearance, drug content and in-vitro drug release study at regular interval of 15 days. No major changes were observed in physical appearance, drug content and in – vitro drug release profile when stored at room temperature. Also, at 40 ºC ± 2^{o}C/75 ± 5 % RH storage condition, no major changes were observed in physical appearance as well as in drug

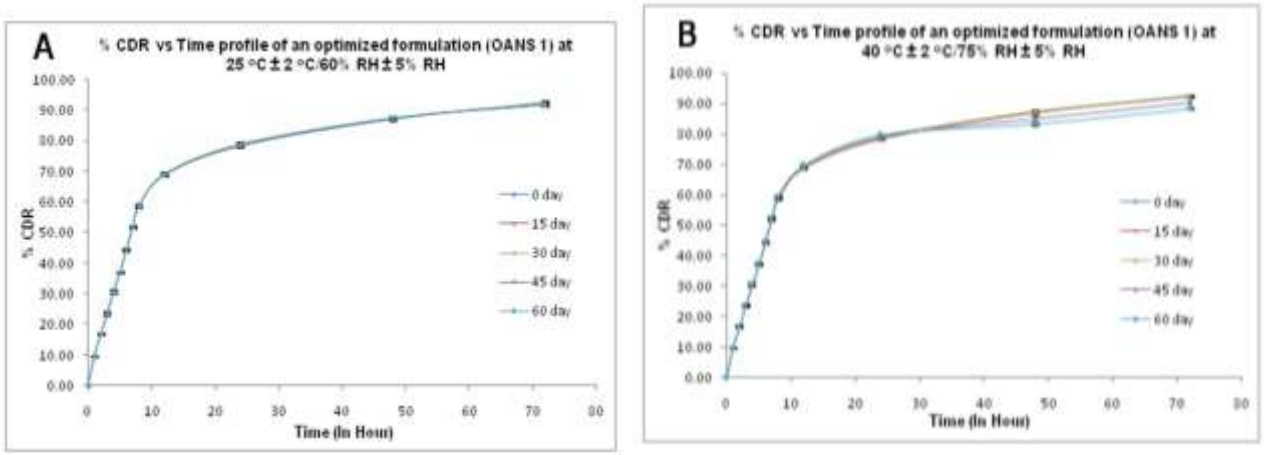

Figure 8: % CDR vs Time profile of optimized formulation (OANS1) at different storage condition A: 25 ^{o}C ± 2 ^{o}C/60 ± 5 %RH, B: 40 ^{o}C ± 2 ^{o}C/75 ± 5 % RH

Table 4: Physical appearance and % drug content of optimized nanoparticulate formulation at various storage conditions

Number of Days	25 ^{o}C ± 2 ^{o}C/60% RH ± 5% RH		40 ^{o}C ± 2 ^{o}C/75% ± 5% RH	
	Physical Appearance	% Drug Content	Physical Appearance	% Drug Content
0	No Change	4.28 ± 0.13	No Change	4.28 ± 0.13
15	No Change	4.28 ± 0.12	No Change	4.27 ± 0.13
30	No Change	4.26 ± 0.14	No Change	4.24 ± 0.12
45	No Change	4.26 ± 0.13	No Change	4.20 ± 0.10
60	No Change	4.26 ± 0.10	No Change	4.18 ± 0.11

content given in table 4, while very minor decline were observed in in-vitro release data at the end of 60 days study as shown in figure 8. It indicates that the formulation were stable at various ICH storage condition for longer period.

CONCLUSION

Chitosan–TPP nanoparticles anastrozole were prepared by ionic cross-linking method. RSM is a useful tool for optimization, which shows goodness of fit. The study confirms that the ionic gelation technique was suitable for the preparation of anastrozole loaded nanoparticles. This formulation approach, with hydrophilic polymer like chitosan, may increases the life span of nanoparticles in systemic circulation by preventing the opsonization of nanoparticles, which generally takes place when a formulation is given thorough an intravenous route. In addition, with these formulations better patient compliance is provided as they shown sustained release behavior.

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