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Optimization And Characterization of Amikacin Loaded Chitosan Nanoparticles Using Full Factorial Design.

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ABSTRACT

The study deals with the implementation of full factorial design of experimental and statistical analysis for the optimization of amikacin loaded chitosan nanoparticles. The influence of independent variables concentration of chitosan (X1), STTP (X2) and stirring speed of magnetic stirrer (X3) on dependent variables particle size (Y1) and entrapment efficacy (Y2), was investigated. As per the design, eleven runs of nanoparticles were prepared by modified ionic gelation method. The particle size and entrapment efficacy were found in the range of 168 – 297 nm and 86.23 – 97.51 % respectively. A statistical analysis was performed using Design expert software with respect to ANOVA and regression values. The cuboidal plot, contour plot and 3D response surface plots showed visual representation of relationship between the experimental response and the independent variables of the formulation. Regression model equations were validated by a numerical and graphical optimization method. Further optimized amikacin loaded chitosan nanoparticles showed positive charge of zeta potential indicating storage stability. SEM reports the rough and porous surface of the nanoparticles and TEM analysis confirms the spherical shape of the nanoparticles. The optimised formulation resulted the non-fickian diffusion (n=0.51), i.e., the release of drug is burst at initial followed by the sustained release confirming the drug release from polymeric system. The optimized chitosan nanoparticles show a better activity against gram-negative bacteria and moderate activity against gram-positive bacteria. The activity of amikacin chitosan nanoparticles is double to that of the amikacin in pure.

Keywords: Chitosan; Amikacin; Nanoparticles; Full factorial design; Ionic gelation; Antibacterial activity.

INTRODUCTION

The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100nm) materials for the development of science. Nanoparticles have an essential role in oral, systemic, transdermal, pulmonary, and other routes for their site-specific action, enhanced bioavailability, and drug stability by altering the molecular weight, polymer ratio, particle size, and fabricating conditions a lot of drug-releasing profiles can be attained(Ealias, 2017; Kumari et al., 2010; Mu & Feng, 2003; Patra et al., 2018).

Chitosan is a natural poly-cationic biopolymer and is the composition of 1 → 4-linked 2-amino-2-deoxy-β-D-glucose. Chitosan is a deacetylated chitin derivative that is obtained from the shells of marine crustaceans and the fungi cell-wall (Sanpui et al., 2008). Chitosan, which contains free amino acids, is insoluble in neutral or basic pH conditions and is soluble in acidic pH conditions. Chitosan is most commonly solubilized in 1-3 % acetic acid solutions. Primary amine groups of chitosan exhibit specific properties that produce useful pharmaceutical applications. Chitosan consists of a positive charge responsible for mucoadhesive, when compared to several other natural polymers (Berscht et al., 1994; Sannan et al., 1976). Chitosan nanoparticles that are formulated by using sodium tripolyphosphate (TPP) as a chemical cross-linking agent were used as potential nanocarriers in the pharmaceutical field. The amino groups of chitosan are crosslinked with the negatively-charged phosphate groups of TPP to form positively charged chitosan nanoparticles (Anand et al., 2018).

Antibiotics are the compounds which either kill or inhibit the growth of microorganisms without significant toxicity to the host. The majority of antibacterial compounds are moderately small molecules having an atomic weight of less than 2000 atomic mass unit (Bisen

et al., 2012). Antibiotics are useful for treating bacterial infections, protozoal infections and for immunomodulation. Aminoglycoside type of antibiotics consists of a pharmacophoric 1,3-diaminoinositol derivative: 2-deoxystreptamine, spectinamine, or streptamine. Some of these alcoholic activities are replaced with distinctive aminosugars via glycosidic linkages to produce pseudo-oligosaccharides. The aminoglycosides are water soluble at all pH levels, are basic and form acid addition salts, are not absorbed in substantial amounts from the gastrointestinal system, and are eliminated in their active state in relatively high concentrations in the urine(Foye, W.0, Lemke, T.L. & Williams, 1995; HORSPOOLs et al., 1994).

Amikacin is a semi-synthetic antibiotic derived from kanamycin that has improved potency and spectrum. Amikacin has potent activity against gram-negative bacteria and retains its antimicrobial activity in the presence of approximately 90% of the enzymes that destroy other aminoglycosides, making amikacin the least susceptible to degradation by bacterial enzymes. It is used for bacterial infections like sepsis, meningitis, pneumonia, joint infections urinary tract infections and intra-abdominal infections. It is also used in multidrug resistant tuberculosis. It is given in the form of injections to veins or muscles. Usage of amikacin causes deafness, renal damage, and paralysis(Bauman et al., 2015; Galanakis et al., 2006; Ovalles et al., 2005). Amikacin is reabsorbed in the proximal tubule after being completely eliminated via glomerular filtration. In human patients with normal renal function, the drug has a half-life of about 2 hours. Amikacin's tissue distribution is comparable to that of other aminoglycosides, with the exception that it appears to be more concentrated in adipose tissue. The primary mechanism of action of amikacin is to bind to bacterial 30S ribosomal subunits and interferes with mRNA binding and tRNA acceptor sites, interfering with bacterial growth. This leads to disruption of normal protein synthesis and production of non-functional or toxic peptides leading to cell death(Caudle et al., 1983; Gingerich et al., 1983).

The design of experiments (DOE) has proven to be a useful tool in research and development, and it is used to determine the relationship between independent and dependent variables. DoE is a critical and methodical tool used in the quality by design approach to draw statistical interpretations using the fewest number of experiments possible in order to estimate design space. In pharmaceutical research and development, many sorts of designs are utilised to optimise formulations with fewer runs, traces problems, and statistically desired solutions. A factorial design is useful for determining the effect of two or more independent factors on dependent variables, as well as for determining how variables interact(Antony, 2014; de Pinho Neves et al., 2014; Zhang & Mao, 2016).

In present study amikacin loaded chitosan nanoparticles were prepared by ionic gelation method using full factorial method and analysing the relationship between independent variables such as concentration of chitosan, sodium tyripolyphosphate (STTP) and stirring speed of magnetic stirrer with the response's particle size and entrapment efficacy and an optimised formulation is determined. These nanoparticles are used to evaluate the antibacterial activity towards both gram negative and gram-positive bacteria.

MATERIALS AND METHODS

Materials:

Amikacin is procured as a gift sample from Dr. Reddy's Laboratories Pvt., Ltd., Hyderabad. Chitosan, STTP, acetic acid and acetone is procured from the Sisco Research Laboratories Pvt., Ltd. All the chemicals used in the study were of analytical grade.

Compatibility studies

Compatibility study of pure amikacin, chitosan, sodium tripolyphosphate and amikacin loaded chitosan nanoparticles were determined by Fourier Transform InfraRed Spectroscopy using SHIMADZU, IRTRACER 100. The pellets are made by gently mixing the samples with potassium bromide under high pressure. The scanning range used is 400 to 4000 cm^{-1}. The spectra of the drug and polymer in the formulations were compared to the spectra of the pure drug and polymer.

Experimental design by design expert

Design Expert 13.0.5 software used to design experimental runs following full factorial method. With a minimum of 8 runs, full factorial design was used to evaluate three variables at two levels. The factors screened for the study are chitosan (X_1), STTP (X_2), and magnetic stirrer RPM (X_3). The factor chitosan, STTP and magnetic stirrer RPM were set at two levels of 2.0 & 4.0 mg/ml, 1.0 & 2.0 mg/ml and 500 & 1000 RPM respectively. To facilitate maximum encapsulation, a ratio of approximately 3:1 for chitosan and TPP was maintained in the combinations.to improve the statistical significance three central points are added to the design. The concentration of center points is 0.3mg/ml, 0.15mg/ml & 750 RPM for X_1, X_2 & X_3 respectively. The real and coded experimental levels based on a three factorial two-level design was shown in table 1(Guthrie, 2010; Parhi et al., 2015).

FORMULATION OF AMIKACIN LOADED CHITOSAN NANOPARTICLES

Low molecular weight chitosan is dissolved in 50ml of 1% acetic acid solution by keeping it on a magnetic stirrer and filter it by using a Millipore membrane to remove impurities. Sodium tripolyphosphate is dissolved in 20 ml distilled water. To the STTP solution amikacin is added and dissolved completely. The STTP-Amikacin solution is added slowly dropwise manner to the Chitosan solution kept on a magnetic stirrer. This solution is kept for stirring overnight. The formed

nanoparticles are separated by centrifuging the resultant suspension at a speed of 10000 RPM for 10 mins. The resulted chitosan nanoparticles are washed thoroughly three times using distilled water to remove the residues. Finally, the chitosan nanoparticles are freeze dried using lyophilizer for further characterization. The formed nanoparticles are stored in an air tight container. The formulation table of amikacin loaded chitosan nanoparticles is given in table 2(Vaezifar et al., 2013).

Table 1: Coded and Actual values of formulation variables

Factor		**Low Level**	**Med-Level**	**High Level**
	Coded Values	**-1**	**0**	**+1**
Amount of Chitosan X_1 (mg/ml)		2	3	4
Amount of TPP X_2 (mg/ml)		1	1.5	2
Magnetic stirrer speed X_3 (RPM)		500	750	1000

Table 2: Formulation table of Amikacin loaded Chitosan nanoparticles

Run	**Amikacin (mg)**	**Chitosan (mg/ml)**	**STTP (mg/ml)**	**Magnetic stirrer speed (RPM)**
1	50	2	1	1000
2	50	2	2	1000
3	50	2	2	500
4	50	2	1	500
5	50	3	1.5	750
6	50	3	1.5	750
7	50	3	1.5	750
8	50	4	1	1000
9	50	4	2	1000
10	50	4	2	500
11	50	4	1	500

Scanning electron microscope (SEM)

The shape and morphology of the chitosan loaded chitosan nanoparticles is identified by using Thermosceintific Apreo S scanning electron microscope. The lyophilized Amikacin loaded chitosan nanoparticles were brushed on the aluminium stub followed by gold sputtering to the thickness of 40 A^o in the chamber and the SEM images were captured by functioning at an accelerating voltage of 15 KV electron beam(Pardeshi et al., 2013).

Transmission electron microscope (TEM)

The morphology of the amikacin loaded chitosan nanoparticles were analysed by JEOL Japan, JEM-2100 Plus transmission electron microscope with an accelerating voltage of 80 KV. Amikacin loaded chitosan nanoparticles are diluted in distilled water and a drop of suspension is placed on the copper-coated 400 mesh copper grids. The grid was dried at room temperature for TEM analysis(Cho et al., 2014).

Zeta potential, Particle size and polydispersity index

The mean particle size, polydispersity index and zeta potential of the amikacin loaded chitosan nanoparticles are analysed by using Horiba Scientific SZ-100 nanopartica Zetasizer. Amikacin loaded chitosan nanoparticles were diluted in the Milli Q water and were analyzed at room temperature(Liu et al., 2015).

Entrapment Efficacy and Loading Capacity

Entrapment efficacy and loading capacity of the Amikacin loaded nanoparticles is calculated by dissolving 10 mg of nanoparticles was dissolved in methanol, and this dispersion is centrifuged at 15,000 RPM for 45 mins. The supernatant and filtrate were

diluted suitably and analyzed for UV-Spectrophotometric method. Entrapment efficacy and loading capacity is calculated by using following formulas(Poovi & Damodharan, 2018):

$$EE\ (\%) = \frac{\text{Amount of Amikacin added} - \text{Amount of free Amikacin in supernatant}}{\text{Amount of Amikacin added}} \times 100$$

$$LC\ (\%) = \frac{\text{Amount of Amikacin added} - \text{Amount of free Amikacin in supernatant}}{\text{Weight of nanoparticles}} \times 100$$

Statistical Analysis and Optimization by DoE

The relationship between the formulation factors and responses were studied by employing the full factorial design. The factors used in the study are the chitosan concentration(X_1), STTP concentration(X_2) and magnetic stirrer speed(X_3) and the responses studied are particle size(Y_2) and entrapment efficacy(Y_1). The polynomial equation used to fit the mean values of the data is shown below

$Y= \beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_3X_3+\varepsilon$

Where, Y represents the predicted response, β_0 is the intercept, β_1, β_2, β_3 and ε are main effects and model residual respectively. X1, X2. X3 corresponds to independent variables. The significance of each coefficient term was determined using an ANOVA and a P-value with a 95% confidence range. Similarly, the 95 % confidence interval was used to evaluate the lack of fit of the recommended model. The regression coefficient (R^2) and adjusted regression (R_a^2) were calculated to measure the extent to which experimental data might be fitted. For measuring model adequacy, graphs displaying anticipated vs. actual values and residual vs. experimental runs were created(Kakkar et al., 2011; Tsai et al., 2011).

***In-vitro* drug release**

In-vitro release patterns were studied using conventional dialysis technique using phosphate buffer pH 7.4 as dissolution medium. In this method Amikacin-loaded Chitosan nanoparticles (equivalent to 10 mg) was placed in a dialysis bag which is soaked in double distilled water for 24 hours before using and after transferring nanoparticles both sides are clamped. The dialysis bag was immersed in a beaker containing 600 ml of phosphate buffer pH 7.4. Acceptor compartment was agitated continuously using a magnetic stirrer at 37 ±1° temperature at a speed of 100 RPM. At different time intervals, 5 ml of the sample is removed by filtration and replaced with fresh medium to maintain the sink conditions and analyzed using UV Visible spectrophotometer. The result of *in vitro* drug release study of nanoparticles was fitted with various kinetic models(Jain et al., 2011).

***In-vitro* Antibacterial activity**

Well diffusion test is used to determine the antibacterial activity of the Amikacin loaded chitosan nanoparticles. Muller-Hinton agar was used as the growth media. To the sterile nutrient agar plates 100 µL of the test organism was aseptically transferred and spread using a sterile L-shaped glass rod. The organism was allowed to settle on the medium for 5 min. Wells were punched on the surface of the bacteria inoculated plates and 50 µL of the nanoparticle preparation is injected into the wells. The plates were incubated for 24 h at 37°. The zones of inhibition were measured using standard HiMedia scale. Wells injected with pure Amikacin is used as positive control and chitosan nanoparticles as negative control(Soleimani et al., 2015).

RESULTS AND DISCUSSION

The present study was researched to prepare the amikacin loaded chitosan nanoparticles using ionic gelation method.

Compatibility studies

Drug- excipient interaction studies were performed by using the FTIR Spectroscopy. The FTIR spectra of pure amikacin showed characteristic peaks at 2910.63 cm^{-1} indicating C-H stretch related to methyne group, 1634.70 cm^{-1} indicating C=C stretch related to alkenyl group and 1595.16 cm^{-1} indicating C=C-C Aromatic ring stretch. The spectra of drug-excipients and the formed chitosan nanoparticles does not show any interactions and the nanoparticles provided are stable.

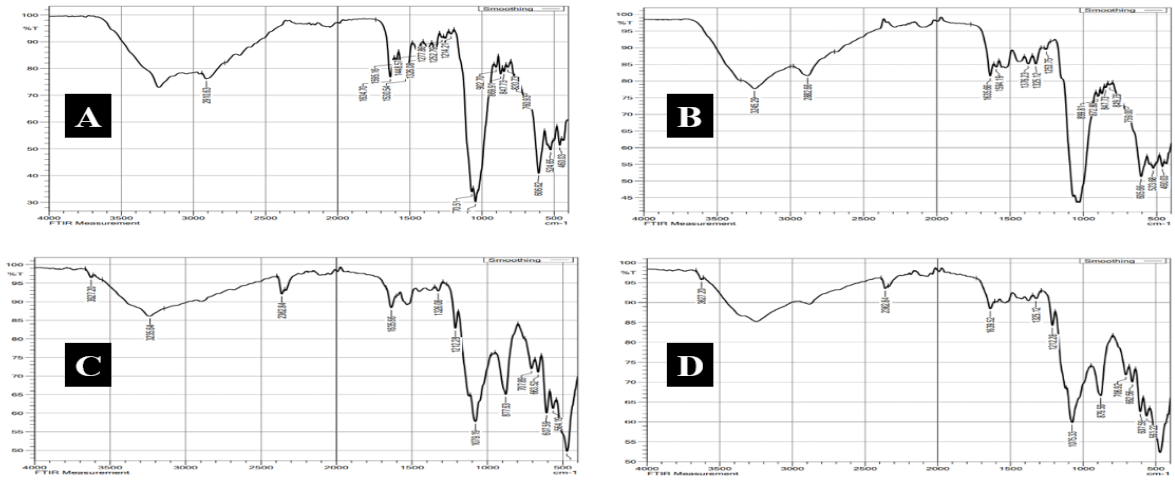

Figure 1: FTIR spectra of A) Pure Amikacin B) Chitosan C) STTP D) Formulateded nanoparticles.

Scanning electron microscopic (SEM)

SEM examination of amikacin-loaded chitosan nanoparticles revealed spherical nanoparticles with rough and porous surfaces. The SEM analysed pictures of amikacin loaded chitosan nanoparticles are represented in figure 2.

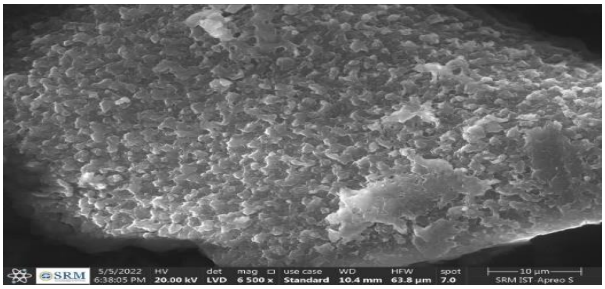

Figure 2: SEM image of Amikacin loaded chitosan nanoparticles

Transmission electron microscopic (TEM)

The TEM analysis of the Amikacin loaded chitosan nanoparticles shown the spherical shaped nanoparticles well dispersed and separated from each other. The TEM analysed pictures of amikacin loaded chitosan nanoparticles are represented in figure 3.

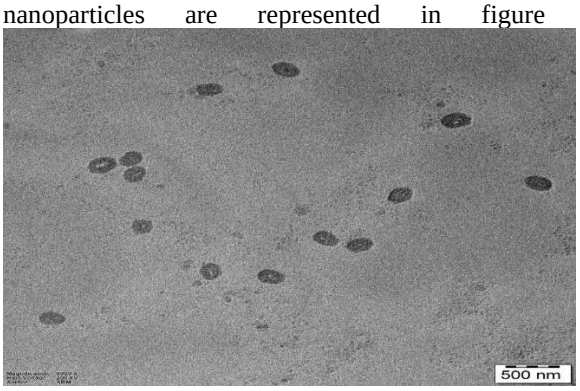

Figure 3: TEM image of Amikacin loaded chitosan nanoparticles.

Entrapment efficacy and Loading capacity

In the evaluation of nanocarrier drug delivery system Entrapment efficacy and Loading capacity are two important factors to be considered. All the experimental runs were disclosed the better entrapment efficacy and loading capacity. Several other investigations indicate the maximum drug entrapment at 3:1 ratio of chitosan and STTP in the formation of chitosan nanoparticles and the ratio is maintained in developing the all-experimental runs. The maximum entrapment efficacy has been recorded was 97.51% in experimental run 9 with a Chitosan and STTP concentration at 4 mg/ml and 2 mg/ml respectively in ratio of 3:1 respectively. The % EE and % LC has shown in table 3.

Particle size and polydispersity index

Zeta sizer is used to analyse the particle size and polydispersity index of chitosan nanoparticles and the results were represented in table . All the experimental runs shown the particle size ranging from 168 to 297 nm with moderate levels of polydispersity index. The minimum particle size is obtained in the run with the minimum concentration of chitosan and STTP at high RPM of magnetic stirrer. Particle size of the nanoparticles is further discussed in optimization of size by DoE model. The particle size, PDI results were shown in table 3.

Zeta Potential

Zeta potential is analyzed by the zeta potential analyzer. All the experimental runs show the positive zeta potential ranging from +34.65mV to +43.24mV were shown in table 3. The positive zeta potential of the runs exhibits the greater stability of nanoparticles in the formulation. The positive nature of the nanoparticles is due to the presence of positively charged amine group of the major component chitosan. Positive charge of the nanoparticles is increased with the increase in concentration of the chitosan in the formulation. The negatively charged

phosphate groups in the STTP has a tendency to lower the positive charge of the formulation.

Table 3: Entrapment efficacy, loading capacity, particle size, PDI and zeta potential of Amikacin loaded chitosannNanoparticles

Run	Entrapment Efficacy (%)	Loading Capacity (%)	Particle size (nm)	PDI	Zeta potential (mV)
1	86.23	27.80	168	0.356	+ 34.65
2	87.29	24.91	183	0.452	+ 37.25
3	88.29	25.26	205	0.392	+ 36.57
4	88.85	27.75	192	0.574	+ 35.27
5	93.62	21.08	236	0.367	+ 37.26
6	93.85	21.03	225	0.388	+ 39.67
7	93.54	20.87	241	0.346	+ 39.34
8	94.26	17.84	265	0.285	+ 41.26
9	97.51	17.13	272	0.511	+ 43.24
10	95.86	16.98	297	0.630	+ 42.34
11	96.21	18.08	264	0.278	+ 40.23

Statistical analysis by DoE:

Using full factorial run design total 11 runs were carried out for the preparation of Amikacin loaded chitosan nanoparticles and investigate the effects of three independent factors Amount of chitosan (X_1), Amount of STTP (X_2) and magnetic stirrer speed (X_3) on two dependent variables particle size (Y_1) and entrapment efficacy $(Y)_2$. Descritive statistics of model represented in table 4.

Table 4: Experimental design of 2^3 Full factorial design With Responses Y_1 and Y_2

Run	Chitosan X_1 (mg/ml)	STTP X_2 (mg/ml)	Magnetic stirrer speed X_3 (RPM)	Particle Size Y_1 (nm)	Entrapment Efficacy Y_2 (%)
1	2	1	1000	168	86.23
2	2	2	1000	183	87.29
3	2	2	500	205	88.29
4	2	1	500	192	88.85
5	3	1.5	750	236	93.62
6	3	1.5	750	225	93.85
7	3	1.5	750	241	93.54
8	4	1	1000	265	94.26
9	4	2	1000	272	97.51
10	4	2	500	297	95.86
11	4	1	500	264	96.21

The experimental data was analyzed by applying multiple regression analysis and fitted to various models. Linear model was adopted due to higher regression values (R^2), low P-value and better descriptive statistics to establish an empirical model to facilitate the interrelation between independent variables and particle size. The final polynomial equation achieved in the coded formulation variables was given below.

Particle size $(Y_1) = +230.75+435.75X_1 + 8.50X_2 - 8.75 X_3$

Entrapment efficacy $(Y_2) = +91.81 + 4.15X_1 + 0.4250X_2 - 0.4900X_3$

ANOVA analysis of the particle size and entrapment efficacy was found to be significant with a F-value of 86.29 and 46.24 respectively. The model P-value of less than 0.0001 indicate the developed linear model is more significant on particle size. The lack of fur F-value of 0.9272 implies the Lack of Fit is not significant relative to the pure error. The results of ANOVA and lack of fit tests for particle size and entrapment efficacy are given below in table 5&6 respectively.

Table 5: ANOVA test for Particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	16503.00	3	5501.00	86.29	< 0.0001	significant
A-Chitosan	15312.50	1	15312.50	240.20	< 0.0001	
B-STTP	578.00	1	578.00	9.07	0.0237	
C-Magnetic Stirrer Speed	612.50	1	612.50	9.61	0.0211	
Curvature	23.05	1	23.05	0.3615	0.5697	
Residual	382.50	6	63.75			
Lack of Fit	248.50	4	62.12	0.9272	0.5779	not significant
Pure Error	134.00	2	67.00			
Cor Total	16908.55	10				

Table 6: ANOVA for selected factorial model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	140.98	3	46.99	46.64	0.0001	significant
A-Chitosan	137.61	1	137.61	136.57	< 0.0001	
B-STTP	1.45	1	1.45	1.43	0.2763	
C-Magnetic Stirrer Speed	1.92	1	1.92	1.91	0.2166	
Curvature	7.53	1	7.53	7.47	0.0340	
Residual	6.05	6	1.01			
Lack of Fit	5.99	4	1.50	57.86	0.0171	significant
Pure Error	0.0518	2	0.0259			
Cor Total	154.55	10				

According to the descriptive statistics of the model, increasing the concentration of chitosan and STTP while lowering the magnetic stirrer's stirring speed increases entrapment efficacy. The design model's Half normal plot and Pareto chart imply that chitosan concentration had a significant impact on nanoparticle particle size and entrapment efficacy. Model diagnostic plots, such as the predicted value vs actual value graph, were useful in illustrating the relationship between experimental and predicted values and determining the model's suitability. An internally studentized residual vs experimental runs plot was constructed to ensure that the established model fit well.

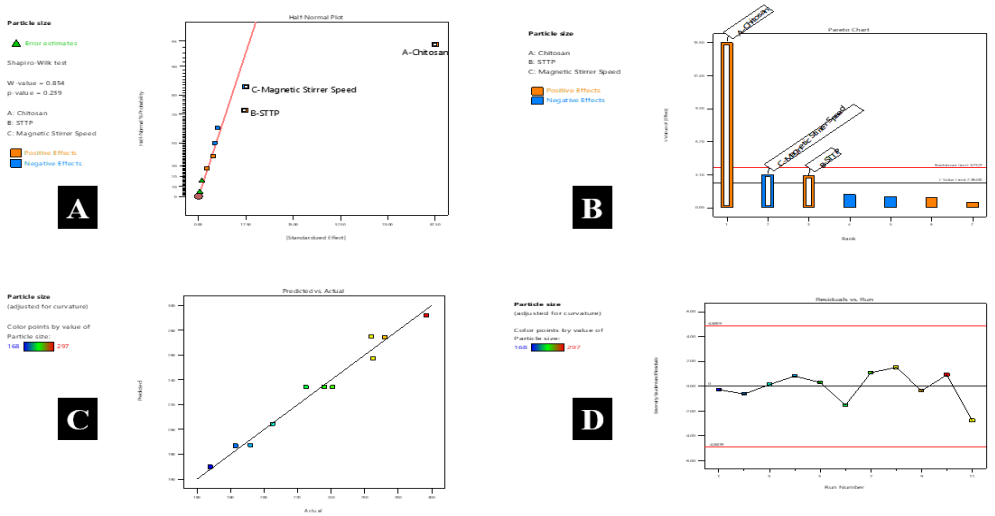

Figure 4: Model analysis A) Half normal chart B) Pareto Chart and Model diagnostic C) Predicted vs Actual D) Residual vs Run Plot of response particle size.

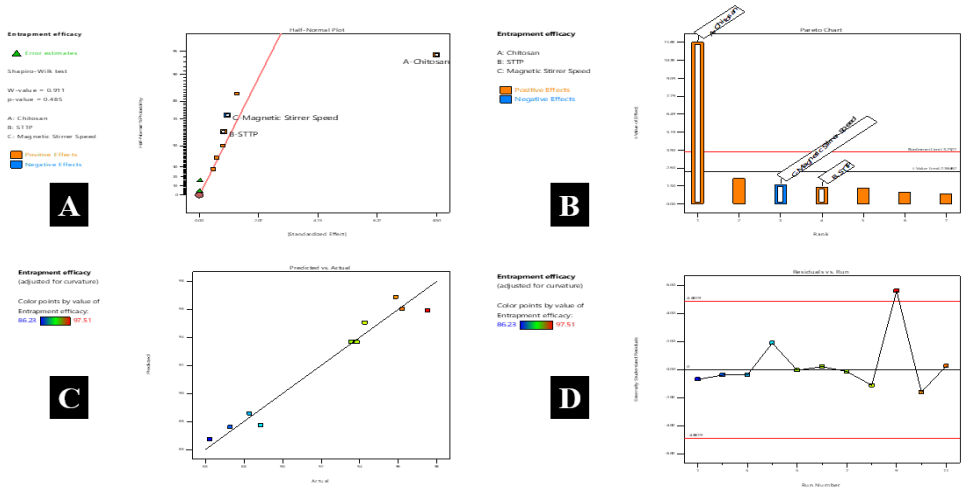

Figure 5: Model analysis A) Half normal chart B) Pareto Chart and Model diagnostic C) Predicted vs Actual D) Residual vs Run Plot of response entrapment efficacy.

To depict the relationship between responses and formulation factors, model graphs such as the cuboidal plot and 3D response surfaces figure 6&7 were constructed to analyze the individual and interaction impacts on the response. From these plots it is shown increase in the independent factor's chitosan and STTP concentration increases the particle size and increase in stirring speed decreases the particle size. Increases in the independent variable's chitosan and STTP concentration increase entrapment efficacy, while increases in stirring speed reduce entrapment efficacy, as demonstrated in these figures

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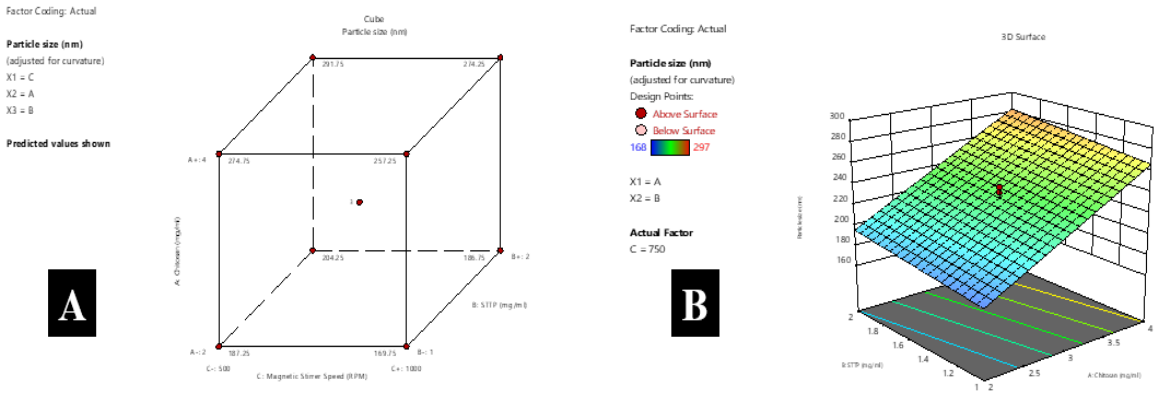

Figure 6: A) Cuboidal plot and B) 3D Response surface plot for particle size

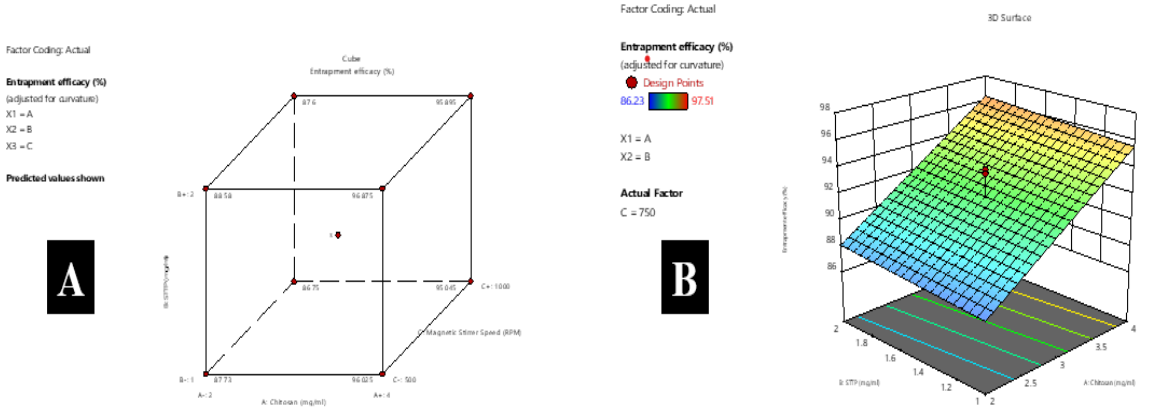

Figure 7: A) Cuboidal plot and B) 3D Response surface plot for entrapment efficacy.

The statistical investigations gave sufficient evidence in the form of adjusted and predicted regression coefficient (R^2) values with a sufficient precision value to examine the design space. Table shows the results of regression analysis for all the responses particle size and entrapment efficacy. The results of regression analysis were represented in table 7.

Table 7: Results of Regression Analysis

Response	R^2	Adjusted R^2	Predicted R^2	Adequate Precision
Particle Size	0.9773	0.9660	0.9233	22.6637
Entrapment efficacy	0.9589	0.9383	0.8361	14.9609

The difference between the predicted R^2 and adjusted R^2 values was less than 0.2, indicating that they were in reasonable agreement. Adequate precision values were larger than 4.0, which is ideal. The model is significant for all two responses in the design space, according to these results.

Optimization of amikacin loaded chitosan nanoparticles

Regression model developed in this study was used to identify out the optimal condition to prepare the nanoparticles of 240 nm particle size and 90% entrapment efficacy. The corresponding experimental parameters for X_3, X_2 and X_3 were 3.61 mg/ml, 1 mg/ml and 1000 RPM respectively. Under these optimum conditions the predicted particle size and entrapment efficacy was 240 nm and 93.42% respectively. Experiments were performed in triplicate and particle size and entrapment efficacy was found to be 234.51 nm and 92.21% respectively and experimental values were close the match to that of predicted values.

Table 8: Validation of optimized formulation by Experimental design

Variable	Unit	Actual value
Chitosan	mg/ml	3.61
STTP	mg/ml	1
Magnetic Stirrer Speed	RPM	1000
Characterization		
	Particle Size	**Entrapment efficacy**
Predicted value	240 nm	93.42 %
Experimental value	234.51 nm	92.21 %

Drug Release Kinetics of optimized formulation

Drug release study was conducted for the optimized formulation and was fitted for several kinetic models. Correlation coefficient was chosen to define the approximation accuracy of the kinetic models and acceptable correlation was achieved when R^2 value was greater than or equal to 0.9 ($R^2 = 0.9$). The drug release pattern was best explained by the Korsemayer-peppas model, that had the best fit and the highest R^2 value ($R^2 = 0.992$). Non-fickian drug release was indicated by the n-value (n=0.51 at pH 7.4), which is common in control release systems involving polymers. This was the case in the current study, with initial rapid dissolution followed by a slower dissolution rate due to the hydrolytic behavior of chitosan.

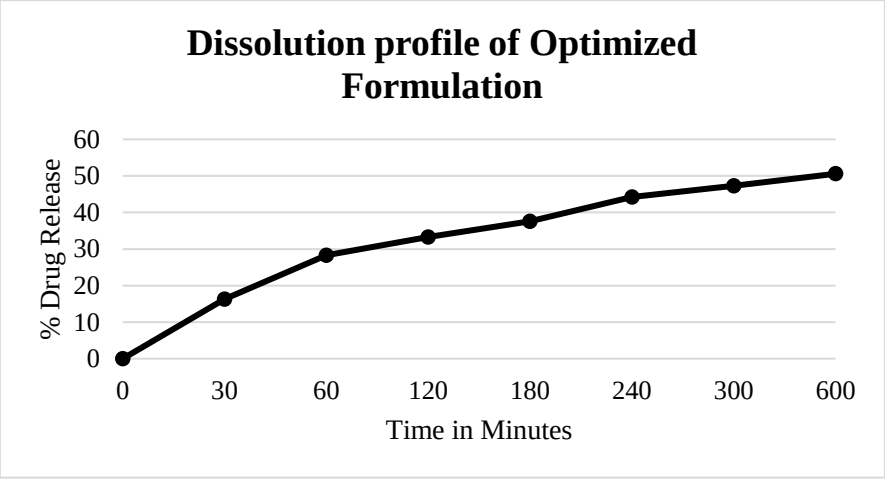

Figure 8: Dissolution profile of Optimized Formulation

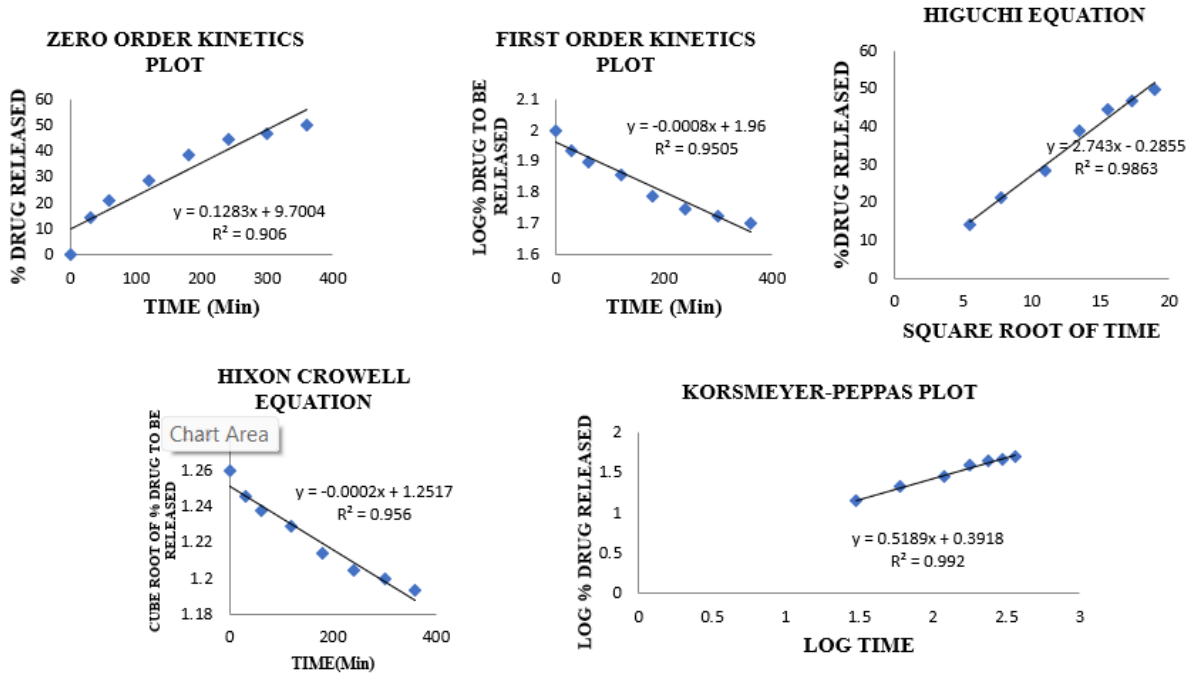

Figure 9: Various kinetic model plots for drug release profile of optimized formulations

***In-vitro* Antibacterial activity:**

The *in-vitro* antibacterial activity of optimized formulation of amikacin loaded chitosan nanoparticles is investigated by well diffusion technique. Pure amikacin is used as a positive control and chitosan nanoparticles are used as negative control. The formation of the zone of inhibition is observed clearly around the wells containing pure Amikacin and Amikacin loaded chitosan nanoparticles. Amikacin shows a better activity against gram-negative bacteria when compared to that of the gram-positive bacteria. Amikacin loaded chitosan nanoparticles exhibit the double fold activity against the bacteria when compared to that of the pure amikacin. The amikacin loaded chitosan nanoparticles penetrate into the bacterial cell well and ceases the growth of bacteria by killing them.

Table 9: Antibacterial activity of amikacin loaded chitosan nanoparticles

S. No	Name of the sample	Zone of inhibition (mm)			
		Gram-Positive Bacteria		Gram-Negative Bacteria	
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Proteus*
1.	Pure Amikacin	11 ± 0.12	13 ± 0.24	15 ± 0.38	22 ± 0.33
2.	Chitosan nanoparticles	-	-	-	-
3.	Amikacin Loaded Chitosan nanoparticles	21 ± 0.14	23 ± 0.35	26 ± 0.24	28 ± 0.34

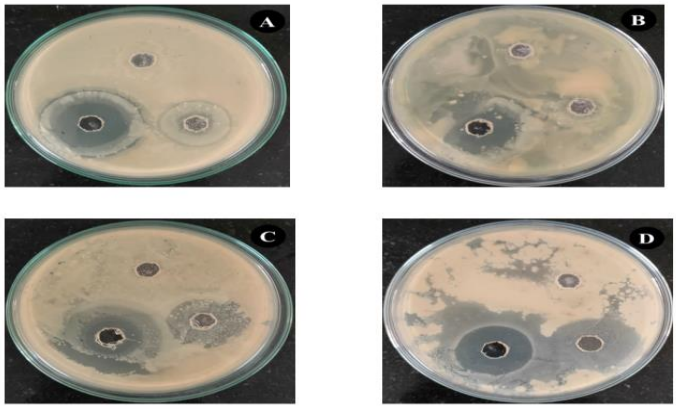

Figure 10: Zone of inhibition of the bacterial mediated amikacin loaded nanoparticles against pathogenic bacteria

a) ***Staphylococcus aureus*** **b)** ***Bacillus subtilis*** **c)** ***Escherichia coli*** **d)** ***Proteus*****.**

CONCLUSION

In the present study amikacin loaded chitosan nano particles were successfully prepared by ionic solution method using full factorial method using full factorial design. The compatibility studies of drug and excipient has shown the interlinking bond between the characters in and STTP and the formation of nano particles is proved. The statistical analysis of particle size and entrapment efficacy has shown their dependence on concentration of chitosan and STTP. The experimental values particle size and entrapment efficacy of optimized formulation were near to that of predicted values that is 234.51 nm and 92.21% respectively. The drug release kinetics of the optimized formulation shows the non-fickian diffusion that is absorbed commonly in polymeric formulations. The prepared chitosan nanoparticle shows better anti-bacterial activity against both gram-positive and gram-negative bacteria. The prepared nanoparticles shown double the antibacterial activity when compared to that of pure amikacin.

Conflicts of Interest

The authors certify that they have no conflicts of interest.

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