Development of Chitosan nanoparticle of Ondansetron using Box-Behnken statistical Design based Optimization

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ABSTRACT

The purpose of this research was to use the ionic gelation method to optimise and develop ondansetron (OND) loaded chitosan nanoparticles (ONDNP) using the Box–Behnken design (BBD). 3 self-directed variants like drug to polymer ratio (X_1), concentration of sodium tripolyphosphate (X_2), and RPM (X_3) were considered to investigate their effect on the dependent variables viz., particle size (Y_1) (PS), Polydispersibility index (Y_2) (PDI), and Zeta potential (Y_3) (ZP). The optimized formula was determined by regression analysis of the output data which was further evaluated morphological, in vitro release, In vitro cell cytotoxicity, Ex vivo histopathology studies and in vivo distribution for brain targeting followed by nasal administration. The designed nanoparticles have average particle size, PDI, ZP from190.5 ± 6.45, 0.426 ± 0.024, 52.40 ±7.20 respectively. No significant toxicity and structural damage was found in nasal mucosa on histopathology examination. The AUCs of ONDNP in plasma and brain was found to be 562.34 ± 14.81 ng h/mL in plasma and 324.36±15.28 ng h/mL in brain.It has a much In the brain, a higher brain blood ratio and nasal bio-availability than the OND pharmaceutical solution.The current investigation is expected to yield promising results that will push us to design a non-invasive way to address the disadvantages of oral brain delivery.

Keywords: Chitosan nanoparticles, Box-Behnken experimental Design, Ondansetron, particle size, zeta potential, Polydispersibility index, cytotoxicity.

Introduction

Ondansetron HCl, a prototype of a brand new elegance of anti-emetic pills controls chemotherapy triggered vomiting.OND is a specific antagonist of the 5-HT3 8,9,10 serotonin receptor sub type. The serotonin release from (5-HT) from small intestine enterochromaffin cells is responsible for cytotoxic chemotherapy and radiotherapy, likely inducing a vomiting reflex by stimulating Vagal afferents have 5-HT3 receptors 8,9,10. Ondansetron has been shown to inhibit the beginning of this response.Chemotherapeutic drugs may promote the production of Antiemetic characteristics of ondansetron Hydrochloride (OND), which suppresses the depolarizing impact of 5HT via 5HT3 receptors in the GIT [8]. The floor of the fourth ventricle, the chemoreceptor trigger zone of the region postrema, may also release serotonin when vagal afferents are activated 8,9,10. Ondansetron antiemetic action is most likely due to specific inhibition of Neurons in both the peripheral and central nervous systems have 5-HT3 receptors 8,9,10.

OND has a plasma half lifestyles of three-4 hours might to be prolonged to six-eight hours inside the aged 9,10 as a results requires frequent management to hold powerful therapeutic awareness. Ondansetron is absorbed through the GI system and undergoes some regulated first-pass metabolism. 8. Bioavailability in healthy people is alarmingly high at 60%. 8,9,10. Ondansetron is a weak alkali (pKa=7.4) that is water soluble in acidic circumstances. Ondansetron hydrochloride solutions have a pH range of four.5 to 4.6 in their normal state [10, 11]. In solutions, the solubility is significantly reduced. that the pH scale bigger than or adequate vi As a result, at higher pH scale, poor absorption is predicted.When OND was distribute intravenously, the start of the effects is because of the risk of unpleasant responses and needle phobia, extremely In addition, quick and channel administration is not a patient-friendly option.Ondansetron nano suspension for nasal delivery was formulate to avoid the primary pass metabolism with improved therapeutic potency in treatment of nausea and unconditioned reflex as an alternate medical care to parental. The limitation of oral drug delivery like because of poor absorption at the target web site of BBB. A well-defined route of administration with associate degree acceptable dose kind would be necessary to enhance bioavailability.Taking under consideration of those Oral or intravenous administration of OND has restrictions, as does nasal administration. of OND might be thought of as an alternate route to stop nausea and unconditioned reflex related to such medical care.

Numerous innovative invasive and noninvasive nano formulations have been used to cross the BBB. However the invasive methods have a drawback of disruption of barrier integrity which is risky and not patient friendly whereas the noninvasive methods facilitate the direct transport of drugs across the brain by avoiding various surgical interventions.

In recent years the intranasal drug delivery has attracted the interest of the researcher by exploring non-invasive route To the brain the delivery of drugs has gotten a lot of interest as a possible option for routes of delivery are oral and parentral. Drugs distributed via nasal route can reach the CNS for brain targeting, travel along the olfactory and trigeminal nerves.Large absorptive surface, rich vascular structures and bypass of BBB makes the nasal route as a possible brain targeting route Nanoparticles a novel carriers administered through a non invasive route via nasal route offers a attractive strategy to improving the pharmacokinetic profile of pharmaceuticals that are poorly water soluble, bio-available, and toxic. The use of novel nano particulate nasal therapeutic agents is enormously approached for its enviable characteristics such as biodegradable, bio compatible and ease in the method of preparation.further it allows access to across BBB by increasing the diffusion through biological membrane that prevents from the biological, chemical and enzymatical degradation. Chitosan, a biodegradable polymer has it as positive charges at neutral pH that interact particle with the salicyclic acid negatively charged mucosal membranes of negative charges residues on the mucus. The chitosan has mucoadhesive properties that provides extended contact duration for drug transport through nasal mucosa, preventing formulation removal as a result, chitosan nanoparticles have been thoroughly tested for use in a nasal nanoparticle medication delivery system.The goal of this study is to create an OND nano particle system that will improve OND brain delivery using a non-invasive nasal medication delivery route. The ionic gelation method was used to create OND nanoparticles, which were then optimised using the Box-Behnken design. The ONDNP was developed and tested for morphological, physicochemical, solid state characterisation, cytotoxicity, and analytic properties. Further it was subjected to pharmacokinetic studies to measure the efficiency of brain targeting and percentage of drug distribution after the intranasal administration.

Material and techniques

Substances

Ondansetron changed into received as gift samples from Hetero pills,Hyderabad. Sodium tripolyphosphate (STPP) and Chitosan (CHI) Sigma-Aldrich sold it to me (Bangalore, India). S.D. excellent chemical compounds were used to make acetic acid.All of the other chemicals and reagents were of the highest quality.

Software applications and units

A scanning electron microscope was used to regulate the particle size and shape of the nanoparticles produced (SEM, 5kV, version 54160, Hitachi, facts point spac Japan) Cam Spec Co. is a company that specialises in camouflage provided the UV-visible spectrophotometer (version, M350). The UV–VIS spectra area has 1nm fact point spacing. A diffuse reflector is included in the Nicolet infrared spectrometer (Madison, USA). FTIR spectra had been recorded in four hundred-4000cm-1 3.85 cm-1 information point spacing in the spectral neighbourhood The WINFIRST software model three.57 was used to digitize the spectra. The sizes of nanoparticles from SEM were determined using Nahamin Pardazan Asia version 1.zero of the micro shape dimension software. This software programme employees picture process to determine the nanoparticles of particle size based on the appearance recorded. Design-professional software programme version eight.zero.zero, Stat-Ease, Inc., Minneapolis, MN, USA, was used to complete the optimization technique.

High-performance liquid chromatography analysis of OND

Chromatographic separations were carried out two LC20AD solvent delivery modules were used in a Shimadzu HPLC (Tokyo, Japan) type. a Rheodyne injector (model 7125, United States of America) valve outfitted with in 20 micro litre loop, and SPD M20A Photo diode array detector, chromatographic device changed into managed by the use of a individual (SCL- 10A) and a machine with chromatographical data station installed on it. A Phenomenex C18 analytical column (150 x 4.6mm, 5m) linked to a Phenomenex C18 guard cadridge was used for chromatographic separations (4mm x 3mm i.d., 5m).Earlier analysis, the mobile phase was filtered through a 0.22 µm filter (Gelman science, Mumbai, India) and Branson Sonicator was used to degas the product (Branson Ultrasonics, USA). The experiment was carried out in a laboratory with air conditioning (20 2°C). Ondansetron was separated chromatographically using a mobile phase containing ACN:MeOH:Ammonium acetate buffer (20:40:40) at pH 4.0 on a

Phenomenex cyano analytical column (150x4.6mm, 5m) (adjusted with acetic acid). Deliveries of solvent mixes were made at 1.5 mL/min flow rate and analyte peak was detected at 222 nm. The R 2 In the concentration range of 0.2-50 g/ml, a value of 0.9999 was found to be linear.

Preparation of chitosan nanoparticles (CHINP)

Ionic gelation of CHI with STPP was used to make CHINP. To make chitosan gel, CHI was disseminated in glacial acetic acid (1 percent 20 ml) (as shown in Table 2) and swirled continuously for 2 hours. The STPP were filtered after being dissolved in distilled water To generate CHI nanoparticles, the poly anionic STPP solution was injected dropwise to the polycationic chitosan gel using a Magnetic stirring for 2 hours at room temperature with a 1 mL micro syringe. To eliminate any remaining STPP, the solution was filtered using a membrane filter (0.45m). Ultracentrifugation at 12,000rpm at 4 degree Celsius for twenty minutes (Remi cooling Cent) concentrated the resulting nanoparticles. A Ultra violet-Visible Spectrophotometer (UV-1601, Shimadzu, Japan) was used to determine the amount of free drug contained in the supernatant. To further characterise the nano suspension, it was lyophilized with mannitol.

The Box-Behnken approach was used to optimise the chitosan nanoparticles CHINP

The preliminary optimization was carried out and investigated to find the effect of particle size, zeta potential and PDI, various trial were performed and its effect was studied. Various trials were performed to determine the characteristics of the ONDNP formation process Three factors Chitosan concentration (X_1), STPP concentration (X_2), and RPM (X_3) has major effect on properties of ONDNP. Particle size (Y_1), Polydispersibility index (Y_2) and zeta potential(Y_3) were set as a fix parameters which as independent variables for further optimization of ONDNP by using BBD. Design-Expert software Version 8.0.0 chose a BBD design with three elements for the optimization of ONDNP. Minneapolis, MN, USA, and Stat-Ease, Inc.

The quadratic response surfaces are explored and 2nd order polynomial models are constructed using this BBD approach. The design consists of two centre points that are located near the midpoint of each multi-dimensional cube's edge, defining the region of interest clearly. This model generates the following second-degree polynomial equation: $Y=\beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_3X_3+ \beta_{12}X_1 X_2+ \beta_{13}X_1 X_3+ \beta_{23}X_2 X_3+ \beta_{11}X^2_1 + \beta_{22}X^2_2+ \beta_{33}X^2_3$ Where Y is the interdependent variant, β_0 arithmetic mean response of the runs, where β_i (β_1 toβ_3) are expected regression constant for the related part Xi (X_1 to X_3).The interdependent and self-directed variables were selected as shown in Table 1 along with their advanced, moderate and down levels. It is feasible to estimate

the linear, quadratic, and interaction impacts of the self-directed variables on the answers using this equation. Design-Expert was used to do Using a regression model, conduct a statistical analysis of the data and plot the responses on surface graphs. Case statistics, full analysis of variance, and prediction equations are all part of the analysis of variance (ANOVA). ANOVA aids in the identification of a significant model for the studies. ANOVA used Fisher's test to determine the Using a p-value, determine the importance of each coefficient (less than 0.05).The correlation coefficient and the adjusted covariance were used to evaluate the expected values and experimental parameters. In order to find the best formulation, numerical and grid searches both were employed in all experimental zones, has a minimal and maximum zeta potential and particle size respectively. The observational answers were compared to the expected values to measure the model's precision (obtained from the equation.)

Model validation and data optimization

With the goal of assuring the necessary quality of ONDNP, For the building of design space, BBD was used to enquire the effect of each self-directed variable on dependent variables. Using desirability (numerical) and overall desirable plot (graphical) criteria, the optimization was carried out with the goal of achieving smaller particle size, PDI, and optimum ZP.

Characterization of ONDNP

Optimized ONDNPs were created and characterised As part of the characterization, physicochemical, solidness, morphological and nasal distribution features were examined.process. Blank NP and ONDNP were lyophilized with mannitol for solid state characterisation.

Particle size, PDI, and Zeta Potential are all factors to consider.

The Zetasizer using photon correlation spectroscopy (Nano-ZS90, Malvern Instruments Ltd, Malvern, Worcestershire, UK) to measure particle size, PDI, and zeta potential.The sample volume for the analysis was kept invariable at for zeta potential measurements, pour 1 mL of formulation into polystyrene cuvettes and use disposable folding capillary cells at 25 °C for particle size and PDI studies.

%EE

The % EE of compounded ONDNP was observed using the centrifuge method. The materials were placed in centrifuge tubes at room temperature and spun at 15000 rpm for 30 minutes to yield OND-NP pellets.The supernatant was collected after centrifugation and UV examined for free drug distribution. The percent EE was computed using the following equation:

$$\%\mathrm{EE} = \frac{(\mathrm{A} - \mathrm{B})}{\mathrm{A}} * 100$$

Where A is the total amount of ondansetron, B is the free amount of ondansetron.

Calorimetry via Differential Scanning (DSC)

DSC Q20 was used to execute OND, CHI, OND + CHI, and OND-NP (TA instrument, USA, V24.9 build 121). Samples weighing 2 to 5 microgram were closed in standard aluminium. The flow rate of 50 ml/min for nitrogen atmosphere, pans were heated at a rate of 10°C/min from 25 to 200°C and analysed at a rate of 10°C/min from 25 to 200°C. An empty sealed aluminium pan was used as a reference.

Infrared spectroscopy using the Fourier transforms (FTIR)

OND, CHI, OND+CHI, and ONDNP FTIR spectra were acquired using an FTIR (Fourier transform infrared) spectrophotometer (photometer) (Spectrum GX, Perkin Elmer, U.S.A.). The potassium bromide disc method was used to create the samples, which were then scanned.at sizes ranging from 4000 to 400 cm1. To explore the relationship between NP and OND, the IR spectrums were compared.

Study of X-ray diffraction (XRD)

OND, CHI, OND + CHI, and ONDNP were measured using an X-ray diffractometer featuring an X-ray setup and a copper electrode, as well as a Xe-filled plate as a detector (X'PERT MPD, Philips, and Holland). The analysis was carried out with a measure sized of 0.017° and an angular range of 3° to 50°, in continuous mode (2). The sample was placed on a specific instrument prior to the measurement. Diffractograms were acquired and reviewed prior to interpretation.

Analysis using scanning electron microscopy (SEM)

SEM (LEO manufacture, 440i, UK) was used to determine the form and surface features of ONDNP using a gold sputtering process. On aluminium the freeze-dried ONDNP was then applied to double-sided tape. The A cold sputter coater was used to coat stubs carrying the gold

sample (Fision SC 7610, Quorum Tech., UK). At a 10 KV accelerated voltage, photomicrographs were taken and 0.6 mm Hg chamber pressure.

Analysis using electron microscopy via transmission (TEM)

A TEM (Philips, Tecnai 20, Holland) was used to examine the morphology of optimized ONDNP at a magnification of 50000x and a 200 kV acceleration voltage Analysis® software was used to calculate the size of the ONDNP (Reutlingen, Germany-based Soft Imaging Systems). After being diluted for ten times in distilled H_2O, a drop of weak ONDNP was utilized as a control. After that, the material was dyed with a 1% phosphotungstic acid aqueous solution and left to adsorb. When the sample had dried, it Images were shot while focusing on a layer of photographic film grid.

Drug Release Studies in Vitro

The in-vitro drug release profile of OND from ONDNP was studied according to the procedure .The OND release profile was carried out by using PBS (pH 7.4) maintained at 37°C and the results were shown in **Fig. 1**

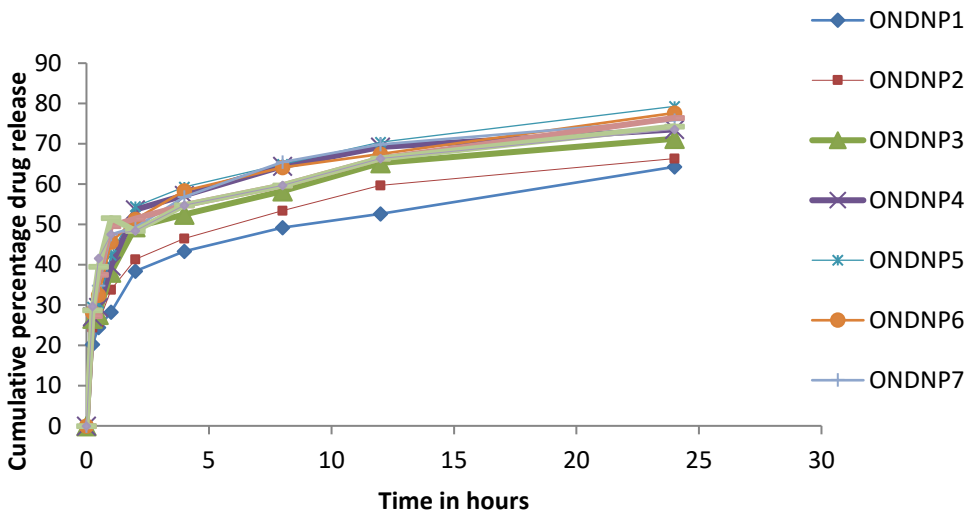


Fig. 1 *In vitro* drug release of ondansetron from ONDNP

The biphasic release pattern of ondansetron-loaded chitosan nanoparticles was observed. Within 30 minutes, after the initial burst release, the remaining medication was released in a constant stream for the next 24 hours. The first burst release of nanoparticles was due to drugs adsorbing

on the particles' surfaces, which swiftly dissolve when exposed to the environment.The ondansetron loaded chitosan nanoparticles (ONDNP8) showed an initial release of 22.8% over the period of 30min, After that, there will be a gradual release for up to 24 hours. The swelling feature of the polymer was responsible for the enhanced release in aqueous media from 22.8 to 71.2 percent. There was no other significant news to report. Various sizes of nanoparticles were shown to be responsible for the different drug release rates. The particle size of ONDNP8 was smaller; therefore surface area tends to become larger and results in faster drug release.

Release Kinetics

To propose a release mechanism of drug from nanoparticles, Data collected from in-vitro drug release trials was plotted and multiple release models were fitted. (0 order, 1st order, Higuchi, Hixson Crowell and Korsemeyer Peppas) **(Gandhi et al 2014 and Li et al 2014).** The release kinetics models were shown in **Figure 2, Figure 3, Figure 4, Figure 5,** and **Figure 6.** Release kinetics data of ondansetron nanoparticles were given in **Table 1**.

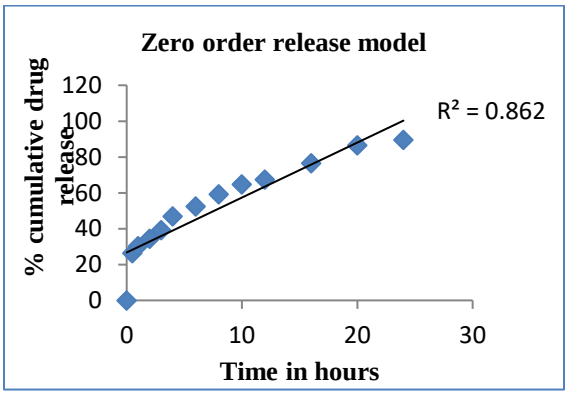


Fig. 2 Zero order release of ONDNP

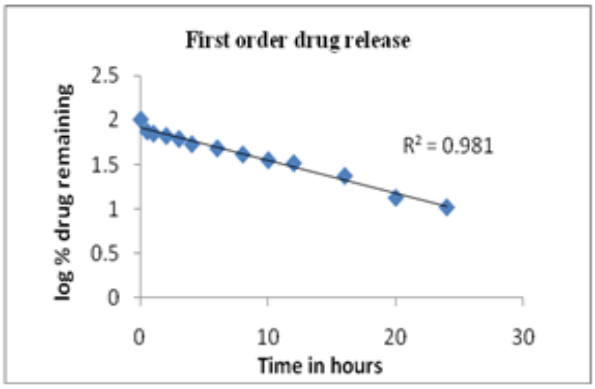


Fig. 3 First order release of ONDNP

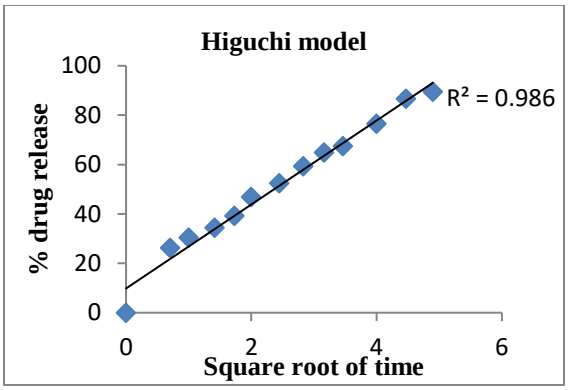


Fig. 4 Higuchi model of ONDNP

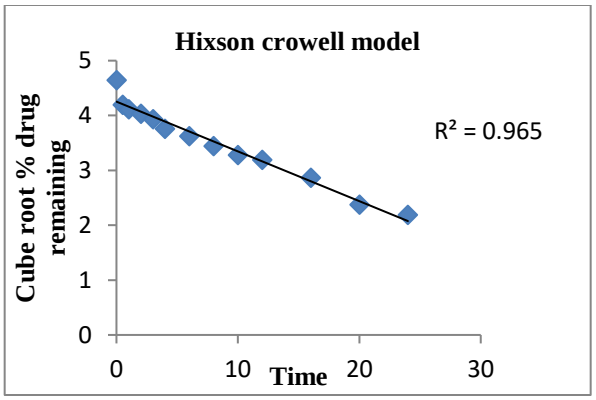


Fig. 5 Hixson Crowell model of ONDNP

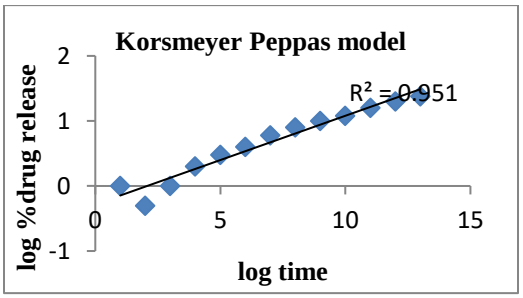


Fig. 6 Korsmeyer peppas model of ONDNP

Table 1 Release kinetics data's of ondansetron nanoparticles

Release Model	Zero order	First order	Higuchi plot	Hixson Crowell	Korsmeyers Peppas	
Regression of ONDNP8	R^2	R^2	R^2	R^2	R^2	N
	0.862	0.981	0.986	0.965	0.951	0.536

Among various release models, the Higuchi model's coefficient of correlation (R2) was near to unity (i.e, 986), therefore the first set form for OND release from the NP was found to be Higuchi model.

Ex Vivo Permeation Study of Ondansetron Nanoparticles

In the present study, the ex vivo nasal permeation survey was carried out in separated porcine nasal mucosa as it's histological and biochemical aspects are closely resembles to human. OND drug solution and ONDNP permeability in vivo were shown in **Fig. 7**.

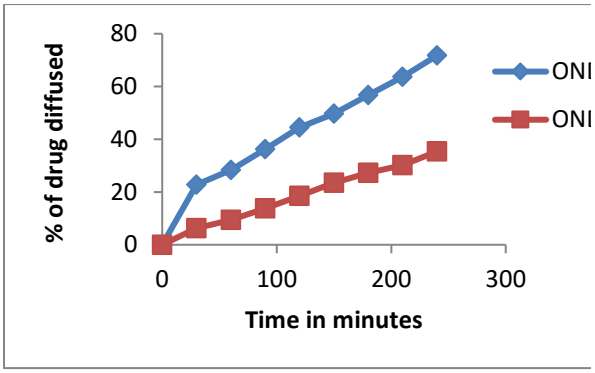


Fig. 7. *Ex vivo* permeation of OND from plain solution and ONDNP across sheep nasal mucosa using a Franz diffusion cell using phosphate buffer solution (pH 6.4) The values represent mean ± SD (n=3) of three batches

Table: 2. Ex vivo permeation of ONDNP and ONDS

Time in min	ONDNP (%)	ONDS (%)
0	0	0
30	22.82±3.26	6.24±1.45
60	28.34±6.42	9.36±4.62
90	36.26±3.87	13.84±6.38
120	44.52±4.18	18.52±3.41
150	49.67±2.52	23.45±5.12
180	56.78±7.10	27.28±4.84
210	63.61±6.06	30.14±3.12
240	71.72±5.78	35.36±5.63

All values were triplicate, expressed in average ± SD (n=3)

The permeation-enhancing action of CHI could explain the important change in diffusion profile (P0.05) of the formulation ONDNP8. In 240 minutes, maximum permeation was found to be 71.72 percent, whereas ONDS was only 35.36 percent (Table 2).A positively charged amino group, CHI, interacts with negatively charged sites on the mucosal membranes could explain the increased penetration of ONDNP. Finally, ONDNP8 was chosen as a promising formulation for further study due to its smaller particle size, higher entrapment efficiency, and improved loading capacity with a relatively enhanced permeation profile. The diffusion exponent (n) and diffusion constant (k) derived from the Peppas plot were 0.266 and 9.555, respectively, while the R2 was

found to be 0.962 in the drug permeation investigation. The n value in this case was between 0.45 and 0.89, showing that ex vivo diffusion of OND from ONDNP followed a Non Fickian diffusion pattern.

Stability Study of ONDNP

The stability of the formulated OND nano formulation was determined as per ICH guidelines. For long-term stability, the OND nano formulation was stored for 6 months at 5±3^0 C. The accelerated stability of OND nano suspension was determined by storing for 6 months at 25±2 ^{0} C and 60 ± 5 % RH and results were summarized in **Table 3.**

Table: 3. Stability study of ondansetron nanoparticles

Sampling (after no.of days)	**particle size (nm)**		**physical appearance**		**zeta potential**	
	Long-term 5± 3^{0}C and 60 ± 5 % RH.	Accelerated 25± 2^{0}C and 60 ± 5 % RH.	Long-term 5± 3^{0}C and 60 ± 5 % RH.	Accelerated 25± 2^{0}C and 60 ± 5 % RH.	Long-term 5± 3^{0}C and 60 ± 5 % RH.	Accelerated 25± 2^{0}C and 60 ± 5 % RH.
0	142.34	140.45	NC	NC	48.1	42.6
30	146.24	141.12	NC	NC	45.3	40.5
60	149.26	154.82	NC	NC	44.8	42.6
90	152.54	168.24	NC	NC	45.1	42.4
120	156.30	169.62	NC	NC	45.2	41.1
150	168.16	172.80	NC	NC	44.2	42.4
180	170.45	181.24	NC	NC	45.4	40.8

NC no change

The stability of the OND nano formulation was ascertained by monitoring the physical appearance, zeta potential and particle size after retention at 5±3^0 C and 25± 2^{0}C for a period of 6 months. Further, no dramatic increase in particle size was observed. However a marginal reduction in the zeta potential of OND nano formulation was observed. There was no change in physical appearance of the nano suspension indicates the stability of the nano formulation.

Methods

Using the Box-Behnken approach to optimize the chitosan nanoparticles production process Ionic gelation was used to make chitosan nanoparticles. DOE was used to optimize the method of preparing chitosan nanoparticles by using Box-Behnken response surface methodology. The relationship between the researched parameters and their impact on the size of chitosan nanoparticles can be investigated using the Box-Behnken experimental design as a response surface method. Three parameters (chitosan (mg/ml) (X1), STPP (mg/ml) (X2), and zeta potential (Y3)) influenced the measured response particle size (Y1), polydispersibility index (Y2), and zeta potential (Y3) (X2). The amount of chitosan polymer (1-8 mg/ml), the amount of STPP as a cross linking agent (0.1-0.4 mg/ml), and the RPM are all factors to consider. all play a role in the modified spontaneous ionic gelation process (1000-2000). The levels of the factors were labelled as -1 and 1.Table 1 shows the code ranges of independent variable levels generated by 16 experiments were produced using the Box-Behnken design. Table 4 shows the model's design matrix for the findings coded and predicted

Table: 4 Variables that were used in the Box-Behnken experiment.

Independent variables	**Symbols**	**Levels**	
		-1	**+1**
Chitosan concentration mg/ml (polymer)	X_1(A)	1	8
STPP mg/ml (CLA)	X_2(B)	0.1	0.4
RPM	X_3(C)	1000	2000
Dependent variables	**Unit**	**Constraints**	
Particle size (PS)	nm	Minimize	
Polydispersibility index (PDI)	-	<1	
Zetapotential (ZP)	-	Maximize	

Chitosan nanoparticles formulation

Chitosan (100 mg) was disseminated in glacial acetic acid (1 percent twenty ml) and endlessly spun for two hours to produce chitosan gel. The STPP were distributed in 10 mL of water. The STPP solution was dropped into the chitosan gel with a 1 mL micro syringe and stirred for 2

hours at room temperature. To eliminate any remaining STPP, the solution was filtered using a membrane filter (0.45m).The nanoparticles were concentrated by ultracentrifugation at 12,000rpm for 20 minutes at 4°C (Remi cooling Centrifuge C-24, Mumbai, India). A UV-Visible Spectrophotometer was used to determine the amount of free medication in the supernatant. (UV-1601, Shimadzu, Japan) at 248nm after the supernatant was removed. The ondansetron-loaded chitosan nanoparticles were lyophilized using sucrose as a cryoprotectant, resulting in a dry powder. The following is a second degree polynomial model for this response:$Y=\beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_3X_3+ \beta_{12}X_1 X_2+ \beta_{13}X_1 X_3+ \beta_{23}X_2 X_3+ \beta_{11}X^2_1 + \beta_{22}X^2_2+ \beta_{33}X^2_3$ (1)

The regression coefficients 1 to 3 are determined using observed experimental Y values from observational runs, and the X1, X2, and X3coded even indicate components A, B, and C, severally. There are three types of equations: quadratic, linear and interaction. Impacts of the self-directed variant on the solutions can be estimated using this equation. Design-Expert was used to do the data was analyzed statistically using a surface graphs of the responses were plotted using the regression model. Case statistics, full analysis of variance, and prediction equations are all part of the analysis of variance (ANOVA). ANOVA aids in the identification of a significant model for the studies. ANOVA used Fisher's test to determine using a p-value to determine the relevance of each coefficient (less than 0.05). The correlation coefficient and the modified correlation coefficient were used to evaluate the expected values and experimental parameters. In order to discover the optimal formulation, both quantitative and electrode searches were used in all observational area, with the requirements that the particle size is maintained at a nominal and the zeta potential be kept at a maximum. To assess the precision of the mode, that the particle size is kept as small as possible and the zeta potential is as high as possible. The experimental answers were compared to the expected values to determine the precision of the mode (obtained from the equation).

Table: 5 Experiments with Box-Behnken designs in several runs and the results

Design points	Factor levels			Responses		
	X_1 chitosan	X_2 STPP	X_3 RPM	Particle size	Polydispersibility index	Zeta potential
1	1.00	0.40	1500.00	310	0.437	28
2	1.00	0.25	2000.00	211	0.336	42
3	4.50	0.25	1500.00	158	0.534	46
4	4.50	0.40	2000.00	152	0.416	52
5	1.00	0.25	1000.00	278	0.37	41
6	8.00	0.40	1500.00	314	0.426	50
7	4.50	0.25	1500.00	158	0.534	46
8	4.50	0.10	1000.00	290	0.474	54
9	8.00	0.25	1000.00	316	0.482	44
10	4.50	0.25	1500.00	158	0.534	46
11	4.50	0.25	1500.00	158	0.534	46
12	1.00	0.10	1500.00	280	0.42	56
13	8.00	0.25	2000.00	340	0.482	51
14	4.50	0.40	1000.00	262	0.416	48
15	8.00	0.10	1500.00	354	0.384	44
16	4.50	0.10	2000.00	160	0.527	53

Preparation of ondansetron-containing chitosan nanoparticles

The experimental design provides the best synthesis method was used to make ondansetron-containing chitosan nanoparticles. In a 100ml beaker, chitosan gel (20ml) was added, and Magnetic stirring was used to dissolve ondansetron in the gel. Drop by drop, the STPP solution was added to the chitosan gel containing the medication, and mixed at room temperature for 2 hours.

Ondansetron nanoparticles release ondansetron in vitro

To compare the drug release from the optimized nano particle formulation and to analyze the release pattern, in vitro dissolution studies were conducted. The identical amount of ondansetron (5 mg) was collected from both the nanoparticulate suspension and the medication solution. The dialysis bag approach was used to conduct the in vitro drug product study. Himedia, Mumbai, cellulose dialysis bag (MWCO 12,000 g/mole) was filled with nanoparticles containing 5 mg of medicine, and Before the bag was closed on both ends, a little amount of dissolving media was added. In a receptor compartment dialysis bag was dipped, which contained the dissolving average phosphate buffer mixture (PBS) pH scale 7.4, whirling at 100 rpm and maintained at 37°C.At regular intervals, sampling were taken out and replaced with fresh dissolving media in the same amount. A UV spectrophotometer set at 248 nm was used to measure the samples.

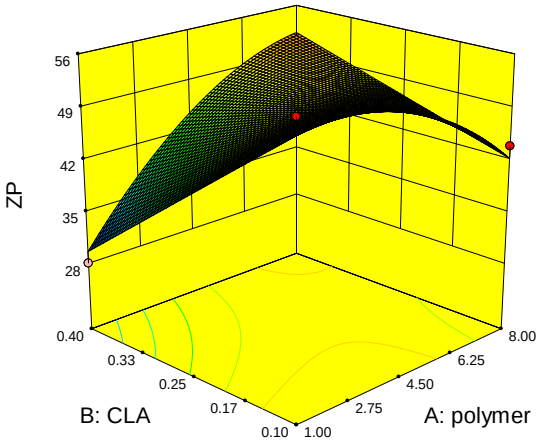
Discussion of the findings

Effects of factors on chitosan nanoparticle size, polydispersibility index, and zeta potential

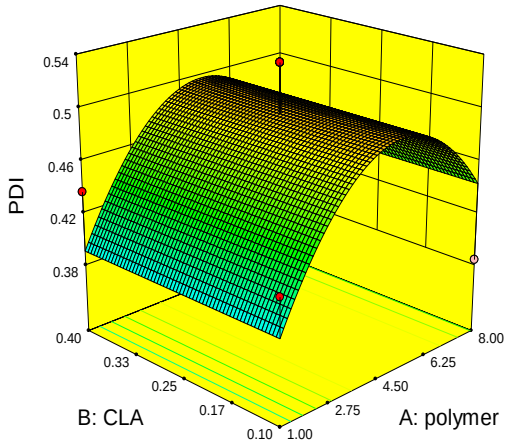
The combined effect of three factors can be predicted using experimental design. At the middle level, one variable is deemed fixed because the quadratic model has three independent variables [13].The response surface plot (Fig. 1) depicts the effects (a) The average particle size of chitosan nanoparticles as a function of numerous variables, including the concentration of chitosan and the concentration of STPP, shown as a surface plot. The response surface plot (Fig. 1) depicts the effects (a). Particle size of chitosan nanoparticles on average as a function of many variables, including chitosan concentration and STPP concentration, shown as a surface plot. As a result, increasing the chitosan polymer concentration reduces particle size. If the average amount of STPP cross linking agent is applied, the particle size will be minimum values, as shown in Fig.1 (a). According to X1 and X2 show diminishing and rising impacts on particle size in polynomial coefficient and variance analyses.It was discovered that X3 had no effect on particle size. The response surface plot of the Polydispersibility index in Fig.1 (b) demonstrates that there are no significant changes with variations in chitosan polymer and cross linking agent STPP concentrations. The reaction surface plot of zeta potential is shown in Fig. 1 (c). The zeta potential of the improved formulation indicates that it is fairly stable. This measurement is a crucial factor in assessing the nanoparticle suspension's stability. A high absolute zeta potential value indicates that the drug-loaded nanoparticles have a high electric surface charge, which can produce strong repellent forces between particles, preventing aggregation. As a result, there is a

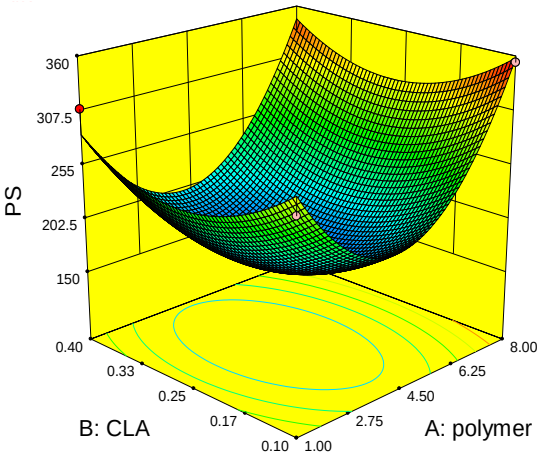
trade-off between nanoparticle size and maximum stability. As a result, the following personality of several parameters on the synthesis of chitosan nanoparticles were describe

(a)



(b)





(c)

Fig.7. Predicted response surfaces plot corresponding to (a) Particle size (b) Polydispersibility index (c) Zeta potential

Optimization of many criteria

Because changes in experimental settings did not have the same effect on the selected responses, a reasonable compromise between nanoparticle size and maximal stability had to be found. In this investigation, the two answers with different goals were optimized. Derringer's the desire function (D) was utilized, which is the geometric mean of all desirability functions, weighted or

$$D = \left[d_1^{p1} \times d_2^{p2} \times d_3^{p3} \times \ldots\ldots \times d_n^{pn} \right]^{1/n} \quad \text{not.} \qquad (2)$$

Where pi is the response's weight, n is the di is the desire function of each participant, and n is the number of responses. The modification of each experiment's individual reaction yields a response, Derringer's desirability function is defined as follows: The individual desirability function has a scale that ranges from 0 to 100 di = 0 (totally undesirable reaction) to di = 1 (entirely desirable answer).The weights might be anywhere from 0.1 to ten. Weights less than one place a lower priority on the goal, whilst weights more than one place a higher priority on the target (Di varies non-linearly in both cases as it approaches the target value).With a weight of one kilo gramme on the other hand, varies linearly. We chose 1 as the weight for each of the six responses in this report. A amount of D other than zero indicates that all replies are in a desirable limit at the same time, and a value of D close to 1 indicates that the accumulation of the opposite criteria is globular optimum, resulting in response values that are close to target values. Table 2 lists the criteria for each individual response's optimization. A set of criteria has been presented for selecting the ideal experimental conditions for the formulation of ondansetron chitosan nanoparticles. In order to obtain optimal in vitro performance, As stated under criterion, the nanoparticle response particle size was selected and kept to a minimum. In addition, the zeta potential was determined. Figure 2 depicts the desirability plot for the global desirability function. RPM, Chitosan (1.95mg/ml), and STPP (0.14mg/ml) were the coordinates that produced the highest desire value (D=0.873) (2000). Table 6 shows the projected response values corresponding to the latter value of D. The model's prediction efficiency was confirmed by running the experiment under ideal conditions. Table 6 shows the agreement between experimental and projected responses for the predicted optimum. With a difference of 1-4 percent, the errors for particle size, polydispersibility index, and zeta potential were found to be 1.60, 2.11, and 1.72 percent, respectively. The particle size analysis and in vitro drug release research of the improved formulation were assessed.

Table 6

comparison of observed and predicted values of different objective functions under optimal conditions

Chitosan	STPP	RPM	PS	PDI	ZP
Desirability value (D) = 0.873					
1.95	0.14	2000			
Predicted value			193.63	0.435	51.50
Experimental value			190.5	0.426	52.40
Average error (%)			1.60	2.11	1.72

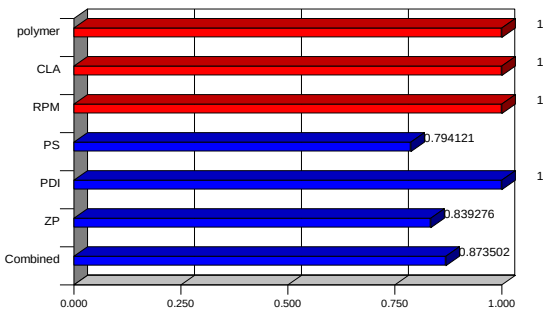


Fig.8. Graphical representation of overall Desirability

Analysis of particle size

SEM was used to find out the particle size and shape of synthesized chitosan nanoparticles. SEM was used to determine the size of optimal chitosan nanoparticles containing the ondansetron medicine developed in this experiment, and the findings are displayed in Figure. 8. The average particle size is 37nm, with a spherical shape morphology, according to SEM images.

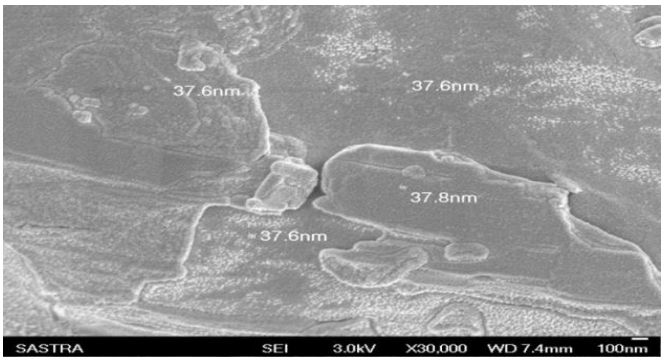


Fig.9. SEM photomicrographs of ondansetron chitosan nanoparticles

FTIR (Fourier transform infrared) spectroscopy

Figure9 shows the FTIR analysis of optimised ondansetron chitosan nanoparticles.

The drift spectrums of the optimised chitosan nanoparticles samples were collected. Chitosan with ondansetron has carbonyl, hydroxyl, and free alkane series teams. it's Because of the O-H stretch and H-bonded referencing aromatic ring in its structure, it peaks at 3405.16. At 2934.13, it reaches its highest point.counsel the existence of alkanes as a result of C-H stretch. The C=O stretch is seen at 1626.63. The C-C stretch in rings that refers to aromaticity is visible at 1577.91, 1481.68, and 1420.69. Peaks at 1090.74 and 960.96 severally show C-N stretch and =C-H bend in its structure.Ondansetron nanoparticles had the useful cluster of chitosan nanoparticles, and there was no modification in any distinctive peaks, indicating that there was no chemical interaction between the drug and also the chemical compound within the ondansetron chitosan nanoparticles.

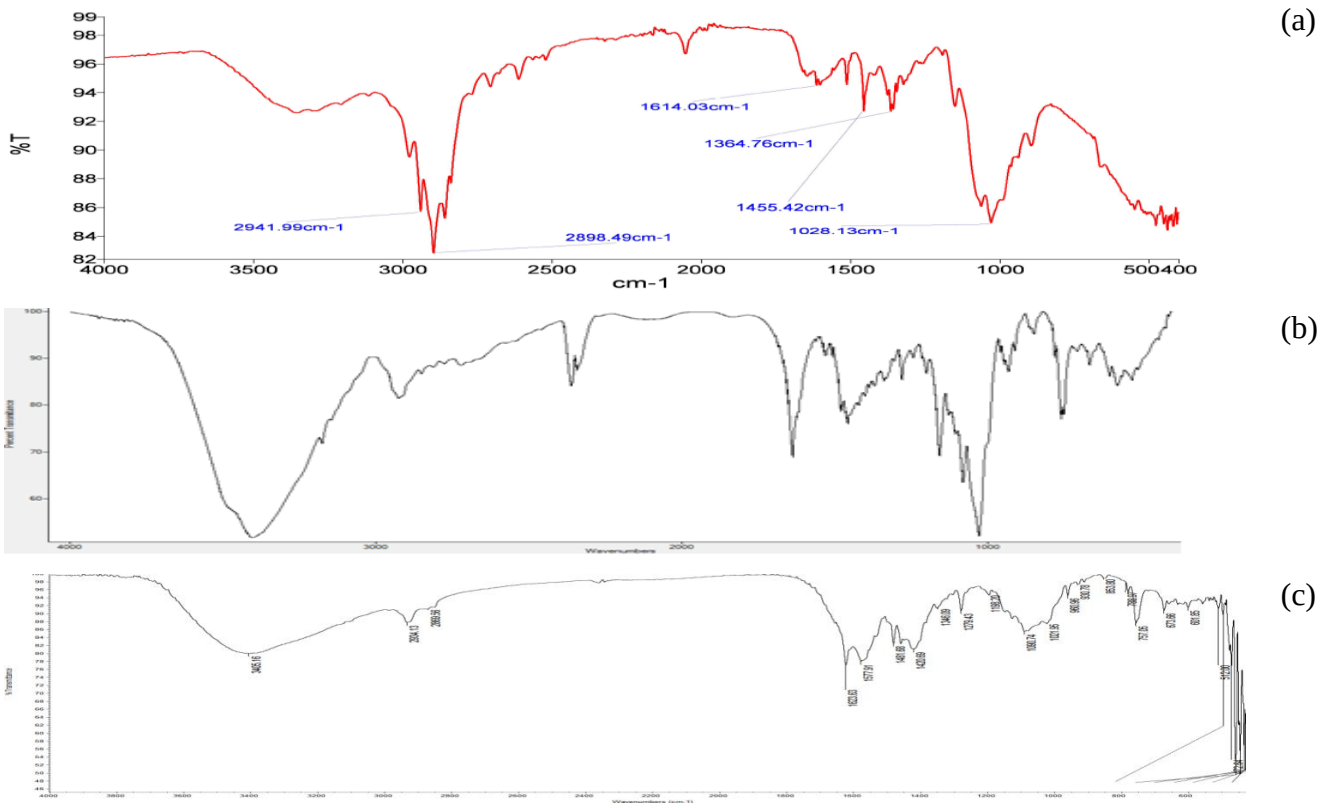


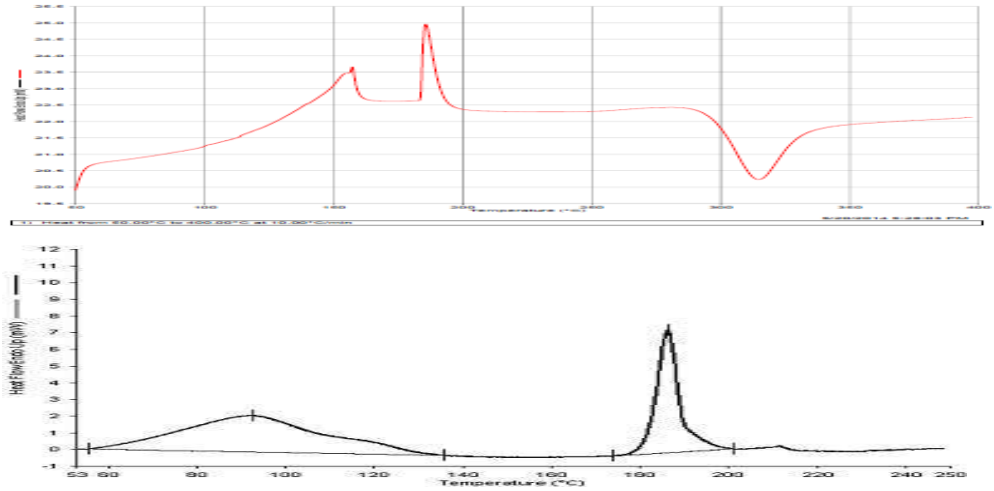
Fig. 10.FTIR of (a) chitosan, (b) ondansetron (c) ondansetron chitosan nanoparticles

Analyzed using DSC

DSC is a useful tool for determining a formulation's thermal characteristics and obtaining information on the drug's physicochemical state within the system In the absence of interaction, a formulation's thermogram will display patterns that are similar to those of the separate components. If an interaction occurs, it may result in the development of one or more new peaks, the loss of one or more peaks matching to those of the components [14], or a shift in peaks [15]. Figure 10 shows the DSC thermograms of pure chitosan, ondansetron, and ondansetron loaded chitosan nanoparticles. The DSC profile of Chitosan reveals 2 heat-absorbing and one chemical reaction (s). With a peak height of 157.20 degrees Celsius, the initial endothermic peak was identified between 153.64 and 159.38 degrees Celsius.The highest height was 0.6884 mW, while the peak area was 43.626 mJ. H was computed from this, and it was found to be 32.0781 J/g. Another endothermic peak, with a peak height of 185.68 C, was interpreted between 183.62 C and 191.61 C. 72.309 mJ and 2.3670 mW were discovered to be the peak area and peak height, respectively. H was found to be 53.1682 J/g. Between 296.83 C and 332.60 C, an exothermic

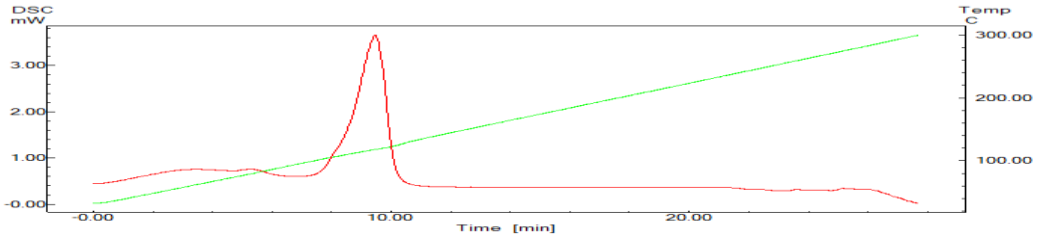
peak was recorded, with a peak height of 314.10 C. 1.9476 mW and 270.049 mJ were discovered to be the peak height and area for this. 198.5652 J/g was calculated as the H value. As a result, the chitosan had gone through three temperature changes, accelerating its thermal properties. The first endothermic peak could be due to water evaporation, while the second endothermic peak could be due to compound decomposition. The disintegration of its elements caused the exothermic peak.

The DSC of ondansetron displays associate exoergic peak at 257.68 C. The DSC curve of Chitosan discovered associate endothermic temperature of 317.26 degrees stargazer associated an exoergic peak of 257.68 degrees stargazer.Between 8 and 10 minutes of operation, the DSC of ondansetron nanoparticles displayed its curve, which corresponded to a temperature range of 80 to 280 degrees Celsius.This curve is due to the release of water molecules and the subsequent disintegration of ondansetron nanoparticles reaching their melting point. It reveals that the exothermic peak crystallisation has broadened, indicating that the medication has been incorporated into the nanoparticle matrix.



(a)

(b)



(c)

Fig. 11. DSC of (a) Chitosan. (b) Ondansetron. (c) Ondansetron hydrochloride chitosan nanoparticles

Ondansetron release from chitosan nanoparticles in vitro

In vitro, over the course of three hundred minutes, the additive proportion unharness of ondansetron nanoparticle suspension varied from 22.8% to 71.2%. The biphasic release pattern (Figure 12) of ondansetron-loaded chitosan nanoparticles was observed.Within 20 minutes, the first ruptured release impact happen, and the leftover amount of medication was released over a 300-minute period. Particles adsorbing on the nanoparticles' surface cause the first burst release, which disintegrates instantly when the particles come into touch with the release medium.

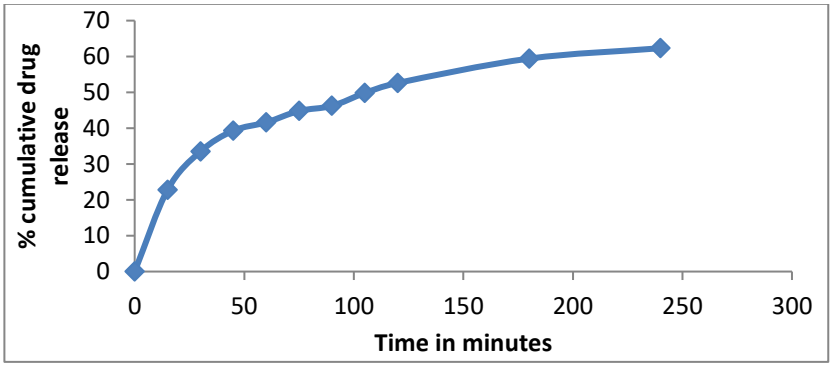


Fig.12 : *in vitro* drug release of ondansetron from chitosan nanoparticles

Conclusion

The purpose of this work was to form and optimise chitosan nanoparticles as delivery systems for the medication medication ondansetron. variety of parameters, as well as the number of chemical compound chitosan utilized, the concentration of STPP utilized as a cross-linking agent, and also the method parameter rate, would possibly alter the dimensions of those particles. The Box-Behnken method was used for statistically optimizing parameters and investigating the many effects of freelancing factors on particle size..SEM, FTIR, DSC, and in

vitro drug unharness were wont to analyse the improved formulation for particle size, alphabetic character potential, and morphological options.The morphology of the spherical form was disclosed by SEM investigation. A biphasic pattern of drug unharness was seen in vitro, with a burst unharness result followed by continuous unharness.These information imply that chitosan nanoparticles loaded with ondansetron square measure acceptable for medication delivery.

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Conflict of Interest:

The authors declare no conflict of interest.

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