

**PREPARATION AND EVALUATION OF BUCCAL FORMULATION FOR
ONDANSETRON HYDROCHLORIDE*****Abdul Naim, Dr. Rama Bukka, Dr. Shachindra L. Nargund, Dr. Sharavan L. Nargund**

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ABSTRACT

the polymeric nanoparticles of ondansetron hydrochloride were prepared by cross-linking method. The polymeric nanoparticles were evaluated for particle size, zeta potential, entrapment efficiency and percentage *in-vitro* drug release by linear model. The particle size and entrapment efficiency of the nanoparticle was found to increase with increase the Sodium Tripolyphosphate (STPP) concentration and decrease the STPP concentration increase the drug release. the optimised concentration for independent variables Chitosan (A) and STPP (B) was found to be 75mg and 0.5% respectively, with a desirability of 0.514 by numerical optimization method. The optimized formulation was the added to buccal patch formulation. Buccal patch was prepared by solvent casting method. The buccal patch was evaluated weight uniformity, folding endurance, thickness, drug content, mucoadhesive strength. Dissolution study, tensile strength, percentage elongation, *in-vitro* drug release and *ex-vivo* studies.

KEYWORDS: Polymeric nanoparticle: Ondansetron hydrochloride, Chitosan, STPP, Buccal Patch: Sodium alginate, HPMC K-4M, PEG-400.**INTRODUCTION**

Nanotechnology applied in drug delivery can be optimized to maximize drug delivery efficiency by tailoring matrix, structure and property of the carriers, and to increase the efficacy of treatment. At the same time, nanoparticles can be used in a variety of administrations, such as oral delivery, subcutaneous injection, transdermal administration and mucosal administration.

Among which, mucosal administration is an important way to study local administration in recent years. The mucosa is highly vascularized, resulting in rapid absorption and administration of drugs in the mucosa route. The specific structure can also avoid degradation in the gastrointestinal tract and first-pass metabolism in the liver. However, the nano carrier and mucous membrane in the mucosal administration should have ideal features, such as adhesion and permeability.^[1] Nanoparticles can also improve the mucoadhesive property of dosage forms because the surface groups on the nanoparticles interact with the buccal mucosa via hydrogen bonding. The potential of aggregation and

inhomogeneous distribution of large particles may affect mechanical films and mucoadhesive properties; therefore, the particle size is reduced to the nanoscale to overcome those limitations.^[2]

Ondansetron hydrochloride is a highly effective 5-HT₃ receptor antagonist widely used to prevent nausea and vomiting associated with chemotherapy, radiation therapy, and surgery. However, conventional oral formulations of ondansetron face several limitations, such as low bioavailability due to first-pass metabolism, variable gastrointestinal absorption, and a delayed onset of action. These challenges can reduce the effectiveness of the drug and complicate treatment, especially for patients who need quick relief.^[3]

MATERIALS AND METHODS**MATERIALS**

Ondansetron Hydrochloride (Neon laboratories Ltd. Mumbai), Chitosan (ultra pure lab chem industries LLP), Acetic Acid (Molychem.pvt. Ltd. Mumbai), STPP (Sisco research laboratories pvt. Ltd), Sodium alginate (S d fine-chem Ltd, Mumbai), HPMC K-4M (S D fine chem

limited. mumbai), PEG-400(Merck Ltd, Mumbai), Methanol (S.d.fine.chem.Ltd.Mumbai).

METHODS

Formulation of Ondansetron HCl polymeric Nanoparticles

Ondansetron Hydrochloride with chitosan and STPP. polymeric nanoparticles, were prepared by ionic cross linking method. Nine different formulation i.e. F1, F2, F3, F4, F5, F6, F7, F8 AND F9 were prepared, changing the amount of chitosan (50, 75, 100 mg) and cross-linking agent STPP (0.5,0.75,1%). Fixed quantity of drug (50mg) was added with the chitosan and 10ml acetic acid (0.75%) and stir on a magnetic stirrer for 2-hours to get

clear solution of polymer and drug, then add 5ml STPP of different concentration into polymer solution as per requirement of formulation and keep on stir for 2 hours continuously. The particles become shrink and turbid due to interaction of chitosan polymer with STPP ions. The pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was continued for 30 minute to get the crosslinking.^[4]

Table no. 1: Formulation chart of Ondansetron HCL nanoparticles (F1-F9).

Formulation Code	Drug (mg)	Chitosan(mg)	STPP (%)
F-1	50	50	0.5
F-2	50	50	0.75
F-3	50	50	1
F-4	50	75	0.5
F-5	50	75	0.75
F-6	50	75	1
F-7	50	100	0.5
F-8	50	100	0.75
F-9	50	100	1

Evaluation of Nanoparticles

Entrapment Efficiency: (indirect method)

The Entrapment efficiency of prepared sample was determined by indirect method by measuring the concentration of the drug in the dispersion medium. The free drug of Ondansetron HCl was determined by adding 2 ml of Ondansetron HCl loaded polymeric nanoparticles which is added with 2ml of methanol transferred to centrifuge tubes and then, dispersion was centrifuged

(Eppendorf Centrifuge 543OR) at 7000 rpm for 30 minutes at 4°C.^[5]

1ml of supernatant was taken and appropriately diluted with phosphate buffer pH 6.8 and absorbance measured by UV spectrophotometrically at 309nm in triplicate. (n=3)

The percentage entrapment efficiency was calculated by given equation.

$$\% \text{Entrapment Efficiency} = \frac{\text{Total drug content}(W) - \text{Free drug content}(w)}{\text{Total drug content}} \times 100$$

Drug Content

1 ml of the dispersed polymeric nanoparticles were added with 6% acetic acid solution which dissolves both the polymer and the drug. After that 1 ml of solution was diluted with 10 ml of pH 6.8 phosphate buffer solution to get the concentration in the standard graph range and the drug content was determined by using UV-Spectrophotometry at 309 nm. The test was repeated 3 times and average readings was noted(n=3).^[6]

Particle size distribution and Polydispersity index

The average particle size and size distribution are important parameters because they influence the physicochemical properties and biological fate of the Polymeric Nanoparticles after *in-vivo* administration. Particle size of the Ondansetron HCl polymeric NP's and Polydispersity index were determined by using dynamic light scattering (DLS) technology, HORIBA Scientific

(nano-partica) NANO PARTICLE ANALYZER SZ-100 at a fixed angle of 90° at 25 °C.

1ml of Ondansetron HCl polymeric NP's were taken and diluted with 10 ml of double distilled water, vortexed for 2 minute after diluting followed by keeping it in polystyrene cuvette for the evaluation of particle size and Polydispersity index.^[7]

Zeta potential

The surface charge (zeta potential) was determined by measuring the electrophoretic mobility (electric charge) of the nanoparticles. It helps to predict the storage stability of the colloidal dispersions. At higher ZP, less PS aggregation occurs due to electrostatic repulsions.^[8] ZP was measured by using a HORIBA zeta sizer. 1ml of prepared Ondansetron HCl Polymeric nanoparticle was taken and diluted with 10 ml double distilled water,

transferred into the cuvette which has electrode and the electrophoretic mobility was measured.

In-vitro drug release studies

The *in-vitro* drug release of Ondansetron HCL polymeric nanoparticles were determined by using dialysis membrane bag diffusion techniques, which is regenerated with seamless(smooth) cellulose tubing; membrane is partially permeable and has molecular weight cut off between 12000-14000Da. Pore size 2.4 nm; ideal for filtration and diffusion work, 4 cm of the dialysis bag were cut and soaked overnight in pH 6.8 phosphate buffer to become fully hydrated, achieving its optimal porosity and flexibility.

This is essential for proper diffusion of solutes across the membrane, this helps to avoid changes in the membrane dimensions during the experiment, which could alter its permeability. Ondansetron HCL polymeric NPs formulation 1 ml equivalent to 4 mg was taken in dialysis bag and both end of the bag was tied on glass rod and placed in a beaker containing 100 ml of pH 6.8 phosphate buffer (glass rod was used to immerse the dialysis bag to avoid the floating of the bag), which acted as receptor compartment. The dissolution medium was continuously stirred at 100 rpm at 37 °C using magnetic stirrer for 24 h. Definite aliquots each of 5 ml volume of sample were withdrawn at regular time intervals and replaced with equal volume of dissolution medium was added to maintain a sink condition.

The amount of drug was analyzed by UV-Vis Spectrophotometer at 309 nm. The study was repeated 3-times for each formulation mean and standard deviation were noted down(n=3).^[9]

Scanning Electron Microscopy of optimized formulation (F-4)

Optimized formulation F-4 was prepared and centrifuged at 7000 rpm for 30 minutes the supernatant was discarded and decant was dispersed in 10ml double

distilled water and subjected to lyophilized. Lyophilized sample was subjected for SEM analysis.^[10]

Surface morphology was analyzed using SEM, in which the powders were imaged by a scanning electron microscope (SEM) run at an accelerating voltage of 10kV using Hitachi SU 3500. The powder in few µg were fixed on to stub by a double sided sticky carbon tape and kept inside the SEM chamber and analyzed at different magnification such as 60X, 200X, 500X. 1.10X and 2.50X respectively to obtain better clarity on the particle morphology.

Preparation Of Nanoparticle Based Buccal Patch By Solvent casting method

The matrix-type buccal patches containing NPs Ondansetron hydrochloride were prepared by solvent evaporation method using 1:1 ratio of hydroxy propyl methyl cellulose K-4M(HPMC-K4M) and Sodium Alginate. The given ratios of polymers were weigh and dissolved separately in 10 ml of water respectively, after soaking for 2 hours, sodium alginate slowly added and mixed with HPMC K-4M. Propylene glycol 0.6 ml (28%v/w) was added as plasticizer and also as permeation enhancer and stirred well for 30 minute. Then, nanoparticles containing Ondansetron hydrochloride equivalent to 196 mg drug was added into the polymer and kept for stirring 1- hour. Make casting mold area of 49 cm² was prepared using aluminium foil.

The above prepared polymers gel was casted over the foil such that every 1 sq.cm of the film contains Ondansetron HCl equivalent to 4 mg. Then, casting solvent mixture was allowed to evaporate for 12 hrs. in hot air oven at 50 °C.

Table 2: Formulation chart of Ondansetron HCl nanoparticles patch.

SI. No	Ingredients	P
1	NPs of Ondansetron hydrochloride equivalent to	196 mg
2	Sodium Alginate	300 mg
3	HPMC K-4M	300 mg
4	Polyethyleneglycol-400 (ml)	0.6 ml
5	DD water	20 ml

Evaluation of buccal patch

Buccal patches were evaluated for following characters

Physical appearance: All the prepared patches were visually inspected for colour, clarity, uniformity, flexibility, and smoothness.

Weight uniformity: To ensure weight consistency, three patches of formulation were individually weighed using

an electronic weighing balance. The average weight of the patches was calculated.

Determination of the thickness of patch: Vernier callipers was used to determine the thickness of randomly selected patches. Three patch was measured at five different points and average of three readings was taken.^[11]

Folding endurance test: Folding endurance of the patches was determined by repeatedly folding one patch at the same place till it broke or folded up to 300 times

manually. The number of times that patch could be folded at the same place without cracking gives the value of folding endurance.

Surface pH determination

Table. No.3 Formula for preparation of simulated human saliva.

Ingredients	Required Quantity
NaCl	0.126
KCl	0.964
KSCN	0.189
KH ₂ PO ₄	0.655
Urea	0.200
Distilled Water	1-Litre

Surface pH determination Patches were left to swell for 3 h on agar plate. Agar suspension was prepared by dissolving 2% (w/v) agar in simulated human saliva of pH 6.8 under stirring and then pouring the solution into a Petri dish until gel is formed at room temperature. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch.^[12]

Drug content uniformity: Three patches of formulation was cut into 1 cm² pieces which contains 4mg drug and added into 100 ml of pH 6.8 phosphate buffer. Then medium was stirred with the help of magnetic stirrer for 24hrs. Then the solution was filtered and diluted suitably with buffer, analysed at 309nm with the help of UV spectrophotometer. The average value of drug content of all patches was taken as final readings.^[13]

Percentage moisture content: Three patches of formulation were cut into 1cm² and weighed individually and placed in a desiccator containing activated aluminium oxide at room temperature for 24 hrs.^[14] Each patch was weighed repeatedly after a specified interval until it shows a constant weight and the percentage moisture content was calculated using the given formula.

$$\text{Percentage moisture content} = \frac{(\text{initial weight} - \text{final weight})}{(\text{initial weight})} \times 100$$

Swelling index

To determine the swelling capacity, the patches were weighed and placed on a 2% agar gel plate. The agar gel plate was prepared by mixing 2 g of agar powder with 98 ml of hot distilled water. A 25 ml portion of the agar solution was poured into a petri plate and left at room temperature until it solidified. three patch formulation was placed on the agar gel plate. At regular intervals, the patches were removed, excess surface water was carefully eliminated using a filter paper, and the swollen patches were reweighed.^[14]

The degree of swelling was calculated using the following formula.

$$\text{Swelling index} = \frac{\text{Wet weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Drug Content

To determine the drug content of the ondansetron hydrochloride loaded buccal patch equivalent to a dose of 4 mg, it was dissolved in 50ml of pH 6.8 buffer. The solution was sonicated for 10 minutes, filtered through Whatman filter paper, and then brought up to a volume of 100 ml using phosphate buffer. 1 ml of the filtrate was diluted to 10 ml with pH 6.8 buffer. The absorbance of the resulting solution was measured using a U.V spectrophotometer at 309 nm, and the drug content was calculated. (n=3).

Tensile strength and Percentage Elongation

The tensile strength of the patches was determined using an apparatus designed as shown in the figure.no 4.3. The instrument was constructed indigenously, consisting of a wooden frame with a fixed scale horizontally placed on top.

Two clips were attached to the frame to hold the patches under study, with one clip fixed and the other movable. The instrument also included a pulley to hold the weight of the patch, with one end of the pulley attached to the weight and the other end attached to the fixed clip.

Care was taken to ensure that the wooden platform did not dislocate during the test. Dried Patches with dimensions of 1x2 cm² were cut for the study, attached the patch both side with the clips of the instrument and the thickness of the patches was noted. The rate of stress changes was kept constant by adding 0.5 gm per 1-minute weight gradually added to pan till patch was broken.

The weight necessary to break the patch was noted as breaking force. The elongation was observed, and the total weights applied was considered for calculation of the tensile strength using the appropriate formula.^[15]

$$\text{Tensile strength (T)} = Fb/A$$

Fb- break at force (kg)

A- initial cross section area of sample

Percentage elongation

$$= \frac{\text{Final length of strips} - \text{Initial length of strips}}{\text{Initial length of strips}} \times 100$$

Mucoadhesive strength

To determine the ex-vivo Mucoadhesive strength, we employed Porcine buccal mucosa as a biological membrane due to its similarity to human buccal tissue. We utilized a modified physical balance method for this study. Fresh porcine buccal mucosa was obtained from a local slaughterhouse and used within 2 hours of slaughter.

The mucosal membrane was prepared by removing the underlying fat and loose tissues using blunt forceps and scissors, followed by dipping the tissue in hot water. The collected tissues were then washed with distilled water, placed in a normal saline solution at 4°C to maintain freshness, and immediately used for the experiment. The tissue was cut into approximate sizes of the Mucoadhesive patches using scissors.

To modify the physical balance, we replaced the left-hand side pan with a glass block that had a flat surface below it. The height of the glass block was adjusted so that when balanced, it would exactly touch the fat surface of the 100ml beaker, which was placed in an inverted position directly below the hanging glass block. Before the study, we made both sides of the balance equal by placing a 5 g weight on the right side.

Next, we attached the porcine buccal mucosa to the bottom flat surface of the 100ml beaker using cyanoacrylate adhesive, ensuring that the mucosal surface faced upward. The beaker containing the mucosal membrane was placed below the left-hand setup of the balance. The buccal patch was then stuck to the lower flat side of the hanging glass assembly with cyanoacrylate adhesive glue. The exposed surface of the patch was moistened with distilled water and left for 30 seconds for initial hydration and swelling. Subsequently, the platform was slowly raised until the patch surface made contact with the mucosa.

A weight of 5g was removed from the right-hand pan, causing the pan to lower along with the patch over the mucosa. The balance was kept in this position for a contact time of 3 minutes.

We then gradually added water to the glass kept on the right-hand pan until the patch detached from the mucosal surface. The force required for detachment provided the Mucoadhesive strength of the buccal patch, measured in grams.^[16]

The force of adhesion was calculated using an equation.

$$\text{Force of adhesion (N)} = \frac{(\text{Mucoadhesive strength (g)} \times (9.8))}{1000}$$

In-vitro Residence Time

In order to determine the *in-vitro* residence time, using modified USP disintegration test apparatus was utilized. A 1×2 cm² segment of porcine buccal mucosa was cut

and fixed on a glass slab using acrylic glue and attached vertically to the apparatus.

The buccal patch was attached with the porcine buccal mucosal membrane and hydrated on one surface using 0.2 ml isotonic phosphate buffer, and the hydrated surface was brought into contact with the mucosal membrane.

The glass slab was fixed to the apparatus and allowed to move up and down in 800ml isotonic buffer pH 6.8, maintained at a temperature of 37°C. This ensured that the patch was fully immersed in the buffer solution at the lowest point and exposed at the highest point.

The time taken for complete erosion or detachment of the patches from the mucosal surface was recorded (n=3).^[17]

In-vitro dissolution studies

Throughout the study, we utilized a modified version of the USP Dissolution apparatus type 1 (Basket). Each formulation's patch, with a diameter of 1cm² (which contains 4 mg drug), was securely fixed to the base of the central shaft without the basket. 100ml of phosphate buffer at pH 6.8 was used as dissolution media, 100 ml beaker was placed in a dissolution jar and the dissolution beaker height was adjusted by keeping petri plate below the 100 ml of dissolution beaker in dissolution jar.

The shaft was dipped into the depth of 2cm of dissolution media. The release study was conducted at a temperature of 37 ± 0.5°C, with a rotation speed of 50 rpm, and lasted for a duration of 10 hours. At the end of each hour, 3.5ml sample was withdrawn from each beaker, and the same volume of the fresh dissolution medium was added back to the beaker to maintain sink condition.

Each withdrawn sample was filtered, 1 ml sample was diluted in a 10 ml volumetric flask using pH 6.8 phosphate buffer, and subsequently analysed spectrophotometrically at a wavelength of 309nm. Three patches were tested, and the average release was calculated.^[18]

In-vitro diffusion study

The *in-vitro* diffusion study was performed by modified Franz diffusion cell across a dialysis membrane. Phosphate buffer solution (PBS) with a pH of 6.8 was used as the receptor compartment, dialysis membrane was treated as mentioned under medium for the diffusion study.

Optimized formulation NPs loaded Patches measuring 1x1cm² were placed on the membrane of donor compartment, which was positioned between the donor and receptor compartments of the Franz diffusion cell.

The dialysis membrane was brought into contact with PBS of pH 6.8 that was filled in the receptor compartment. Taking care to avoid the entry of air

bubbles. The temperature was maintained at 37°C with stirring using a magnetic stirrer. A sample of 1ml was withdrawn from the receptor compartment at predetermined intervals and replaced with fresh PBS of pH 6.8.

The samples were measured for absorbance at 309nm using a UV-visible spectrophotometer after suitable dilution. (n=3).^[19]

Ex-vivo permeation study

The Ex-vivo buccal permeation study of Ondansetron HCl through porcine buccal mucosa was conducted using a modified Franz type glass diffusion cell at a temperature of 37±1°C.

Tissue preparation

The porcine buccal tissue was obtained from a local slaughterhouse immediately after the pig was slaughtered. The buccal tissue of freshly slaughtered pig was immediately placed in cold kerb's buffer and transferred to our laboratory.

The buccal mucosa was carefully separated from fat and muscles using micro-dissecting forceps and scissors. The permeation studies were started within 2 hours of isolating buccal epithelium.^[22]

The drug permeation from the solution was studied using the Franz diffusion cell. The buccal epithelium was carefully mounted in between the two compartments of a Franz diffusion cell with internal diameter of 2.1 cm (3.46 cm² areas) with a receptor compartment volume of 135 ml. pH 6.8 phosphate buffer was placed in the receptor compartment and taking care to avoid the entry of air bubbles, maintained by stirring with a magnetic stirrer at 50 rpm. The buccal epithelium was allowed to equilibrate with the receptor for one hour to enhance the permeation.

The mucosa was mounted between the donor and receptor compartments, with the outer smooth surface of the mucosa facing the donor compartment.

Ex-vivo permeation study was carried out with 3 formulations.

1. Pure drug solution containing 4 mg of drug
2. F-4 optimized NPs equivalent to 4 mg of drug
3. The buccal patch equivalent dose of 4 mg of drug

The receptor compartment was filled with phosphate buffer (pH 6.8), and taking care to avoid the entry of air bubbles, maintained by stirring with a magnetic stirrer at 50 rpm. At predetermined time intervals 0.5,1,2,4,6,8,10,12,14 hours, a 3.5 ml sample was withdrawn from diffusion cell and after each sample withdrawal, pre-warmed (37±0.5°C) buffer was introduced to the diffusion cell and withdrawn sample was analysed using a UV-visible spectrophotometer at 309 nm. After filtering through Whatman filter paper.

1. Blank permeation study was carried by placing blank NPs in the donor compartment.
2. At each time interval from the absorbance of the studies, blank absorbance was subtracted to know actual amount of drug diffused (to eliminate the influence of tissue component permeated during the diffusion study)

For the purpose of calculating ex-vivo drug permeation, the procedure was performed in triplicate (n = 3). Absorption is a passive diffusion process that can be described using Fick's law equation.

The steady-state flux of Ondansetron HCl through the porcine buccal mucosa was calculated from the slope of the linear portion of the cumulative amount permeated through the membrane per unit area vs. time plot.

The steady state permeability coefficient (kp) of the drug through porcine, buccal mucosa was calculated by using the following equation $k_p = J/C$, where 'J' is the drug flux and 'C' is the initial concentration of Ondansetron HCl in donor cell.

RESULT AND DISCUSSION

Formulation Studies of Ondansetron HCL loaded polymeric NPs

Ondansetron HCL loaded polymeric NPs were successfully produced by ionic cross linking method. Different formulations were optimized in order to obtain uniform Nano-sized particles

Table no. 4: Evaluated parameter for Ondansetron HCl loaded polymeric nanoparticles as per CCD design. (F1 to F9).

Formulation Code	Particle Size (nm)	Polydispersity Index	Zeta potential(mV)	EE %	In-vitro % DR at 8 th hour
F1	302.1±0.042	0.529±0.028	-27.8±0.071	64±0.004	69.1±0.70
F2	341.1±0.024	0.559±0.010	-21.8±0.063	69.3±0.011	64±0.54
F3	373.4±0.049	0.502±0.038	-19.7±0.014	70.5±0.018	61±0.07
F4	399.5±0.014	0.514±0.035	-28.0±0.007	70.8±0.017	60.7±0.32
F5	496.5±0.056	0.437±0.014	-15.0±0.015	73.9±0.015	57.1±0.26
F6	564.4±0.063	0.790±0.021	-20.6±0.073	75.2±0.020	54±0.78
F7	635.7±0.035	0.489±0.046	-23.2±0.067	81.7±0.010	55±0.64
F8	701.9±0.070	0.673±0.013	-27.9±0.056	83.8±0.017	52±0.41

F9	718.4±0.063	0.33±0.070	-30.4±0.089	89.9±0.040	47±0.22
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Evaluation of nanoparticles by DOE

Particle Size

The particle size of obtained nanoparticles was ranged from 302.1nm to 718.4nm,

The ANOVA (Analysis of Variance) for linear model was used to analyse the particle size of the prepared nanoparticles. The p-value and R^2 value was found to be <0.0001 and 0.9779 respectively, showing the model is significant.

The Model F-value of 132.99 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A and B is a significant model term.

Table No. 5. Fit Statistics of Particle size.

Std. Dev.	27.22	R^2	0.9779
Mean	503.70	Adjusted R^2	0.9706
C.V. %	5.40	Predicted R^2	0.9513
		Adeq Precision	28.8146

The Predicted R^2 of 0.9513 is in reasonable agreement with the Adjusted R^2 of 0.9706; i.e. the difference is less than 0.2.

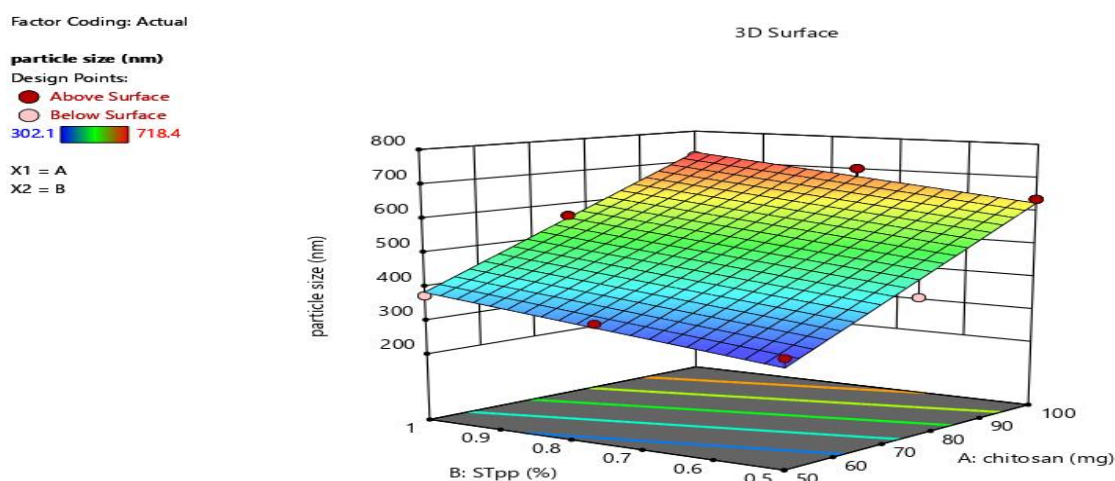


Fig.1 3-D graph of particle size.

Final equation in term of coded factors

Particle size = $503.7 + 173.233 * A + 53.15 * B$

Where, A=Chitosan and B= STPP

According to the coded factor equation of particle size, chitosan positively affects size; as its concentration increases, particle size also increases increasing, the cross linking agent also increases the particle size. this is attributed to cross-linking formulation and particle aggregation with higher chitosan content, thus increasing particle size.

lower concentration of STTP resulted in finer chitosan particles due to a decline cross-linking bonding is responsible for particle formulation as well as particle aggregation.

The distribution curve (PDI) show that the particle became smaller with decreasing concentration of STTP.

Our result showed that the particle size of Chitosan NPs increased at higher concentration of chitosan and STPP, and higher ratios of chitosan to STPP.^[20]

Entrapment Efficiency.

The results showed that the entrapment efficiency of the Ondansetron HCl loaded polymeric nanoparticles ranged from 64 % to 89.9%.

ANOVA for the linear model of entrapment efficiency showed the p-value of 0.0002 and R^2 value of 0.9429 respectively showing the model is significant.

DOE Results for Entrapment Efficiency

The Model F-value of 49.56 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms.

Table No. 6. Fit Statistics of EE.

Std.Dev	2.26	R ²	0.9429
Mean	75.46	Adjusted R ²	0.9239
C.V%	2.99	Predicted R ²	0.8735
		Adeq Precision	18.0924

The Predicted R² of 0.8735 is in reasonable agreement with the Adjusted R² of 0.9239; i.e. the difference is less than 0.2.

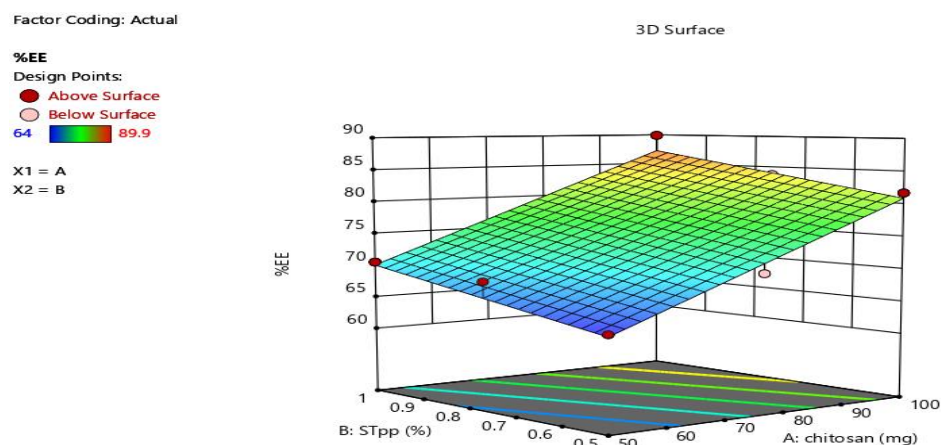


Fig. 2. 3-D graph of Entrapment Efficiency.

Final Equation in terms of coded factors

Entrapment Efficiency % = 75.4556 + 8.6 * A + 3.18333 * B

According to this equation, as the concentration of chitosan increases entrapment efficiency also increase. This is because chitosan and STPP enhancing EE in capsulation process. Chitosan, is a biopolymer with positive charges makes strong interaction with negatively charged compound leading to improved encapsulation, STPP as a crosslinking agent forms a stable network with chitosan enhancing structural integrity and minimizing leakage Cross-linking increases EE while maintaining control release properties.

In this work, we have maintained the drug concentration constant and the amount of chitosan and STPP was varied among the formulations. The formulation had shown the highest encapsulation. This may be due to the higher TPP content in this formulation, which may have helped interact with a large number of chitosan molecules permitting more drug in the nanoparticle core.^[21]

Table No. 7 Fit Statistics for Drug release at 8th hour.

Std. Dev.	0.7278	R ²	0.9911
Mean	57.77	Adjusted R ²	0.9882
C.V.%	1.26	Predicted R ²	0.9803
		Adeq Precision	49.8960

The Predicted R² of 0.9803 is in reasonable agreement with the Adjusted R² of 0.9882; i.e. the difference is less than 0.2.

In-vitro drug release study

% In-vitro drug release from polymeric NPs at 8th hour

The in-vitro drug release of obtained nanoparticle at 8th hour was ranged from 47% to 69.1%. The ANOVA (analysis of variance) for Linear model was used to analyzed the in-vitro drug release of the prepared nanoparticles. The p-value and R² value was found to be <0.0001 and 0.9911 respectively, showing the model is significant.

DOE Results for in-vitro drug release at 8th hour

The Model F-value of 334.74 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms.

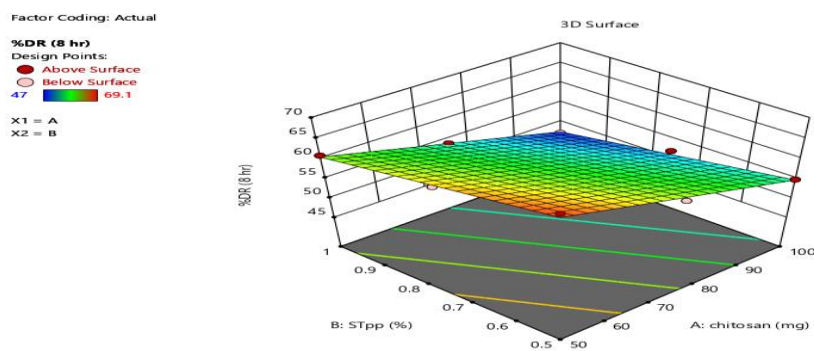


Fig no. 3 3-D graph of drug release at 8th hour.

Equation in Terms of Coded Factor

Drug release at 8th hour = $57.7667 - 6.68333 * A - 3.8 * B$
According to the equation above, both the factors (chitosan and STPP) negatively affects drug release as, as its concentration decreases, drug release increases. Due to high concentration of polymer and cross linking agent leading to more drug entrapment and extended release profile.

The lower concentrations of STPP, increase drug release from polymeric nanoparticles. This is because decreased chitosan levels reduce particle size, thereby increasing

surface area and solubilization in the recipient medium. Above formulation shows initial burst release followed by a controlled release. Finally, it can be concluded that the different drug release rates may be attributed to different sizes of the formulation of the NPs. It is expected as the particle size of chitosan nanoparticle is smaller, their surface area will be more and the drug release is faster.^[24]

Scanning Electron Microscopy Of Optimum Formulation F-4.

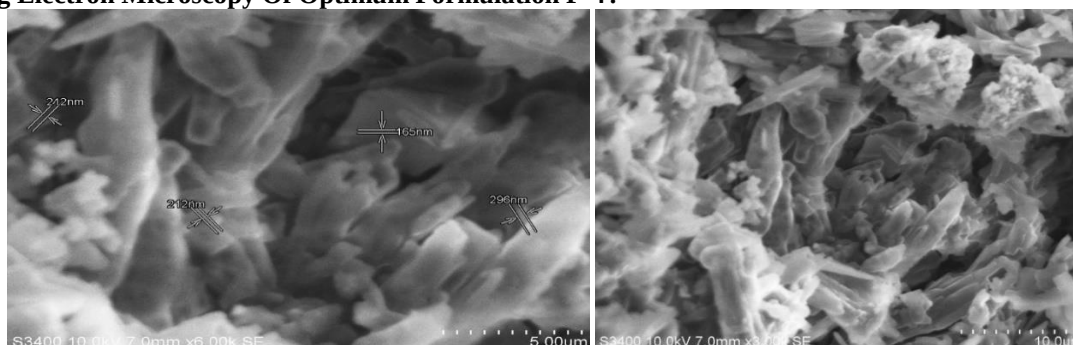


Fig 4. Scanning Electron Microscopy.

The surface morphology and particle size of the polymeric nanoparticles is done by scanning electron microscopy (fig no.4) and particle size was found to be

296 nm and the morphology was found like rod in shape and aggregation.

Evaluation of Ondansetron HCl NPs Buccal patch

Table No. 8. Evaluation of Ondansetron Buccal Patch.

Formulation code	Thickness (mm) (N=3)	Folding endurance (N=3)	Weight uniformity(mg) (N=3)	%Moisture content (N=3)
P-1	0.31±0.015	≥300±0.043	118.4±0.41	33±0.001

Table No. 9: Evaluation of Ondansetron Buccal Patch.

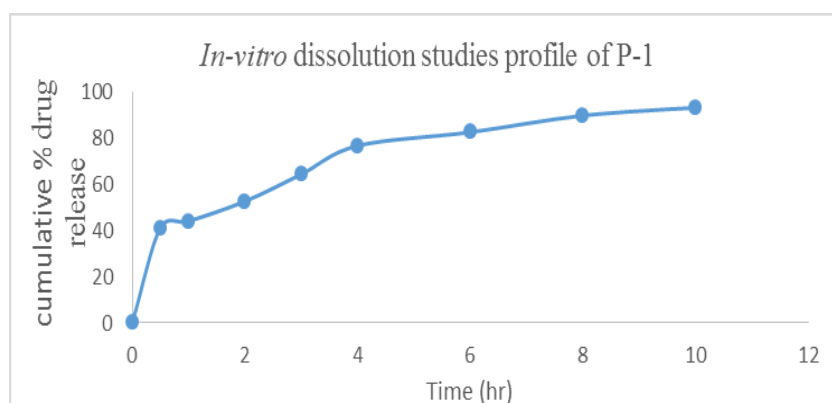
Formulation code	Swelling Index (in4 hr) (N=3)	Surface pH determination(N=3)	Drug content (%) (N=3)	Drug content uniformity (N=3)
P-1	263.5±0.032	6.7±0.057	97.2±0.37	94.4±1.217

Table No. 10: Evaluation of Ondansetron Buccal Patch.

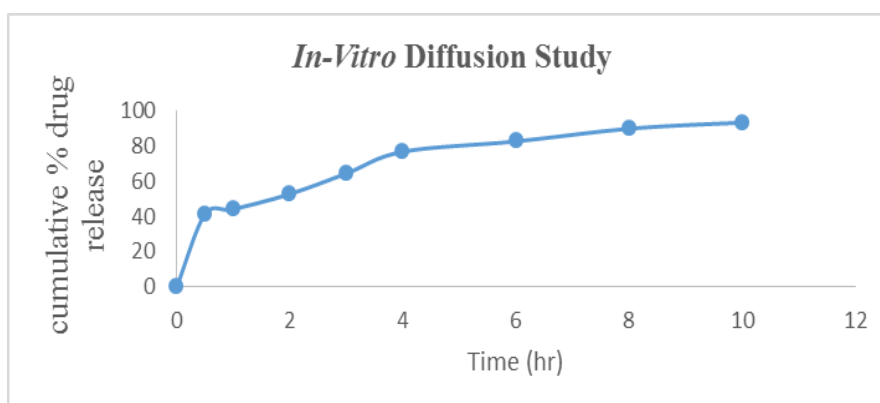
Formulation code	Mucoadhesive strength(gm) (N=3)	In-Vitro residence (time in minute) (N=3)	Tensile strength (kg/mm ²) (N=3)	Percentage Elongation (N=3)
P-1	20.8±0.029	240±0.213	4.4±0.05	30±0.057

In-vitro dissolution studies**Table no 11: In-vitro dissolution studies profile of P-1.**

% CUMULATIVE DRUG RELEASE	
TIME (hrs)	P-1
0	0
0.5	19.5±0.123
1	27.4±0.098
2	42.2±0.137
3	49.7±0.084
4	56.9±0.083
5	63.1±0.087
6	68.9±0.097
8	74±0.098
10	85.7±0.116
12	91.7±0.112

**Fig No 5. In-vitro dissolution drug release study of the optimized formulation patches (P-1) Optimized patch F-1 showing highest in-vitro drug release at 12 hours, the drug release was found to be 91.7%.****In-Vitro Diffusion Study****Table no: 12 In-vitro diffusion studies for optimized patch.**

Time (hr)	% Drug Diffused
0	0
1	41.18
2	44.06
3	52.66
4	52.66
6	82.55
8	89.74
10	93.20

**Fig No 6. In-vitro diffusion drug release study of the optimized formulation patches (P-1).**

Optimized patch F-1 showing *In-vitro* diffusion study was found to be 93.20% at 10 hours.

1. Weight uniformity

The patches were subjected to mass variation by individually weighing dried patches of 49 cm². The weight of the prepared patches ranged between 77.75±0.74 mg indicating that the patches were uniform in weight.^[22] (table no.8)

2. Thickness

The thickness of the patch of P-1 formulation varied from 0.31± 0.015mm, which indicates that the patch was of nearly uniform thickness. The thickness and weight of the patch depends on the concentration of polymer used in the formulation. An excessive increase in the concentration of the polymer could lead to an increase in weight and thickness of the patch.^[22] (table no.8)

3. Folding endurance

Folding endurance of P-1 formulations was found to be more than 300±0.43, hence results indicated that the patches did not break and maintained their integrity with buccal folding when applied and it measures the ability of patch to withstand rupture.^[22] (table no.8)

4. Surface pH determination

Surface pH is an important parameter since the surface of the mucoadhesive formulation is directly in touch with the buccal mucosal membrane. Highly acidic or basic pH may cause pathological irritation. So, the value near to normal range is theoretically more acceptable. The normal pH of saliva is around pH 6.7 and this value varies in the normal range limit of 6.2-7.6

Results of salivary pH of mucoadhesive formulations have been listed (Table no.9). The range of pH was 6.7±0.05, respectively for P-1. These values occur in between the aforesaid normal salivary pH. So, it can be conferred that the found pH of the mucoadhesive formulation was mimicking the physiological pH.^[23]

Drug content uniformity

The Drug content uniformity of P-1 formulation was found to be 94.4±1.217% (Table.no.9). Concentration of polymer increases the drug loading capacity of the patch. The % drug release will largely depend on the concentration and type of polymer used hence; the concentration of polymer used must be carefully considered when formulating the film.

5. Percentage moisture content

The percentage moisture content of P-1 formulation was found to be 33±0.001% (Table no.8). The lower moisture content within the buccal patches is well appreciable to avoid the chances of microbial contaminations. In addition, it can help to preserve the stability enough from being dried and brittle.

6. Swelling index

Swelling index of P-1 formulation was found to be 263.5% at 4 hours (Table no.9)

7. Drug content

The drug content of formulation P-1 was found to be 97.2± 0.37 % (Table No.9). The polymer, plasticizer, and permeation enhancer used to prepare the patch is capable of giving drug content.

8. Tensile strength

The tensile strength provides insight into the patch's overall strength and flexibility. All three patches of P-1 formulations exhibited improved tensile strength and elongation percentages. The measured tensile strength ranged from 4.4±0.05 kg/mm². There was no notable variation in the tensile strength across in formulation P-1 patches. Percentage elongation provides insight into the patches overall strength and flexibility P-1 patch formulation exhibit improved % elongation was found to be 30±0.057 (table no. 10)

9. Mucoadhesive strength

Mucoadhesive strength was determined by using modified physical balance method. The procedure was followed by using porcine buccal mucosa. P-1 formulation patches had shown good mucoadhesive strength with the range of 20.8±0.029 gm (Table no.10). Formulation P-1 having concentration of HPMC K-4M and Sodium alginate shows mucoadhesive strength.

10. In-vitro residence time

In-vitro residence time test was carried out using modified USP disintegration test apparatus. *in vitro* residence time of mucoadhesive patch was shown the range 240 minutes. (Table no.10)

In-vitro dissolution studies

In vitro dissolution study was carried out using modified version of the USP Dissolution apparatus type 1 (Basket) using PBS of pH 6.8 as medium. At the end of 12th hour of the dissolution study, the % drug diffused from optimized patch P-1 was 91.7±0.112 (Table no.11) (fig no.5)

In-vitro diffusion study

In vitro diffusion study was carried out using modified Franz diffusion cell across cellophane membrane using PBS of pH 6.8 as medium. At the end of 10th hour of the diffusion study, the % drug diffused from optimized patch P-1 was 93.20 (Table no. 12) (fig no. 6)

Ex-vivo permeation study

The *ex-vivo* drug permeation study of Ondansetron HCl utilizing a modified Franz diffusion cell revealed important insights into the effectiveness of different formulations. The drug-loaded patch (p-1), NPs-suspension (F-4) and Pure drug suspension (D-1) showed a cumulative amount per sq. cm. of 89.6 ± 3.12, 82.6 ± 1.02 and 72.7 ± 2.14 µg/cm² after 14 hours.

Table no. 13: The study state flux and permeability coefficient.

Formulation	Permeation flux(Fss) (J) ($\mu\text{g}/\text{cm}^2/\text{h}$) $\pm$ SD	Permeability coefficient (Kp) (cm/h) $\pm$ SD
NPs Suspension	82.4 $\pm$ 0.5675	20.6 $\pm$ 0.016
Drug loaded patch	89.6 $\pm$ 0.481	22.4 $\pm$ 0.003
Pure drug suspension	72.7 $\pm$ 0.2481	18.175 $\pm$ 0.013

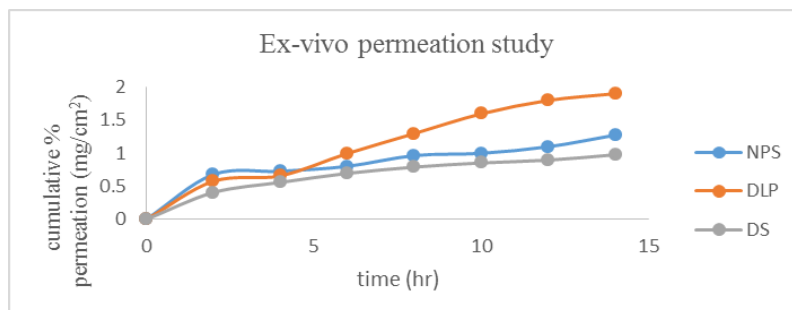


Fig no. 7. Ex-vivo permeation study

The study demonstrates the significant enhancement in the permeation of Ondansetron HCl through buccal mucosa when formulated as a drug-loaded patch compared to both pure drug suspension and NPs-suspension. The permeation flux of the drug-loaded patch (P-1) was notably higher ($22.4 \pm 0.003 \mu\text{g}/\text{cm}^2/\text{h}$) than that of the NPs suspension (F-4) and pure drug suspension (D-1), which were $20.6 \pm 0.016 \mu\text{g}/\text{cm}^2/\text{h}$ and $18.1 \pm 0.013 \mu\text{g}/\text{cm}^2/\text{h}$, respectively. This marked increase, four times higher than the pure drug solution and NPs-suspension, indicates that the patch formulation effectively enhances drug delivery.

The incorporation of sodium alginate, HPMC K-4M, chitosan, and propylene glycol in the patch formulation appears to play a critical role in improving mucosal drug penetration. This is likely due to the solvent-drag effect, which enhances the solubility of Ondansetron HCl (a drug with poor water solubility) and increases its partitioning into the buccal mucosa. This mechanism contributes to the higher permeation flux observed for the drug-loaded patch, making it a more efficient drug delivery system compared to the other formulations.

The sustained drug accumulation within the buccal mucosa from the drug-loaded patch suggests that it can provide prolonged release, improving therapeutic outcomes. The smaller sizes and superficial charge of the nanoparticles in the patch also likely facilitate more effective drug targeting, further enhancing the delivery efficiency.

The study underscores the importance of formulation strategies, such as the use of chitosan as a cross-linking agent and propylene glycol as an enhancer, in improving the bioavailability and therapeutic potential of Ondansetron HCl.

The results highlight the advantages of advanced drug delivery systems, particularly drug-loaded patches, in

enhancing drug permeation and therapeutic efficacy, offering a promising alternative for buccal drug delivery.

CONCLUSION

The study aimed to formulate and evaluate Ondansetron hydrochloride nanoparticles for buccal drug delivery. The polymeric nanoparticles were prepared using ionic cross-linking method and incorporated into buccal patches with HPMC K-4M, sodium alginate, and propylene glycol. The optimized formulation (F4) showed good physicochemical properties and controlled release. The ex-vivo drug permeation study showed that the drug-loaded patch provided the highest permeation flux compared to the NPs suspension and pure drug suspension. The drug-loaded patch had the highest permeability coefficient, indicating better buccal absorption. The SEM analysis of the lyophilized polymeric nanoparticles confirmed their uniform structure and suitability for drug delivery applications. The study concluded that Ondansetron HCl-loaded chitosan nanoparticles offer a promising approach for controlled buccal drug delivery with potential clinical applications in anti-emetic therapy.

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