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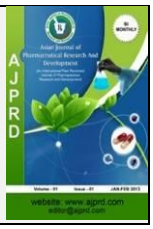

Open Access Research Article

Formulation and In-vitro Characterisation of nasal In-Situ Gel for treatment of Anti-migraine Drug

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ABSTRACT

The present study aimed to develop and evaluate a temperature-dependent nasal in situ gel of Rizatriptan benzoate for enhanced nasal residence time and improved bioavailability in migraine therapy. Rizatriptan benzoate undergoes extensive first-pass metabolism, resulting in low oral bioavailability. To overcome this limitation, a thermosensitive nasal in situ gel system was prepared using polymers such as Chitosan HCl, Sodium β-Glycerophosphate, Sodium alginate, and Gellan gum. The formulations were evaluated for physicochemical parameters including pH, gelation temperature, viscosity, mucoadhesive strength, drug content, and in vitro drug release. The optimized formulation FF5 showed satisfactory gelation at nasal temperature, good mucoadhesion, and sustained drug release over 8 hours. Drug release kinetics revealed diffusion-controlled release following the Higuchi model. Stability studies indicated that the optimized formulation remained stable under different storage conditions. The developed temperature-dependent nasal in situ gel demonstrated potential as an effective and patient-friendly approach for sustained nasal delivery of Rizatriptan benzoate.

Keywords: Rizatriptan Benzoate, Nasal in-situ gel, Migraine, Thermosensitive polymer, Drug delivery.

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INTRODUCTION

Thermosensitive in situ forming drug delivery systems are liquid aqueous formulations at room temperature that undergo sol-to-gel transition upon exposure to physiological temperature (≈32–37 °C). This temperature-triggered gelation occurs due to changes in polymer solubility and micellar structure, leading to the formation of a three-dimensional gel network. Commonly used thermosensitive polymers include Chitosan HCl and Sodium beta glycerol phosphate, which exhibit reversible gelation behavior and are widely employed due to their biocompatibility and ease of formulation. These systems do not require organic solvents, chemical cross-linking agents, or external stimuli, making them safe, convenient, and patient-friendly. Thermosensitive nasal in situ gels have gained significant attention as they allow easy administration in liquid form and prolonged residence at the site of absorption after gelation.[1-3]

Conventional routes of drug administration, such as oral and parenteral, present several limitations. Oral delivery is often unsuitable for drugs that undergo degradation in the gastrointestinal tract or extensive first-pass hepatic metabolism, resulting in reduced bioavailability and delayed onset of action. Although parenteral administration provides rapid drug delivery, it is invasive and less suitable for chronic therapy due to poor patient compliance. Transdermal delivery offers a non-invasive alternative but is limited by the low permeability of many drugs across the skin barrier.[3-7]

To overcome these limitations, non-parenteral routes such as nasal, buccal, and pulmonary delivery have been extensively explored. Among these, the nasal route is particularly advantageous due to its rapid onset of action, rich vascularization, high permeability for small molecular weight drugs, and avoidance of first-pass metabolism. Additionally, it enables self-administration and improves patient compliance. The nasal route is especially beneficial when rapid therapeutic response is required, such as in the

treatment of migraine, and also provides a potential pathway for delivering peptides and proteins that are otherwise difficult to administer orally.[6]

Rizatriptan benzoate, a selective serotonin (5-hydroxytryptamine; 5-HT$_1$B/1D) receptor agonist, is widely used in the management of migraine. Although it is well absorbed from the gastrointestinal tract, its absolute bioavailability is approximately 45% due to extensive first-pass metabolism by monoamine oxidase-A (MAO-A), leading to the formation of an inactive metabolite. Furthermore, it has a short elimination half-life of approximately 2–3 hours, necessitating frequent dosing and resulting in fluctuating plasma drug levels.[8-9]

Therefore, the present study aims to formulate and evaluate a thermosensitive polysaccharide-based in situ nasal gel of rizatriptan benzoate. This approach is expected to undergo gelation at nasal physiological temperature, thereby prolonging residence time, enhancing drug absorption, bypassing hepatic first-pass metabolism, and ultimately improving therapeutic efficacy and patient compliance.[10]

MATERIALS AND METHODS:

Reagent and Chemicals Rizatriptan Benzoate was obtained as a gift sample from Dhamtec Pharma, Mumbai. All the materials used in the study are Chitosan HCL Merck Pharma, Ahmedabad, Gujarat, HPMC K4M Colorcon Asia Pvt. Limited, Goa, Carbopol 934 Dr. Reddy's Research & Development, Hyderabad, Sodium alginate, Gellum gum and Sod. B-Glycerophosphate Himedia Lab. Pvt. Limited.

Instruments:

The various apparatus used were like Electronic Balance (Model No.AW-220 and BX –6205 Pioneered (OHAUS), USA.), FTIR Spectrophotometer (Model - 84005, Shimaduzu Asia Pacific Pvt Ltd. Singapore.), Brookfield Viscometer (Brookfield RVDE 230), Assembly for gel Strength Measurement, Assembly for Mucoadhesion Force Measurement, Franz Diffusion Cell (Laboratory fabricated assembly).

Standard Calibration Curve of Rizatriptan Benzoate in Saline Phosphate buffer pH 7.4:

From solution having concentration 100 ug/ml aliquots of 1, 2, 3, 4, and 5ml were pipette out into volumetric flasks. The volume was made up to the mark with Saline Phosphate Buffer 7.4 use as (Blank) to get the final concentration of 2, 4, 6, 8, and 10ug/ml respectively. The absorbance of each concentration was measured at 225 nm. Results are shown in a graph of absorbance versus concentration was plotted. The graph obtained shows a straight line indicate that the calibration curve obeys Beer's law. Shown in Graph No. 1

Table 1: Standard calibration curve of Rizatriptan Benzoate Standard calibration curve

Sr. No.	Concentration (µg/ml)	Absorbance	Standard Deviation
1	0	0	0
2	1	0.26	0.195
3	2	0.49	0.045
4	3	0.69	0.00014
5	4	0.91	0.043
6	5	1.16	0.210

Selection of Method:

Preparation of Phase Transition System was done by Thermo-sensitive method. Prepare nasal in situ gel with good consistence.

Preparation of Nasal gel formulations:

Thermosensitive nasal in situ gel of Rizatriptan Benzoate was prepared as follows-

Accurately weighed quantities Chitosan hydrochloride of were gradually added to cold distilled water (maintained at 4°C) with continuous stirring to prevent lump formation. The solution was then refrigerated overnight to ensure complete hydration and dissolution of polymers.

Accurate amount of Sodium alginate and Gellan Gum were weighed, add into another beaker with small amount of distilled water. Then add Sodium β-Glycerophosphate of the specified quantity to it.

To this, accurately weighed **Rizatriptan Benzoate** was added and mixed thoroughly until a clear solution was obtained.

The drug-polymer solution was then slowly incorporated into each other with continuous stirring and suitable preservatives were added, if required.

The final volume was adjusted to 10 ml using cold distilled water. The formulation was maintained at low temperature (4°C) throughout the preparation to retain it in liquid form.

Finally, the pH of the formulation was adjusted to **5.5–6.5**, suitable for nasal administration.

The prepared formulation undergoes sol-to-gel transition at nasal physiological temperature (35–37°C), forming a gel upon administration.

Preliminary Batch for Formulation of Phase Transition System for Nasal Drug Delivery System:

Preliminary studies were performed to determine the factors which will affect the Formulation Phase transition system. Also their appropriate concentrations were determined and some of these were kept fixed for further optimization of the product.

Table 2: Preliminary Batch Of The Formulation

Ingredients	PF1	PF2	PF3	PF4	PF5	PF6	PF7	PF8
Drug (mg)	150	150	150	150	150	150	150	150
Chitosan (mg)	100	100	100	100	100	100	100	100
HPMC K4M (mg)	25	50	–		–	–	25	50
Carbopol 934 (mg)	25	50	25	50			–	–
Sodium Alginate (mg)	–	–	25	50	25	50		
Gellan Gum (mg)					25	50	25	50
Sodium β-Glycerophosphate (mg)	306.11	306.11	306.11	306.11	306.11	306.11	306.11	306.11
Water (mL)	10	10	10	10	10	10	10	10

Factorial Batch: Factorial batch is used to evaluate two or more factor simultaneously. The treatment is combination of level of factors. The advantages of factorial designs over one factor at-a-time experiments include theirs efficiency and deletion of interaction. Intervention studies with two or more categorical explanatory variable leading to numerical outcome variable are called factorial designs. A factor is simply a categorical variable with two or more value refers as levels. A study in which there are two factors with theirs three levels called 3^2 factorial designs. A factor for the present work 3-factorial was elected. The two independent variable were selected Sodium alginate and Gellan gum and the nine formulations formulated as per experimental design.

Table 3: Amount of Variable in 3^2 Factorial Design Batches

Coded Value	Sodium Alginate (X_1)	Gellan Gum (X_2)
-1	20 mg	20 mg
0	30 mg	30 mg
+1	40 mg	40 mg

Table 4: Factorial Batch of The Formulation

Ingredients	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8	FF9
Rizatriptan Benzoate (mg	150	150	150	150	150	150	150	150	150
Chitosan (mg)	100	100	100	100	100	100	100	100	100
Sodium Alginate (mg)	20	30	40	20	30	40	20	30	40
Gellan Gum (mg)	20	20	20	30	30	30	40	40	40
Sodium β-Glycerophosphate (mg)	306.11	306.11	306.11	306.11	306.11	306.11	306.11	306.11	306.11
Benzalkonium Chloride (mg)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Water (mL)	10	10	10	10	10	10	10	10	10

Evaluation of Phase Transition System

1. Appearance [11]

Appearance test for the prepared formulations has done by visual inspection under black and white background. There was no evidence of contamination, the entire formulations Passes appearance test.

2. Measurement of pH [12]

1ml quantity of each formulation was transferred to a beaker and diluted by using distilled water to make 25ml. pH of the resulting solution was determined using digital pH meter.

3. Drug Content Estimation [13,14]

1ml of formulation was taken in 50 ml volumetric flask, diluted with distilled water and volume adjusted to 50 ml. One ml quantity from this solution was again diluted with 10 ml of distilled water. Finally the absorbance of prepared solution was measured at 226.0 am by using Shimadzu-1800 UV visible spectrophotometer.

4. The Gelation Time [15]

Transition systems solution (2.0mL), was added into a tube (10ml) with a diameter of the gelation time at at 37°C was determined by test tube inverting method. The Phase 1.0 cm and kept in a water bath at 37°C. The tube was taken out every 1 min and inverted to observe the state of the sample. The gelation point was determined by flow or no-flow criterion over 30 sec with the test tube inverted.

5. Gelation Temperature [16]

The gelation temperature was estimated by heating the solution (about 1-2°C/min) in a test tube with gentle stirring until gel formed. Gel formation was taken as the point when there was no flow, when the container was overturned. The temperature was noted and designated as gel formation temperature. Three replicates of each measurement were done and results were reproducible within the range. Stirring speed and heating cooling rates have no significant effect on the transition temperature.

6. Viscosity Measurement [17,18]

The viscosity measurements were carried out by using Brookfield DV-III Ultra Programmable Rheometer. The LV-4 i.c. spindle no. 64 was used and rotated at 0.2 rpm. The temperature of sample was maintained with the help of temperature control unit. The Measurement was taken at 37°C.

7. Gel strength determination [19]

This test was performed by using Gel strength apparatus. A 50 ml sample was placed in 100 ml graduated measuring cylinder and placed into water bath at 37° C. The sample was converted into gel. Then marking at upper meniscus level was done as a starting point and measured 5 cm distance from that point and marked as a end point: Stopwatch was kept ready. Then piston was placed onto the gel which having weight 35 g and measure the time in seconds which required for moving the piston 5 cm down through the gel. It is indication for the viscosity of the nasal gel at physiological temperature.

8. In-vitro Diffusion Study [20,21]

The in-vitro diffusion study is a critical parameter for evaluating mucoadhesive in situ gel formulations. The study was performed using a glass Franz diffusion cell with a diffusion area of 2.0 cm diameter and a receptor compartment capacity of 15 ml, maintained with a water jacket system. A dialysis membrane (110 LA 395-1 MP, HiMedia Laboratories Pvt. Ltd., Mumbai) was used as the diffusion barrier.

Prior to the experiment, the membrane was soaked in phosphate buffer (pH 7.4) for 24 hours to ensure proper hydration. The receptor compartment was filled with phosphate buffer (pH 7.4). The soaked membrane was mounted between the donor and receptor compartments. The donor compartment was carefully positioned so that the membrane remained in direct contact with the diffusion medium.

Table 5: Parameter for In-vitro Diffusion study

Parameter	Details
Diffusion Apparatus	Franz Diffusion Cell
Diffusion Membrane	Dialysis Membrane (110 LA 395-1 MP)
Diffusion Medium	Phosphate Buffer (pH 7.4)
Volume of Diffusion Medium	25.0 ml
Sample Size	1.0 ml
Temperature	37 ± 0.5°C
Time Interval	8 Hours

Kinetics of Drug Release [22,23,24]

In vitro dissolution studies play a crucial role in drug development as they help in understanding the release mechanism and rate of drug from the dosage form. To analyze the drug release kinetics of the formulated dosage forms, the dissolution data obtained from in vitro studies were fitted into various kinetic models such as **Zero-order, First-order, Higuchi, and Korsmeyer–Peppas**.

The model that best describes the drug release mechanism was selected based on the **correlation coefficient (R^2 value)**.

Analysis of Data by Design Expert Software [25,26]

A 3^2 full factorial design was selected to evaluate the effect of two independent factors at three different levels using Design-Expert Software by Minitab version 12.2.

Response Surface Plot

The obtained data were further analyzed using 3D response surface methodology to study the interaction between the independent variables and their effect on the responses.

Optimization

Optimization was carried out using Design-Expert software to obtain the optimized formulation batch. The results of numerical and graphical optimization are presented.

Stability Study: [27,28]

Stability testing is performed to evaluate the effect of various environmental factors such as temperature, humidity, and light on the quality of a drug substance or formulation over time. It provides essential information regarding recommended storage conditions, re-test periods, and shelf life of the product.

As per the guidelines of the International Conference on Harmonization (ICH), stability studies are conducted under different conditions, including long-term testing at 25 ± 2°C/60 ± 5% RH for 12 months, intermediate testing at 30 ± 2°C/65 ± 5% RH for 6 months, and accelerated testing at 40 ± 2°C/75 ± 5% RH for 3 months.

In the present study, stability studies of the optimized formulation were carried out at room temperature and

under accelerated conditions (40 ± 2°C/75 ± 5% RH) for a period of 3 months.

RESULTS & DISCUSSION:

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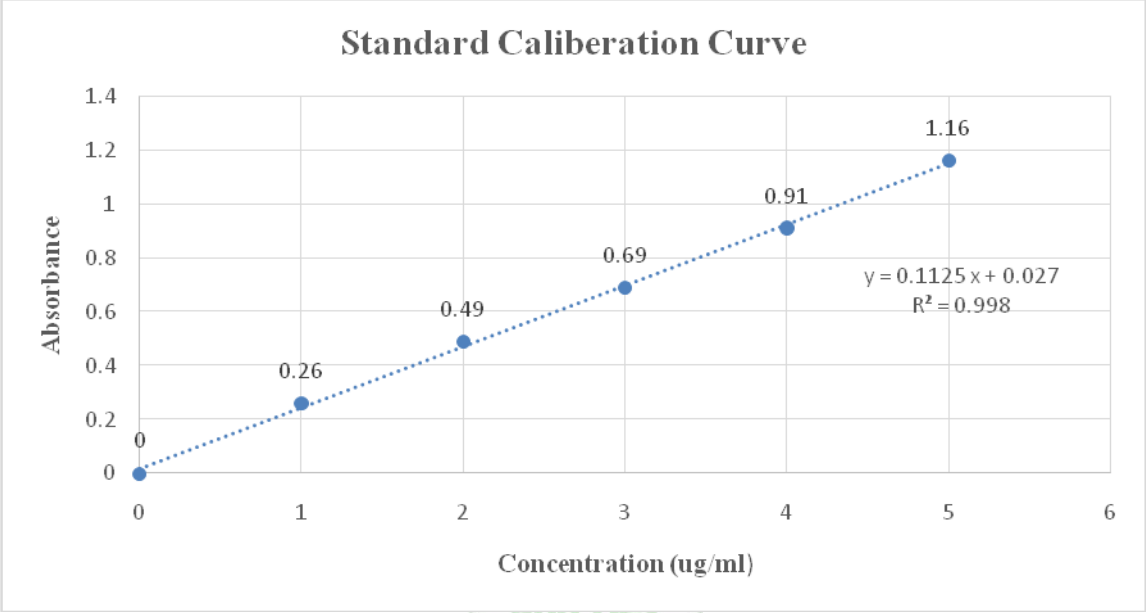
Melting Point Determination:

The melting point of the Rizatriptan benzoate drug sample was found to be 179°C which is within the reported range of 178 to 180°C. It complies with the purity of the drug sample and literature values.

Table 6: Standard calibration curve of Rizatriptan Benzoate by Spectrophotometric Analysis

Sr. No.	Concentration (µg/ml)	Absorbance	Standard Deviation
1	0	0	0
2	1	0.26	0.195
3	2	0.49	0.045
4	3	0.69	0.00014
5	4	0.91	0.043
6	5	1.16	0.210



Graph 1: Standard calibration curve of Rizatriptan Benzoate

Results For Preliminary Batch Conclusion: It was concluded that formulation containing polymers HPMC K4M, Sodium Alginate, Carbopol 934, Gellan gum for In- situ Gelling System has shown good drug content in Preliminary batch.

Evaluation Results For Factorial Batch:

Table 7: Results For Factorial Batch

Batch	pH	Drug Content (%)	Gelation Time (secs)	Viscosity Before Gel (cp	Viscosity After Gel (cp)
FF1	7.05 ± 0.05	97.82 ± 1.42	4.60 ± 0.45	69.50 ± 2.75	73.20 ± 1.10
FF2	7.18 ± 0.12	98.10 ± 1.60	4.85 ± 0.52	71.80 ± 2.10	76.90 ± 2.45
FF3	7.21 ± 0.06	97.75 ± 1.38	3.10 ± 0.66	72.40 ± 2.05	74.60 ± 2.20
FF4	7.19 ± 0.10	98.52 ± 1.70	3.85 ± 0.58	74.30 ± 1.90	77.10 ± 2.05
FF5	7.06 ± 0.06	97.88 ± 1.45	3.05 ± 0.48	75.20 ± 1.80	79.30 ± 1.95
FF6	7.22 ± 0.02	97.92 ± 1.50	3.30 ± 0.42	77.10 ± 1.50	81.20 ± 1.60
FF7	7.14 ± 0.06	98.40 ± 1.55	4.50 ± 0.61	78.90 ± 1.55	83.80 ± 1.75
FF8	7.20 ± 0.02	98.36 ± 1.48	4.7 ± 0.57	80.10 ± 1.60	84.90 ± 1.85
FF9	7.17 ± 0.08	98.05 ± 1.52	3.85± 0.79	82.30 ± 1.70	86.40 ± 1.90

Discussion:

It was observed that Gelation time of formulation from (FF1 to FF9) was in range of (3.05 ±0.48) to (4.7 ±0.57) min. All formulation has good Gelation time.

It was observed that Viscosity before gel of formulation from (FF1 to FF9) was in Range of (69.50 ± 2.75) to (83.30

± 1.70) Cp. All formulation has good Viscosity before Gel properties.

It was observed that Viscosity after gel of formulation from (FF1 to FF9) was in Range of (73.20± 1.10) to (86.40 ± 1.90) Cp All formulation bas good Viscosity after Gel Properties.

Table 8: Resuts of In Vitro Drug Release Study

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	21.77 ± 0.60	20.29 ± 0.79	23.25 ± 1.39	17.33 ± 0.71	24.74 ± 0.69	20.29± 0.71	20.29 ± 0.85	26.22± 1.37	23.25± 0.74
2	38.16 ± 1.21	39.63 ± 1.51	35.20 ± 0.73	46.58 ± 0.67	61.43 ± 0.87	49.56 ± 0.88	52.52 ± 0.66	64.40 ± 0.57	55.50 ± 1.03
4	50.16± 1.92	56.09 ± 1.85	51.64± 1.38	62.88 ± 1.72	74.76 ± 0.98	56.97 ± 0.39	62.89± 0.71	71.80 ± 0.81	64.38 ± 1.92
6	69.62 ± 0.57	74.09 ± 1.13	71.10± 1.13	82.14 ± 1.58	86.61 ± 0.82	70.30 ± 1.25	73.26 ± 1.15	86.62 ± 1.28	77.72 ± 0.69
8	81.75 ± 0.88	84.75 ± 1.40	80.27 ± 0.89	93.99 ± 0.64	96.98 ± 0.51	82.15 ± 1.42	83.63 ± 0.81	95.51 ± 1.31	91.05 ± 0.55

Discussion :

The in vitro drug release study of formulations F1–F9 demonstrated a gradual and sustained release pattern over 8 hours. Among all formulations, F5 showed the highest cumulative drug release (96.98 ± 0.51%) followed by F8 (95.51 ± 1.31%) and F4 (93.99 ± 0.64%), indicating better drug diffusion and optimized polymer concentration. Formulations F1, F2, F3, F6, and F7 exhibited moderate drug release ranging from 80–85% after 8 hours, while F9 showed comparatively higher release (91.05 ± 0.55%). The initial release at 1 hour was controlled in all formulations, confirming sustained release behavior. Overall, formulation F5 was considered the optimized batch due to its maximum and consistent drug release profile with low standard deviation, suggesting uniformity and effective formulation performance

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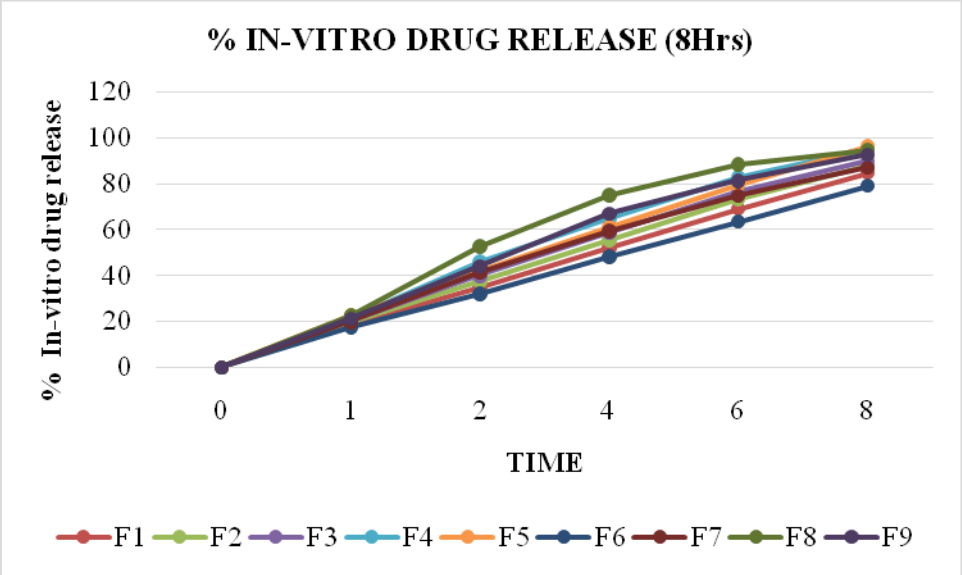


Graph 2: % IN-VITRO DRUG RELEASE (8Hrs) F1-F9

Permeation Study

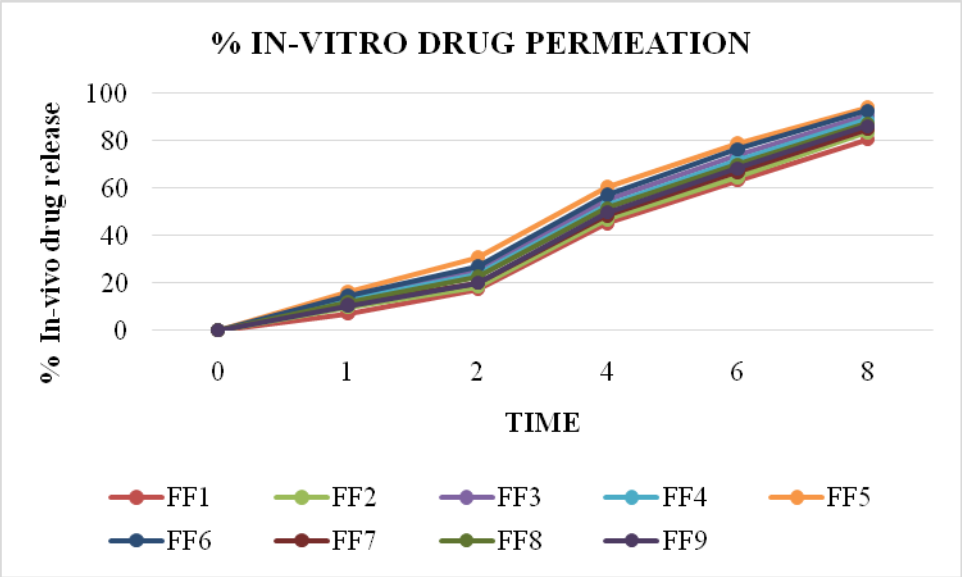
Table 9: Permeation Study of Prepared Batches

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	7.20 ± 0.55	9.85 ± 0.88	13.40 ± 1.45	12.85 ± 0.58	16.20 ± 0.62	14.75 ± 1.10	11.30 ± 1.80	11.92 ± 1.05	10.40 ± 0.60
2	17.50 ± 1.00	18.30 ± 0.55	25.20 ± 1.60	23.70 ± 0.70	30.80 ± 1.45	27.10 ± 1.50	19.80 ± 0.55	22.50 ± 0.90	20.10 ± 0.40
4	45.20 ± 2.80	46.90 ± 1.00	54.80 ± 1.00	52.90 ± 1.00	60.50 ± 1.80	57.20 ± 0.40	48.50 ± 0.90	51.60 ± 1.70	49.80 ± 0.70
6	63.40 ± 1.35	64.80 ± 1.48	74.10 ± 0.60	71.80 ± 1.00	78.90 ± 0.65	76.50 ± 0.73	66.90 ± 1.10	70.20 ± 0.72	68.50 ± 1.25
8	80.50 ± 1.03	83.60 ± 1.66	90.60 ± 1.70	88.90 ± 1.80	94.20 ± 1.20	92.80 ± 0.70	84.90 ± 1.85	87.40 ± 0.11	86.20 ± 0.72

Discussion :

The permeation study of nasal in situ gel formulations FF1–FF9 showed sustained and controlled drug release over 8 hours. Drug release increased gradually with time due to diffusion through the gel matrix. Among all formulations, FF5 showed the highest cumulative drug

release (94.20 ± 1.20%), followed by FF6 and FF3, indicating optimized polymer concentration and efficient drug diffusion. Formulations with higher viscosity showed comparatively slower release. Overall, FF5 was considered the optimized nasal in situ gel formulation due to its maximum and consistent drug release profile suitable for prolonged nasal drug delivery.



Graph 3: % In-vitro drug permeation FF1-FF9.

Kinetics Study

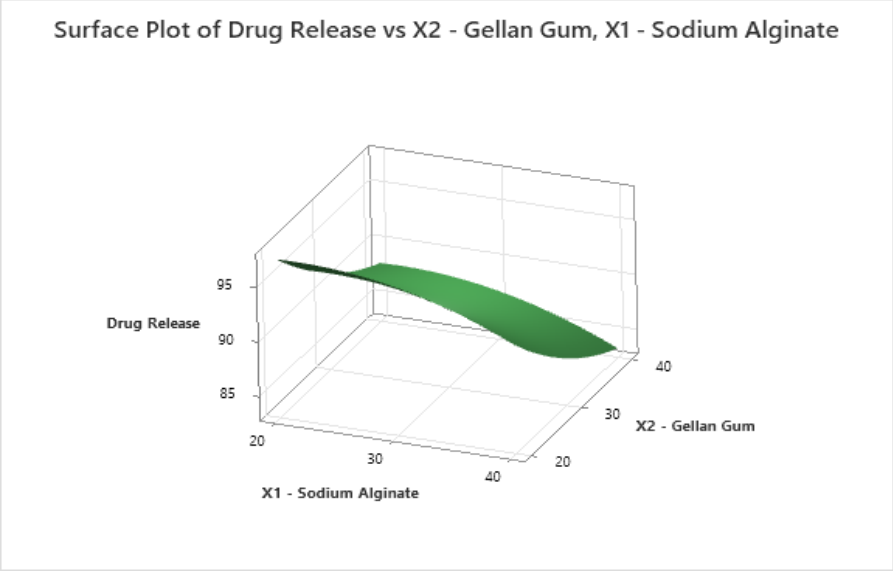
The drug release kinetics study for the optimal formulation was calculated and the results obtained are present in table no. 10.

Table 10: Kinetics modeling of drug release

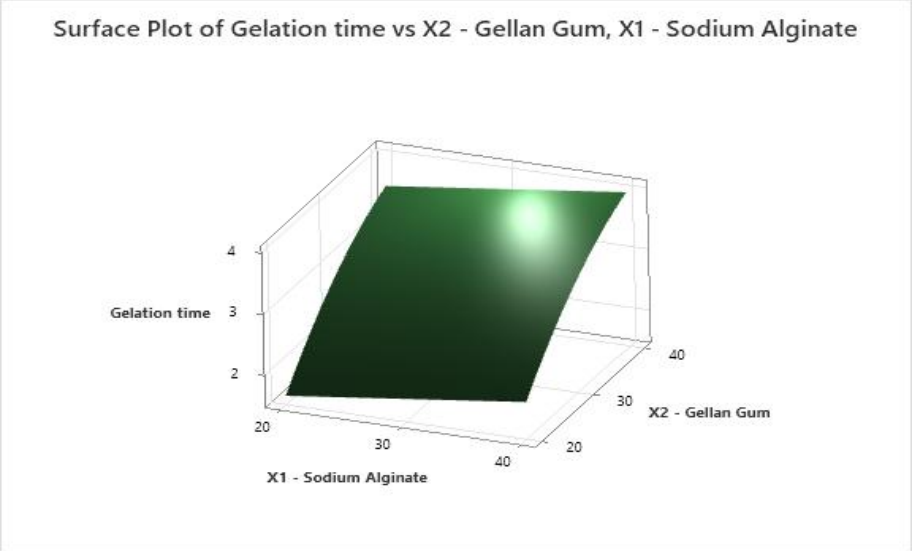
Formulation Batch (Optimized)	Zero order	First Order	Higuchi model	Korsemeyer peppas model
F5	0.9846	0.8391	0.9997	0.9909

Results and discussion:

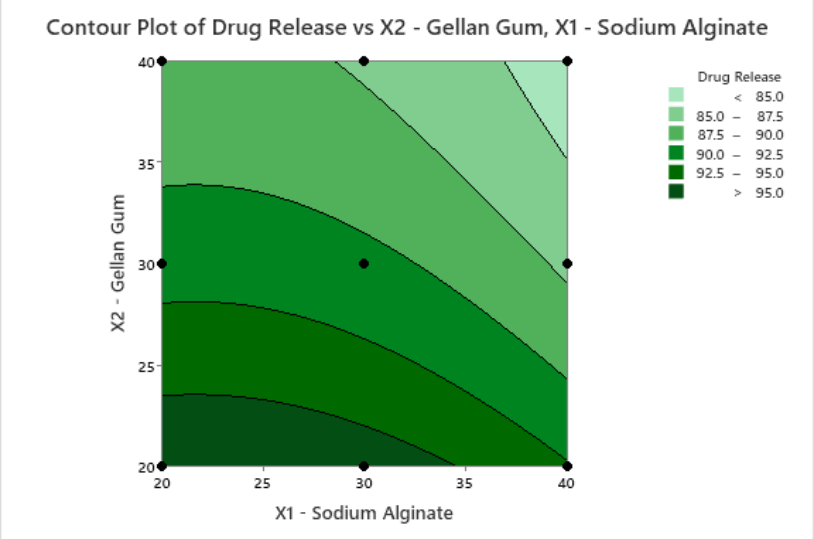
The in-vitro drug release data of formulation F5 were fitted into different kinetic models to determine the release mechanism. Among all models, the Higuchi model showed the highest correlation coefficient (R^2 = 0.9997), indicating diffusion-controlled drug release from the nasal in situ gel. The Korsmeyer–Peppas model (R^2 = 0.9909) suggested anomalous non-Fickian transport involving both diffusion and polymer relaxation. The Zero-order model also showed good linearity (R^2 = 0.9846), confirming sustained and controlled drug release, while the lower R^2 value of the First-order model (0.8391) indicated that drug release was not concentration-dependent. Overall, formulation F5 exhibited controlled and sustained drug release suitable for nasal drug delivery.



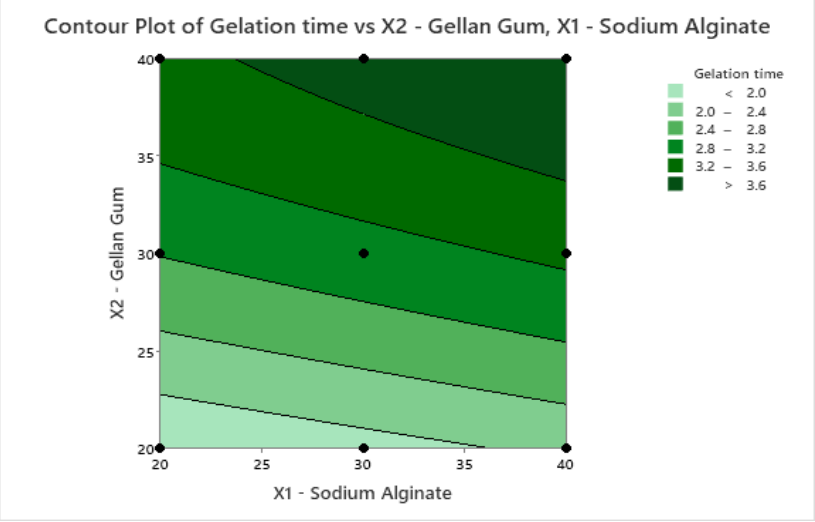
Graph 4: Response Surface Graph for Drug Release



Graph 5: Response Surface Graph for Gelation Time



Graph 6: Optimization for % Drug Release



Graph 7: Optimization for Gelation time

Stability Study of F5 Optimized Batch from Factorial Batch:

Table 11: Stability Study of F5 Optimized Batch From Factorial Batch (Accelerated stability study)

Time Interval 25 ± 2°C / 75 ± 5% RH	Drug Content (%)	Gelation Temperature (°C)	Mucoadhesion Strength (dyne/cm²)	% Cumulative Drug Release After 8 Hours
0 Day	98.56 ± 0.32	48.02 ± 0.55	32.59 ± 0.78	83.02 ± 0.787
1 Week	98.88 ± 0.86	47.05 ± 0.96	32.80 ± 0.25	83.49 ± 0.110
2 Week	98.59 ± 0.75	47.10 ± 0.53	32.25 ± 0.55	83.13 ± 0.125
3 Week	98.45 ± 0.25	47.40 ± 0.85	32.70 ± 0.50	83.34 ± 0.421
4 Week	98.65 ± 0.15	47.62 ± 0.42	35.10 ± 0.32	83.45 ± 0.258

Table 12: Room Temperature Stability Study of Optimized Batch F5

Time Interval 40 ± 2°C / 75 ± 5% RH	Drug Content (%)	Gelation Temperature (°C)	Mucoadhesion Strength (dyne/cm²)	% Cumulative Drug Release After 8 Hours
0 Day	98.90 ± 0.23	48.03 ± 0.77	32.55 ± 0.78	83.42 ± 0.225
1 Week	98.74 ± 0.42	47.55 ± 0.25	32.74 ± 0.25	83.59 ± 0.289
2 Week	99.00 ± 0.77	47.00 ± 0.21	35.41 ± 0.55	83.93 ± 0.248
3 Week	98.35 ± 1.33	47.00 ± 0.56	35.00 ± 0.50	83.44 ± 0.116
4 Week	98.77 ± 0.89	47.10 ± 0.59	35.10 ± 0.10	83.25 ± 0.545

Table 13: Refrigerated Stability Study of Optimized Batch F5

Time Interval 10 ± 2°C / 75 ± 5% RH	Drug Content (%)	Gelation Temperature (°C)	Mucoadhesion Strength (dyne/cm²)	% Cumulative Drug Release After 8 Hours
0 Day	98.74 ± 0.35	48.03 ± 0.37	32.40 ± 0.132	83.02 ± 0.605
1 Week	98.21 ± 0.55	48.55 ± 0.080	32.96 ± 0.554	83.00 ± 0.281
2 Week	98.05 ± 0.72	47.50 ± 0.20	32.71 ± 0.481	83.23 ± 0.228
3 Week	98.43 ± 1.32	45.20 ± 0.45	33.25 ± 0.452	83.84 ± 0.453
4 Week	98.77 ± 0.42	46.15 ± 0.50	32.62 ± 0.352	83.245 ± 0.607

Discussion

The stability studies of the optimized formulation (F5) were carried out for a period of one months under different storage conditions, namely 40 ± 2°C / 75 ± 5% RH (accelerated condition), 25 ± 2°C / 75 ± 5% RH (room temperature condition), and 10 ± 2°C / 75 ± 5% RH (refrigerated condition)**.**

The results revealed that there was only a slight reduction in drug content during the storage period. However, no significant changes were observed in percentage drug content and percentage cumulative drug release after 8 hours under all storage conditions.

These results indicate that the formulation maintained its physical and chemical stability throughout the study period. Therefore, the optimized formulation F5 remained stable during the one-month stability study**.**

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