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FORMULATION AND EVALUATION OF NASAL IN- SITU GEL FOR THE TREATMENT OF MIGRAINE

Roshani Sawarkar[1], Shrikant Pande[2]

[1,]M. Pharm, [2]Vidyabharti college of Pharmacy, Amravati, C. K. Naidu Road, Camp, Amravati, Maharashtra, India

Abstract:

The aim of the present study is to overcome the limitations of nasal cavity like low residence time by using in situ gel forming nasal drug delivery system prepared from polymers that exhibit phase transition (Sol-Gel) and pseudo plastic behaviour to minimize interference within the mucociliary clearance. It the increasing of the delivery residence of the delivery system and enhancing bioavailability. Rizatriptan benzoate is a selective serotonin (5-hydroxytryptamine; 5-HT) type 1B and 1D receptor agonist ("triptan") commonly used for the treatment of a migraine and completely absorbed from GIT, but absolute bioavailability (as conventional tablet) is 45%, indicating the first-pass metabolism, which is due to the metabolism of the drug by monoamine oxidase A isoenzyme (MAO-A) to an inactive indole acetic acid metabolite with the advent of new era of pharmaceutical dosage forms, nasal drug delivery system has established itself as an integral part of novel drug delivery system. Nasal gel is prepared by using gelling agent such as Chitosan HCL, HPMC K4M, Carbopol 934, Sodium alginate, Gellun gum and Sod. β-Glycerophosphate and other excipients. Optimized Batch PF9 was observed to be the best batch.

Keywords: *Rizatriptan benzoate, Chitosan HCL, Sod. β-Glycerophosphate , Sodium alginate.*

Corresponding author:

Roshani Ashok Sawarkar,
M.Pharm (Pharmaceutics)
Vidyabharati College of Pharmacy, Amravati
Camp, Amravati, Maharashtra, India
roshsawarkar999@gmail.com
9168505078

QR code

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INTRODUCTION:

In situ forming systems are liquid aqueous solution before administration, but gel at physiological conditions as like solvent exchange, UV radiations, ionic cross linkage, pH[1,2] change,temperature modulations[3,4]. pH triggered system (e.g. carbopol [5,6],cellulose acetate phthalate [7]), ion activated system. (e.g. gelrite [8,9] sodium alginate [10]). In this system do not require any organic solvents, copolymerization agents or an externally applied triggered for gelation, have gained increasing attention, such as thermo sensitive nasal in situ gel[3].Traditional drugs are administered by oral and parenteral routes. Oral administration is unsuitable for some drugs like the drugs undergoes significant degradation in gastrointestinal tract or metabolized via liver in high degree and gives undesirably slow effects[11]. For this parenteral route is preferred but it is undesirable or impractical if drug is intended for the treatment of chronic disease, so alternative route is preferred; also transdermal route is used for drug delivery but its use is limited due to low permeability of the skin to many drugs[12]. To overcome these problems non parenteral routes are used includes nasal, buccal, pulmonary route. These no parenteral routes have some advantages like self-administration is possible in an ambulatory setting. The nasal route offers rapid onset of action, high absorption of small molecular weight hydrophobic drugs, high bioavailability, avoid the first metabolism, patient compliance[13]. Nasal route has been used for treatment of nasal congestion, allergy and infections. Nasal route mat be when rapid onset of action is required and small molecular weight polar drugs, peptide and proteins are not easily administered via other routes than by injection[14].

Rizatriptan benzoate is a selective serotonin (5-hydroxytryptamine; 5-HT) type 1B and 1D receptor agonist ("triptan") commonly used for the treatment of a migraine and completely absorbed from GIT, but absolute bioavailability (as conventional tablet) is 45%, indicating the first-passmetabolism, which is due to the metabolism of the drug by monoamine oxidase A isoenzyme (MAO-A) to an inactive indole acetic acid metabolite. Rizatriptan benzoate has also relatively short elimination half-life (about 3 h) and the prolonged drug release is needed [15, 18].The aim of this research was, to formulate and evaluated rizatriptan benzoate loaded polysaccharide based In-situ gel for the delivery via the nasal route with aim to avoid hepatic first-pass metabolism, and enhance residence time.

MATERIALS AND METHODS:

Reagent and Chemicals

Rizatriptan Benzoate was obtained as a gift sample from Nosch Labs Hyderabad. Gujarat all the materials used in the study are Chitosan HCL Merk Pharm, Ahmedabad, Gujarat, HPMC K4M Colorcon Asia Pvt. Limited, Goa, Carbopol 934 Dr. Reddy's Research & Development, Hyderabad, Sodium alginate, Gallum gum and Sod. βGlycerophosphate 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).

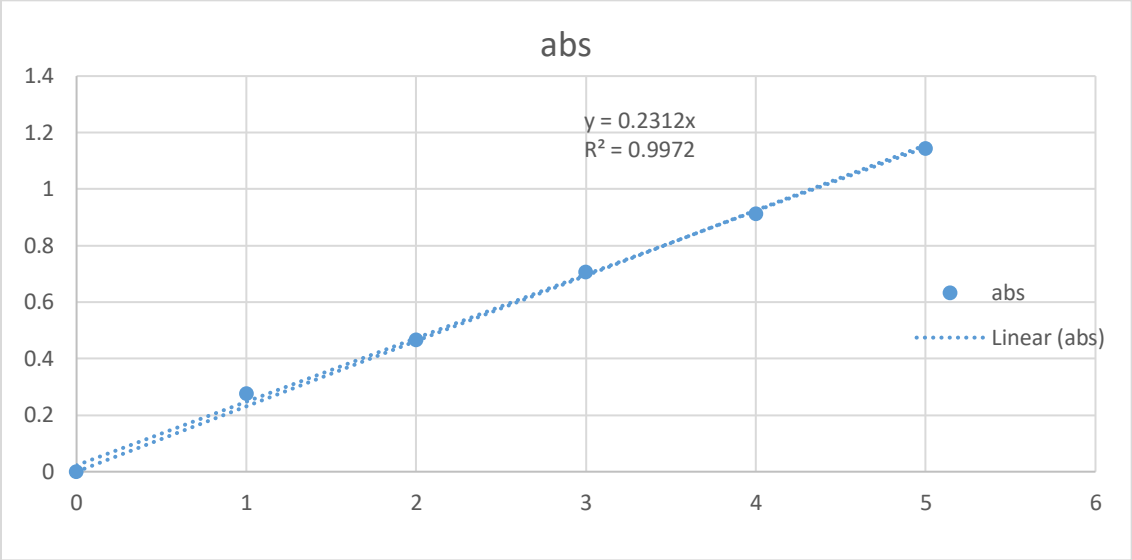
Standard Calibration Curve of Rizatriptan Benzoate in Saline Phosphate buffer pH 7.4:

From solution having concentration100 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 218 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 No. 1 Standard calibration curve of Rizatriptan Benzoate

Standard calibration curve

Sr. No	Concentration (u g /ml)	Absorbance	Standard deviation
1	0	0	0
2	1	0.2763	± 0.01695
3	2	0.4676	± 0. 01364
4	3	0.7083	± 0.00825
5	4	0.9149	± 0.01014
6	5	1.1441	± 0.00654



Graph No. 1 Standard calibration curve of Rizatriptan Benzoate

Selection of Method:
Preparation of Phase Transition System was done by pH depended method. Prepare nasal in situ get with good consistence.

Preparation of Nasal gel formulations :
Aqueous nasal gel was prepared by using the Cold method described The formulation of phase transition system were prepared by employing hydroxyl propyl methyl cellulose K4M, gallan gum, carbopol934, sod alginate and using Chitosan as mentioned in the Table No. 5 was prepared by dissolving the chitosan hydrochloride and hydroxy propyl methyl cellulose in the distilled water and to that resultant solution, weighted quantity of drug Rizatriptan Benzoate dissolved and cooling up to 4°C. To this solution sodium glycerophosphate solution was added drop by drop with continuous stirring and the volume was made upto 10 ml. The final pH of the formulation was adjusted 7.0-7.2

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 concentration were determined and some of these were kept fixed for further optimization of the product.

Table No. 2 Preliminary Batch Of The Formulation

	Formulation Code With Their Quantity							
Name Of Ingredient	**PF1**	**PF2**	**PF3**	**PF4**	**PF5**	**PF6**	**PF7**	**PF8**
Drug (mg)	7.265	7.265	7.265	7.265	7.265	7.265	7.265	7.265
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
Sod. B-Glycerophosphate (mg)	432	432	432	432	432	432	432	432
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 3^2factorial 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 No. 3 Amount of Variable in 3^2 Factorial Design Batches

Coded Value	Actual Value	
	SODIUM ALGINATE (X1)	**GELLAN GUM (X2)**
-1	20 mg	20mg
0	30mg	30mg
+1	40mg	40mg

Table No. 4 Factorial Batch Of The Formulation

	Formulation Code With Their Quantity								
Batch code	**PF1**	**PF2**	**PF3**	**PF4**	**PF5**	**PF6**	**PF7**	**PF8**	**PF9**
Drug (mg)	7.265	7.265	7.265	7.265	7.265	7.265	7.265	7.265	7.265
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
Sod. B-Glycerophosphate (mg)	657	657	657	657	657	657	657	657	657
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:

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:

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 :[17]

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. Viscosity Measurement :[17, 19, 20]

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.

5. Gel strength determination :[17,18]

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 370 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

6. In-vitro Diffusion study: [21-28]

In vitro diffusion study is one of the important criteria for mucoadhesive in situ gel. This study was carried out by using Franz diffusion cell having 2.0 cm diameter and 25 ml capacity and water jacketed which was fabricated with glass. For this study, dialysis membrane 110 LA 395-1 MP (Hi Media laboratories Pvt. Ltd. Mumbai) was used as diffusion membrane. Before starting the experiment, these pieces of dialysis membrane were soaked in phosphate buffer having pH 6.8 for 24hrs. The receptor compartment of diffusion cell was filled with phosphate buffer pH 6.8. The dialysis membrane was mounted in between donor and receptor compartment of the diffusion cell. The position of the donor compartment was adjusted so that the membrane just touches to diffusion medium.

Table No.5: Parameter for In-vitro Diffusion study,

In-vitro Diffusion Apparatus	Franz Diffusion Cell
Diffusion Membrane	Dialysis Membrane 110 LA 395-1 MP
Diffusion Medium	Phosphate Buffer pH 6.8
Volume of Diffusion Medium	25.0 ml
Volume of Sample size	1.0 ml
Temperature	37^0 C
Time interval	8 Hours

Stability Study[29] :

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and enables recommended Storage conditions, re-test periods and shelf lives to be established.
International Conference on Harmonization specifies the length of study and storage conditions:

Long term testing: 25°C +2°C/60 % RH +5% RH for 12 months.

Intermediate testing: 30°C +2°C/65% RH +5% for 6 month

Accelerated testing: 40°C +2°C/75 % RH 5% RH for 3 months

In the present study, stability studies were carried out at Room Temperature and Accelerated testing: 40°C 2°C / 75 % RH+ 5% RH for 3 months for the optimized formulation.

RESULT & DISCUSSION:

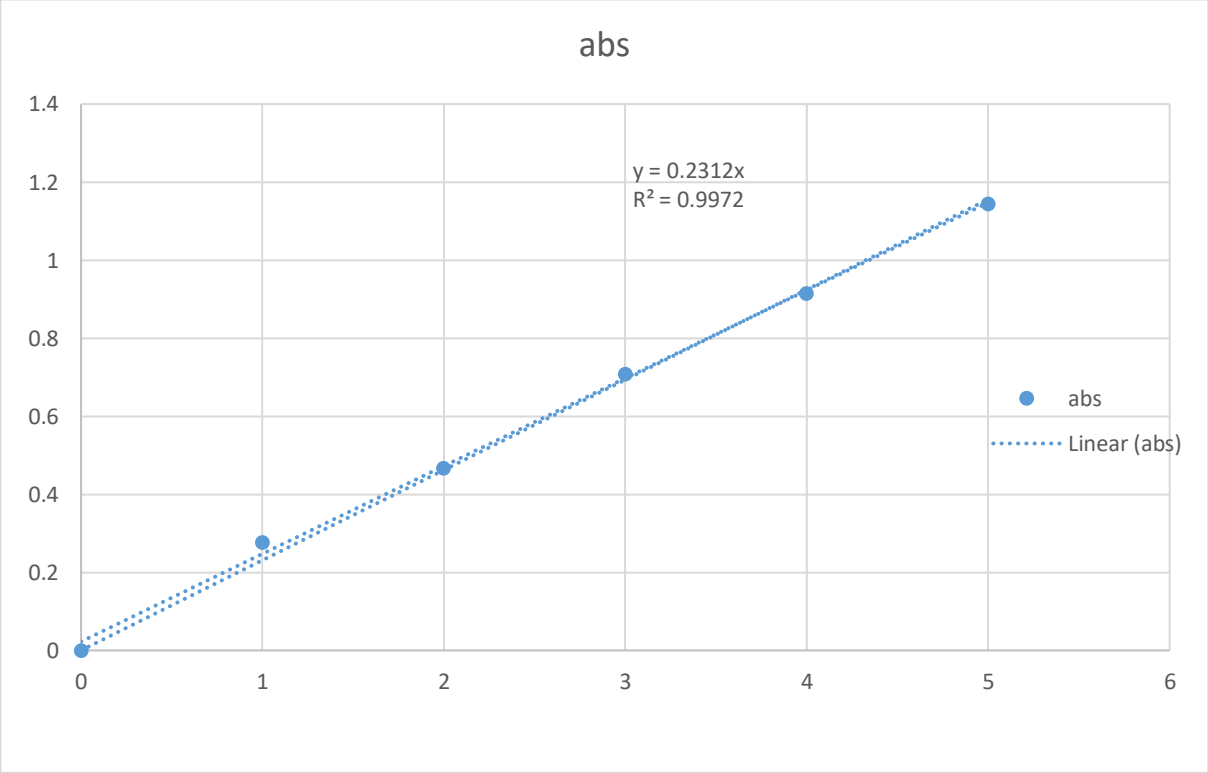
Melting Point Determination:

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 No. 6 Standard calibration curve of Rizatriptan Benzoate

Sr. No	Concentration (u g /ml)	Absorbance	Standard deviation
1	**0**	**0**	**0**
2	**1**	**0.2763**	**± 0.01695**
3	**2**	**0.4676**	**± 0. 01364**
4	**3**	**0.7083**	**± 0.00825**
5	**4**	**0.9149**	**± 0.01014**
6	**5**	**1.1441**	**± 0.00654**

Standard calibration curve



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**

5.9.1 Evaluation:

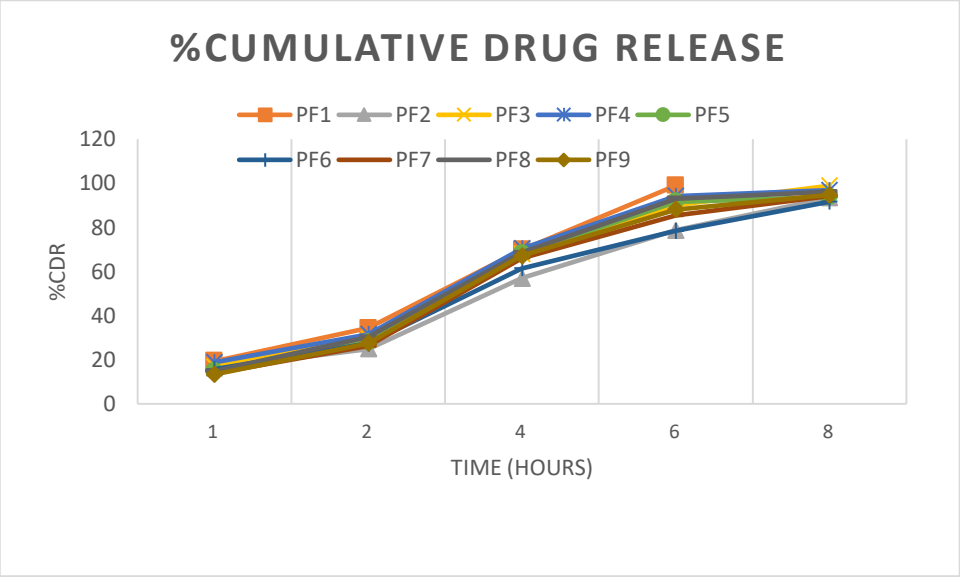
Table No.7 Result For Factorial Batch

Batch Code	pH	Drug Content	Gellation on time (min)	Viscosity Before Gel (cp)	Viscosity After Gel (cp)
PF1	7.033 ± 0.054	97.66 ± 1.5272	1.54 ± 0.4479	70.00 ± 2.98	73.66 ± 1.00
PF2	7.23 ± 0.1155	98.00 ± 1.7320	1.70 ± 0.5084	72.00 ± 1	77.33 ± 2.5166
PF3	7.23 ± 0.0577	97.63 ± 1.5257	2.04 ± 0.6683	71.33 ± 2.0816	73 ± 2.
PF4	7.2 ± 0.1	98.68 ± 0.5773	2.95 ± 0.4834	70.43 ± 1.1547	74.00 ± 2
PF5	7.03 ± 0.0577	97.65 ± 1.1547	3.12 ± 0.4129	75.66 ± 1.5275	85.00 ± 2
PF6	7.2 ± 0.01	97.66 ± 1.5275	3.42 ± 0.6087	79.66 ± 1.547	84.00 ± 1.7320
PF7	7.13 ± 0.0577	98.33 ± 1.547	3.58 ± 0.7769	74.66 ± 2.5166	78.00 ± 2.6457
PF8	7.2 ± 0.01	98.33 ± 1.5275	3.78 ± 0.6089	75.66 ± 3.5118	80.00 ± 1.5275
PF9	7.16 ± 0.1528	98.00 ± 1	3.97 ± 0.787	81.66 ± 1.1547	85.66 ± 0.5773

In Vitro Drug Release Study:

Cumulative % drug Release of phase transition (PF1- PF9) was found to be range (97.88 ±0.38766) (8 hours) to (83.14±0.5486) (8 hours). It was observed that cumulative % drug release of phase transition system depend on concentration of polymer (sodium alginate, gellan gum). Here, as concentration of polymer increases % drug release time of formulation also decreases. Maximum cumulative Drug i.e. **(97.88 ±0.3984) (8 hours)** was found to be for (PF1) and prolong cumulative % Drug Release was **(83.14 ± 0.5486) (8 hours)** found to be (PF9). Here polymer show concentration dependence controlled release behaviour for these gum.

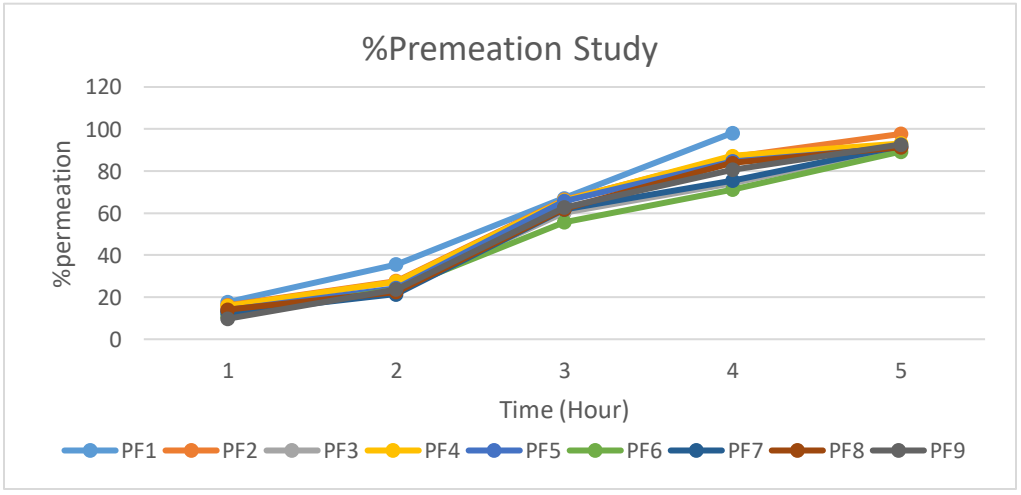
Graph No. 2 Cumulative % Drug Release Of Phase Transition



Permeation Study

Drug permeation of In-Situ Gelling system **(PF1-PF9)** was found to be range **(92.38 ±1.1885)** (8 hours) to **(78.42 ± 1.0274)** (8 hours), it was observed that cumulative % drug release of depend on concentration of In-Situ Gelling system polymer (sodium alginate, gellan gum). Here, as concentration of polymer increased % Drug release time of formulation also decreases.Also, it was observed that transition system nasal gel (PF9) a formulation has best fitted to order release. In-Situ Gelling system of nasal gel (PF9) with r^2 value (0.9343) n value (1.1566).

From evaluation of In- Situ system nasal gel formulation for factorial batch, formulation (PF9) has shown pH (7.161.528), drug content (98±1). Gelation time (9.72 ±0.4787), viscosity before gel (85.66+1.1547), viscosity after gel (85.66 0.5773), drug permeation time (78.42 ±1.0274), drug release time (83.14 ± 0.4358), have good controlled release behaviour. Hence, from above it was calculated that In-situ gel system nasal gel formulation containing sodium alginate and gellan gum which could be most promising formulation for Rizatriptan Benzoate.



Graph No. 3 Drug permeation of In-Situ Gelling system

Stability Study of FF9 Optimized Batch from Factorial Batch

Table No 8.: Stability Study of FF9 Optimized Batch From Factorial Batch (Accelerated stability study)

Stability (25 ± 2^0,75±5%RH)	Drug Content (%)	Gelation Temperature (°C)	Mucoadhesion Strength (dyne/ cm^2)	% 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 ±.096	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

n=3

Table No 9.: Stability Study of FF9 Optimized Batch From Factorial Batch (Room Temperature stability study)

Stability (40 ± 2^0,75±5%RH)	Drug Content (%)	Gelation Temperature (°C)	Mucoadhesion Strength (dyne/ cm^2)	% 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 ±.025	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 ±0.100	83.25 ± 0.545

n =3

TableNo.10 : Stability Study of FF9 Optimized Batch From Factorial Batch

Stability (10 ± 2^0,75±5%RH)	Drug Content (%)	Gelation Temperature (°C)	Mucoadhesion Strength (dyne/ cm^2)	% 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 ±.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

n=3

- The stability studies of optimum formulation (PF9) revealed that there is slightly reduction in Drug content was observed over period of 3 month. no significant change was observed on % Drug content and % cumulative drug release (after 8hrs) at various storing condition**(40 ± 2^0,75±5%RH)**,**(25 ± 2^0,75±5%RH)**and**(10 ± 2^0,75±5%RH)**. Hence formulation (PF9) was found to be stable for 3 months
- In optimized batch (PF9) containing polymers Gellan Gum and Sodium Alginate shows that good stability at various storing condition. **(40 ± 2^0,75±5%RH)**, **(25 ± 2^0,75±5%RH)**and**(10 ± 2^0,75±5%RH)**Hence formulation (FF9) was found to be stable for 3 months.

SUMMERY AND CONCLUSSION:

The aim of the present study was to formulate and evaluate the nasal in situ nasal gel containing Rizatriptan Benzoate. The approach is pH dependent which is used for designing the in situ nasal gel. The different polymers like Carbopol934, HPMC KAM, Gellan Gum, Sodium Alginate were used for the studies. The preformulation parameters like visual inspection, Melting point, detection of wavelength were evaluated and FT-IR shady was carried out to rule out any possible interactions between the drug and

the excipients, this confirming the compatibility between the selected range of the drugs and the polymers pH dependent in situ nasal gel of Rizatriptan Benzoate were prepared by dispersing the polymer sodium alginate and Gellan gum in different concentrations in a water, hydrating it for sufficient period of time. The pH dependent in situ nasal gel were evaluated for their pH, viscosity, drug content, pH, in-vitro diffusion studies, , gelation time in vitro drug release. All the gels were transparent, homogenous, and they were found to be uniform with their drug content. Sodium alginate and Gellan Gum for controlling the release of Rizatriptan Benzoate from the gel. The in-vitro drug release of in situ nasal gel formulations were carried out using Franz diffusion cell. The data obtained was analysed From all the prepared formulations, the best formulation was selected based on different parameters and in vitro release studies. The best formulation FF9 was selected because it produced the optimum gelling strength and %CDR at the end of 8 hours. In evaluation stage, the viscosity of gel was evaluated using Brookfield viscometer Viscosity of optimized formulation was found to be 81.66cps before gel 85.66 after gel and. In-vitro release study of drug was carried out for 8 hrs through the cellophane membrane and release of optimized formulation was found to be about 83.14% Temperature dependant nasal in situ gel of rizatriptan Benzoate was prepared by cold method in which the polymer like sodium alginate, gellan gum were used in different concentration and then dissolved in cold water at 40c .Hydrating it for sufficient period of time . the temeperature dependant nasal in- situ gel were evaluated for their gelling time, viscosity, drug content,pH, in vitro diffusion study. The vitro drug release of Nasal in situ gel formulations were carried out using diffusion well. The data obtained was analyzed. From all the prepared formations, the best formulation was selected based on different parameters and in vitro release studies. The best formulation FF9 was selected because it produced the opti mucoadhesive strength, time, gelling strength and %CDR at the end of 8 hour In evaluation stage, the viscosity of gel was evaluated using Brookfield viscometer. Viscosity of optimized formulation was found to be 81.66cps. In vitro drug release was carried out for 8 hrs through the cellophane membrane and release of optimized formulation was found to be about 83.14 % and the gelling time was found to be 2 min, gelling strength 42 sec, gelling temperature 34 °C and time & hour the ex vive release was Found to be 44.98%. The best formulation FF9 showed negligible change in mucoadhesive force, gelling temperature, clarity, viscosity percentage drug content and diffusion profile on subjecting to stability study for a period of 3months.

FUTURE SCOPE

There is a scope for further studies using animal like rabits and rats for in-vivo evaluation of the prepared formulations. The formulation can also be further studied on human volunteers. It can be studied by observing how much drug reaches to the brain through nasal cavity. The histopathological studies on the nasal mucosa can be done and the toxicity level on the nasal mucosa and limitation by the formulation to nasal cavity can be studied. Checking of the nasal gamma scistography of the prepared formulation can be done to know the actual mocoadhesion time of the prepared formulation in nasal cavity.

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