

FORMULATION AND IN-VITRO EVALUATION OF THERMOREVERSIBLE RIZATRIPTAN BENZOATE NASAL GEL

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ABSTRACT

Rizatriptan undergoes hepatic first pass, hence it shows poor bioavailability. In this study attempt has been done to improve bioavailability by formulating thermoreversible in-situ gel; formulation was developed to reduce the mucociliary clearance by using Mucoadhesive polymer in gel, thereby increasing the contact of formulation with nasal mucosa and hence improving the absorption of drug. The in-situ gelation was achieved by the use of pluronic F127, which exhibit thermoreversible gelation property. Gels were prepared by previously reported cold technique and characterized by Gelation Temperature, Permeation Studies, Histopathological Evaluation, PH, Drug Content, Rheological studies, gel strength, drug polymer interaction, Stability study. The gelation temperature of all studied gel formulations are found in range of 32-42°C. In in-vitro drug release study was found 37.45-97.04%, PH of gel is in range of 4-5, drug content 98.2-100.02%. Rheological study of gel formulation indicated that increase in polymer concentration increases the viscosity, gel strength was found in range of 110-130 sec. Spectral study revealed no interaction between drug and polymer as there is no shifting of drug λ_{max} . Stability study indicates that there was no significant change in the Rizatriptan benzoate. Rizatriptan benzoate formulated as bioadhesive thermoreversible Poloxamer solution for nasal administration could have potential to avoid first-pass effect than oral route, thus improve bioavailability of drug and as a safe and sustained release nasal delivery system to control migraine.

Keywords: Mucoadhesive, Nasal gel, Pluronic F127, Carbopol, Migraine,

INTRODUCTION

Chemically Rizatriptan Benzoate is N, N-dimethyl- 5-(1H-1, 2, 4-triazol-1-ylmethyl)-1H-indole-3-ethanamine monobenzoate and is a selective 5-hydroxytryptamin (5-HT) 1B/1D receptor agonist. It has a weak affinity for other 5-HT receptor subtypes and was launched in 1998 for the acute treatment of migraine in adults. Rizatriptan benzoate have good water solubility (42 mg/mL) but it have poor bioavailability (45%) due to hepatic first pass effect. The suggested mechanism of anti-migraine action of Rizatriptan Benzoate is through activation of post synaptic 5-HT 1B receptors within cerebral and dural vessel walls, causing vasoconstriction of perivascular nerve terminals.

For systemic therapy, drugs are traditionally administered by oral and parenteral routes. However in many instances, oral administration is unsuitable when the drug undergoes significant degradation in the gastrointestinal tract or is metabolized to a high degree via the first pass effect in the liver. Researchers resorted to the parenteral route as an easy solution to this problem. But parenteral route is undesirable or impractical if a drug is intended for the treatment of chronic diseases. Therefore an alternative route of administration would be preferred. For the past few decades, the transdermal route has been explored for a number of drugs, but its use is limited due to low permeability of the skin to many drugs. Nonparenteral routes for drug delivery include nasal, buccal, pulmonary and transdermal routes. All these application routes are suitable for self-administration in an ambulatory setting. The nasal application is the only route of administration, which offers rapid onset of action; high absorption of small molecular weight hydrophobic drugs, relatively high bioavailability, avoidance of first pass effect and ease of administration by patients favors this route of application. Traditionally, the nasal route has been used for delivery of drugs for local treatment such as nasal congestion, allergy and infections. More recently, nasal drug delivery has generated widespread interest among the scientific community as an alternative route for the administration of drugs and biomolecules that are susceptible to enzymatic or acidic degradation and first pass hepatic metabolism or incompletely absorbed in the gastrointestinal tract or gives undesirably slow effects when administered orally. However nasal delivery of drug offers many advantages over other delivery routes still possess some drawbacks like nasal mucociliary

clearance which results in reduced absorption of drug. Several strategies were tested to improve nasal absorption of drug. To improve the nasal absorption of drug it is necessary to increase the nasal residence time. One method to lengthen nasal residence time has been to include a bioadhesive in the formulation. The use of bioadhesive polymers can increase nasal absorption of drugs with several ways such as increasing the residence time of drug in the nasal cavity. Carbopol derivatives are bioadhesive polymers used in attempt to formulate Mucoadhesive drug delivery system for application to nasal mucosa. Poloxamers or pluronics are triblock copolymers which form micelles at low concentration and clear, thermoreversible gel at high concentrations. The concentrated solutions (15-30%) are transformed from low viscosity transparent solutions at 5°C to solid gel on heating to body temperature. By modulating the gelation temperature of different PF127 solutions, liquid bases for nasal use can be formulated that form a gel in nasal cavity at body temperature resulting in enhancement of residence time in the nasal cavity. Possible strategy used to decrease the mucociliary clearance by use of gel/ Mucoadhesive formulation to prolong nasal residence time. Nevertheless, the most prominent advantage of the *in-situ* gel over the silent gel is that it is fluid like prior to contact with nasal mucosa, a feature that offers the convenience of administration for patients since it can be easily instilled as a drop allowing accurate drug dosing. The objective of present study is to develop in situ gel of rizatriptan benzoate, with favorable gelation, rheological and release property. This may give patient friendly, needle free dosage form.

MATERIAL AND METHOD

Rizatriptan Benzoate was obtained as a gift sample from Cipla Ltd, Kurkumbh, Pune. Carbopol (C934P) was supplied from Hi-Media Lab Pvt Ltd. (Bombay, India) propylene glycol and preservative benzalkonium chloride purchase from research lab, fine chemical industry Mumbai.

Determination of λ_{max} of rizatriptan benzoate¹

A stock solution of 100 µg/ml of rizatriptan benzoate was prepared by dissolving 10 mg in 100 ml of distilled water then dilution of stock solution were prepared in range of 1-10. The resulting solution was scanned between 200 nm to 400 nm using UV visible spectrophotometer UV 1400, Shimadzu.

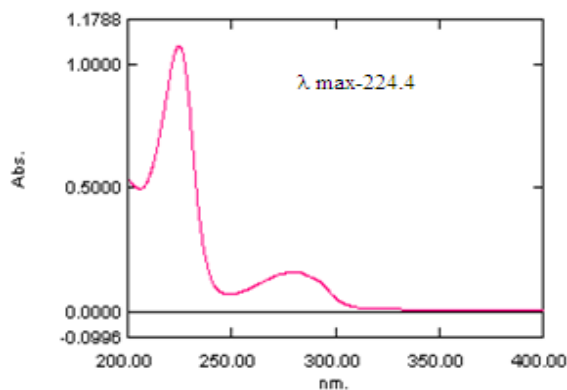


Fig. 1: It shows UV spectrum of Rizatriptan benzoate

Preparation of Mucoadhesive Polymer-based Thermoreversible Nasal Formulations

Pluronic gels were prepared by cold technique, reported by Schmolka. 1% Propylene glycol was added in the 0.25% w/v solution of drug in distilled water. To this, PF127 were added in 16%

to 20% w/v ranges. This solution was then stirred until PF127 completely dissolves in it. After the complete dissolving of PF127, Carbopol 934 was added in 0.5-1.5 %w/v. These resulting formulations were then kept at 4°C overnight until clear gel obtained. Various PF127 and Carbopol 934 concentrations used in Formulation are given in table 1.

Table 1: Table shows Thermo responsive Nasal gel Formulations containing various ranges of Poloxamers and Carbopol

Sr. no.	Formulation code	Carbopol %	Poloxamers %	Propylene Glycol %	Benzalkonium Chloride %	Water Q.S.
1	F1	0.5	16	1	0.1	50
2	F2	1	18	1	0.1	50
3	F3	1.5	20	1	0.1	50
4	F4	0.5	18	1	0.1	50
5	F5	1	20	1	0.1	50
6	F6	1.5	16	1	0.1	50
7	F7	0.5	20	1	0.1	50
8	F8	1	16	1	0.1	50
9	F9	1.5	18	1	0.1	50

Evaluation of Gels¹⁰⁻¹⁵

Measurement of Gelation Temperature by Visual Inspection^{9, 12-14}

It was determined by using method described by Miller and Donovan technique. A 2ml aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer moves upon tilting through 90°.

pH of the gels^{5, 12, 14}

Each formulated batch pH was measured using pH meter (manufactured by Toshwan Industries Ltd, ajmer.) which was previously calibrated using standard buffers of pH 4 and pH 7

Drug Content^{12, 14}

Gel equivalent to 0.25% w/v of rizatriptan benzoate was dissolved in distilled water, suitably diluted and analyzed by UV spectrophotometer at 224.4 nm. Formulation concentrations were calculated using standard curve as shown in Fig 1

Rheological studies^{12, 14}

Gels Viscosity was measured by using Brookfield programmable Lv pro-II model.

The gel were exposed to helipath spindle (Lv62), rotated at various speed ranging from 0.5-100 rpm and repeated by reversing speed from 100-0.5 rpm. The apparent viscosity values of studied gels were carried out at 4 ± 1° C and 37 ± 1° C temperatures.

Measurement of gel strength¹²

Gel strength was measured by method reported by Choi *et al.* 50 g of gel was placed in a 100 ml graduated cylinder and gelled at 37°C using thermostat. A weight of 35g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by weight to sink 5cm was measured.

Determination of Mucoadhesive Strength^{9, 14}

Mucoadhesive Strengths of gel was determined by using the modified method reported by Choi *et al.* Nasal mucosal tissues, obtained from the local slaughterhouse, were carefully removed from the nasal cavity of goat and mounted on glass surface using adhesive tape while another mucosal section was fixed in inverted position to the cylinder. 50mg of gel was placed on mucosal surface. The glass mounted mucosal surface with gel formulation and mucosal surface attached to cylinder were held in contact with each other for 2min to ensure intimate contact between them (as shown in Fig 2). In second pan, the weights were kept rising until two mucosa get detached from each other. The nasal mucosa was changed for each measurement.

The Mucoadhesive force expressed as the detachment stress in dynes/cm² was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

$$\text{Mucoadhesive Strength (dynes/cm}^2\text{)} = \frac{mg}{A} \text{----- (1)}$$

Where,

- m = weight required for detachment in gram,
- g = Acceleration due to gravity (980cm/s²),
- A = Area of mucosa exposed.

Drug polymer interaction study²⁷

Interaction study was carried out using uv- visible spectrophotometer and interpreted in terms of λ_{max} shifting. Rizatriptan benzoate $2.97 \times 10^{-5}M$ solution ,gel solutions containing equivalent $2.97 \times 10^{-5}M$ of rizatriptan benzoate prepared in 6.4 PH phosphate buffer and scanned for λ_{max} . The overlay spectra shown in fig 5.

In-vitro permeation study⁹

Fresh nasal tissues were carefully removed from the nasal cavity of goat obtained from the local slaughterhouse. Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 1.76 cm². 7 ml of 6.4 pH phosphate buffer saline (PBS) was added to the acceptor chamber and agitated with magnetic stirrer at 34°C. After pre-incubation time of 20 min, pure drug solution and formulation equivalent to 0.25%w/v of rizatriptan benzoate was placed in the donor chamber. From the acceptor compartment, 0.2-mL sample aliquots were withdrawn at predetermined time intervals up to 6 hrs, replacing the sampled volume with 6.4 pH PBS after each sampling, filtered and analyzed by UV spectrophotometer at 224.4 nm. (Linearity range = 1 µg/mL to 10 µg/mL, R² = 0.998).

Data Analyses of Permeation Studies

The effective permeability coefficients (P_{eff}) (cm s⁻¹) under steady-state conditions across excised goat nasal mucosa can be calculated (table-6) according to Equation

$$P_{eff} = (dC/dt)_{ss} VA C_D$$

Where (dC/dt)_{ss} (µg mL⁻¹ s⁻¹) is the time-dependent change of concentration in the steady-state; A (cm²) is the permeation area; V (mL) the volume of the receiver compartment; and C_D(µg mL⁻¹) is the initial donor concentration

Histopathological Evaluation of Mucosa⁹

In Histopathological evaluation, tissue incubated in the diffusion chamber with gel formulation was compared with freshly collected tissue incubated in PBS (pH 6.4). Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with xylene and eosin. Sections were examined under a light microscope, to detect any damage to the tissue during in vitro permeation by a pathologist blinded to the study.

Stability study¹²

Stability studies of Rizatriptan nasal gel was performed under actual storage condition (refrigeration condition). Gels are stored in clean, dry, airtight, moisture proof bottles, kept away from light. The gel samples were withdrawn at 15, 30, 45, days intervals and evaluated for rizatriptan content.pH.

RESULTS AND DISCUSSION

Rizatriptan benzoate exhibited λ_{max} at 224.4 nm. Linearity was observed in the range of, 1 to 10 µg/ml with the r² value of 0.998

Gelation Temperature

It was previously proved that pluronics undergo thermal gelation or sol-gel transition at a temperature of about 25 to 37°C. Below the transition temperature Pluronic solutions allow a comfortable and precise delivery in the nasal cavity where thermogelation occurs. Immediate gelling increases residence time and enhances bioavailability of drug. The gelation temperature of all batches is shown in Tables 2

In Pluronic gels, gelation studies in 20-24 % (w/w) concentration showed that gelation temperature decreases with increase in gel melting temperature as Pluronic concentration increases. Gelation of PF-127 was found dependent on aqueous solubility of the polymer. The enthalpy of gelation process will depend on the type and extent of interaction occurred. Above 16% w/w concentrated aqueous solutions PF 127 exhibit reverse thermal gelation. As the temperature increases, micellar entanglement is promoted, leading to gel formation and hence overall increase in bulk viscosity.

Researchers have investigated the gelation of PF 127 as a result of body-centered cubic packing of spherical micelles. Temperature plays an important role in the micelle formation of PF 127 through the temperature-dependent hydration of the ethylene oxide units. Water is good solvent for PEO as well as PPO chains of polymer at low temperatures. However, at higher temperature the solubility of PPO is reduced and micelle formation occurs

The decrease in the gelation temperature exhibited as PF- 127 concentration increased. It may be attributed to the higher number and volume occupied by micelles at lower temperature. As the concentration of PF-127 increases, the gel structure becomes more closely packed with the arrangement in a lattice pattern. In turn, the disruption of the lattice melting of gel occurs at higher temperatures. This is indicated by the negative value of enthalpy of gel melting. These results were supported by Ultrasonic velocity and light scattering studies. A pronounced decrease in critical micellar concentration accompanied the micellar association. Aggregates were asymmetric at low temperature but spherical above 25°C. Temperature dependent changes in micellar properties have been related to the reversible thermal gelation of PF 127. The 13C NMR studies reveals that, increased temperatures produced conformational changes in the methyl group of the polyoxypropylene within the hydrophobic micellar region and also in the motion of the hydrophilic end chains. The subsequent dehydration and increased end chain friction causes the gel formation. In attempt to increase the residence time we used Mucoadhesive Carbopol 934 polymer in study. In presence of carbapol 934 polymer, rizatriptan benzoate pluronic gel formulation showed high gelation temperature.

It was observed that formulation F4 containing low level (0.5%w/v) of Carbopol showed 36°C as gelation temperature of whereas F9 containing high level of Carbopol (1.5%w/v) showed T1 of 40°C. It indicates, that the Mucoadhesive polymer used i.e. Carbopol 934 has significant effect on gelation temperature. The phenomenon may be mediated through modification of micellar association of the PF127 molecule.

Table 2: Table shows Gelation Temperature

Sr. no	Formulation	Gelation temperature
1	F1	42°C
2	F2	38°C
3	F3	32°C
4	F4	36°C
5	F5	38°C
6	F6	40°C
7	F7	30°C
8	F8	38°C
9	F9	40°C

Drug Content

Drug content of all formulation was found to be in range of 98-100%Table 3

pH of the gels.

It is known that the normal physiological pH of nasal mucosa is 4.5 – 6.5 however the nasal mucosa can tolerate solutions within pH range of 3-10. The pH of all gel formulation was found to be in a range of 4-5.that is between physiological range of pH of nasal mucosa. Table 3

Table 3: Table shows pH and %drug content

Sr. no	Formulation code	pH	Drug content %
1	F1	4.82	98.22 ±.013
2	F2	4.7	99.2±.014
3	F3	4.17	98.30±.011
4	F4	4.66	100.02±.015
5	F5	4.27	98.43±.011
6	F6	4.08	87.24±.012
7	F7	4.52	98.16±.001
8	F8	4.17	98.00±.014
9	F9	4.13	99.15±.013

Rheological studies

Viscosity measurement of the formulations at 4°C and 37°C temperatures showed that there was increase in viscosity with increase in temperature. This indicated the formation of temperature induced gel structure of poloxamer. In addition to this Carbopol 934 showed viscosity enhancing effect. At constant concentration, abrupt changes in viscosities were observed due to sudden rise in micellar concentration. At low temperature region the liquid shows a very slight decrease in viscosity which was attributed to the dehydration of PPO blocks of the unimers. With rise in temperature the unimers start to form spherical micelles causing increase in intrinsic viscosity as a result of extremely high solvation in the micellar shell. At 1°C temperature increase causes 10% increase in the micellar concentration and 3.3% decrease in the intermicellar distance as well as two-fold increase in viscosity. Viscosity of all formulation at 4°C and 37°C showed in table 4.

Table 4: Table shows measurement of viscosity

Sr. no	Formulation	Viscosity (cp) at 4°C	Viscosity(cp) at 37°C
1	F1	23.4	144
2	F2	33.0	947
3	F3	81.6	185000
4	F4	28.5	309.5
5	F5	42.3	2879
6	F6	51.3	164000
7	F7	27.3	287.9
8	F8	36.9	1236
9	F9	69.1	356000

Measurement of gel strength

It is very important that the nasal gel formulation must have suitable gel strength. The gel strength of nasal gel formulation at 37°C, increased as the concentration of Carbopol and Poloxamers increased. The mechanism of the increase gel strength might be related to hydrogen bonding between Pluronic and bioadhesive polymers in the nasal gel. Table 5

Table 5: Table shows Measurement of gel strength and bioadhesive force

Sr. no	Formulation	Gel strength in seconds	Bioadhesive force(dynes/cm ²)
1	F1	110	2496.81±0.10
2	F2	117	4369.42±0.113
3	F3	130	8114.64±0.118
4	F4	115	2746.49±0.11
5	F5	120	3745.22±0.12
6	F6	120	5617.83±0.115
7	F7	118	2496.81±0.12
8	F8	117	4993.63±0.11
9	F9	125	8738.85±0.113

Mucoadhesive Strength

Mucoadhesive strength was determined in term of detachment stress i.e. force required to detach the formulation from mucosal surface. Results indicated that the variation in concentration of Carbopol 934 and Poloxamers 407 showed changes in Mucoadhesive strength. The gradual increase was observed in Mucoadhesive strength as the level of Carbopol 934 increased (Table 5). Our findings are similar with previously reported work with Carbopol polymer. In this review, bioadhesion was observed due to the availability of carboxyl group. Carbopol has very high percentage of (58%-68%) of carboxyl group that undergoes hydrogen bonding with sugar residues in oligosaccharide chain in mucus membrane, resulting in strengthened network between polymer and mucus membrane. The stronger the Mucoadhesive force is, the more it can prevent the gelled solution coming out of the nose.



Fig. 2: It shows Mucoadhesive Strength apparatus

In-vitro drug release

Diffusion studies were carried out using the Franz diffusion cell, it was obvious that the release of rizatriptan benzoate was not only affected by Pluronic concentration but also by the type of bioadhesive used. The bioadhesive polymer retarded the drug release from nasal gel, the retarding effect of the bioadhesive polymers could be attributed to their ability to increase the overall product viscosity as well their ability to distort or squeeze the extracellular aqueous channels of Pluronic micelles through which the drug diffuses thereby delaying the release process. Formulation F4 showed highest drug release after 6 hr as shown in fig-3.

All formulation drug release data showed peppas best fit model having n >1 indicate that super case II mechanism. This indicated that drug release depends on swelling, relaxation and erosion of polymer with zero order release kinetic.

Permeation Studies

This study confirms the importance of chelating calcium for enhancing permeability of rizatriptan. Anionic polymers such as polycarbophil or Carbopol are reported to demonstrate permeation enhancing properties. These polymers were shown to express a high Ca²⁺ binding ability. Therefore, it was important to address the question as to what extent an increase in in-vitro permeation across the nasal membrane could be attained by thermoreversible gels of PF127 and Carbopol. Presence of Carbopol results in very rapid dissolution and release of highly soluble drug due to rapid swelling and dissolution of Carbopol at pH 6.4. However, the presence of PF127 in the gel retards the drug release rate slightly owing to reduction in dimension of water channels resulting for enhanced micellar structure. Addition of 0.1% Carbopol to PF127 had no permeation enhancing effect owing to insufficient concentration of Carbopol (0.1%) to enhance drug dissolution from the gel by circumventing PF127 gel network. Although addition of 0.2% Carbopol had permeation enhancing effect as, it was not significantly greater than that obtained with pure drug solution. The addition of 0.3% and 0.5% Carbopol enhanced the permeation of drug from gel significantly. This result could be attributed to increase in concentration of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of the ionized carboxyl group results in decoupling of the polymer chain, resulting in the relaxation of the polymer network. At this stage, drug is rapidly dissolved and released from the gels as a result of very high swelling (or fast dissolution) of the ionized Carbopol. Increase in permeation of the drug from the formulation can be further explained on the basis that increase in Carbopol concentration will not only result in an increase in Ca²⁺ binding sites but also in an increase in interaccessibility of Ca²⁺ binding sites owing to relaxation of the polymer network. Formulation f4 showed highest permeability table 6.

Table 6: Table shows Permeation Studies

Sr. no	formulation	R value	n-values	Permeability coeff, after 6 hr(10^{-4})
1	F1	0.9719	1.403	1.47
2	F2	0.8651	1.057	1.04
3	F3	0.9663	1.624	2.5
4	F4	0.9008	1.580	2.9
5	F5	0.9171	1.585	2.2
6	F6	0.9358	1.480	1.8
7	F7	0.9409	1.757	2.2
8	F8	0.9552	1.843	2.5
9	F9	0.9391	1.800	2.2

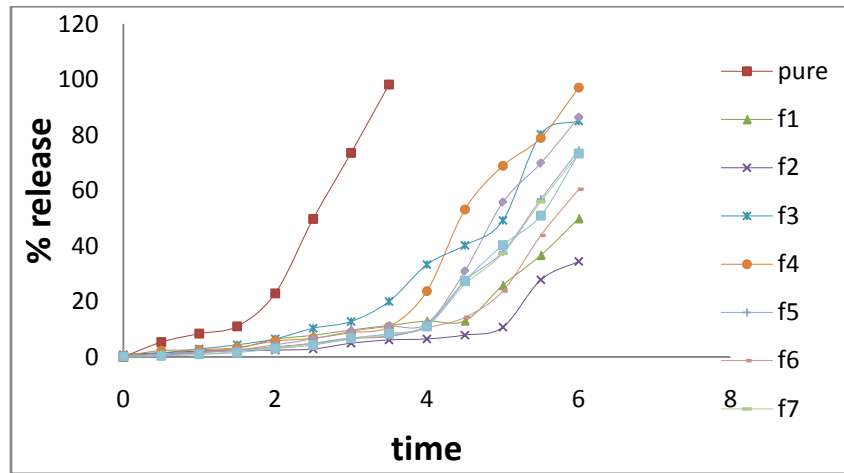


Fig. 3: It shows In-vitro drug release

Histopathological Evaluation of Mucosa

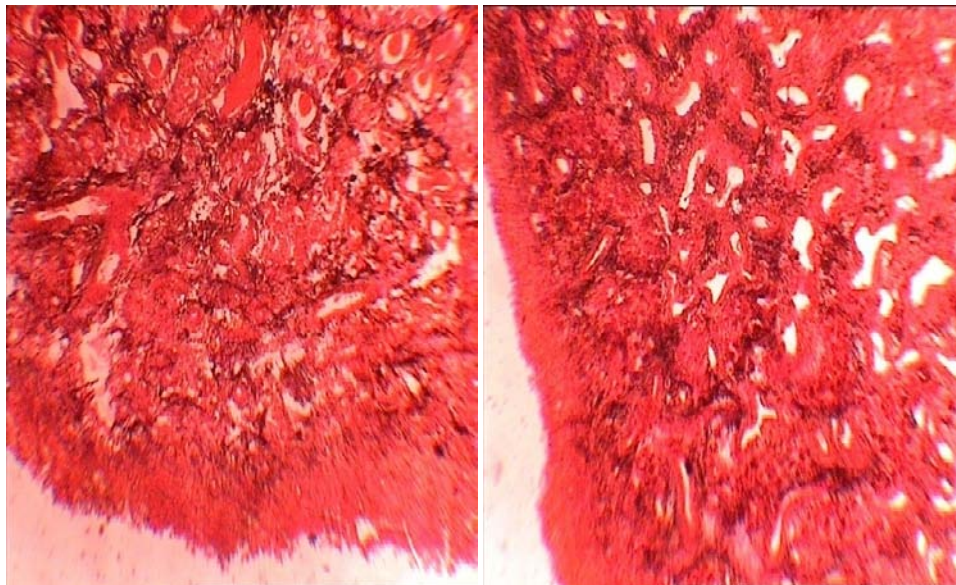


Fig. 4: It shows Histopathological Evaluation

Photomicrograph of goat nasal mucosa after the permeation studies were observed for Histopathological changes in the comparison with PBS treated mucosa . The microscopic observations indicate that the optimized formulation F4 has no significant effect on the microscopic structure of mucosa. As shown in fig 4 neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of gel. The epithelium layer was intact and there were no alterations in basal membrane and superficial

part of sub mucosa as compared with PBS-treated mucosa. Thus, gel formulations seem to be safe with respect to nasal administration.

Drug polymer interaction study

In comparison of UV spectra, it was observed that there was no shifting of λ_{max} of Rizatriptan and gel formulation as shown in Fig 5. This study revealed that non existence of interaction between drug and polymer.

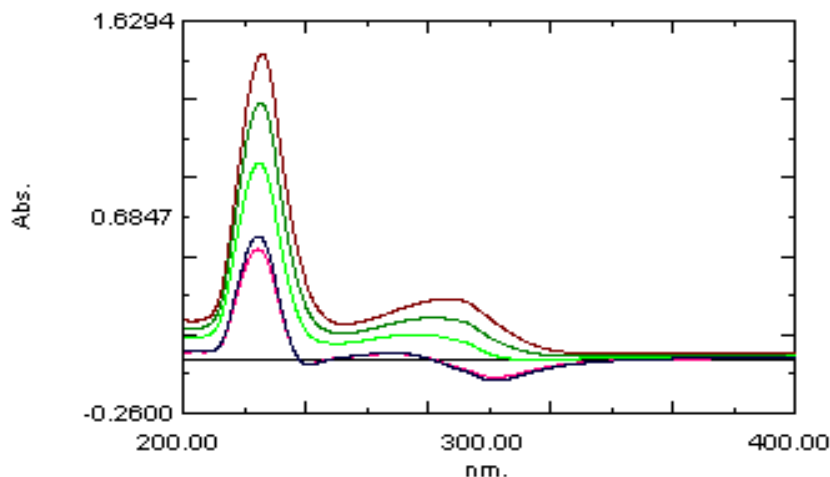


Fig. 5: It shows drug polymer interaction study

Stability study

Table 7: Table shows Stability study

Sr. no	days	% drug contain	pH
1	0	100.02	4.66
2	15	99.93	4.61
3	30	99.93	4.6
4	45	99.77	4.58

Stability study indicates that there was no significant change in the Rizatriptan benzoate after 45 days when compared with the initial value. The results indicated that the formulation did not show any change in % drug contain, pH during the stability testing period.

CONCLUSION

Rizatriptan benzoate formulated as bioadhesive thermoreversible poloxamer solution for nasal administration could have potential to avoid first-pass effect than oral route, thus improve bioavailability of drug and as a safe, sustained release nasal delivery system to control migraine. Bioadhesion that allows stagnation of gel in the nose may be advantage to protect the drug from drain out. As increasing the concentration of polymer, the bioadhesive force, gel strength and retardation of drug release increases. Thus it can be concluded that nasal gel containing 0.5% carbopol & 18% poloxamer concentration is an ideal gel for controlled nasal drug delivery system with respect to its evaluation parameters like gelation temperature, drug release.

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REFERENCES

1. Khedkar amol*, rajendra vivek, kulkarni alpna, dehghan mohdhassan, saifee maria, lahoti swaroop Spectrophotometric method for analysis of rizatriptanbenzoate IJPS 307-309
2. Goadsby P. Serotonin 5-HT1B/1D Receptor Agonists in Migraine: Comparative Pharmacology and Its Therapeutic Implications. CNS Drugs. 1998; 10: 271. 286.
3. Sachin S Jagtap*, CL Gopu , Kakasabeb R Mahadik, Mahadev V Mahadik Stability indicating reversed-phase high-performance liquid chromatographic method for the determination of rizatriptan benzoate in bulk powder and in pharmaceutical formulations Research Journal of Pharmaceutical, Biological and Chemical RJPBCS Volume 1 Issue 2 Page No. 385

4. Jadhav KR, Gambhire MN, Shaikh IM, Kadam VJ, Pisal SS. Nasal Drug Delivery System-Factors Affecting and Applications. Current Drug Therapy 2007; 2: 27-38.
5. Behl CR, Pimplaskar HK, Sileno AP, Xia WJ, Gries WJ, deMireles JC, et.al. Optimization of systemic nasal drug delivery with pharmaceutical excipients. Advanced Drug Delivery Reviews 1998; 29: 117- 33.
6. Kim DD. Drug Absorption Studies. Vol.VII. Part 2. Springer US, 2008; 216-234.
7. Dondeti P, Zia H, Needham TE. Bioadhesive and formulation parameters affecting nasal absorption. Int.J.Pharm 1996; 127:115-33.
8. Zaki NM, Awad GA, Mortada ND, AbdElHady SS. Enhanced bioavailability of metoclopramide HCl by intranasal administration of a mucoadhesive in situ gel with modulated rheological and mucociliary transport properties. European Journal of Pharmaceutical Sciences 2007; 32:296-307.
9. Majithiya RJ, Ghosh PK, Umrethia ML, Murthy RSR. Thermoreversible-mucoadhesive Gel for Nasal Delivery of Sumatriptan. AAPS PharmSciTech. 2006; 7(3)
10. Jones DS, Woolfson AD, Brown AF, Coulter WA, McClelland C, Irwin CR. Design, characterization and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease. J Control Release. 2000;67:357-368.
11. Ch'ng HS, Park H, Kelly P, Robinson JR. Bioadhesive polymers as platforms for oral controlled drug delivery II. Synthesis and evaluation of some swelling water-insoluble bioadhesive polymers. J Pharm Sci. 1985;74:339-405.
12. Chaudhari Praveen*, Ajab Amit, Malpure Prashant, Kolsure Pramod, Sanap Devidas Development and In Vitro Evaluation of Thermoreversible Nasal Gel Formulations of Rizatriptan Benzoate Indian J.Pharm. Educ. Res. 43(1), Jan-Mar, 2009
13. Miller SC, and Donovan MD. Effect of poloxamer 407 gels on the miotic activity of pilocarpine nitrate in rabbits, Int. J. Pharm., 1982, 142-152.
14. Varsha Gaikwad et al. Formulation and evaluation of In-Situ gel of metoprolol tartrate for nasal delivery / Journal of Pharmacy Research 2010, 3(4),788-793

15. Pisal SS, Paradkar AR, Mahadik KR, Kadam SS. Pluronic gels for nasal delivery of Vitamin B12. Part I: Preformulation study. *Int J Pharm* 2004; 270: 37-45.
16. Charlton S, Jones NS, Davis SS, Illum L. Distribution and clearance of bioadhesive formulations from the olfactory region in man: Effect of polymer type and nasal delivery device. *European Journal of Pharmaceutical Sciences* 2007; 30: 295-302.
17. Nirmal H.B.*, Bakliwal S.R., Pawar S.P. In-Situ gel: New trends in Controlled and Sustained Drug Delivery System *International Journal of PharmTech Research CODEN (USA): IJPRIF Vol.2, No.2, pp 1398-1408, April-June 2010*
18. Renuka Chand , Anuja a. Naik, Hema a. Nair Thermoreversible biogels for intranasal delivery of rizatriptan benzoate date of publication 3 feb 2010 Year : 2009 Volume : 71 Issue : 6 Page : 723-725
19. Tanaji Nandgude*, Rahul Thube, Nitin Jaiswal, Pradip Deshmukh, Vivek Chatap, Nitin Hire B.R. Nahata Formulation and Evaluation of pH Induced In-situ Nasal Gel of Salbutamol Sulphate Allana College of Pharmacy, 2390-k, B. Hidayatullah road, Azam campus, Pune-411 001
20. Patel Meenakshi*, Thakkar Hetal, Kasture P. V. preparation and evaluation of thermoreversible formulations of flunarizine hydrochloride for nasal delivery *International Journal of Pharmacy and Pharmaceutical Sciences* 2010 Vol 2, Issue 4,
21. Choi HG, Jung JH, Ryu JM, et. al; Development of in situ gelling and mucoadhesive acetaminophen liquid suppository; *Int.J. of pharm.* 1998;165:33-44.
22. Pisal S, Shelke V, Mahadik K, Kadam S; Effect of organogel compound on In vitro Nasal Delivery of Propranolol HCl; *AAPS Pharma Sci Tech*;2004; 5(4)
23. Pisal S, Reddy P, Paradkar A R, Mahadik R, Kadam S; Nasal melatonin gels using pluronic-P127 for chronobiological treatment of sleep disorder. *Indian. J. Biotech.* 2004; 3:369-377.
24. Grace Rathnam*, Narayanan N, Ilavarasan R preparation and evaluation of carbopol based nasal gels for systemic delivery of progesterone *International journal of pharma. Research & development online IJPRD*
25. Schmolka IR. Artificial Skin I. Preparation and properties of Pluronic F-127 gels for treatment of burns. *J. Biomed. Mater. Res* 1972; 6:571-82.
26. Pisal SS, Paradkar AR, Mahadik KR, Kadam SS. Pluronic gels for nasal delivery of Vitamin B12. Part I: Preformulation study. *Int. J Pharm* 2004; 270: 37-45
27. K. Venkates kumar, N. Arunkumar, Prp. Verma, C. Rani Preparation and in vitro characterization of Valsartan solid dispersions using skimmed milk Powder as carrier *international journal of pharmtech research Coden(usa): ijprif Vol.1, no.3, pp 431-437, july-sept 2009*
28. Nahla S. Barakat1 In Vitro and In Vivo Characteristics of a Thermogelling Rectal Delivery System of Etodolac ,2 *AAPS PharmSciTech*, Vol. 10, No. 3, September 2009 724-731
29. Jaya raj Kumar.K*, Jayachandran.E, Srinivas.GM, Giridhar.B, Rahul Nair, Jayakandan A Novel Thermo-Sensitive Sol-Gel Reversible Buccal Adhesive Property of Fluconazole in Situ Gel For Oral Thrush. *M S.C.S College of pharmacy, Harapanahalli (KA). J Biomed Sci and Res.*, Vol 2 (2), 2010, 100-109
30. S. B. Waikar et al Preformulation and thermodynamic study of rizatriptan benzoate nasal gel formulation *Journal of Pharmacy Research* 2009, 2(5), 986-990
31. Vamshi krishna T*, Madhusudan rao Y formulation and evaluation nasal insulin gel *International Journal of Pharmacy and Pharmaceutical Sciences* vol 2, issue 3, 2010 ,161-164.