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Formulation and Evaluation of pH Dependent Zolmitriptan in-Situ Nasal Gel

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ABSTRACT

Development of pH sensitive zolmitriptan*in-situ*nasal gel was aimed to improve absorption and patient compliance. In the present research work, mixture of Carbopol 940 and Hydroxypropylmethylcellulose K100 were used to confer pH sensitive gelation property. Different formulation was prepared by varying the concentrations of Carbopol 940 and HPMC K100.These formulations were evaluated for parameters like pH, drug content, viscosity, mucoadhesive strength, gel strength, in-vitro drug release, in-vitro permeation and drug excipients compatibility. In this formulation the release profile depend on the concentration of Carbopol 940 and HPMC K100. A 3^2 factorial design was applied to see the effect of variables Carbopol 940 (X_1) and HPMC K100 (X_2) on the various models to ascertain kinetic of drug release. Regression analysis and analysis of variance were performed for dependent variables. The results of the F-statistics were used to select the most appropriate model. Formulation containing Carbopol 940 (0.1%) and HPMC K100 (0.2%) was found to be optimum. The study indicate that the formulation was effective in providing in-vitro release of drug and the mucoadhesive formulation.

Keywords: Anti-migraine, Carbopol 940, HPMC K100, In-situ Gelling system.

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INTRODUCTION

A migraine is a relatively common medical condition that can severely affect the quality of life of the sufferer and his or her family and friends. Migraine is most commonly experienced by both men and women between the ages of 25 and 39.There are two different types of migraines, migraine without aura and migraine with aura[1].A migraine without aura is a condition characterized by moderate to severe thrombing and unilateral pain with the following symptoms. Nausea, loss of appetite and/or vomiting, Photophobia (increased sensitivity to light), Phonophobia (increased sensitivity to sound). On average, untreated migraine episodes last from 4 to 72 hours.A migraine without aura involves any number of different sensations that range from visual disturbances to physical sensations[2]. The aura symptoms usually occur in alternating body sites during different attacks. Almost always proceeding, a feeling of strangeness a day or two before the attack begins. Prodromes are characterized by mood changes, food cravings, feeling tried or hyperactive, or excessive yawning. Migraine is a condition, in which a person experiences tremendous headache. Generally, this headache affects only one side of the head and body[3]. Migraine attacks are more common to those people who take too much of stress or are work alcoholic. In such people, the blood flow in the brain muscles drops, as a result of too much load, squeezing the arteries. When the person suddenly relaxes, these tight brain muscles expand, stretching the blood vessel walls[4]. The blood pumped with each heartbeat, then, pushes the vessels further, causing immense pain. Though the exact cause of migraine has not been identified, there are a number of factors that can trigger the severe headache. The typical migraine headache is unilateral and pulsating, lasting from 4 to 72 hours. Migraine is a neurological syndrome characterized by altered bodily perceptions, headaches, and nausea. Physiologically, the migraine headache is a neurological condition more common to women than to men. In other words Migraine is a familial disorder characterized by recurrent attacks of headache widely variable in intensity, frequency and duration. Attacks are commonly unilateral and are usually associated with anorexia, nausea and vomiting[5,6]. Zolmitriptan, 4S-4-({3-[2-(dimethylamino) ethyl]-1H-indol-5-yl} methyl)-1,3-oxazolidin -2-one, is a second –generation triptan prescribed for patients with migraine attacks, with or without an aura, and cluster headaches[7]. It is currently available as a conventional tablet, an oral disintegrating tablet and nasal spray (2.5 mg and 5.0 mg per dose). The nasal dose is claimed to be absorbed rapidly, with detectable plasma zolmitriptan concentration within 2 min after administration. In contrast, plasma zolmitriptan concentrations are generally not detected until 15-20 min after administration of a tablet formulation [8, 9]. The absolute bioavailality

of zolmitriptan is up to 40% for both oral and nasal dosage forms. The faster clearance of the drug from the nasal cavity could explain the low bioavailability for nasal formulation. It has a selective action on serotonin (5-HT1B/1D) receptors and is very effective in reducing migraine symptoms, including pain, nausea, and photo or phonophobia[10]. Carbopol also called as Carbomer are high molecular weight polymers of acrylic acid cross-linked with allyl ethers of pentaerythritol, It is a viscosity increasing agent, release modifying agent, bioadhesive and Hydroxypropylmethylcellulose also called as hypromellose contains methoxy and hydroxypropoxy groups which is viscosity increasing agent. It is available in several grades that vary in viscosity and extent of substitution. Grades may be distinguished by appending a number indicative of the apparent viscosity. Both polymer are also have pH sensitivity as the pH increase the viscosity of the solution increase and as pH decrease the viscosity of the solution also decrease. So that these two polymer are used in the preparation of pH sensitive formulation[11]. This research work is undertaken to develop the zolmitriptan *in-situ* nasal gel using Carbopol 940 grade and bioadhesive HPMC K100 polymers. The pH of the prepared solution were maintained in between 7.3 and 7.4 which was reported as being an acceptable range to ensure gelation at physiological pH without leakage after administration. The gel strength, bioadhesive force was determined and the drug release from the prepared nasal gel formulation finally the nasal mucosa was examined histopathologically.

MATERIALS AND METHODS

Zolmitriptan was a gift sample from Glenmark pharmaceutical, Sinner Nasik. , India. Carbopol 940, Hydroxypropylmethylcellulose K100 was kindly supplied by Research-Lab Fine Chem. Industry, Mumbai, India. Benzalkonium chloride and Sodium Metabisulphite was procured from, Research-Lab Fine Chem. Industry Mumbai, India. All other chemicals were of research grade. The formulations were prepared by cold method (Reported by Shmolka). The drug containing PEG, gelling polymer and mucoadhesive polymers were hydrated separately in calculated amount of distilled water at room temperature and cooled and stored at 4^{o}C. Both polymeric solutions was mixed slowly on ice bath, Preservative was added slowly with continuous stirring in polymer solution. Both solutions (drug and polymer) were mixed with each other by gentle stirring. The final dispersion was then stored in a refrigerator until clear solution was obtained. Different formulies of gel were prepared by using ingredients mentioned in (Table -1). In this formulation concentration of Carbopol 940 was ranged between 0.1 to 0.5 %, concentration of HPMC in between 0.1 to 0.3%. Drug was dissolved in mixture of distilled water and PEG; both the polymers

were hydrated separately. Kept solutions at room temperature over night. Preservative was added in polymeric solution. Mixing of drug and polymeric solution was done at cold condition[12].

Table no-1: Compositions of formulations

Formulation	Zolmitriptan	Carbopol 940	HPMC K 100	Benzalkonium chloride	Sodium Metabisulfite
F1	0.5	0.1	0.1	0.01	0.02
F2	0.5	0.25	0.1	0.01	0.02
F3	0.5	0.5	0.1	0.01	0.02
F4	0.5	0.1	0.2	0.01	0.02
F5	0.5	0.25	0.2	0.01	0.02
F6	0.5	0.5	0.2	0.01	0.02
F7	0.5	0.1	0.3	0.01	0.02
F8	0.5	0.25	0.3	0.01	0.02
F9	0.5	0.5	0.3	0.01	0.02

EVALUATION OF NASAL GEL

Physical parameter[13, 14]

Determination of Clarity (visual appearance) and pH

The formulations were visually checked for the clarity.

pH of each formulation was determined by using Digital pH meter (Digital pH meter 335). This was previously calibrated by pH 4 and pH 7. The pH values were recorded immediately after preparation.

Compatibility Study[15]

Compatibility study was carried out by using Fourier transform infrared spectrophotometer (Cary 630, Agilent technology USA). FTIR study was carried on pure drug and physical mixture of drug and polymers. Physical mixtures were prepared and samples kept for 1 month at 40^0C. The infrared absorption spectrum of zolmitriptan and physical mixture of drug and polymers was recorded with a KBr disc over the wave number 4000 to 400 cm^{-1}.

Rheological study

Viscosity[16, 17]

The rheological properties ofgels were determined by the Brookfield viscometer; type DV-II + PRO using spindle SC4-18 .Viscosity of the formulations were taken at room temperature and the 37^0C with varying shear rate. Also the effect of pH condition on viscosity of gel was determined and the pH conditions were modified by using triethanolamine. The viscosity of the gel at respective pH range between 5.9-6.5 and after adjusting the pH to 7.4 was determined.

Measurement of the gel strength[17]

A sample of 25 ml of the gel was put in a 50 ml graduated cylinder. A weight of 14.33 g was

placed on the gel surface. The gel strength, which is an indication for the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel. All measurements were performed in triplicate (n=3). The apparatus used for measuring gel strength is shown in Figure.1.

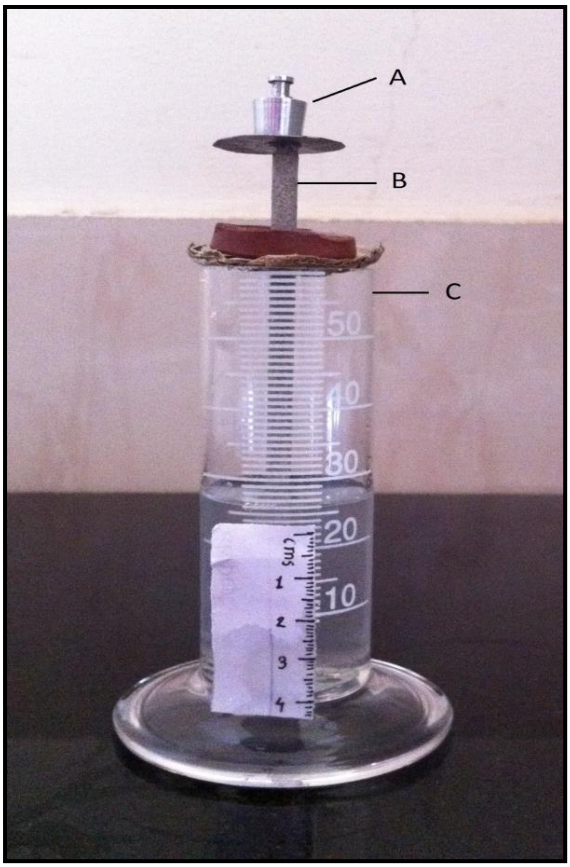

Figure. 1: Gel strength measuring device

(A) weights (B) device (C) Graduated cylinder (D) gel

Mucoadhesive Strength[18]

Detachment Stress is the force required to detach the two surfaces of mucosa when a formulation/gel is placed in between them". The detachment stress was measured by using a modified analytical balance. *In vitro* mucoadhesion studies were conducted using modified mucoadhesion test assembly described by Gupta et.al

Fabrication of equipment: The equipment was fabricated by us in the laboratory as shown in figure 3. A double beam physical balance was taken, both the pans were removed. The left pan was replaced with a brass wire, to which was hanged a polypropylene disc (A), also locally fabricated. The dimensions are 2 cm height and include an expanded cap of diameter 3.8 cm and thickness 2 cm. Another propylene disc of 2 cm height and 1.5 cm diameter was placed right below the suspended disc upon the base of the balance. The right pan (C) was replaced with a

lighter pan so that, the left pan weighs 5.25 gm more than the right pan. The lower polypropylene block was intended to hold the mucosal tissue (D) of goat nasal mucosa and to be placed in a beaker containing simulated nasal solution pH 6.7. (E).

Measurement of adhesion force

The following procedure was used for all the test formulations using the above equipment. The nasal mucosa was removed from refrigerator and allowed to attain equilibrium with ambient conditions in the laboratory. The goat nasal mucosa was carefully excised, without removing connective and adipose tissue and washed with simulated nasal solution. The tissue was stored in fresh simulated nasal solution. Immediately afterwards the membrane was placed over the surface of lower polypropylene cylinder (B) and secured. This assembly was placed into beaker containing simulated nasal solution pH 6.7 at 37 ± 2°C. From each batch, some quantity of gel was taken and applied on the lower surface of theupper polypropylene cylinder. The beaker containing mucosal tissue secured upon lower cylinder (B), was manipulated over the base of the balance so that, the mucosal tissue is exactly below the upper cylinder (A). The exposed part of the gel was wetted with a drop of simulated nasal solution, and then a weight of 10 Gms was placed above the expanded cap, left for 10 minutes. After which the gel binds with mucin. The weight was removed. Then slowly and gradually weights were added on the right side pan till the gel separates from the mucosal surface/ membrane. The weight required for complete detachment is noted (W1) (W1-5.25G)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

Calibration of test equipment

Initially, a gel from the same batch was taken ten times and individual force required for complete detachment was noted and S. D.was calculated.

Force of adhesion(N) = (bioadhesive strength/1000) × 9.81

Bond strength (N/m^2) = force of adhesion (N)/surface area of disk (m^2)

The Apparatus for Bioadhesive study is shown in (Figure.2).

Drug Content[19]

1 ml of solution was taken in 100 ml beaker; in that beaker 50 ml phosphate buffer 7.4 pH was added. Aliquot 01 ml from this solution was diluted up to 10ml with water to get the final concentration of 10 µg/ml and further dilutions where prepaid. The absorbance of prepared solution was measured at 283 nm by using UV visible spectrophotometer.

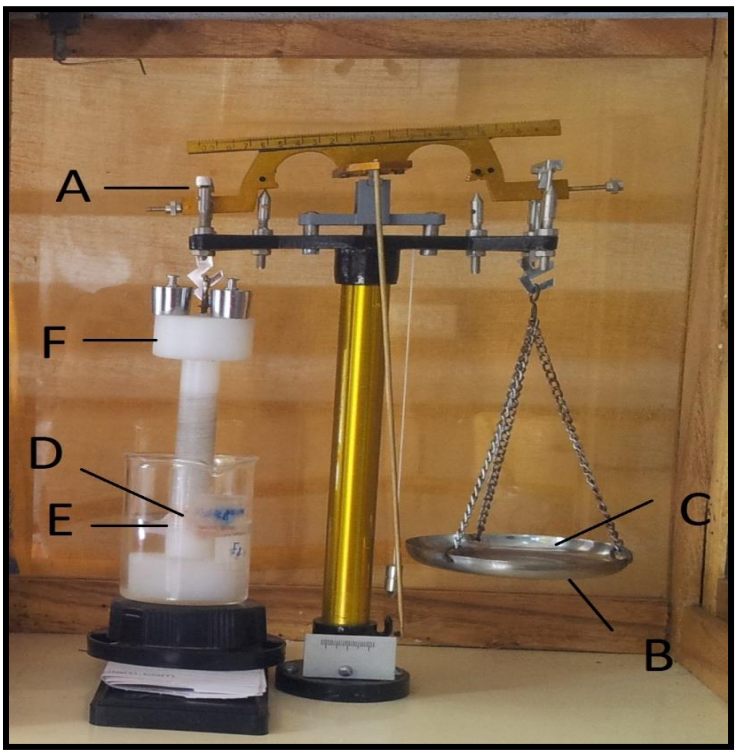

Figure 2: Modified balance for bioadhesive study

A: Modified balance, B: Weighing pan, C: Weight, D: Gel, E: Nasal mucosa F:polypropylene cylinder.

***In-vitro* Drug Release Study [20]**

In-vitro release study of the formulated in-situ gel was carried out by using diffusion cell through egg membrane as a biological membrane. Diffusion cell with inner diameter 1.4cm was used for the study (figure 3). The formulation 1 ml were placed in donor compartment and Freshly prepared 100 ml simulated nasal electrolyte solution (sodium chloride 0.745gm, potassium chloride 0.129 gm, calcium chloride dehydrated 0.005gm, distilled water q.s. 100ml) in receptor compartment. Egg membranes were mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 2ml of sample is withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7 & 8 hrs and same volume of fresh medium is replaced. The withdrawn samples was diluted to 10ml in a volumetric flask with 7.4 pH phosphate buffer and analyzed by UV spectrophotometer at 283 nm.

Figure-3: Laboratory designed diffusion cell. A- Test tube containing formulation. B- Egg membrane. C-beaker containing simulated nasal solution. D- Magnetic stirrer.

***In-vitro* permeation study** [21]

Natural membranes are utilized to determine in vitro permeation study to mimic the in vivo permeation patterns. In this experiment goat nasal mucosa was utilized because the respiratory area of goat is large and it is easy to get. Fresh mucosal tissue was removed from the nasal cavity of goat. The tissue was placed on the diffusion cell with permeation area 0.786cm^2. The acceptor chamber of the diffusion cell (laboratory designed)with a volume capacity 100ml was filled with simulated nasal fluid (SNF) containedaccurately7.45mg/ml NaCl, 1.29mg/ml KCl and 0.32 mg/ml $CaCl_2 \cdot 2H_2O$. 0.5(10 mg equivalent) ml of formulation was placed in donor compartment .At predetermined time intervals of 30 min, 1,2,3,4,5,6,7,and 8 hrs 1ml of sample was withdrawn from the acceptor compartment replacing the sample removed with simulated nasal fluid after each sampling for period of 8 hrs .Then samples were specifically diluted and absorbance was noted at 283 nm. Permeability coefficient (p) was calculated by the following formula: 90

$$P = (dQ/dt) / (C0 \times A)$$

Where, dQ/dt is the flux or permeability rate (mg/h), C_0 is the initial concentration in the donor compartment, and A is the effective surface area of nasal mucosa.

Stability studies[22]

The formulations were stored at room temperature with 60±5 % RH. The formulations were evaluated mainly for their physical characteristics at the predetermined intervals of 6 months like appearance/clarity, pH, viscosity and drug content

RESULT AND DISCUSSION

Visual appearance and pH

On careful visual inspection against dark and white background, all the prepared nasal gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be clear.The pH of all the formulations from F1 to F9 was found to be in the range of 6.5 to 6.8.pH values of formulations shown in (Table -2).

Table-2: Evaluation parameters

Formula	Observed pH (±S.D)	Gel Strength (sec)(±SD)	Detachment Force (N)(±S.D.)	Drug Content (%)(±SD)	Cumulative Drug Release (%)(± SD)
F1	6.04±0.0808	0.35±0.07211	0.222±1.21	99.42±0.02	99.72±0.03
F2	6.09±0.0152	0.39±0.0450	0.3169±1.85	95.8±0.01	98.76±0.18
F3	6.1±0.0115	0.43±0.03055	0.4799±0.85	99.88±0.00577	87.54±0.13
F4	6.09±0.4647	0.39±0.0435	O.2939±3.06	101.4±0.01	99.30±0.18
F5	6.11±0.01	0.43±0.023	0.4173±2.477	98.4±1.0115	98.39±0.18
F6	5.98±0.0152	0.49±0.01	0.5724±1.84	102.4±0.00577	85.36±0.139
F7	6.08±0.0230	0.41±0.0351	0.6383±4.26	96.2±0.02	98.145±0.13
F8	6.09±0.0173	0.48±0.02	0.7671±4.19	94.44±0.0152	97.146±0.27
F9	6.02±0.0404	0.51±0.01	0.9330±4.65	96.48±0.0057	81.426±0.18

Compatibility Study

Infra-red spectra of drug and polymers showed matching peaks with the drug spectra. The characteristic peaks of drug were also present in the spectrum of all drug- polymer combinations is shown in Figure.4 and Figure 5.

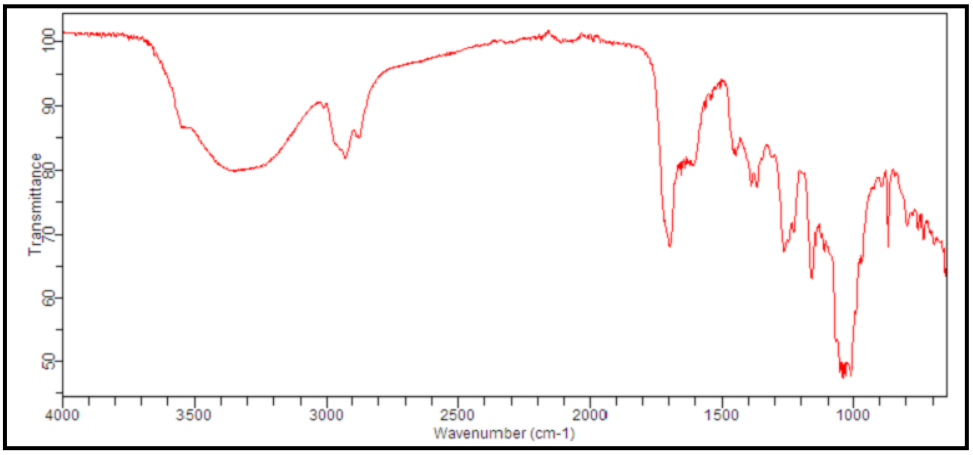

Figure.4. Infra-red spectrum of drug with polymer.

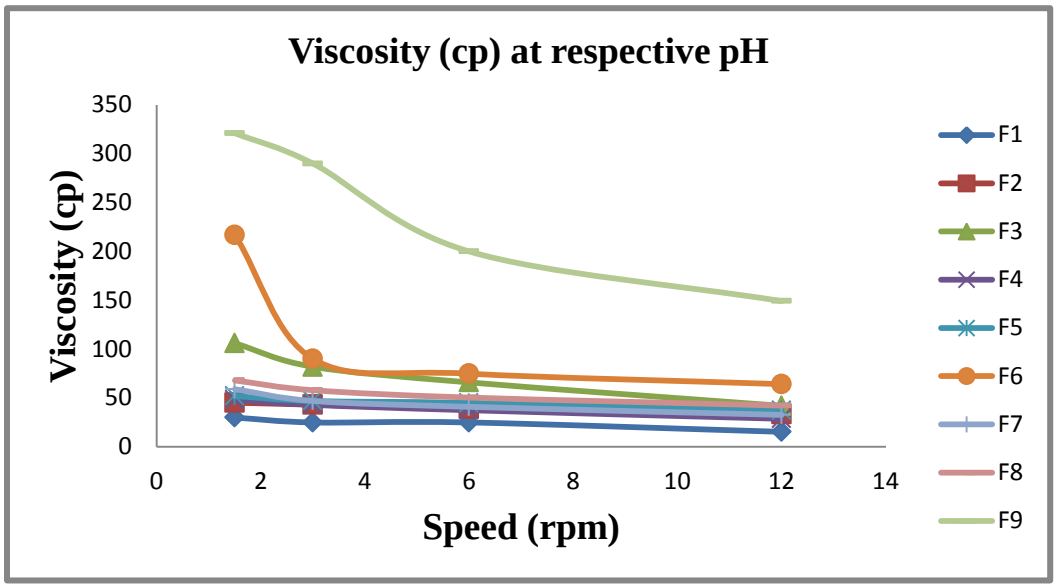

Figure 5: Viscosity profile of formulations at respective pH

Rheological study

Viscosity

The viscosities of formulations at respective pH and at pH 7.4 are shown in (Table-3) and (Table-4) respectively. The Viscosity profile of formulations at respective pH and at pH 7.4 are shown in(Figure.6) and(Figure.7)respectively**.** Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased which indicate that gel has the pseudo plastic flow. As pH was increased the increased in viscosity was observed. Concentration of Carbopol was a major factor affecting viscosity of formulations. In combination with HPMC, Carbopol has shown considerable increases in viscosity when concentration of HPMC is 0.1% w/v to 0.3 %w/v.

Table no 3. Viscosity of formulations at respective pH

Viscosity (cp) at respective pH									
Rpm	**Formulation code**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1.5	30	45.2	105.98	47.99	51.99	216.9	58.99	67.99	320.99
3	25	43.2	81.98	42.99	46.99	90.1	46.99	57.99	289.9
6	24.99	39	65.99	37.49	44.99	74.9	40.99	50.49	200
12	15.4	33.24	41.99	28.74	37.25	64.2	32.74	42.25	149.2

Table no 4. Viscosity of formulations at pH 7.4

Viscosity (cp) at 7.4									
rpm	**Formulation code**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1.5	62.5	90.4	210.87	95.89	103.99	435.3	117.98	135.98	641.98
3	55.2	86.5	163.9	92.34	95.2	181.09	94.23	115.76	579.87
6	49.98	70.8	135.89	75.24	90.32	140.45	82.23	101.43	409.32
12	32.6	32.6	85.89	57.87	75.6	129.04	65.48	84.25	298.76

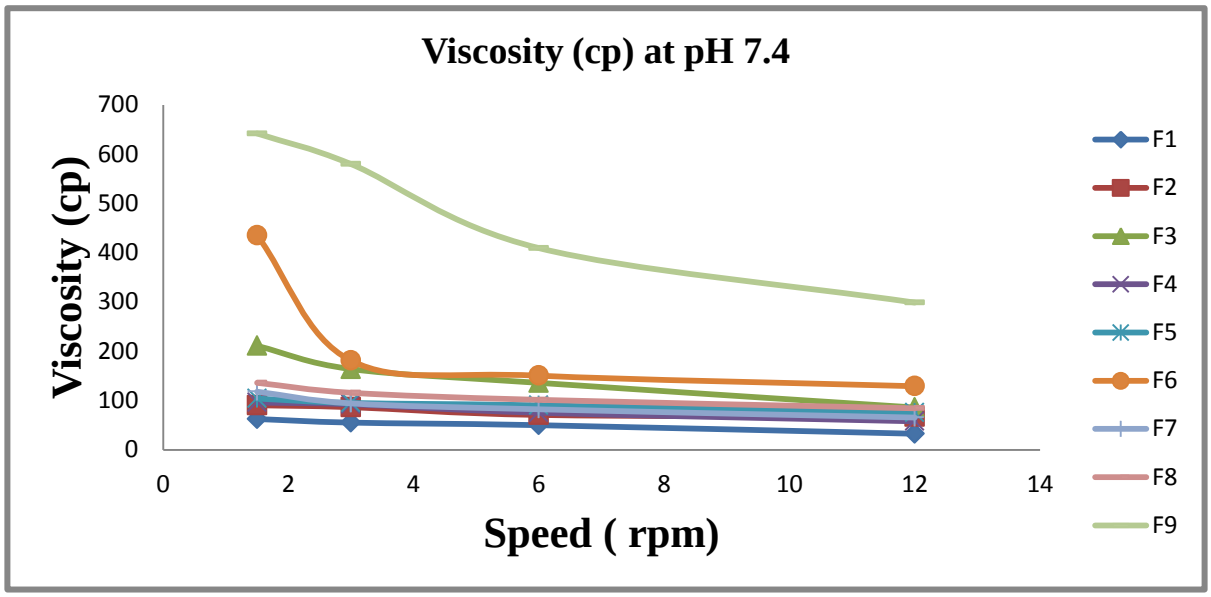

Figure. 6: Viscosity profile of formulations at pH 7.4

Gel Strength

The gel strength was found to be affected by concentrations of gelling and mucoadhesive polymers. Optimal mucoadhesive gel must have suitable gel strength so as to be administered easily and can be retained at nasal mucosal region without leakage after administration. Gel strength of all formulations showed comparable results as that of viscosity results shown in (Table-2).

Mucoadhesive strength

Mucoadhesive force means the force with which gels bind to nasal mucosa. Greater mucoadhesion is indicative of prolonged residence time of a gel and thus prevents its drainage from nose cavity. The mucoadhesion force increased significantly as the concentration of mucoadhesion polymers increased. The Detachment force was determined for nasal gels. Results of this test indicate that the variable Carbopol and HPMC both arehaving effect on mucoadhesive strength. It shows that mucoadhesive force has increased with the increasing concentration of the Carbopol shown in (Table-2)

Drug Content

The percentage drug content of all prepared nasal formulations was found to be in the range of 94.44-102.4%. Therefore uniformity of content was observed in all formulation shown in Table-2.

***In-vitro* Drug Release**

The result shows that with increase in concentration of Carbopol 940 and Hydroxypropylmethylcel lulose K100 the release rates were found to decrease gradually. The result showed that the gels had the ability to extend the release of Zolmitriptan for duration of 8 hrs (Table-2). On the basis of gel strength, viscosity, Mucoadhesive Strength, percent drug content and in-vitro drug release profile

the optimum mucoadhesive gel formulation of Carbopol 940 0.1% and HPMC K100 0 .2% was selected. The F4 batch was selected as optimized and subjected for permeation study. *In-vitro* drug release profile of all formulations are shown in (figure 7).

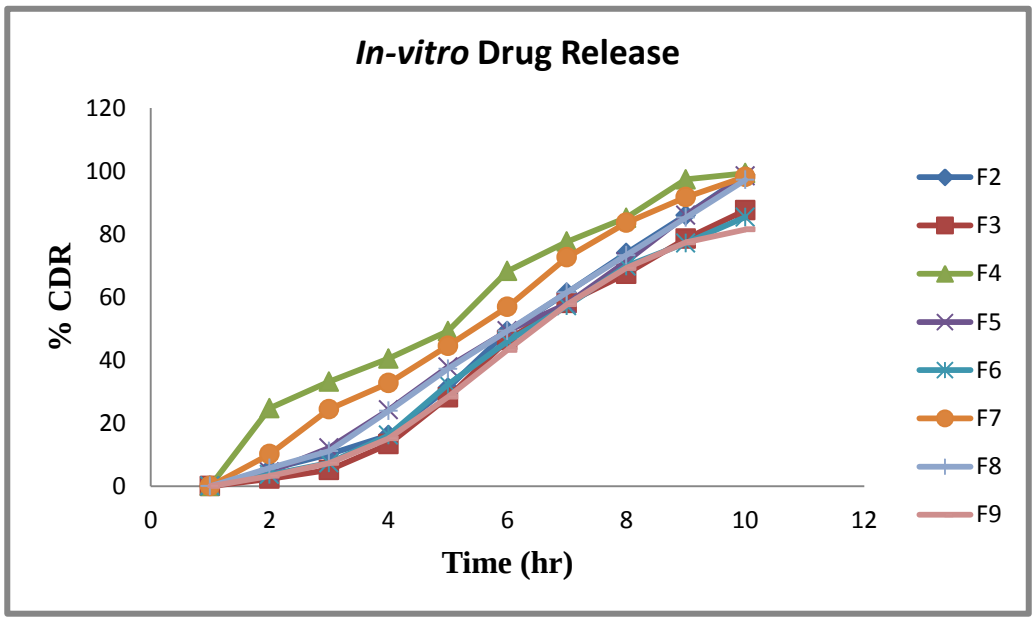

Figure.7. *In-vitro* drug release of formulation

Optimization[23]

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of Carbopol (X_1) and HPMC (X_2) were selected as independent variables and the dependent variable was % drug release. The data obtained were treated using Design expert version 8.0.4.1 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to study the interaction of Carbopol (X_1) and HPMC (X_2) on dependent variable. Table-3. Shows ANOVA for the dependent variable % drug release. The values of X_1 and X_2 were found to be significant at $p < 0.05$, hence confirmed the significant effect of both the variables on the selected responses.From this data optimum concentration of Carbopol 0.1%w/v and HPMC 0.2%w/v was found.

Y1 (% CDR)=107.7408-35.7250*(A)-13.6833*(B)

A= Carbopol

B= HPMC

From design expert version 8.0.4.1 five solutions were found in which optimum batch Carbopol 0.1%w/v and HPMC 0.2%w/v with desirability 1 was found to be optimum. From this data F4 batch was selected as optimum formulation.

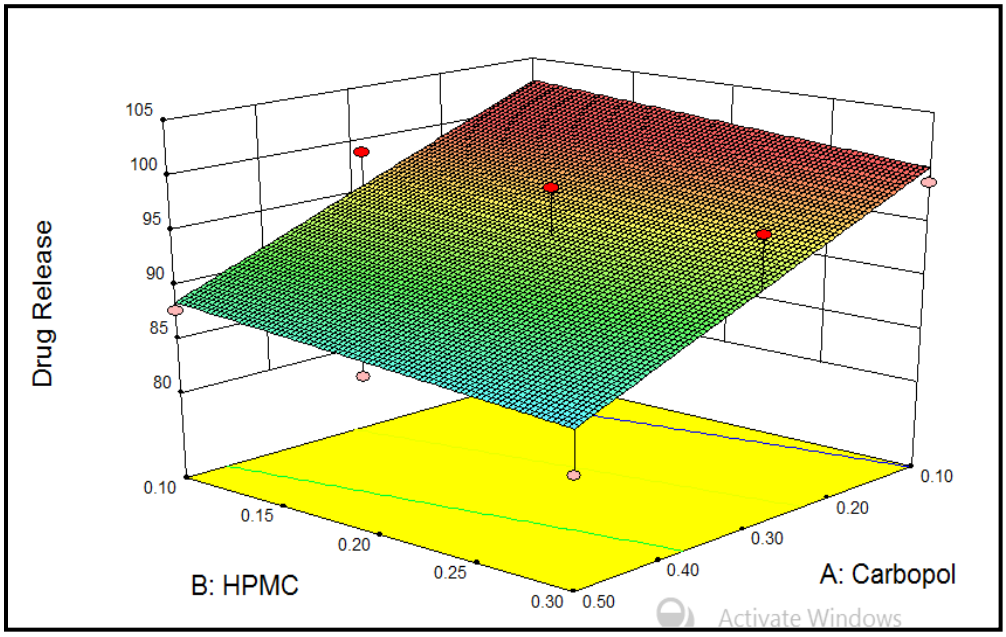

Figure.8: Surface response plot showing effect of Carbopol 940 and HPMC K100 on drug release.

***In-vitro* permeation study**

The In-vitro permeation study of formulation F4 is shown in (Table-5).In-vitro permeation study was performed for the optimized batch using goat nasal mucosa. The percent drug permeated after 8 hr was found to be 85.81% from nasal gel formulation. This suggests that this formulation can act as controlled release for treating migraine (Figure 9).

Table 5: *In-vitro* permeation study for optimized batch F4.

Sr no.	Time (hrs)	Drug permeation rate (mg/cm^2/hr) (± S.D.)	% Cumulative drug permeation (±S.D.)
1	0.5	0.682±0.008461	16.043±0.045092
2	1.00	0.504±0.005686	32.237±0.015676
3	2.00	0.437±0.001801	41.206±0.015112
4	3.00	0.350±0.004349	49.797±0.013797
5	4.00	0.312±0.001044	54.014±0.008249
6	5.00	0.273±0.012423	59.44±0.011294
7	6.00	0.227±0.005012	64.383±0.01106
8	7.00	0.228±0.005865	75.354±0.015772
9	8.00	0.227±0.006543	85.817±0.015383

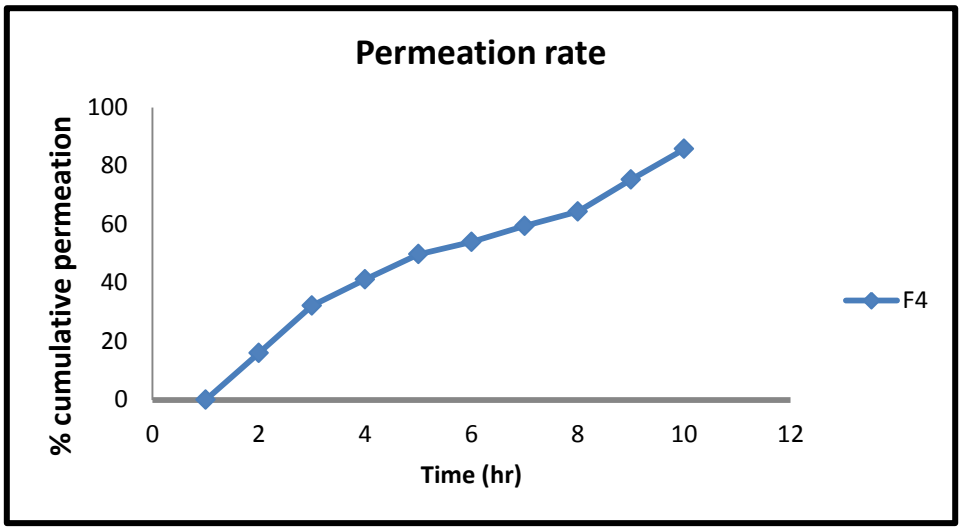

Figure 9: Permeation rate of optimized formulation.

Release Kinetics [24]

In the present study, the drug release was analyzed by PCP Disso version v3 software to study the kinetics of drug release mechanism. The results showed that the factorial design batches followed korsmeyerpeppas model kinetics. The R^2 value of korsmeyerpeppasmodel was found close to one and the drug release kinetics for best fitting optimized batch was calculated and it is shown in (Table-6).The drug release was occurred by non-fickian diffusion mechanism as reflected by its n value 0.684 ($n \leq 0.5$)

Table-6: Drug release kinetics for optimized batch

Sr. No.	Model Fitting	R^2 Value	n	K
1.	Korsmeyer- peppas	0.9929	0.6849	6.2698

Stability Test

Formulation at room temperature was found to be stable upto 6 month. There is no change in drug content, visual appearance, pH and viscosity. All formulation stored at elevated temperature found to be unchanged.

CONCLUSION

Permeation study suggested that such formulation can be a alternative route to the conventional dosgeform of zolmitriptan and it can also be part replacement therapy to the conventional oral administration of zolmitriptan.

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