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Original Article

FORMULATION AND EVALUATION OF ION-SENSITIVE IN-SITU NASAL GEL OF ZOLMITRIPTAN

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ABSTRACT

In situ gel system is novel drug delivery system in which there is the transition of sol to gel on external stimuli like change in pH, temperature or change in ion concentration(sol-gel transition). In the present study various formulations were prepared by using gellan gum as gelling agent and HPMC K100 as controlled or sustained release polymer. All the formulations were evaluated for various parameters like pH, viscosity, drug content, gel strength, mucoadhesive strength and drug release. At minimum concentration of polymer lose their integrity and at maximum concentration stiff gel were formed. At optimized concentration of gelling agent and HPMC K100 showed in situ gelling with all parameter in range. *In vitro* release data revealed that the optimized formulation showed controlled and sustained drug release pattern. The optimized formulation also obeyed korsmer Peppas model equation and which showed the release exponent n value 0.765. Thus, the ex vivo higher bioavailability can be expected from the optimized formulation.

Keywords: In situ gelling, Nasal delivery, Migraine, Polymers, Sustained release, Higher bioavailability etc.

INTRODUCTION

Migraine headache is one of the most common human ailment and the most common complaints of patients evaluated by a neurologist. It is a specific neurological syndrome that has a wide variety of manifestations. In recent years, the Nasal route has received a great deal of attention as a convenient and reliable method for the systemic administration of drugs. The nasal cavity as a site for the systemic absorption of drugs has some advantages which include the relatively large surface area, porous endothelial basement, highly vascularised epithelial layer, high total blood flow/cm^3, avoiding the first pass metabolism and easy access. 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. It is an effective agent in the treatment of acute migraine attack with or without aura. However, oral bioavailability is poor with only 40 % of the dose reaching systemic circulation. This is likely due to extensive presystemic clearance on first pass. As migraine sufferers have markedly reduced functional ability, they would be benefited from acute treatment that helps them to resume their functional activities as quickly as possible.

In situ gel, or in vivo gel, environment sensitive gel, is a new dosage form which has been applied as nasal drug delivery recently. Compared with liquid nasal formulations, nasal in situ gels are instilled as low viscosity solutions into the nasal cavity and upon contact with the nasal mucosa, or nasal composition the polymer changes conformation producing a gel, so it cannot only prolong the contact time between the drug and the absorptive sites in the nasal cavity, but also release drug slowly and continuously. Hence, it is especially very useful for those drugs used chronically. The phase transition can be induced by the presence of cations as for gellan gum a shift in pH as for cellulose acetate phthalate, a shift in temperature as for the thermo gelling Poloxamer 407.

MATERIALS AND METHODS

Zolmitriptan was obtained as a gift sample from Emcure Pharmaceuticals pune India HPMC K100 From Colorcon Goa, Gellan Gum(kelcogel) From Signet Pharma, Benzalkonium chloride from Merck Pharma. All the ingredient used were of LR Grade.

Preparation of standard curve of zolmitriptan

Accurately weighed 10 mg of Zolmitriptan was dissolved in 100 ml of distilled water to get the stock solution of 100µg/ml. From this stock solution aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 ml were withdrawn and further diluted to 10 ml with distilled water to obtain concentrations range of 1 to 5 µl/ml. The absorbance of the solutions was measured at 283.5 nm by using UV-Vis spectrophotometer. A graph of concentration vs. Absorbance was plotted.(Table no.1)

Preparation of nasal formulations

Gellan gum solutions of various concentrations were prepared by adding the gum to deionised water and heating up to 90◦C continuing stirring remi- stirrer. After cooling to below 40◦C, HPMC K100, Zolmitriptan (25% w/v), mannitol (5%, w/v), and Benzalkonium chloride (0.01%, w/v) were added and mixed well. Three various kinds of Zolmitriptan in situ gels were prepared at the concentrations of Gellan gum which were 0.3%, 0.6%, 0.9% and with combination of HPMC K100 concentrations0.10%, 0.13% and 0.0.15% (w/v) respectively.(Table No.2)

Characterization and Evaluation of of Nasal in Situ Gel

Appearance

The developed formulations were inspected visually for clarity, colour in sol and gel form against white background and for particulate matter any if present.

Table 1: Absorbance of Standard solutions at 283.5 nm

S. No.	Conc.(µg/ml)	Absorbance
1	10	0.202
2	20	0.367
3	30	0.567
4	40	0.756
5	50	0.933

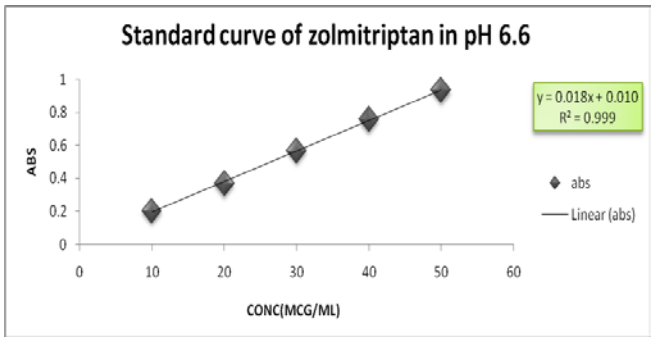

Fig. 1: Calibration curve of Zolmitriptan

Table 2: Composition of Nasal in situ gel formulation of Zolmitriptan

Ingredients (%w/v)	Formulation								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zolmitriptan	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Gellan gum	0.3	0.3	0.3	0.6	0.6	0.6	0.9	0.9	0.9
Hpmc(100)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BK CL%(v\v)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mannitol%	5	5	5	5	5	5	5	5	5
Dis. water	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s

pH of gel

Each formulated batch pH was measured using pH meter which was previously calibrated using standard buffers of pH 4 & pH 7.26

Determination of mucoadhesive strength

The muco adhesive strength of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosal was fixed on each of two glass slides using thread. 50mg of gel was placed on the first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in an inverted position to the underside of the same pan. Both the slides with mucosal section were fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2 min to ensure intimate contact between them. Then the weight was kept rising in second pan until slides get detached from each other. The muco adhesive force expressed as the detachment stress in dynes/cm2 was determined from the minimal weight that detached the mucosal tissue from the surface of each formulation.

$$\text{Mucoadhesive strength (dynes/cm2)} = \frac{\text{mg}}{\text{A}}$$

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

Table 3: Appearance pH gelling capacity and drug content estimation of various formulations

Form. code	Appearance	Gelling capacity	pH	Drug content
Z-1	Transparent Solution	---	5.7 ± 0.265	96.05
Z-2	Transparent and less viscous solution	+	6.1 ± 0.153	97.03
Z-3	Transparent Solution	+++	6.2 ± 0.265	97.32
Z-4	Transparent Solution	+++	6.5 ± 0.200	96.02
Z-5	Transparent and less viscous solution	+	5.8 ± 0.210	96.00
Z-6	Transparent and viscous solution	+++	6.1 ± 0.251	95.00
Z-7	Transparent Solution	+++	6.2 ± 0.265	96.05
Z-8	Transparent and viscous solution	++++	5.7 ± 0.252	96.10
Z-9	Transparent Solution	+++	6.2 ± 0.132	95.02

- No gelation, **+** Gelation occurred in few min and remained for few h, ++ Gelation immediate, remained for few h +++ Gelation immediate, and for extended period, ++++ Very stiff gel.

Rheological studies

Viscosity of the prepared formulations was measured by using Brookfield Cone and Plate Viscometer. The suitable spindle was lowered perpendicularly into the fixed volume of gel which was to be measured. The spindle was rotated at varying speeds and the suitable speed was selected. The temperature was maintained at 25°C and then the viscosity was measured as the system was allowed to cool gradually (fig no.6 and 7).

Gelation study

It is the proportion of cations at which the liquid phase makes a transition to gel. Gelation point was considered as the proportion where formulations would not flow when test tubes were tilted to 90° angle, as the cations ions concentration was gradually increased.

***In vitro* release studies**

In vitro release study of the formulated in situ gel was carried out in two chamber diffusion cells through dialysis membrane-70 with the molecular weight cut off 1200-1400 KDa. Diffusion of diameter 1.5 cm and 20 ml capacity consisted of an upper cylindrical compartment open from above and diffusion membrane at its base. To prepare artificial membrane, pieces of dialysis membrane were soaked in PBS pH6.6 for hrs before mounting on diffusion cell. Dialysis experimental and the content of the receiver compartment

were stirred using magnetic stirrer. The position of the donor compartment was adjusted so that dialysis membrane just touches the diffusion medium. An aliquot of 3 ml was withdrawn from receiver compartment initially after 15 and 30 min and then 1 hr interval and replaced with the same amount of fresh medium. Aliquots withdrawn were suitably diluted and analysed using UV spectrophotometer at 283.50 nm for drug. *In vitro* drug release was carried out for 8 hrs. membrane was in a two chamber cells. In situ gels of gellan gum with drug were placed in the donor compartment. 16 ml of PBS 6.6 was placed in the receptor compartment. The temperature of the receiver compartment was maintained at the 37◦0 C ±1.00C during experimental and the content of the receiver compartment was stirred using magnetic stirrer. The position of the donor compartment was adjusted so that dialysis membrane just touches the diffusion medium. An aliquot of 3 ml was withdrawn from the receiver compartment initially after 15 and 30 min and then 1 hr interval and replaced with the same amount of fresh medium. Aliquots withdrawn were suitably diluted and analysed using UV spectrophotometer at 283.50 nm for drug. *In vitro* drug release was carried out for 8 hrs.

In vitro permeation study

Fresh nasal tissues were carefully removed from the nasal cavity of goat obtained from the local slaughterhouse. Tissue sample was inserted in Franz diffusion cell displaying a permeation area of 1.76 cm2 7 ml of 6.6 pH phosphate buffer saline was added to the acceptor chamber and agitated with magnetic stirrer at 370 C. After pre incubation time of 20 min, pure drug solution and formulation equivalent to 0.25%w/v of Zolmitriptan was placed in the donor chamber. From the acceptor compartment 0.2 ml sample aliquots were withdrawn at predetermined time interval up to 6 hrs replacing the sample volume with 6.6 pH PBS after each sampling, filtered and analysed by UV spectrometer at 283.50 nm

RESULT AND DISCUSSION

Determination of λ max of zolmitriptan

A stock solution of 100µg/ml of Zolmitriptan was prepared by dissolving 10 mg in 100 ml of deionised distilled water. The resulting solution was scanned between 200 nm to 800 nm using double beam UV-visible spectrophotometer (2700-Shimadzu, India.).(fig no.1)

Fourier transforms infrared spectral studies

Fourier transform infrared (FTIR) spectra were taken on FTIR (model-200,Thermo Electron, shimadzu) to investigate any possible chemical reactions between the drug and the polymer. FTIR spectra of the pure drug and physical mixture of drug with polymers were obtained. Pure drug (Zolmitriptan) and polymers were subjected to FTIR studies alone and in Combinations.(1:1) The mixtures were placed in the sample holder and were analyzed by FTIR to study the interference of polymers with the drug.(fig no 2 and 3).

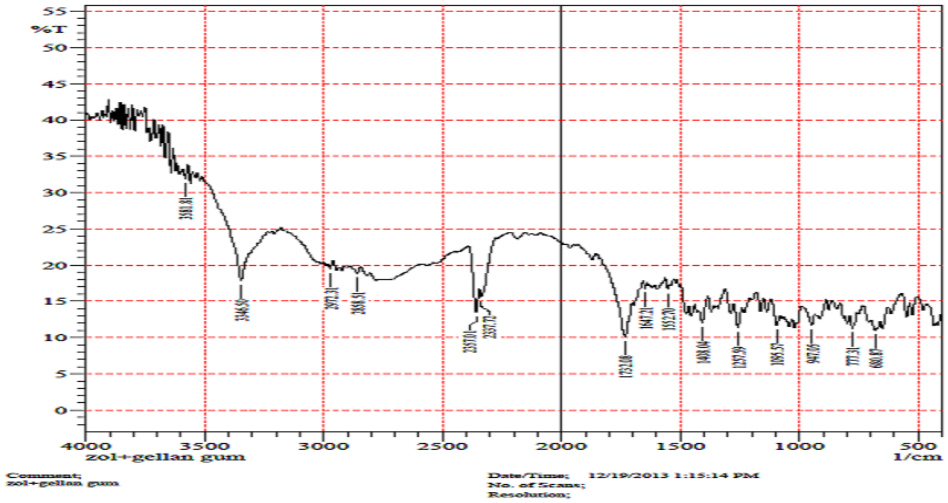

Fig. 2: FTIR spectra of physical mixture of drug and Polymers

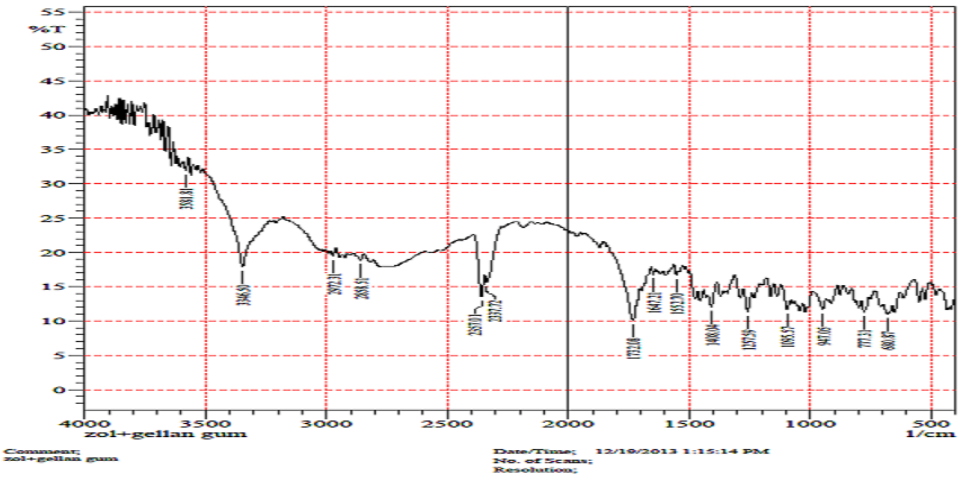

Fig. 3: FTIR spectra of plane of drug Zolmitriptan

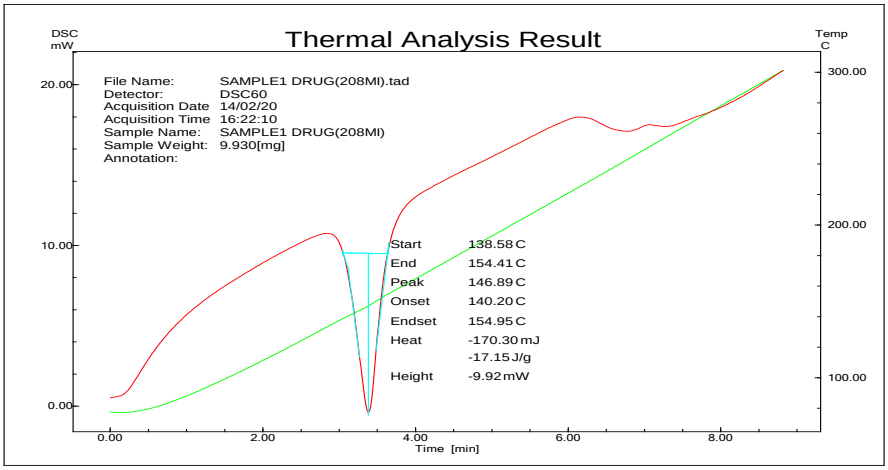

Fig. 4: DSC graph for drug sample

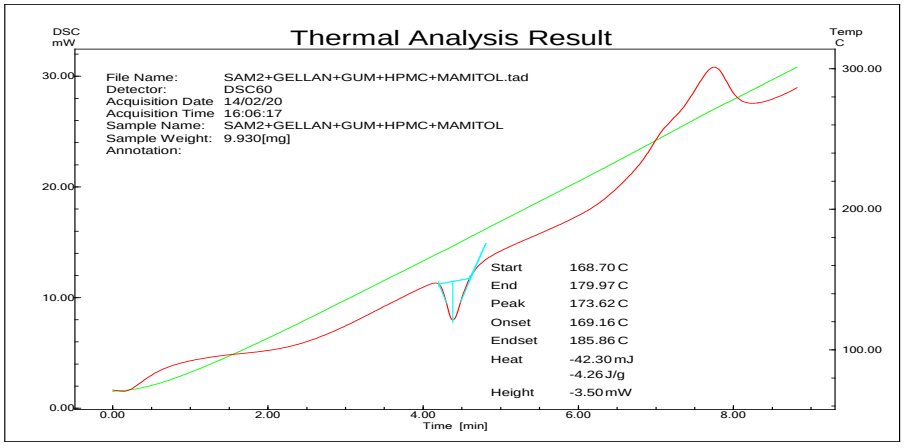

Fig. 5: DSC graph for mixture of polymer and drug

Table 4: Viscosity

Viscosity at (sol)	Viscosity at (Gel)
10	30
12	35
15	38
20	60
22	68
25	75
28	86
30	92
32	105

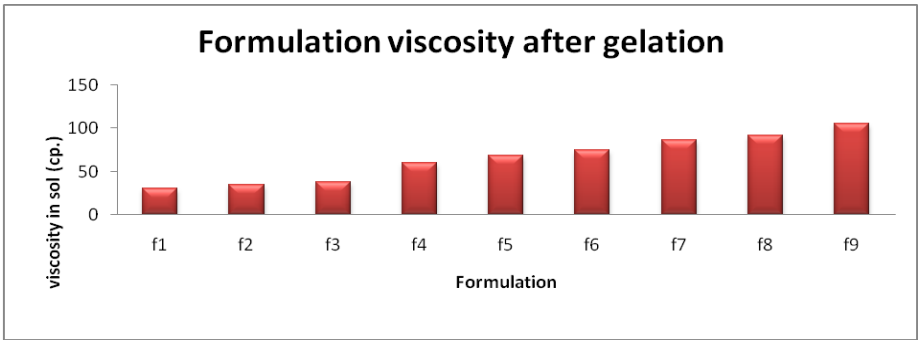

Fig. 6: Rheological studies of formulation after gelation

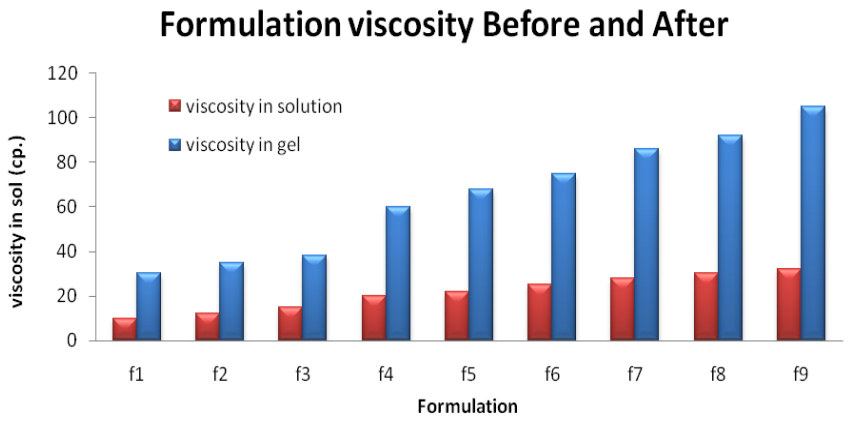

Fig. 7: Rheological Comparative Studies of Formulation

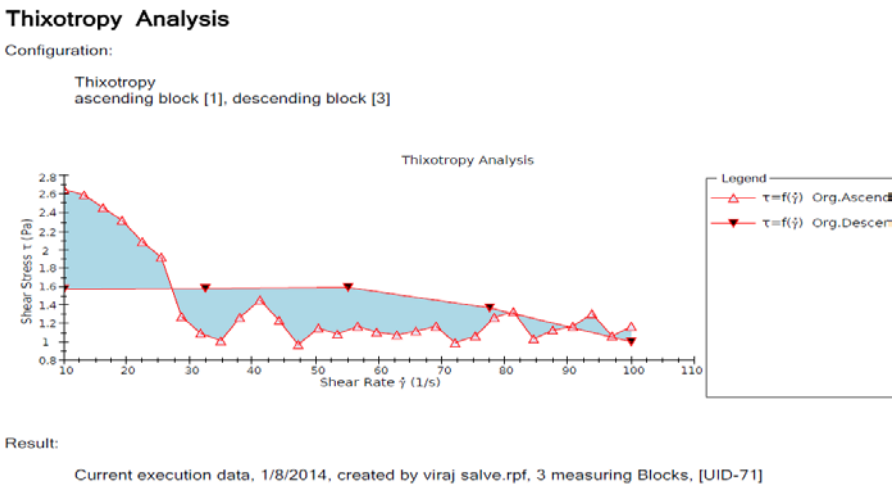

Fig. 8: Thixotropy analysis study of optimised formulation (F3)

Table 5: Evaluation parameter of formulation

Formulation code	pH (mean ± S. D)	Drug content (mean ± S. D)	Mucoadhesive strength (Dynes/cm2)	Gel strength (seconds) (mean ± S. D)	Viscosity (cps)
F1	4.7	96.05	2032 +0.32	18+0.01	30
F2	4.9	97.03	2205+0.22	20+0.5	35
F3	5.1	97.32	2403+0.45	21+0.6	38
F4	5.2	96.02	2606+0.15	25+0.2	60
F5	5.4	96.00	2801+0.10	28+0.3	68
F6	5.5	95.00	2907+0.20	32+0.2	75
F7	6.1	96.00	3005+0.25	38+0.3	86
F8	6.2	96.05	3215+0.35	45+.01	92
F9	6.0	95.02	3510+0.45	48+.05	105

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to evaluate the thermal behaviour of pure drug and physical mixture of the drug and excipients using a DSC-60 (Shimadzu Corporation, Japan.). Samples were weighed and sealed in standard aluminium pans and then scanned over a temperature range from 50 to 300C at a heating rate of 10.00 0C / min.(fig no 4 and 5).

Viscosity of optimised formulation

Solution viscosity and gel viscosity were studied on Brookfield Cone and Plate viscometer using various spindle likeC-25 and C-75. and there viscosities were determind. before and after viscosity are given in below(table no 4).

Drug content estimation

Acceptable range for all the formulations. % drug content was in the range 95- 97 % indicating uniform distribution of drug. (Table no.5) shows the result of percentage drug for all the formulations. The drug content of optimised formulation was found to be in 97.32%.

***In vitro* release studies**

In vitro release study of the formulated in situ gel was carried out in two chamber diffusion cells through dialysis membrane-70 with molecular weight cut off 1200-1400 KDa. Diffusion of diameter 1.5 cm and 16 ml capacity consisted of upper cylindrical compartment open from above and diffusion membrane at its base. To prepare artificial membrane, pieces of dialysis membrane were soaked in

PBS pH 6.6 for hrs before mounting on diffusion cell. Dialysis membrane was in a two chamber cells. In situ gels of Zolmitriptan loaded with drug were placed in the donor compartment. 16 ml of PBS 6.6 was placed in the receptor compartment. The temperature of receiver compartment was maintained at the 37°C ±1.0°C during experimental and the content of the receiver compartment was stirred using magnetic stirrer. The position of the donor compartment was adjusted so that dialysis membrane just touches the diffusion medium. An aliquot of 3 ml was withdrawn from receiver compartment initially after 0 and 30 min and then 1 hr interval and replaced with same amount of fresh medium. Aliquots withdrawn were suitably diluted and analysed using UV spectrophotometer at 283.50 nm for drug. *In vitro* drug release was carried out for 8 hrs.

In vitro release studies

Diffusion studies were carried out using Franz diffusion cell, F3 showed the sustained drug release. F7 showed drug release 79.76% at 8 hrs. Concentration of HPMC increases leads to decrease the drug release. Gellan gum concentration affect on drug release.(Tab no.6)

Table 6: percent drug release from respective formulation

Time in Min	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
30	14.00	15.02	12.60	12.65	13.60	14.06	13.08	12.56	13.06
60	20.97	24.06	25.07	28.09	22.02	22.86	24.09	22.09	20.26
120	30.00	32.07	35.04	35.06	30.12	35.06	33.06	31.00	28.08
180	45.05	46.00	45.07	48.00	44.00	47.70	45.00	42.56	41.86
240	55.07	52.07	53.07	56.02	52.86	50.76	56.00	49.06	48.05
300	68.08	62.83	64.05	62.02	60.72	61.02	62.00	60.05	58.08
360	77.08	70.65	74.02	71.00	70.00	68.02	69.00	68.05	66.08
420	82.06	81.05	84.95	82.05	83.26	74.01	73.05	72.10	73.08
480	97.02	95.00	92.02	86.00	85.36	82.06	80.06	78.86	75.08

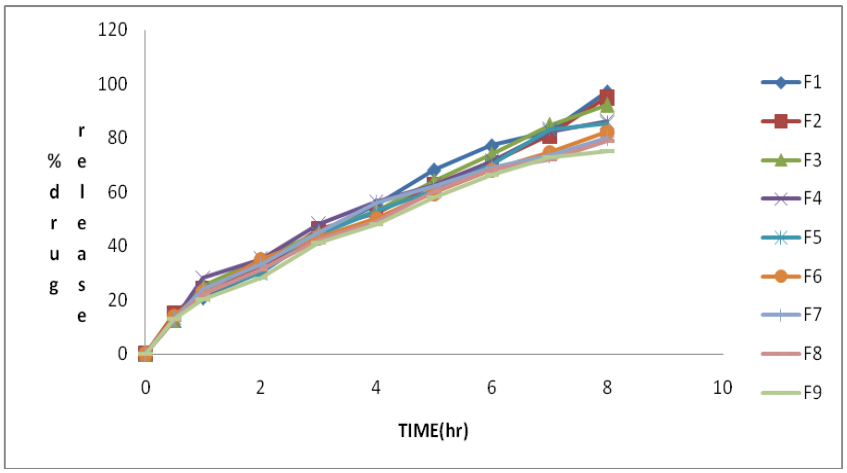

Fig. 9: *In- vitro* drug release

In vitro permeation study

Fresh nasal tissues were carefully removed from the nasal cavity of goat obtained from the local slaughterhouse. Tissue sample was inserted in Franz diffusion cell displaying a permeation area of 1.76 cm2 7 ml of 6.6 pH phosphate buffer saline was added to the acceptor chamber and agitated with magnetic stirrer at 37°0 C. After pre incubation time of 20 min, pure drug solution and formulation equivalent to 2.5%w/v of Zolmitriptan was placed in the donor chamber. From the acceptor compartment 0.2 ml sample aliquots were withdrawn at predetermined time interval up to 8 hrs replacing the sample volume with 6.6 pH PBS after each sampling, filtered and analysed by UV spectrometer at 283.50 nm.

Drug release mechanism

The drug release mechanism was studied by using DD solver software in which various kinetic modelling were studied and it was found that the optimised formulation obey korsmer Pappas model with exponent n value of0.745. which proves that the formulation non anomalous or non fickan release model.(fig no. 2)

Table 7: permeation drug release of selected batch with compare to drug solution

Time min	F3	Drug solution
0	0	0
30	12.60	19.56
60	25.07	25.26
120	35.04	30.85
180	45.07	38.01
240	53.07	40.72
300	64.06	51.86
360	74.02	66.96
420	84.95	85.94
480	92.02	97.94

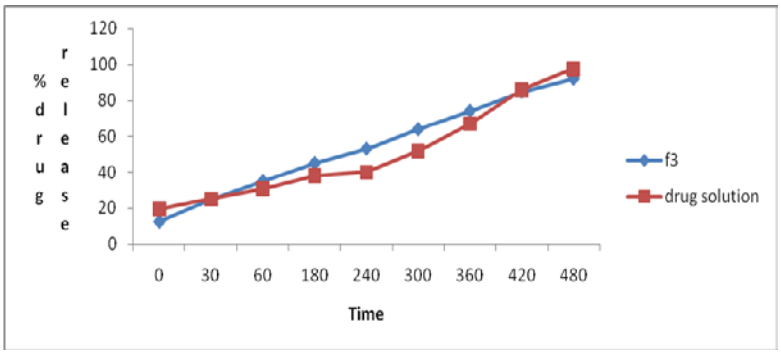

Fig. 10: Ex vivo permeation of selected batch (F3) compare to drug solution

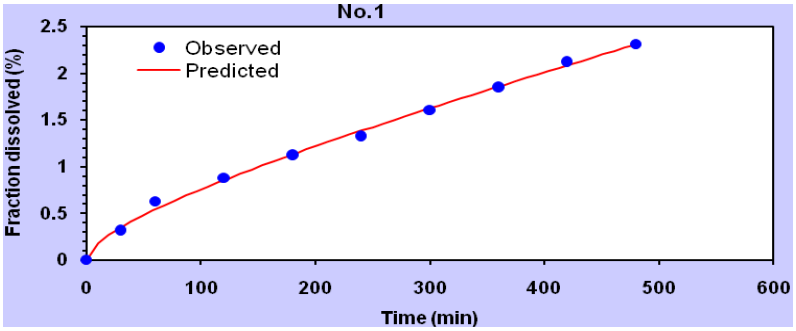

Fig. 11: predicted and observed value of optimised formulation (F3)

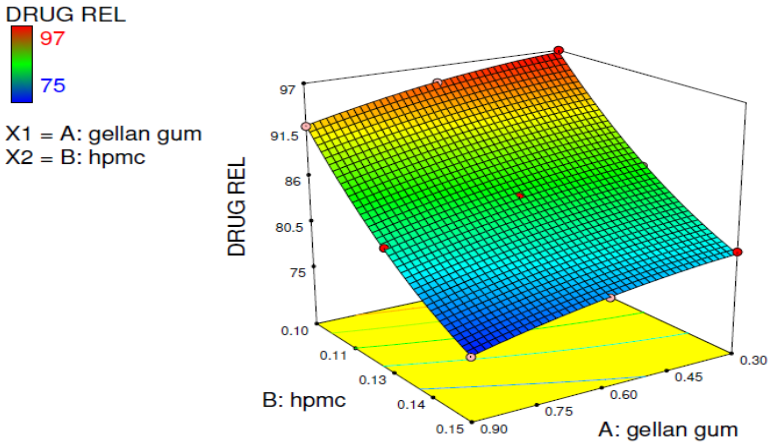

Fig. 12: Anova result for drug release

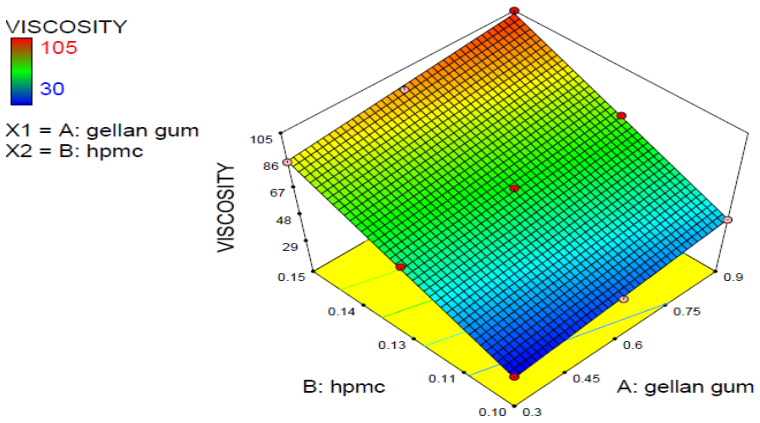

Fig. 4: Anova result for viscosity

Stastistical data

Anova study was used to analysis stastiacal data of research and all parameter were within range and found to be significant (Drug release &Viscosity). fig no.11&12.

RESULTS

In situ nasal gel formulation Zolmitriptan with 0.3 % of Gellan Gum and 0.13% HPMC K100 is a novel promising nasal drug delivery system for a antimigraine drug Zolmitriptan, which would enhance nasal residence time owing to increased viscosity and mucoadhesive characteristics; furthermore it also exhibited a good residence time due to which there will be controlled and sustained release of drug.

CONCLUSION

In conclusion, this study demonstrated that the use of in situ gelling vehicle of gellan gum incorporating mucoadhesive polymer and HPMC K100 could effective and safe. Which eventualy improve the nasal residence time and absorption of Zolomitriptan. Finally from this study it was concluded that use of controlled release polymer(HPMC k 100) ion-sensitive(gellan gum) nasal in situ gel drug delivery was very novel beneficial and effective in In- situ nasal drug delivery system of Zolmitriptan drug.

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