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FORMULATION AND EVALUATION OF THERMOREVERSIBLE IN SITU NASAL GEL OF ZOLMITRIPTAN

Shubhangi C. Daswadkar[1]* and Jyoti D. Gaikwad[2]

[1]Department of Pharmaceutical Chemistry, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune – 411044 India.
[2]Department of Pharmaceutical Quality Assurance Techniques, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-411044 India.

***Corresponding Author: Shubhangi C. Daswadkar**
Department of Pharmaceutical Chemistry, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune – 411044 India.

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ABSTRACT
The aim of present research work was to prolong the residence time at site of absorption by formulating Zolmitriptan thermo reversible mucoadhesive *in-situ* intranasal gel (TMISG) by using cold technique which forms a gel at the nasal mucosal temperature and contains a natural mucoadhesive polymer obtained from *Trigonellafoenum-graecum L.* (FSM) and characterized by gelation time, gelation temperature, pH, rheological studies, mucoadhesive strength, ex-vivo drug release, in-vivo pharmacokinetic study in rats. The study procedure involved extraction of mucoadhesive agent (FSM) which increases the residence time, will enhance the bioavailability of the ZMT. The mucoadhesive strength and viscosity of FSM polymer was found to be higher in comparison to the synthetic polymers, ie HPMC and carbopol 934 which are conventionally used for a similar purpose. The thermoreversible *in-situ* gelation was achieved by the use of poloxamer 407. The optimized nasal *in situ* gel shows gelation temperature 32˚C, and drug release 70.4% after 8 hrs.

KEYWORDS: ZMT, TMISG, CNS, *Fenugreek* seed mucilage, *in situ* gel.

INTRODUICTION
Oral administration is the most commonly used route of administration for systemic effect. But in case of some drugs the systemic effect is not desirable due to lesser oral bioavailability. Nasal drug delivery is used for treatment of various diseases like rhinitis, migraine, cold, nasal congestion and CNS disorders. In recent years it has been proved that many drugs achieved better systemic bioavailability through nasal route rather than oral route.[1,2] Nasal route achieve faster and higher level of drug absorption, due to large surface area, high total blood flow, the avoidance of first pass metabolism and readily accessibility. The appealing advantage of nasal drug delivery is the possibility of targeting central nervous system (CNS) by bypassing blood brain barrier (BBB). The drugs absorbed nasally via olfactory epithelium are reported to enter in olfactory neurons and supporting cells and subsequently into the brain, which reduced not only the systemic toxicity of centrally acting drugs but also enhanced therapeutic efficacy.[3,4] *In situ* gelling system is a new approach has been applied recently in nasal drug delivery. As compared to conventional nasal liquid formulations in situ gels are introduced as low viscosity solutions which upon contact with nasal mucosa, the thermoreversible polymer (poloxamer 407) changes conformation producing a gel. Which increases residence time between drug and absorptive site in the nasal cavity, helps to prolongs drug release.[5] In order to improve bioavailability of drug it is necessary to improve residence time of drug in absorptive site. Most effective method to lengthen nasal residence time is use of mucoadhesive polymer. Nowadays polymers from natural origins are mostly used in pharmaceutical formulations, because of their various benefits such as environment friendly, safety, natural origin, free from side effect, low cost, renewable sources, patient compliance and easily availability.[6,7,8] In the present research work the natural polymer from *Trigonellafoenum-graecum L.* (FSM) is used. Mucoadhesive strength and viscosity of FSM polymer was found to be higher in comparison to the synthetic polymers.[9]

Migraine is a physiological condition, in which a person suffers from tremendous headache. In the present investigation, Zolmitriptan was selected as a model drug in the development of nasal in situ gel. The ZMT is a serotonin (5-HT1) agonist used for the treatment of migraine with or without aura. The absolute oral bioavailability is about 40 to 50%. The half-life of Zolmitriptan is 2.5 to 3 hrs and it undergoes hepatic metabolism.[10,11] Because of these, it was selected as a model drug for the nasal in situ gel. In situ gel was prepared by using FSM as mucoadhesive polymer, and

poloxamer 407 as thermoreversible polymer by cold method. FSM used as mucoadhesive polymer beneficial for the treatment or management of migraine for long period of time by sustaining the drug release & having anti-inflammatory action. It enhances nasal residence time by increased viscosity of gel in the nose which may be advantageous to protect the drug from draining increase bioavailability of drug.

MATERIALS AND METHOD

Materials

Zolmitriptan was obtained as gift sample from Glenmark Pharma Goa.

Fenugreek seeds were purchased from local market, Poloxamer-407,Glycerine, Benzalkonium Chloride were purchased from Reasearch fine lab Mumbai. All the solvents and chemicals used in this study were of analytical-reagent grade.

Methods

Preformulation Studies

Characterization of API

Organoleptic Properties

The organoleptic properties such as appearance, color, odor of drug were evaluated by visual examination, shown in Table.

Determination of Melting Point

Open capillary tube method was used for determining the melting point of the drug. By taking small amount of drug in the capillary tube was closed at one end and inserted into melting point apparatus and reading was recorded. This was performed in triplicate and mean was record.

Solubility Study

The solubility of the drug was performed by using different solvents distilled water, methanol, ethanol, acetonitrile. This practice was performed thrice and a solvent for analysis of drug was finalized.

FT-IR Spectroscopic Determination

The drug was identified by the use of Infrared absorption spectroscopy. The Required quantity of drug was taken and mixed with potassium bromide and packed into the compact disk and spectrum was recorded.

Estimation of Maximum Absorbance Wavelength of Drug

Working standard solution of drugs was prepared for estimation of maximum absorbance wavelength. UV spectrum of drug solutions was recorded in the wavelength range 400-200nm and maximum absorbance was observed at the particular wavelength as shown in "Table 1". This wavelength was further used as a detection wavelength (λmax) for analysis of drug.

Table 1: Concentration of drugs used for determination of λ_{max}

Sr. No.	Name of Drug	Stock solution Concentration	Working std. Concentration	λ_{max} (nm)
1	ZMT	10mg of drug dissolved in 100mL distilled water (100µg/mL)	0.1mL-1ml of stock solution diluted to 10mL with distilled water.	283

The appropriate aliquots of standard stock solution 0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9, 1mL were transferred to a series of 10mL volumetric flask in order to obtain working standard solution of concentration of 1,2,3,4,5,6,,7,8,9,10µg/mL and absorbance was measured at 283nm. The standard calibration curve plotted of absorbance Vs Concentrations.

Isolation and Characterization of Natural Mucoadhesive Polymer

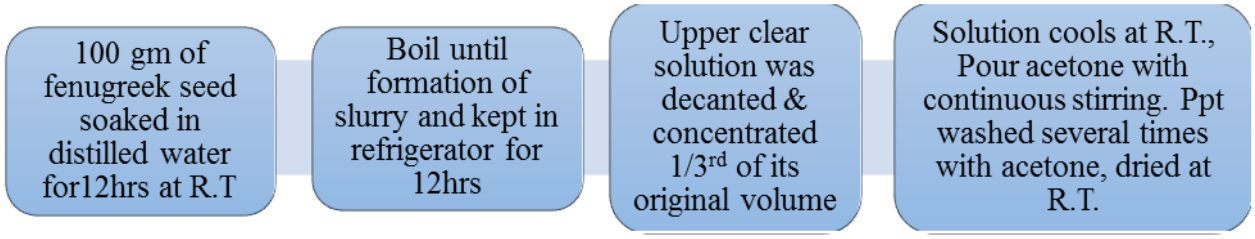


Fig 1: Extraction procedure for fenugreek seed mucilage.

Characterization of Polymer

Organoleptic properties

The Organoleptic properties like physical state, color, odor etc. of the polymers were reported with help of the descriptive terminology.

Percentage Yield

% Yield = Amt. of powder obtained* 100 / Amt. of seeds (gm.)

Solubility studies

Solubility studies carried out in different solvents like ethanol, methanol, and water as per following procedure.

1gm powder dissolve in 100mL respected solvent and stirr for 30min. The solubility measure by stirring the mixture at R.T. and elevated temperature. Gum solution then centrifuged at 6000 rpm for 30 min. then supernatant transfer to Petri dish and oven dried at 105^{0}C for 24hrs. The solubility calculated by weight difference and expressed in dry basis.[12]

Solubility % =C1×100/C2
Where, C1= Supernatant concentration (mg)
C2= Initial concentration (mg)

Swelling Index
1 gm of fenugreek gum placed in 25mL measuring cylinder, add 25 mL distilled water. Shake the mixture every 10 min. for 1 hr and allowed to stand for 24 hrs. At R.T. finally measure the volume occupied by the gum.[13]

$$\text{SWEELING INDEX: -} \quad \frac{\text{Final volume- Initial volume}}{\text{Final volume}} \times 100$$

Viscosity
1gm powder of FSM suspend in 75mL distilled water for 5hrs. Add distilled water up to 100 mL to produce concentration 1% w/v. then the mixture homogenize by mechanical stirrer for 2 hrs. Determine viscosity by Brookfield viscometer at 37°C.[12-13]

Moisture Content:
Sample of FSM weighted into clean dry Petri plate, dried in oven at 105° C for 6-8 hrs. Cool and Weighted.[12]

$$\text{\% MOISTURE:} \quad \frac{\text{W1- W2}}{\text{W1-W0}} \times 100$$

Where,
W0:- weight of petri plate
W1:- weight of petri plate + sample (gm)
W2:- weight of petri plate + dried sample (gm)

Total Ash Value
Weigh about 2 gm of powdered drug into crucible and heat above the flame. Heat till vapours almost cease to be evolved, then lower the dish and heat more strongly until all the carbon is burnt off. Cool in a desiccator and weigh ash and calculate the percentage of total ash.[13,14]

Total ash value = 100(z-x) /y

pH Measurement
Prepare 1% w/v solution of FSM in water, pH of solution determined by digital pH Meter.[13]

FT-IR
The polymer was identified by FT-IR same as described previously. The characteristic IR peaks observed in the spectrum of FSM are reported.

Drug Polymer Compatibility Study
A compatibility study was carried out with potential formulation excipients and drug. API and excipients were mixed in specific quantity and placed in sealed vials for 4 weeks at 40°C ± 2°/75%±5% RH and 25°C±2°/75% ±5% RH. The samples were examined physically for any change in following parameters.

- Discoloration
- Odor or gas formation
- Crystal growth.

The primary physical observations for any change in appearances were recorded.

Table 2: Drug polymer ratio

Sr. no	Ingredients	Ratio	Primary Observation
1	API alone	1	White Powder
2	API + FSM	1:1	Light Brown
3	API + POLOXAMER 407	1:1	White Powder

Further the samples were examined for drug and polymer compatibility study. IR study of pure drug and drug + polymer was performed as prescribed previously and reoprted the findings.

Optimization of Formulation
Different formulation batches were prepared with different concentration of polymers as prescribed in "Table 3"by using cold method.[15]

For preparation of gel, accurately weighed Poloxamer 407 was solubilized in cold water with continuous stirring using magnetic stirrer and stored overnight at 4°C until clear solution obtained and the FSM was separately soaked in warm water overnight for proper solubilisation and swelling. Then FSM solution, excipients and drug were slowly added to the Poloxamer 407 solution with continuous agitation to form formulation. The formed mixtures were stored overnight at 4°C until clear solution obtained.

Table 3: Composition of Mucoadhesive Nasal Insitu Gel.

Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
ZMT	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
FSM	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Poloxamer 407	20	20	20	20	20	20	20	20	20	20
Glycerin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Benzalkonium Chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Distilled Water(qs)	10	10	10	10	10	10	10	10	10	10

Evaluation of Mucoadhesive Nasal Insitue Gel

Clarity

The clarity of formulation was determined by visual inspection under black and white background by using clarity test apparatus and it was graded as follows.[16]
Turbid +, Clear ++, Very clear (glassy):+++.

pH

1 ml quantity of formulation was transferred into 10ml volumetric flask and the pH of formulation was determined by using pH meter which was calibrated using solution of pH 5 and 7 before the measurement.[17]

Viscosity

The viscosity of formulation before and after gelation was measured by using Brookfield Viscometer coupled with S-18 spindle. The prepared formulation was placed in small sample adapter, spindle was lowered perpendicularly into the formulation at 10 rpm and the temperature was maintained at 37±0.5˚C.[18]

Gelation Temperature

It was determined by using method described by Miller and Donovan technique. A 2 mL aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly and left to equilibrate for 5 min at each new setting. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer moves upon tilting through 90˚C.[19]

Mucoadhesive Strength

The mucoadhesive strength of each formulation was determined by measuring the force required to detach the formulation from goat nasal mucosal tissue by using a modified chemical balance.[15,20]

Fig 2: Modified Mucoadhesive Strength Apparatus.

A section of nasal mucosa was cut from the goat's nasal cavity and mucosal side was instantly fixed into each glass vial using a rubber band. The vials with nasal mucosa were stored at 37°C for 5 minutes. Then next vial with a section of mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Fixed amount of sample of each formulation were placed onto the nasal mucosa of first vial. Then the height of second vial was adjusted so that mucosal surfaces of both vials come in intimate contact. Two minutes contact time was given to ensure intimate contact between tissues and the sample. Then weight was increased in the pan until vials got detached.

In vitro Drug Release

In vitro drug release of ZMT nasal in-situ gel was determined using Franz- diffusion cell. The cellophane membrane was mounted between the donar and receptor compartments. The receptor compartment was filled with phosphate buffer (pH 6.4) at 37℃. The solution was stirred at 100 rpm. The ZMT nasal mucoadhesive in- situ gel was placed on cellophane membrane and the compartments were clamped together. One ml of the sample was withdrawn at predetermined time interval for 8 hrs, from receptor compartments and immediately replaced using phosphate buffer (pH 6.4). After filtering through 0.45µm filter and appropriate dilution, the sample were analyzed for drug content at 283nm.[22,23]The % drug release of formulation is shown in "Fig 8".

RESULT AND DISCUSSION

Preformulation Study

Identification of Drug

The sample of ZMT procured for study was identified and estimated for its purity. The sample was identified by organoleptic property, solubility study, UV spectrum, IR spectrum, and observations were compliance with IP, as shown in "Table 4".

Table 4: Observed and reported organoleptic properties.

Sr. No.	Parameter	Observed properties
1.	Appearance	White Amorphous powder
2.	Color	White powder
3.	Odor	Odorless

Melting point

Melting point was determined by using melting point apparatus and result is summarized in "Table 5"indicated purity of the drug.

Table 5: Melting point of pure ZMT.

Reported melting point	Observed melting point
137-141ºC	139-141ºC

Solubility

The ZMT was found to be soluble in Methanol, Ethanol, Acetonitrile, and readily soluble in water which was in accordance with IP.

FT-IR of ZMT

Infrared spectroscopy for pure drug was performed for identification of compound. The IR spectrum is shown in "Fig 3"and the interpretation of IR spectra is shown in "Table 6".

Table 6: IR data of ZMT.

Interpretation	Peak at wave number (cm^{-1})
CH-Aromatic stretching	3351.37
N-H Streching	3050
C=O Streching	1709
C-O Streching of ester group	1233.50

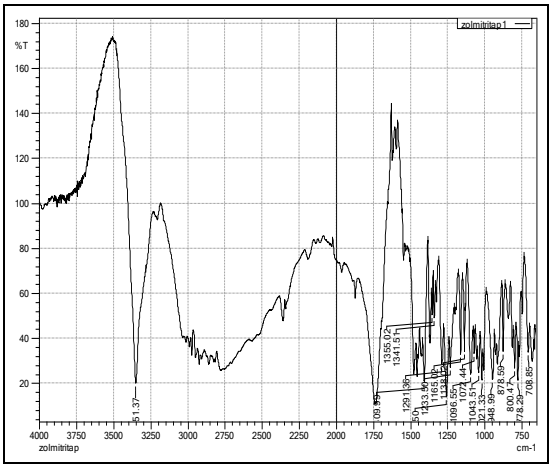


Fig 3: IR spectra of ZMT.

Estimation of Maximum Absorbance Wavelength of Drug

Calibration curve of ZMT in Distilled water

As UV spectroscopy was used for determination of λ_{max} and this wavelength was used for further studies. ZMT showed excellent response at 283 nm when it was scan from 400 to 200 nm in a UV spectrophotometer. So, 283 nm wavelength was selected for analysis of ZMT. The UV spectrum is shown in "Fig 4".

Table 7: Calibration Data of ZMT at λ max 283nm.

Concentration (µg/ml)	Absorbance
1	0.045
2	0.059
3	0.074
4	0.109
5	0.133
6	0.166
7	0.179
8	0.204
9	0.230
10	0.253

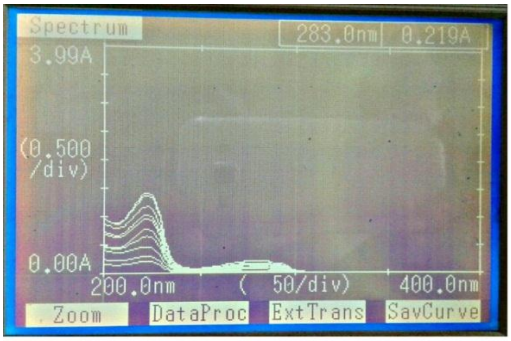


Fig 4 : UV spectra of Zolmitriptan.

The standard solution of ZMT was prepared in distilled water. The regression coefficient was found to be 0.994 and slope 0.024. The result indicated that there was a linear relationship between concentration and absorbance. As shown in "Fig 5".

Table 8:Linear regression data for the calibration plot of ZMT (n=6).

Linearity range (µg/mL)	1-10
Regression equation	y = 0.024x -0.013
Correlation coefficient (R^2)	0.994
Slope ± *SD*	0.024±0.0016
Intercept ± *SD*	0.013±0.0017
Standard error of slope	0.0006
Standard error of intercept	0.0007

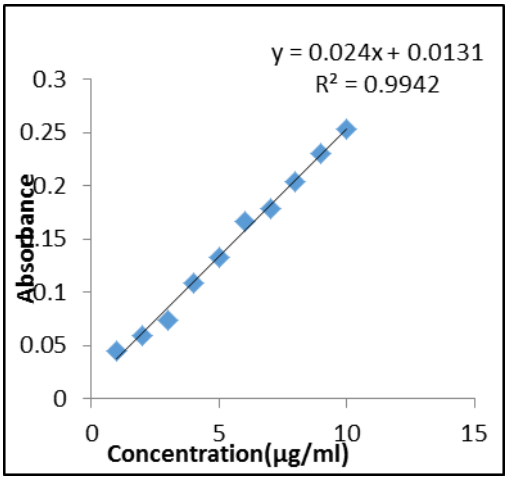


Fig 5: Calibration curve of ZMT.

CHARACTERIZATION OF NATURAL MUCOADHESIVE POLYMER

Organoleptic Properties

The fenugreek seed mucilage was tested for organoleptic properties and observations are given below in "Table 9".

Table 9: Observed organoleptic properties.

Sr. No.	Parameter	Observed properties
1	Appearances	Solid
2	Color	Light brown
3	Odor	Odorless
4	Taste	Tasteless
5	Percentage Yield	10.20%
6	Solubility (Water)	50%
7	Swelling Index	86.66%
8	Viscosity	39cps
9	Moisture Content	3%
10	Total Ash Content	1.67%

FT-IR of FSM

Table 10: IR Data of FSM.

Interpretation	Peak at wave number (cm^{-1})
-OH	3512-3154
-CH Streching	2920
C-O-C Ether Linkage	1450-1400
-CO Streching	1018

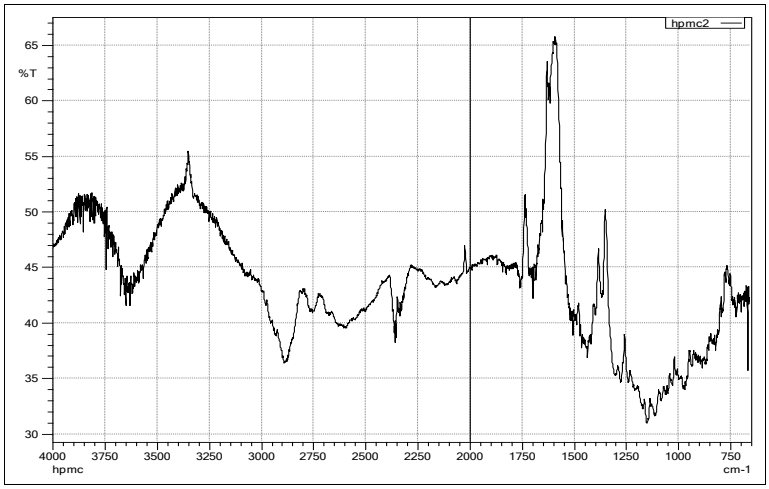


Fig 6: IR Spectra of FSM.

COMPATIBILITY STUDY OF DRUG AND POLYMERS

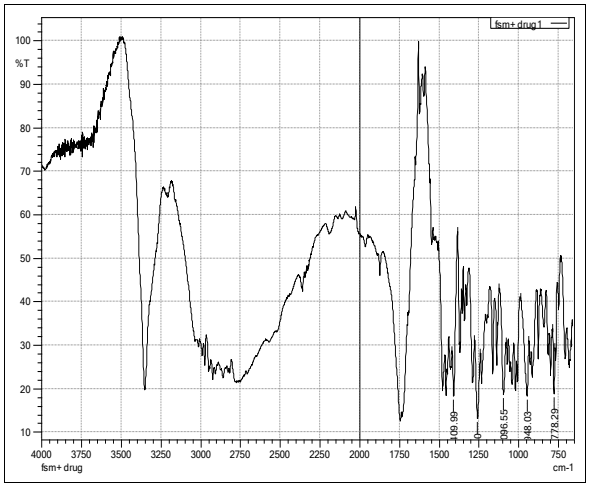
Table 11: Drug-polymer compatibility data.

API/ Polymer Ratio	Parameter	Initial	60°C ±2°C/ 75%±5% RH	25°C±2°/ 75% ±5% RH
API alone (1)	Appearance	White Powder	As initial	As initial
	Color Change	No	No	No
API + FSM (1:1)	Appearance	Light Brown	As initial	As initial
	Color Change	No	No	No
API + Poloxamer 407 (1:1)	Appearance	White Powder	As initial	As initial
	Color Change	No	No	No

a) FT-IR of drug and FSM

Table 12: IR data of ZMT & FSM.

Interpretation	Peak at wave number (cm^{-1})
C-O Streching of ester group	1233
-OH	3512-3154
-CH Streching	2920
Ether Linkage	1450-1400
C=O Streching	1750
N-H Streching	3050



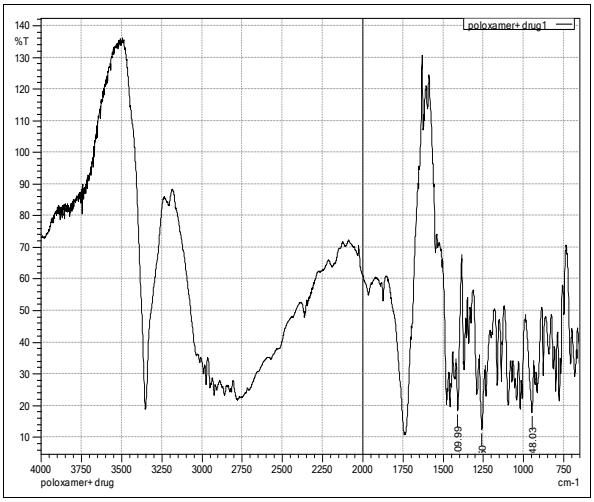


Fig 7(a,b): IR spectra of ZMT & Polymers.

From the spectral analysis it was found that FTIR spectrum of ZMT with Polymers Poloxamer 407, FSM showed all characteristics peaks in combination with no significant changes as shown in **Figure 7.5 (a-b).** Therefore, it could indicate that there was no incompatibility between drug and polymers.

b) FT-IR of drug and Poloxamer 407

Table 13: IR data of ZMT & poloxamer407.

Interpretation	Peak at wave number (cm^{-1})
C=O Streching	1750
N-H Streching	3050
O-H Streching	3496-3481
C-O Streching in C-O-C groups	1233.50

Optimization of Formulation Batch

Table 14: Trial Batches of Formulation

TRIAL BATCHES	POLOXAMER 407 %W/V	FSM %W/V	GELATION TEMP(℃)	GELATION TIME
F1	20	0.1	38	< 1
F2	20	0.2	40	< 1
F3	20	0.3	37	< 1
F4	20	0.4	35	< 1
F5	20	0.5	34	< 1
F6	20	0.6	32	< 1
F7	20	0.7	30	< 1
F8	20	0.8	30	< 1
F9	20	0.9	29	< 1
F10	20	1	28	< 1

From all the trial batches F6 was found to be optimized batch, which showed the gelation temperature 32℃ and gelation time < 1.

Evaluation of Mucoadhesive Nasal Insitue Gel

Clarity

The clarity of formulation was determined by visual inspection under black and white background. The prepared formulation was found to be clear (++).

pH

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. pH of formulation was found to be 6.3±0.1 that is between physiological range of pH (within 5.70-6.78) of nasal mucosa.

Viscosity

Viscosity measurement of the formulation at 4ºC and 32ºC temperature showed that there was increase in viscosity with increase in temperature. This indicated that the formulation of temperature induced gel structure of poloxamer. In addition to this FSM 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.

Gelation Temperature

The physiological range of the nasal mucosal temperature lies between 32-34ºC. Poloxamer 407 undergo thermal gelation or sol-gel transition at a temperature of about 25-37 ºC. In presence study after addition of ZMT and FSM alter the gelation temperature of poloxamer solution. This result was obtained in F6 formulation containing 0.6% FSM.

Mucoadhesive Strength

Mucoadhesive strength was determined in term of detachment stress i.e. force required to detached the formulation from mucosal surface. The stronger the mucoadhesive force is, the more it can prevent the gelled solution coming out of the nose.

In vitro drug release

In vitro drug release carried out of only optimized batch (F6) using cellophane membrane. The cumulative drug release from this nasal in situ gel containing ZMT was 70.40%. Release profile show in "Fig 8".

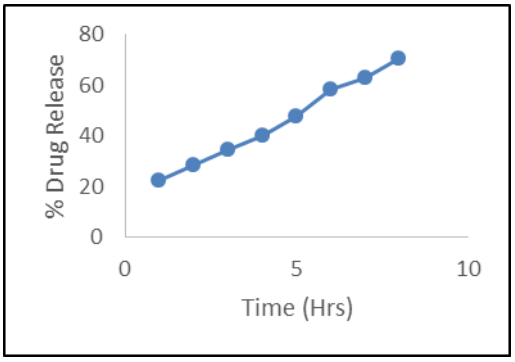


Fig 8: Graph of % Drug Release of F6.

Table 15: Evaluation of Optimized Batch of nasal *in- situ* gel.

Formulation	Clarity	pH	Viscosity At		Gelation Temperature	Mucoadhesive Strength (dyne/cm$^{2)}$	%Drug release
F6	++	6.30±0.1	4ºC 45.80±0.3	32ºC 163±0.85	32-33ºC	2730.89	70.40

CONCLUSION

Mucoadhesive Nasal in-situ gel can be a successful approach to treat the Migraine by providing rapid onset and efficacy. The nasal route has found to be useful in targeting drugs to the CNS. The poor bioavailability and therapeutic response exhibited by the conventional sprays and nasal drops due to rapid nasal elimination can be overcome by the use of in- situ gelling system that are instilled as drops into nasal cavity and undergoes sol to gel transition . The high permeability, high vasculature, and low enzymatic environment of nasal cavity as ell suitable for systemic delivery of drug molecule via nose.

Fenugreek seed mucilage is the most useful polymer for mucosal drug delivery system. It has showed favorable biological properties, low toxicity and high mucoadhesive properties and an important capacity to enhance drug permeability and absorption at mucosal site.

The optimized batch was proven to be a promising nasal drug delivery system for an antimigraine drug ZMT, which would enhance nasal residence time owing to increased viscosity and mucoadhesive strength. The ex-vivo and in-vivo results indicated sustained release for a period of more than 5 hrs. The cumulative drug release from this nasal in situ gel containing ZMT was found to be 70.40%, which would help to achieve longer action of ZMT.

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