Research Article

Formulation and Development of Temperature Sensitive In Situ Gelling System of Desmopressin Acetate for Nasal Drug Delivery

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ABSTRACT

The present work was aimed for the formulation development of stable temperature sensitive thermoreversible in situ gel of desmopressin acetate by cold method using different ratio of Pluronic F127 and Pluronic F68 as thermoreversible polymer. The 3^2 factorial design was employed using concentration of Pluronic F127 and concentration of Pluronic F68 as independent variables and Viscosity and Mucoadhesive strength were selected as dependent variables. The optimized batch was selected using Design Expert software employing overlay plot with desirability approach. The temperature sensitive thermoreversible in situ gel formulation was evaluated for inflection point, gelation temperature, pH, viscosity, % drug content, gel strength, mucoadhessive strength, nasal toxicity study, ex vivo drug diffusion study, stability study and in-vivo study. The composition of optimized formulation consisted of 1 mg of Desmopressin acetate, 1988.67 mg of Pluronic F127, 305.33 mg of Pluronic F68, 1 mg of Benzylkonium chloride and 10 ml of Purified water showing inflection point (32.5˚C), gelation temperature (33 ˚C), viscosity (789.7 cps), mucoadhesive strength (2759.14 dynes/cm^2) and % drug content (99.8 %). Temperature sensitive thermoreversible in situ gel increase the nasal residence time and the drug get released in a sustained and controlled manner thus increased the bioavailability of desmopressin acetate.

Keywords: Desmopressin acetate, thermoreversible In situ gel, Mucoadhesive strength, Pluronic F127, Gelation.

INTRODUCTION

Intranasal drug delivery is one of the focused delivery options for brain targeting, as the brain and nose compartments are connected to each other via the olfactory route and peripheral circulation. One of the major disadvantages to deliver drug through nasal route is the mucocilliary clearance. To avoid it, there are so many strategies and one of these is the use of the mucoadhesive polymer to increase the nasal residence time.

In-situ gel is the formulations, intended to be applied as solutions, sol, or suspension, which undergoes gelation after administration due to the physicochemical environment.[1] It is a polymeric solution which can be administrated as liquid, but upon exposure to the physiological environments, undergoes a phase transition to semisolid gel. The formation of gel depends on the factors like temperature, pH change, presence of ions, etc. The formulations in the liquid state are easy to be administered and on gelation after administration, increases the residence time and the drug gets released in a sustained and controlled manner, and thus the *in-situ* gel incorporates the benefits of both the state.

Desmopressin is taken by oral or parental routes in the treatment of nocturnal enuresis and central diabetes insipidus. It has a low oral bioavailability. IV, IM, or SC administration is an alternative. The intranasal route may be a viable alternative for self-administration. The problem related with nasal delivery of desmopressin is the lower retention time of solution in nasal cavity resulting in poor bioavailability and less transfer of drug directly to the brain through the olfactory pathway. Hence, a desmopressin formulation which may increase residence time in the nasal cavity and increase absorption of the drug would be more beneficial.

To the best of our knowledge, no information is available in the literature on the improvement of desmopressin bioavailability using temperature sensitive thermoreversible in situ gel. The present research was aimed to explore thermoreversible in situ gel formulation development using 3^2 factorial design for bioavailability improvement. The present work described the formulation development of temperature sensitive thermoreversible in situ gel of desmopressin acetate using different ratio of pluronic F127 and F68 on the basis of preliminary trials. The 3^2 factorial design was employed using concentration of Pluronic F127 and concentration of Pluronic F68 as independent variables and viscosity and mucoadhesive strength were selected as dependent variables.

Stability study for optimized thermoreversible in situ gel of desmopressin was performed as per ICH guidelines by keeping at room temperature room temperature (30°C ± 2°C/65% RH ± 5% RH) and in refrigerator temperature (2-8˚C) for 4 months. The optimized formulation was subjected to inflection point, gelation temperature, pH, viscosity, % drug content, gel strength, mucoadhessive strength, nasal toxicity study, ex vivo drug diffusion study, stability study and in-vivo study.

MATERIALS AND METHODS

Materials

Desmopressin acetate was gifted from Sun Pharmaceuticals, Halol for research. Pluronic F127 and Pluronic F68 were gifted from Sigma Aldrich. Water used in the preparation of formulations was purified water, whereas ultra-pure water, used in analyses, was obtained with a Milli-Q apparatus. All other chemicals and reagents used were of pharmaceutical grade or HPLC grade.

Methods

Preparation of Temperature sensitive thermoreversible gel[2]

Thermo reversible gels were prepared using cold method described by Schmolka.[2] This method involved slow addition of polymer pluronic F127 and pluronic F68 in the quantity of 1750, 1850, 1950 mg and 000, 300, 400 mg respectively in cold water with continuous agitation. The formed mixtures were stored overnight at 4°C. The liquid was left at 4˚C until a clear solution was obtained. To the above solution, 1 mg drug and benzaylkonium chloride 1 mg were added.

Preliminary Trials

A preliminary screening was performed to optimize the concentration of pluronic F127 and F68 for the stable formulation of temperature sensitive thermoreversible in situ gel. Viscosity and mucoadhesive strength were measured for the batches.

3^2 factorial design for optimization of formulation parameters of thermoreversible in situ gel of desmopressin acetate[3,4]

The concentration of pluronic F127 and pluronic F68 play important role in formation of temperature sensitive thermoreversible gel. The 3^2 factorial design was employed using concentration of pluronic F127 and pluronic F68 as independent variable X_1 and X_2. The viscosity (Y_1) and mucoadhesive strength (Y_2) were selected as dependent variables. Multiple regression analysis, contour plot and 3D response surface plot were used to study the main and interaction effects of the variables on the responses. The responses were measured for each trial and then either simple linear equation, or interactive equation or quadratic equation model was fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant term.

Microsoft EXCEL was used to identify non-significant terms. A coefficient is significant if ti > tcrit(v), where v denotes the degrees of freedom of residual variance. The refined model may be used for calculating the residuals or for drawing the contour plot.

Measurement of evaluation parameters of thermoreversible in situ gel formulations[5-8]

Inflection point: 2 ml of solution was taken in test tube and placed in the water bath whose temperature was increased gradually. The temperature at which drastic change in the viscosity was observed, measured using a calibrated thermometer, that point was considered as Inflection point.[5,7]

Gelation temperature: 10 ml of solution was taken in a test tube and placed in a water bath, whose temperature was increased gradually. The temperature at which a solid gel formed was measured using a calibrated thermometer. This temperature represented the gelation temperature.[6,7]

pH of the gel: The pH of each batch was measured using pH meter.

Content uniformity: % drug content was determined by dissolving formulation in distilled water and after suitable dilution estimation was carried out using HPLC method and peak due to desmopressin acetate was identified and area was calculated.

Viscosity Study: Viscosity of the prepared gel was studied using Brookfield viscometer.[7]

Gel strength: Gel was placed in the test tubes and gelled in a thermostat at 37°C. The apparatus for measuring gel strength (Weight) was then placed onto the gel. The time taken by the apparatus to sink to a depth of 5 cm through the prepared gel was measured for each formulation.[8]

Determination of mucoadhesive force: The mucoadhesive force of all the batches was determined by modified physical balance.[9,10]

***Ex-vivo* drug diffusion study:** The *ex-vivo* diffusion study was performed with freshly isolated sheep nasal mucosa procured from slaughter house using Franz diffusion cells.[11]

Nasal toxicity study: Freshly excised sheep nasal mucosa was collected from the slaughter house in PBS pH7.0. Three sheep nasal mucosa pieces with uniform thickness were mounted on franz diffusion cells. One mucosa was treated with 0.5 ml of PBS pH 7.0; the other mucosa with 0.5 ml of isopropyl alcohol; third mucosa was treated with thermoreversible insitu gel batch TO for 1 hr. After 1 hr the mucosa rinsed with PBS pH 7.0 and carried to the pathological laboratory in 10% formalin for the preparation pathological slides. The sheep nasal mucosa treated with PBS pH 7.0 and isopropyl alcohol were taken as positive and negative control respectively. The prepared pathological slides were studied under Olympus microscope for any sign of toxicity and the images were stored in the form of photographs.[11,12]

***In-vivo* study for antidiuretic activity:** In-vivo study was performed on adult Wistar albino male rats. A protocol for animal study was approved by Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Antidiuretic activity of desmopressin acetate was observed after nasal administration of thermoreversible insitu gel. Three groups, with six animals in each group were used in the study. Diuresis was induced

by hydrochlorothiazide at a dose of 10 mg/kg given orally.[12,13]

Group I – Control: Animals of control group were given no treatment.

Group II - Hydrochlorothiazide Solution (HCTZ): Animals of second group were given Hydrochlorothiazide Solution orally.

Group III - Nasal administration of thermoreversible in situ gel of DA batch TO: Third group of animals, were administered thermoreversible in situ gel of desmopressin acetate equivalent to 10 µg/kg BW.

Urine samples were collected every 2 h over a period of 24 h in metabolic cage, measured urine volume and effect of intra nasal dose of dry powder containing desmopressin acetate on serum sodium and potassium concentration.

Stability study: The stability of the formulation was assessed under different storage conditions as per ICH guidelines, namely, room temperature (30°C ± 2°C/65% RH ± 5% RH) and in refrigerator temperature (2- 8˚C).[14]

RESULTS AND DISCUSSION

Preliminary Trials

The formulation details of the preliminary trails for temperature sensitive thermoreversible in situ gel of desmopressin acetate were presented in Table 1 with the results.

A preliminary screening was performed to optimize the concentration of pluronic F127 and pluronic F68 as shown in Table 1. Batches (TA to TC) were formulated using pluronic F127 concentration (1750 mg) and pluronic F68 concentration (0, 30, 40 mg), Batches (TD to TF) were formulated using pluronic F127 concentration (1850 mg) and pluronic F68 concentration (0, 30, 40 mg) and Batches (TG to TI) were formulated using pluronic F127 concentration (1950 mg) and pluronic F68 concentration (0, 30, 40 mg). All these batches were evaluated for viscosity and mucoadhesive strength and on the basis of result of preliminary trials batch TF was found satisfactory.

Optimization of thermoreversible in situ gel of Desmopressin acetate using factorial design[3,4]

The 3^2 factorial design was employed using concentration of pluronic F127 and pluronic F68 as independent variable X_1 and X_2. The viscosity (Y_1) and mucoadhesive strength (Y_2) were selected as dependent variables. The coded and actual value of independent variable was shown in Table 2. The runs and responses for factorial batches were presented in Table 3.

Multiple regression analysis was carried out for the responses using MS Excel. The reduced model was obtained by using significant terms ($p > 0.05$ was considered non-significant and such termed were neglected) for all the responses. The contour and response surface plot were constructed using Design Expert version 12 (Demo version).

Viscosity (Y_1) cps

A full model equation for viscosity (Y_{FV}) was written as Equation 1

$$Y_{FV} = 530.555 + 55.333X_1 + 144.833X_2 + 5.666X_1^2 - 0.8333X_2^2 + 18.25X_1X_2 \ldots \text{(Equation 1)}$$

The reduced model equation for viscosity (Y_{FV}) was presented as Equation 2

$$Y_{RV} = 534.211 + 55.466X_1 + 144.75X_2 \ldots \text{(Equation 2)}$$

Mucoadhesive strength (Y_2)

A full model equation of mucoadhesive strength (Y_{FMS}) was written as Equation 3

$$Y_{FMS} = 2961.222 + 228.333X_1 + 671.166X_2 + 2.6666\ X_1^2 - 72.8333X_2^2 + 12.25X_1X_2 \ldots \text{(Equation 3)}$$

The reduced model equation for mucoadhesive strength (Y_{FMS}) was presented as Equation 4

$$Y_{RMS} = 2914.97 + 228.211X_1 + 617.19X_2 \ldots \text{(Equation 4)}$$

Table 1: Preliminary trials for temperature sensitive thermoreversible insitu gel

Batches	Conc. of pluronic F127 (mg)	Conc. of pluronic F68 (mg)	Desmopressin acetate (mg)	BKC (mg)	Purified Water (ml)	Viscosity (cps)	Mucoadhesive strength (dynes/cm^2)
TA	1750	000	1.0	1.0	10	309.3±0.184	1937.3±0.198
TB	1750	300	1.0	1.0	10	334.9±1.923	2021.9±1.673
TC	1750	400	1.0	1.0	10	348.3±2.763	2309.4±2.751
TD	1850	000	1.0	1.0	10	389.2±1.973	2609.1±1.972
TE	1850	300	1.0	1.0	10	401.5±1.082	2930.4±0.162
TF	**1850**	**400**	**1.0**	**1.0**	**10**	**499.9±0.124**	**3188.2±1.723**
TG	1950	000	1.0	1.0	10	597.2±3.098	3392.6±2.910
TH	1950	300	1.0	1.0	10	680.0±1.092	3791.0±0.297
TI	1950	400	1.0	1.0	10	750.2±2.091	3812.3±0.176

Table 2: Factors and levels of independent variables in 3^2 factorial design for formulation of thermoreversible insitu gel of desmopressin acetate

Independent variables	Level		
	Low (-1)	Medium (0)	High (+1)
Pluronic F127 concentration (X_1) (mg)	1650	1850	2050
Pluronic F68 concentration (X_2) (mg)	200	400	600

Table 3: Experimental runs and measured responses of 3^2 factorial design for thermoreversible insitu gel of desmopressin acetate

Batch	X_1	X_2	Viscosity (Y_1) (cps)	Mucoadhesive strength (Y_2) (dynes/cm^2)
T1	-1	-1	342.2	1994.42
T2	0	-1	397.8	2087.51
T3	1	-1	427.7	2576.27
T4	-1	0	498.1	2871.88
T5	0	0	518.4	2990.72
T6	1	0	587.5	3028.58
T7	-1	1	601.2	3196.4
T8	0	1	675.9	3661.83
T9	1	1	759.1	3827.12

Table 4: ANOVA of full model and reduced model

Response Y_1	Model	DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	145628.7	29125.74	108.025	0.9972	0.9944	0.9852
	RM	2	144174.7	72087.34	198.57	0.9925	0.9851	0.9801
Error	**FM**	3	808.8611	269.6204				
	RM	6	2178.16	363.02				
Response Y_2	Model	DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	3026829	605365.7	16.59	0.9823	0.9650	0.9069
	RM	2	3015473	1507736	74.84	0.9805	0.9614	0.9486
Error	**FM**	3	109465.5	36488.50				
	RM	6	120869.5	20144.92				

Contour Plots and Response Surface Plots

Two dimensional contour plots were constructed for all dependent variables i.e. viscosity (Y_1) and mucoadhesive strength for desmopressin acetate temperature sensitive thermoreversible in situ gel and shown in Figure 1, 2. Response surface plots are very helpful in learning about both the main and interaction effects of the independent variables.[13]

Viscosity (cps)

Figure 1 showed contour plot for viscosity at prefixed values. The contour plot was found to be linear, thus the relationship between independent variables for viscosity could be linear.

The response surface plot showed Increase in mucoadhesive strength with increase in the concentration of pluronic F127: concentration of pluronic F68.

Mucoadhesive strength (MS)

Figure 2 showed contour plot for mucoadhesive strength at prefixed values. The contour plot was found to be linear, thus the relationship between independent variables for mucoadhesive strength could be linear.

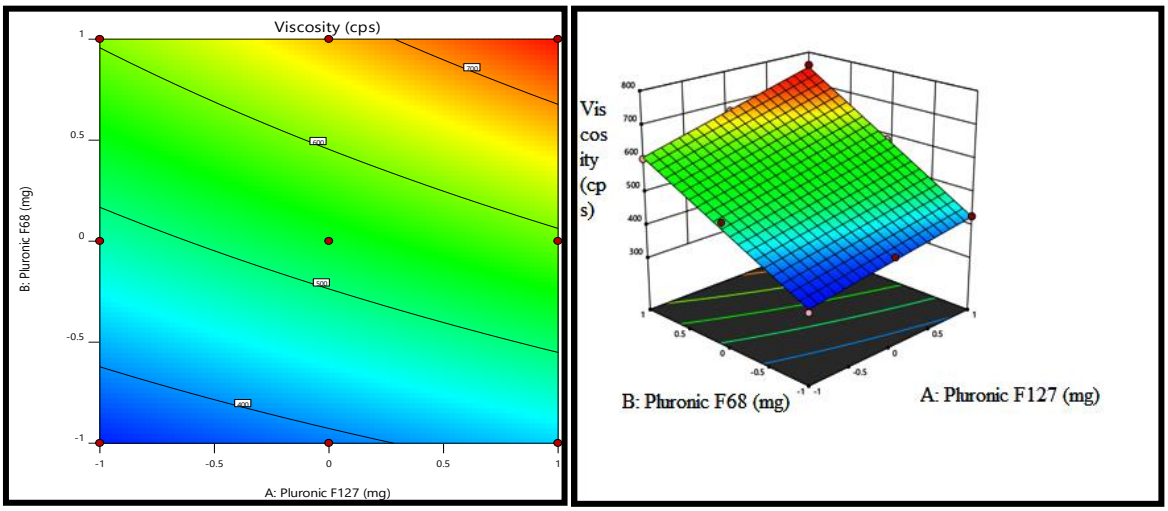

Figure 1: Contour plot and 3D surface plot for the effect on viscosity.

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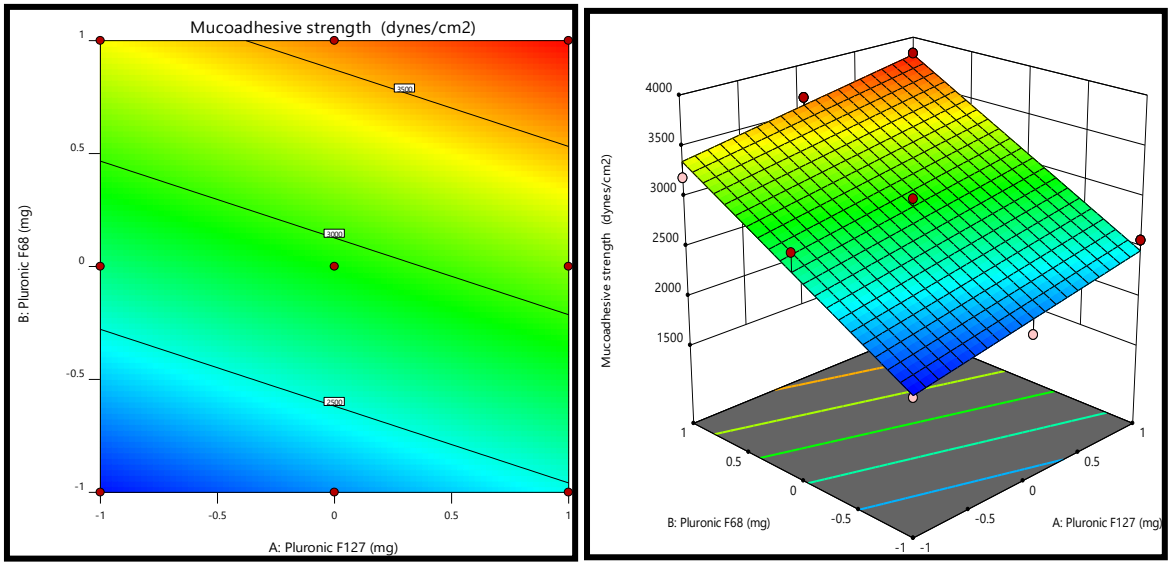


Figure 2: Contour plot and 3D surface plot for the effect on mucoadhesive strength

The response surface plot showed increase in mucoadhesive strength with increase in the concentration of pluronic F127: concentration of pluronic F68.

Optimization of thermoreversible insitu gel Formulation

Optimized formulation was selected by arbitrarily fixing the criteria of 342.2 – 759.1 cps of the viscosity and 1994.42 – 3827.12 dynes/cm^2 mucoadhesive strength for desmopressin temperature sensitive thermoreversible in situ gel. The recommended concentrations of the independent variables were calculated by the Design Expert software using overlay plot with desirability approach (Figure 3). The results gave one optimized solution with theoretical target profile characteristics which were shown in Table 5.

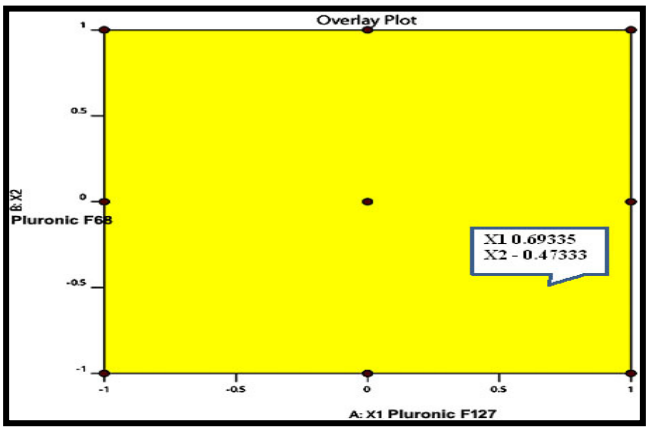


Figure 3: Overlay plot for optimization of thermoreversible in situ gel formulation.

Table 5: Solution proposed by Design Expert

Sol. Run	Conc. Of pluronic F127	Conc. of pluronic F68	Viscosity	Mucoadhesive strength
1	- 0.628	0.536	570.85	3131.302

Figure 3 showed the overlay plot obtained from Design Expert. The plot, yellow area indicated the area in which the optimized formulation can be formulated. In this yellow portion, the values of all variables i.e. viscosity and mucoadhesive strength for desmopressin acetate temperature sensitive thermoreversible in situ gel were selected. The point indicating toggle flag showed the coded value of X_1= 0.693335 and X_2= -0.473326 for optimized formulation. The actual value of X_1 and X_2 was shown in Table 6. The batch TO was prepared (n=3) and responses were measured. The predicted responses were calculated using the reduced model equation. There was no significant difference in the predicted and actual responses.

Evaluation parameters of thermoreversible in situ gel - Batch TO[5-8]

The inflection point, gelation temperature, pH, viscosity % drug content, gel strength and mucoadhessive strength of the various formulations are shown in table 7. It can be seen from the table 7, that there is definite relation of the inflection and gelation point with viscosity. The formulations which exhibited minimum inflection and gelation point had maximum viscosity at 37°C.

Table 6: Optimized formulation of desmopressin acetate thermoreversible in situ gel -Batch TO

Material used	Quantity (mg)	
Desmopressin acetate	1 mg	
Pluronic F127	1988.67 mg	
Pluronic F68	305.33 mg	
Benzylkonium chloride	1 mg	
Purified water	10 ml	
Response	**Predicted**	**Actual**
Viscosity (cps)	498.214	491.41±3.412
Mucoadhesive strength (dynes/cm^2)	2755.04	2759.14±5.613

Table 7: Inflection point, gelation temp., pH, viscosity, % drug content, gel strength, mucoadhessive strength

Batches	Inflection point (˚C)	Gelation Temp. (˚C)	pH	Viscosity (cps)		Drug content (%)	Gel strength (sec)	Mucoadhessive strength (dynes/cm^2)
				25 ˚C	37 ˚C			
T1	33	35	4.55	169	342.2	98.43	21.54	1994.42
T2	32.5	34	4.72	174	397.8	98.71	26.33	2087.51
T3	34	36.5	5.03	190	427.7	98.13	27.65	2576.27
T4	30	32.5	4.98	216	498.1	97.62	31.97	2871.88
T5	31	33	4.77	264	518.4	99.4	35.71	2990.72
T6	32.5	33.5	4.85	284	587.5	99.6	37.91	3028.58
T7	29	31.5	5.01	371	601.2	98.73	42.76	3196.4
T8	30	31	4.93	380	675.9	98.52	47.32	3661.83
T9	30.5	32.5	4.52	401	759.1	98.28	51.43	3827.12
TO	**32.5**	**33**	**4.90**	**491**	**789.7**	**99.8**	**40.1**	**2759.14**

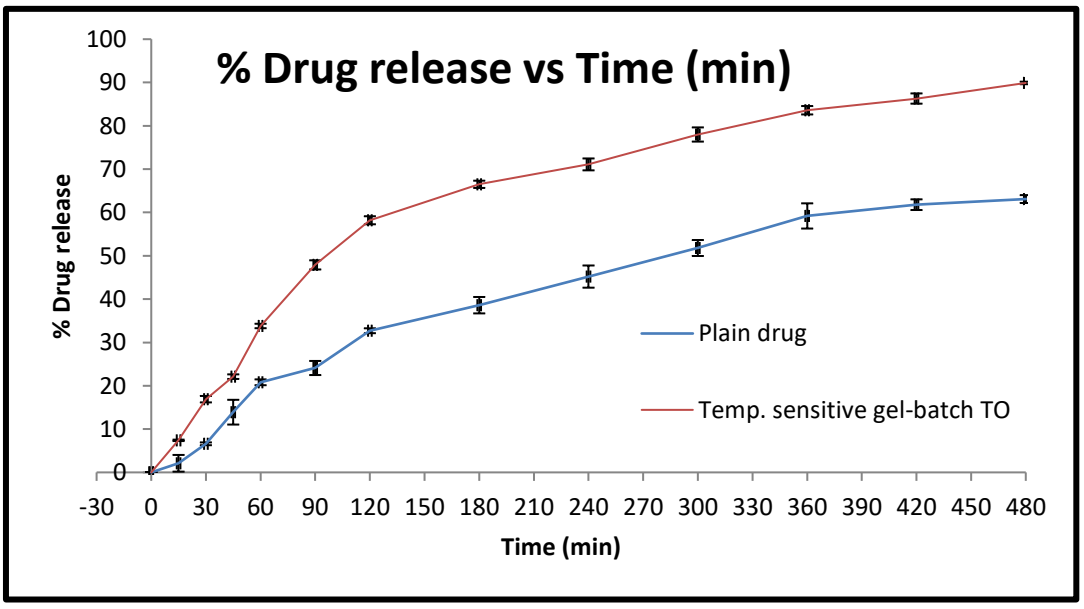


Figure 4: Comparative ex vivo drug diffusion of plain drug and thermoreversible in situ gel of desmopressin acetate

Ex-vivo drug diffusion study[11]

Comparative diffusion study was carried out for plain drug and thermoreversible in situ gel of desmopressin acetate using Franz diffusion cell shown in Table 8.

Table 8: % Drug Diffusion at different time point

Time (min)	% Drug Diffused (Mean±SEM)	
	Plain drug	Batch-TO
0	0.00	0.00
15	2.1±1.927	7.4±0.165
30	6.6±0.321	16.9±0.743
45	13.9±2.856	22.1±0.529
60	20.8±0.672	33.8±0.512
90	24.1±1.624	47.9±1.056
120	32.7±0.662	58.2±0.927
180	38.6±1.892	66.5±0.837
240	45.2±2.561	71.1±1.372
300	51.8±1.824	78.0±1.639
360	59.2±2.912	83.6±0.972
420	61.8±1.231	86.3±1.184
480	63.1±0.912	89.9±0.295

Nasal toxicity study

The mucosa treated with PBS pH 7.0 showed intact epithelial layer without any damage while mucosa treated with isopropyl alcohol (mucociliary toxic agent) showed complete destruction of epithelial layer and even deeper tissues. The nasal mucosa treated with test preparation batch TO showed reversible contraction of epithelial layer after 1 hr washing and no damage to the other parts of mucosa were observed.[11,12]

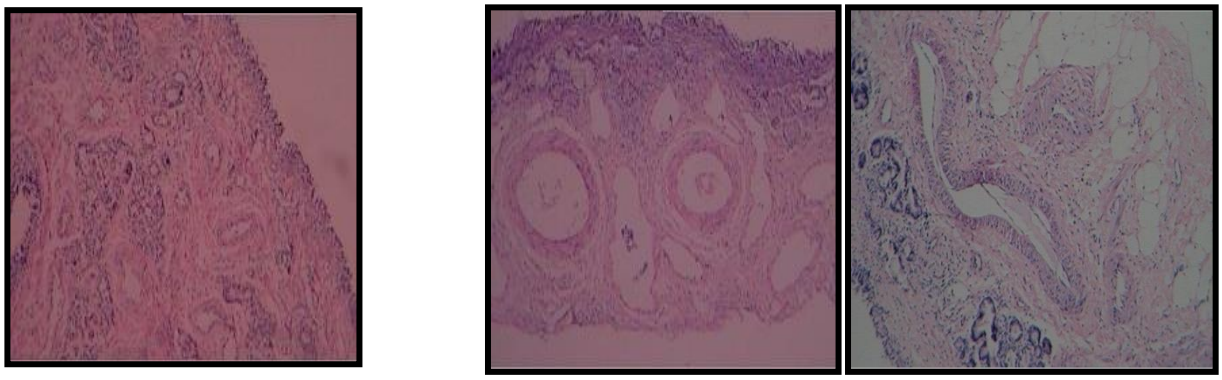
In-vivo study for antidiuretic activity

Effect of thermoreversible in situ gel of desmopressin acetate on urine volume, serum sodium and potassium concentration shown in Table 9.[12,13]

Results demonstrated that after intranasal administration of desmopressin acetate thermoreversible in situ gel urine volume was significantly decreased as compared to control and HCTZ.

Stability study

The stability of the formulation was assessed under different storage conditions as per ICH guidelines and the results obtained are as shown in Table 10.[14]



I II III

Figure 5: I) PBS pH 7.0, II) IPA, III) Thermoreversible in situ gel batch TO

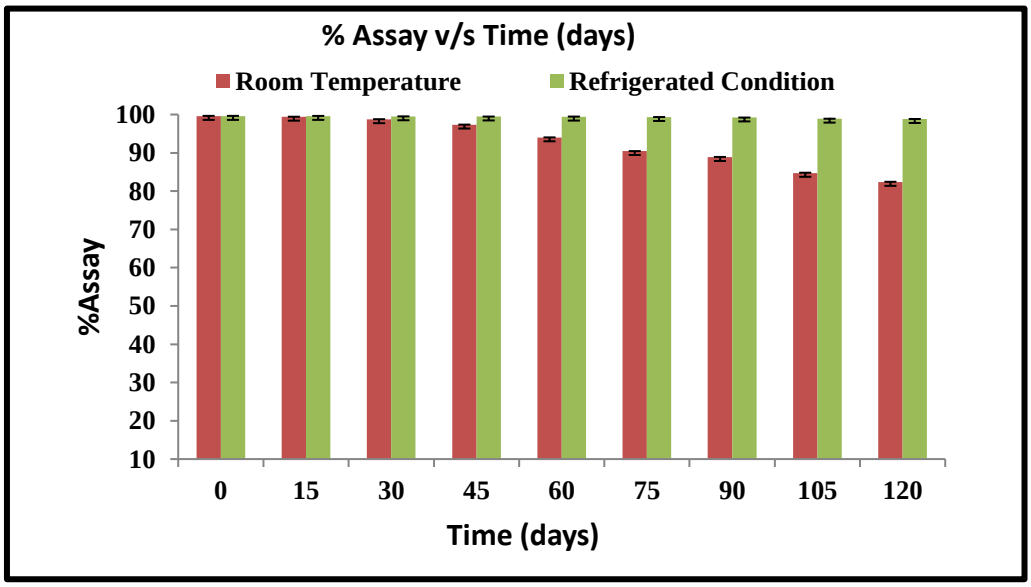


Figure 6: Stability profile of batch TO: % Assay vs. Time (Days)

Table 9: Effect of thermoreversible in situ gel on Urine Volume

Formulation	Urine volume (After 24hr.) ml	Na^+ mmol/lit (after 24 hr.)	K^+ mmol/lit (after 24 hr.)
Group I: Control	2.7 ± 0.28	137.3 ± 0.39	5.1 ± 0.43
Group II: HCTZ	3.4 ± 0.19	156.5 ± 0.14	5.7 ± 0.65
Group III: Temperature sensitive insitu gel batch TO + HCTZ	1.2 ± 0.38	151.9 ± 0.76	5.3 ± 0.62

Table 10: Effect of storage condition on thermoreversible in situ gel of desmopressin acetate

Sampling time (days)	% Drug retain (% Assay) (n=3)	
	Room condition (30 ± 2 °C with 60 ± 5 % RH) Batch TO	Refrigerated condition (2 - 8 °C) Batch TO
0	99.6± 0.053	99.6± 0.053
15	99.4± 0.029	99.6± 0.034
30	98.74± 0.042	99.5± 0.016
45	97.34± 0.027	99.45± 0.022
60	94.01± 0.018	99.42± 0.043
75	90.43± 0.014	99.32± 0.023
90	88.87± 0.054	99.20± 0.011
105	84.73± 0.064	98.89± 0.015
120	82.37± 0.057	98.80± 0.037

From the % assay determination of the thermoreversible in situ gel stored at different temperature conditions, it was found that there is significant decrease in the % assay of the drug when stored at room temperature so optimum storage condition for thermoreversible in situ gel is refrigerated condition (2-8 °C).

CONCLUSION

In-situ gel is a polymeric solution which can be administrated as liquid, but upon exposure to the physiological environments, undergoes a phase transition to semisolid gel. The formation of gel may depend on factors like temperature, pH change, presence of ions, etc. The present study was aimed to explore temperature sensitive thermoreversible in situ gel formulation development using 3^2 factorial design for bioavailability improvement of desmopressin acetate. The 3^2 factorial design was employed using concentration of pluronic F127 and pluronic F68 as independent variables and viscosity and mucoadhesive strength (MS) were selected as dependent variables. Multiple regression analysis, contour plot and response surface plot were used to study the main and interaction effects of the variables on the responses. The optimized batch was selected using Design Expert employing overlay plot with desirability approach. The optimized formulation was subjected to inflection point, gelation temperature, pH, viscosity, % drug content, gel strength, mucoadhessive strength, nasal toxicity study, ex vivo drug diffusion study, stability study and in-vivo study. The optimized formulation was found stable at refrigerated condition (2-8 °C).

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