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FORMULATION AND EVALUATION OF DRY NASAL MUCOADHESIVE POWDER OF DESMOPRESSIN ACETATE BY USING 3^2 FULL FACTORIAL DESIGN

Bhoomita G. Hadiya*[1], Dr. L. D. Patel[2] and Mayur P. Parmar[3]

[1]Ph.D. Scholar, Gujarat Technological University, Sharda School of Pharmacy, Pethapur - 382610, Gandhinagar, Gujarat, India.

[2]Former Principal, Sharda School of Pharmacy, Pethapur - 382610, Gandhinagar, Gujarat, India.

[3]Ph.D Scholar, Gujarat Technological University, Sharda School of Pharmacy, Pethapur - 382610, Gandhinagar, Gujarat, India.

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***Corresponding Author**
Bhoomita G. Hadiya
Ph.D. Scholar, Gujarat Technological University, Sharda School of Pharmacy, Pethapur - 382610, Gandhinagar, Gujarat, India.

ABSTRACT

The present work was aimed for the formulation development of stable dry nasal powder of desmopressin acetate using concentration of mucoadhesive polymer HPMC (Methocel E5) and feed rate on the basis of preliminary trials. The 3^2 factorial design was employed using concentration of HPMC (Methocel E5) and feed rate as independent variables and particle size (PS) and % yield were selected as dependent variables. The optimized batch was selected using Design Expert software employing overlay plot with desirability approach. The dry nasal powder formulation was evaluated for particle size, % yield, mucoadhesive strength, % drug content, scanning electron microscopy, differential scanning calorimetry, ex vivo drug diffusion study, nasal toxicity study, stability study and in-vivo study. The composition of optimized formulation consisted of 15 mg of Desmopressin acetate, 41.32 mg of HPMC (Methocel E5) as mucoadhesive polymer, 1443.68 mg of Mannitol as cryoprotectant and 50 ml of distill water showing particle size (13.23 µm), % yield (65.01), mucoadhesive strength (2709.3 dynes/cm^2) and % drug content (61.3 %). Dry nasal powder with mucoadhesive polymer HPMC (Methocel E5) increase the nasal residence time and dry form of formulation improves the stability of desmopressin acetate.

KEYWORDS: Dry nasal powder, Desmopressin acetate, Spray drying, HPMC Methocel E5.

INTRODUCTION

Many drugs are not delivered effectively and efficiently by conventional drug delivery approach to brain or central nervous system (CNS) due to their complexity. 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 many strategies and one of these is the use of the mucoadhesive polymer to increase the nasal residence time.

Nasal drop is one of the most simple and convenient system for nasal delivery. Its disadvantage is the lack of the dose precision and therefore nasal drops may not be suitable for prescription products. Both solution and suspension formulations can be formulated into nasal sprays, but major disadvantage associated with solution and suspension dosage form is the leakage of formulation from the nasal cavity. The advantages to the nasal powder dosage form are the absence of preservative and superior stability of the formulation.

Desmopressin is taken by oral or parental routes in the treatment of nocturnal enuresis or 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. The use of mucoadhesive polymer can lengthen the residence time and enhance its bioavailability of drugs delivered to the nasal cavity.

In spray drying, fluid mixture is sprayed into a hot dry air as a solvent, emulsion, suspension or dispersion. It is atomized into millions of droplets by a nozzle or a rotary wheel. The solvent is vaporized instantly by the hot air. The product is turned into a powder, granulate or agglomerate within seconds. Spray drying provides the advantage of weight and volume reduction and the powder is a suitable dosage for insufflation into nasal cavity.[1,2]

To the best of our knowledge, no information is available in the literature on the improvement of Desmopressin bioavailability using mucoadhesive polymer HPMC (Methocel E5) by spray drying methodology. The present work described the formulation development of dry nasal powder of desmopressin using mucoadhesive polymer HPMC (Methocel E5). The 3^2 factorial design was employed using concentration of HPMC (Methocel E5) and feed rate as independent variables and particle size and % yield were selected as dependent variables.

Stability study for optimized dry nasal powder containing desmopressin was performed as per ICH guidelines by keeping at room temperature 30°C ± 2°C/65% RH ± 5% RH and accelerated condition 40°C ± 2°C/75% RH ± 5% RH for 4 months.[3] The optimized formulation was subjected to particle size, % yield, mucoadhesive strength, % drug content, scanning electron microscopy, differential scanning calorimetry, ex vivo drug diffusion study, nasal toxicity study and in-vivo study.

MATERIAL AND METHODS

Materials

Desmopressin acetate was gifted from Sun Pharmaceuticals, Halol for research. HPMC (Methocel E5) was gifted from Colorcon Asia Pvt. Ltd., Mannitol was gifted from S.D. Fine Chemicals. Water used in the preparation of formulations was distilled 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 dry nasal powder[4,5]

Solution prepared by dissolving hydroxy propyl methyl cellulose (HPMC Methocel E5), mannitol and desmopressin acetate in distilled water (q.s to 50 ml) to give solid concentration 3%. Spray drying of the solution was carried out using a model LU-227 Advanced lab spray dryer.

Preliminary Trials

Preliminary trials were formulated without drug with varying quantity of HPMC as mucoadhesive polymer at different feed rate and aspirator speed of the spray dryer to select the variables. Mannitol was used as the cryoprotectant. The drug was incorporated in the

selected batches of respective polymer. Particle size and % yield were measured for the batches.

3^2 factorial design for optimization of formulation parameters of dry nasal powder of desmopressin acetate[6,7]

The preliminary trials were carried out using different concentration of HPMC (Methocel E5) as mucoadhesive polymer at different feed rate and aspiration speed. On the basis of results of preliminary trials, concentration of HPMC (Methocel E5) (X_1) and feed rate (X_2) were selected as independent variables and particle size (Y_1) and % yield (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.

Contour Plot[8]

Contour plot is a diagrammatic representation of values of the response and it is helpful in explaining visually the relationship between independent and dependent variables. The reduced model was used to plot two dimension contour plot using demo version of Design Expert 12 software.

Response Surface Plot

Response surface plot is helpful in understanding the main and the interaction effects of variables in the formulation development. The effect of level of independent variable on the response parameter can be understood from the respective response surface plot.

Optimization of dry nasal powder plot by Design Expert software

The desirability function approach is a technique for the simultaneous determination of optimum settings of input variables that can determine optimum performance levels for one or more responses.[8] The optimization of dry nasal powder formulation was performed using Design Expert software employing overlay plot with desirability approach.

Measurement of evaluation parameters of dry nasal powder Formulations

Particle size

Light scattering determination was performed with a Malvern Master Sizer from Malvern Instruments. The dry powder dispersion was filled in the cell and the size was measured. The average particle size was measured after performing the experiment in triplicate.

% Yield

% Yield was calculated using the following formula

$$\% \text{ Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Mucoadhesion test

This test was carried out by using modified physical balance. Sheep nasal mucosa was excised and kept in PBS pH 7.0. Nasal mucosa was cut into required size and paste on both balance plate then apply the formulation and kept it for 10 min. Then add weight on the other side of balance to detach the plate.

% Drug content

% drug content was determined by dissolving weighed quantity of dry powder in distilled water, then estimation was carried out using HPLC method peak due to desmopressin acetate was identified and area was calculated.

Scanning electron microscopy (SEM)

The morphology and size of the dry nasal powder were examined by SEM. Dry nasal powder was placed on the sample holder. Samples were directly examined under a JEOL JSM-5610LV Scanning electron microscope.

Differential scanning calorimetry (DSC)

The dry nasal powder containing the drug, pure drug and polymer were characterized by DSC (Shimadzu, Japan) in the range of 25–300^{o}C at a heating rate of 10^{o}C per minute with average sample weights of 2-3 mg. The transition temperature of polymer as well as the presence of any interaction between the drug and excipient was characterized.

***Ex-vivo* drug diffusion study**

The *ex-vivo* diffusion study was performed with freshly isolated sheep nasal mucosa using Franz diffusion cells.[9] The excised nasal membrane was mounted on Franz diffusion cells

with phosphate buffer pH 7.0, (0.067 M) filled in receptor compartments. The Dry nasal powder was carried out, dissolves in 1.5 ml phosphate buffer and was transferred in the diffusion membrane and maintaining sink condition. At specified intervals of time, 1 ml of the aliquots were withdrawn, filtered and analyzed for drug content by HPLC method.[9]

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 dry nasal powder batch HPO 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.[10]

***In-vivo* study for antidiuretic activity**

In-vivo study was performed on adult Wistar albino male rats. A protocol for animal studies 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 dry nasal powder. 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.[11]

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 dry nasal powder of DA batch HPO: Third group of animals, were administered dry powder containing 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 of optimized batch of dry nasal powder

The stability of the formulation was assessed under different storage conditions as per ICH guidelines, namely, 30°C ± 2°C/65% RH ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH for 4 months. At the interval of 15 days, samples were withdrawn from the vials and subjected for the analysis of size and % drug retained.[3]

RESULT AND DISCUSSION

Preliminary Trials

Table 1: Preliminary trials for dry nasal powder of desmopressin acetate.

Batch Name	HPMC (Methocel E5) (mg)	Mannitol (mg)	Drug (mg)	Distill water (ml)	Feed Rate (ml/min)	Aspirator (RPM)	Avg. Particle Size d(0.9) (µm)	% Yield
HA	15	1485	-	50	6	600	119.3±1.010	44.6±1.926
HB	15	1485	-	50	6	1200	73.51±1.662	51.7±0.278
HC	15	1485	-	50	6	1500	46.6±0.219	33.0±2.821
HD	15	1485	-	50	6	1800	47.2±1.423	36.1±2.109
HE	15	1485	-	50	3	1500	38.9±2.071	21.9±0.151
HF	15	1485	-	50	6	1500	25.4±0.578	53.3±1.020
HG	15	1485	-	50	9	1500	21.9±1.282	39.1±1.519
HH	22.5	1477	-	50	6	1500	4.9±0.802	12.5±2.671
HI	30	1470	-	50	6	1500	6.2±2.208	42.7±0.592
HJ	37.5	1462	-	50	6	1500	13.7±0.125	56.8±1.291
HK	45	1455	-	50	6	1500	14.4±1.134	59.0±1.629
HL	**45**	**1440**	**15**	**50**	**6**	**1500**	**13.23±2.091**	**63.7±1.942**

Batches HA to HL were prepared using varying amount of HPMC. Batches HA to HD were prepared using aspiration speed like 600 rpm, 1200 rpm, 1500 rpm and 1800 rpm and on the basis of effect of aspiration speed on particle size and % yield 1500 rpm was optimized as aspiration speed. Then batches HE to HG were prepared using different feed rate like 3, 6 and 9 ml/min and on the basis of effect of feed rate on particle size and % yield, 6 ml/min was optimized as feed rate. Then, batches HH to HL were prepared using different HPMC (Methocel E5) concentration like 15 mg, 22.5 mg, 30 mg, 37.5 mg and 45 mg on the basis of effect of HPMC (Methocel E5) concentration on particle size and % yield 45 mg HPMC (Methocel E5) amount was optimized. After optimization of all three parameter like HPMC (Methocel E5) concentration, feed rate and aspiration speed, in batch HL drug was incorporated and analyzed for particle size and % yield. On the basis of result of preliminary trials batch HL was found satisfactory.

Optimization of dry nasal powder of Desmopressin acetate using factorial design

The 3^2 factorial design was employed using concentration of HPMC (Methocel E5) and feed rate as independent variable X_1 and X_2. The particle size (PS) (Y_1) and % yield (Y_2) were selected as dependent variables. The coded and actual value of independent variable was shown in Table 3 and the runs and responses were presented in Table 2. Mannitol was used as cryoprotectant.

Table 2: Factors and levels of independent variables in 3^2 factorial design for formulation of dry nasal powder of desmopressin acetate.

Independent variables	Level		
	Low (-1)	Medium (0)	High (+1)
HPMC (Methocel E5) conc. (X_1) (mg)	30	45	60
Feed rate (X_2), (ml/min)	5	6	7

Table 3: Experimental runs and measured responses of 3^2 factorial design for dry nasal powder of desmopressin acetate.

Batch	X_1	X_2	Particle size (PS) (Y_1) µm	% Yield (Y_2)
H1	-1	-1	19.42	44.04
H2	0	-1	18.21	52.42
H3	1	-1	16.59	33.33
H4	-1	0	12.44	57.12
H5	0	0	12.22	64.46
H6	1	0	11.99	53.36
H7	-1	1	12.98	55.48
H8	0	1	11.78	64.65
H9	1	1	11.35	64.12

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).

Particle size (PS) (Y_1) µm

A full model equation of particle size (PS) (Y_{FPS}) was written as Equation 1

$$Y_{FPS} = 12.177 - 0.8183X_1 - 3.018X_2 + 0.300X_1X_2 + 0.0583X_1^2 + 2.838\ X_2^2 \ldots \text{(Equation 1)}$$

The reduced model equation for particle size (PS) (Y_{RPS}) was presented as Equation 2

$$Y_{RPS} = 12.21666 - 0.818333X_1 - 3.01833X_2 + 2.83833X_2^2 \ldots\ldots\ldots \text{(Equation 2)}$$

% Yield (Y_2)

A full model equation of % yield (Y_{FY}) was written as equation as Equation 3

$Y_{FY} = 64.492 - 0.971X_1 + 9.076X_2 + 4.837X_1X_2 - 9.268X_1^2 - 5.973X_2^2$…… (Equation 3)

The reduced model for % yield (Y_{RY}) was presented as equation as Equation 4

$Y_{RY} = 60.51 - 0.971667X_1 + 9.07667X_2 - 9.26833X_1^2 + 4.8375X_1X_2$ …… (Equation 4)

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	75.159	15.032	38.349	0.9922	0.9845	0.9589
	RM	3	74.792	24.930	80.787	0.9898	0.9797	0.9679
Error	**FM**	3	1.1761	0.3920				
	RM	5	1.5429	0.3085				
Response Y_2	Model	DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	836.751	167.35	17.437	0.9832	0.9667	0.9112
	RM	4	765.387	191.34	7.6476	0.9403	0.9421	0.9321
Error	**FM**	3	28.791	9.5973				
	RM	4	100.153	25.038				

Contour Plots and Response Surface Plots

Two dimensional contour plots were constructed for all dependent variables i.e. particle size (PS) and % yield for desmopressin acetate dry nasal powder 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.[8]

Particle size (PS)

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

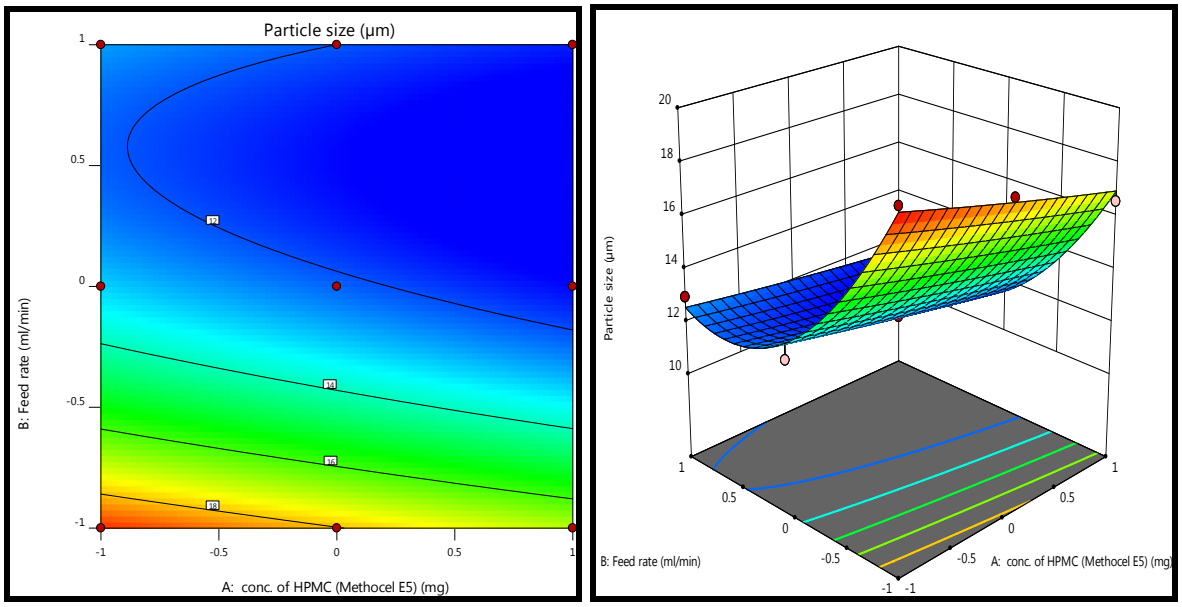


Figure 1: Contour plot and 3D surface plot for the effect on particle size.

The response surface plot showed decrease in particle Size (PS) with increase in the concentration of HPMC (Methocel E5): Feed rate.

% Yield

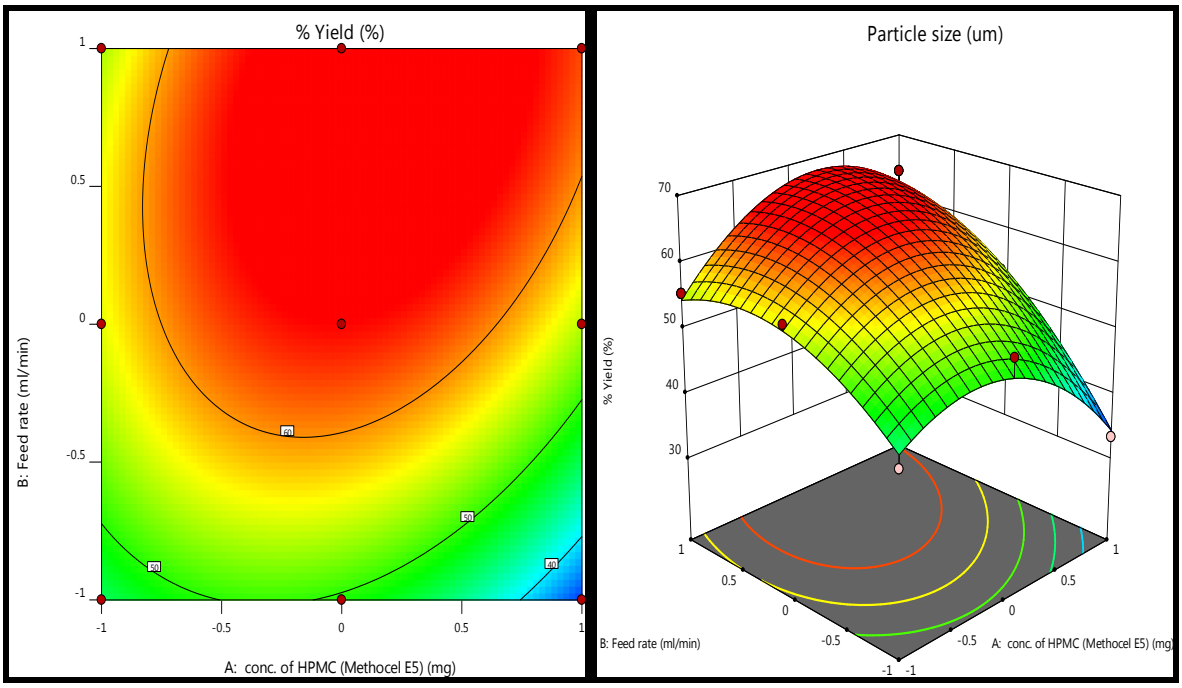


Figure 2: Contour plot and 3D surface plot for the effect on % yield.

The response surface plot showed increase in % yield with increase in the concentration of HPMC (Methocel E5): Feed rate.

Optimization of dry nasal powder Formulation

Optimized formulation was selected by arbitrarily fixing the criteria of 11.35 – 19.92 µm of the particle size (PS) and 33.33– 64.65% yield for dry nasal powder. 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.

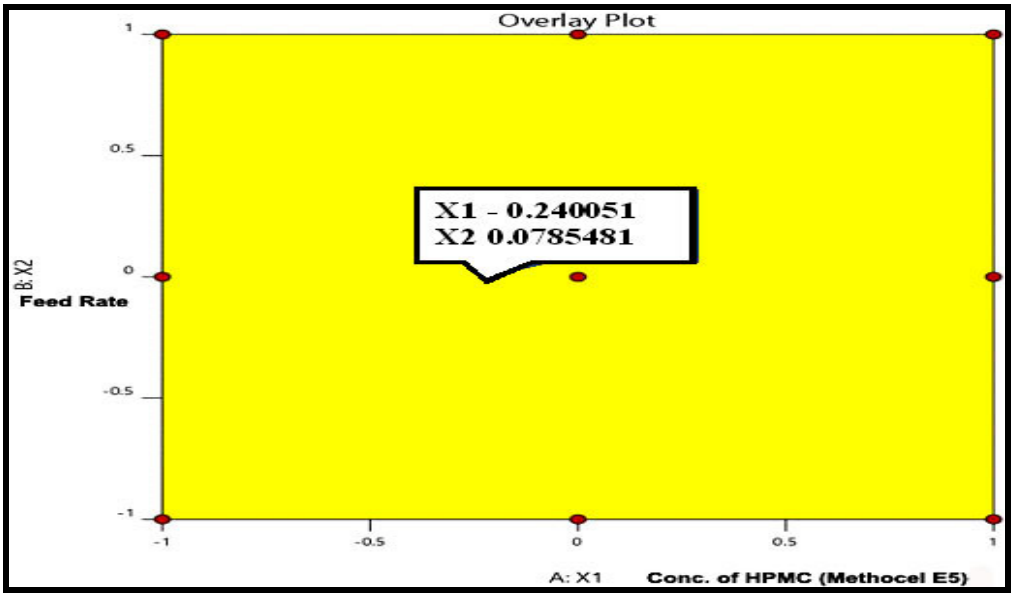


Figure 3: Overlay plot for optimization of dry nasal powder formulation.

Table 5: Solution proposed by Design Expert.

Sol. Run	Conc. Of HPMC (Methocel E5)	Feed rate	Particle size	% Yield
1	0.078	0.656	11.37	67.99

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. particle size and % yield for desmopressin dry nasal powder were selected. The point indicating toggle flag showed the coded value of X_1= -0.240051 and X_2= 0.0785481 for optimized formulation. The actual value of X_1 and X_2 was shown in Table 6.

Table 6: Optimized formulation of desmopressin acetate dry nasal powder-Batch HPO.

Material used	**Quantity (mg)**	
Desmopressin acetate	15 mg	
HPMC (Methocel E5)	41.32 mg	
Mannitol	1443.68 mg	
Distill water	50 ml	
Feed rate	6.08 ml/min	
Aspiration speed	1500 rpm	
Response	**Predicted**	**Actual**
Particle size (um) (Y_1)	12.152	13.23±0.123
% Yield (Y_2)	64.778	65.0123±0.356

The optimized batches were prepared n=3 and responses were measured. There was no significant difference between predicted and observed actual responses. Thus the derived model was validated using the prepared optimized check point batches.

Evaluation parameters of dry nasal powder Formulations -Batch HPO

Particle size

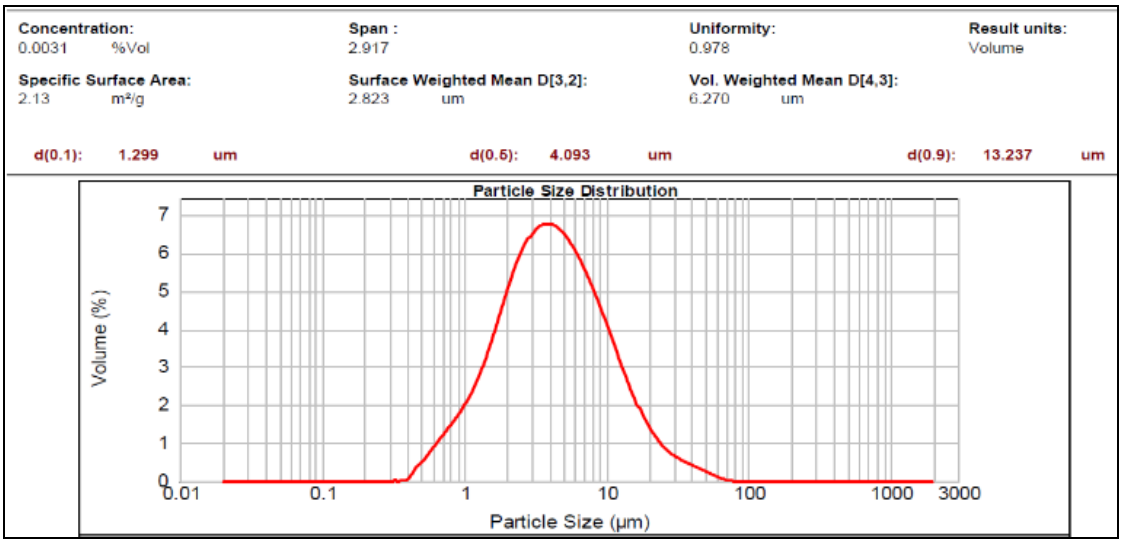


Figure 4: Particle size of batch-HPO.

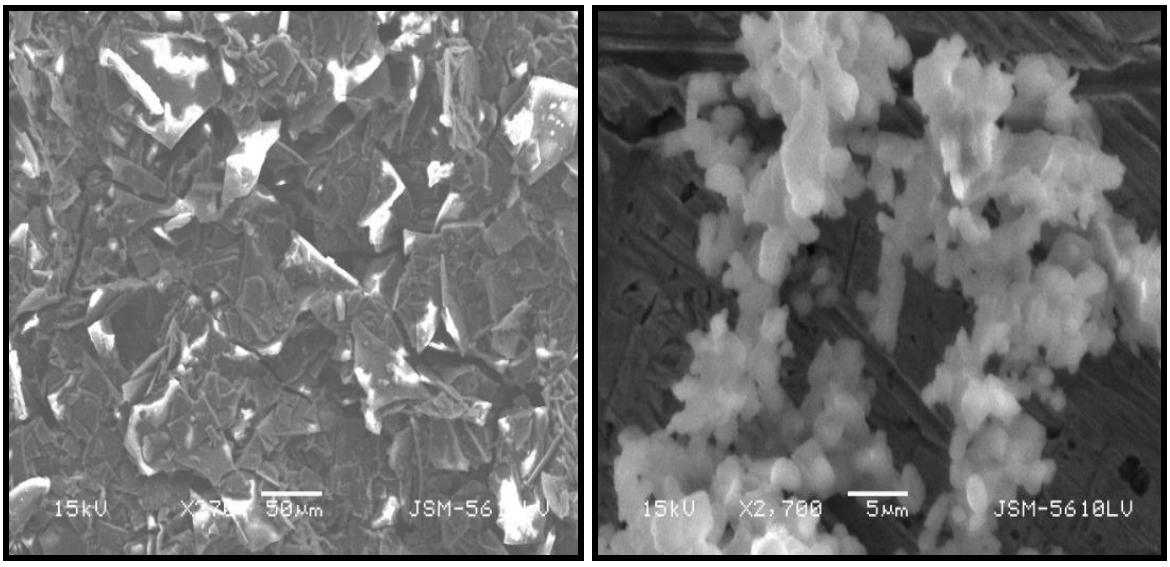
% Yield: % Yield of batch HPO was found to be 65.0123 ± 0.356 %.

Mucoadhesive strength (MS): The MS of batch HPO was found to be 2709.3 dynes/cm^2.

% Drug content: % Drug content of batch HPO was found to be 61.3±1.091%.

Scanning electron microscopy (SEM)

The Scanning electron micrographs in Figure 5 revealed that pure desmopressin acetate shows irregular shape and dry nasal powder containing desmopressin acetate were of good spherical shape.



I **II**

Figure 5: I) SEM Image of Desmopressin acetate, II) SEM image of batch-HPO.

Differential Scanning Calorimetry (DSC)

The DSC thermogram of Desmopressin acetate shows presence of characteristic endothermic peak at 82.82º C indicating melting point of Desmopressin acetate. The Mannitol shows a sharp endothermic peak at 168.31º C indicating its melting point. The DSC thermogram of HPMC shows broad peak around 60.13º C indicating glass transition temperature of polymer. The dry nasal powder showed the presence of sharp endothermic peak around 165.80º C which is of Mannitol. DSC study revealed that peak of drug was absent in DSC thermogram of dry powder indicating conversion of crystalline form of Desmopressin acetate in amorphous form during preparation. The obtained DSC thermograms are reported in Figure 6 (a)-(d).

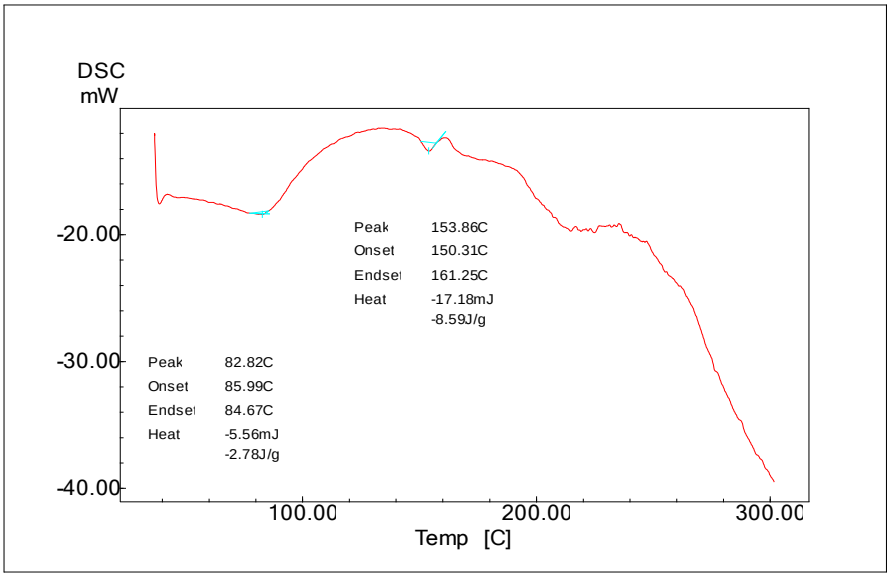


Figure 6a: DSC thermogram of Desmopressin acetate.

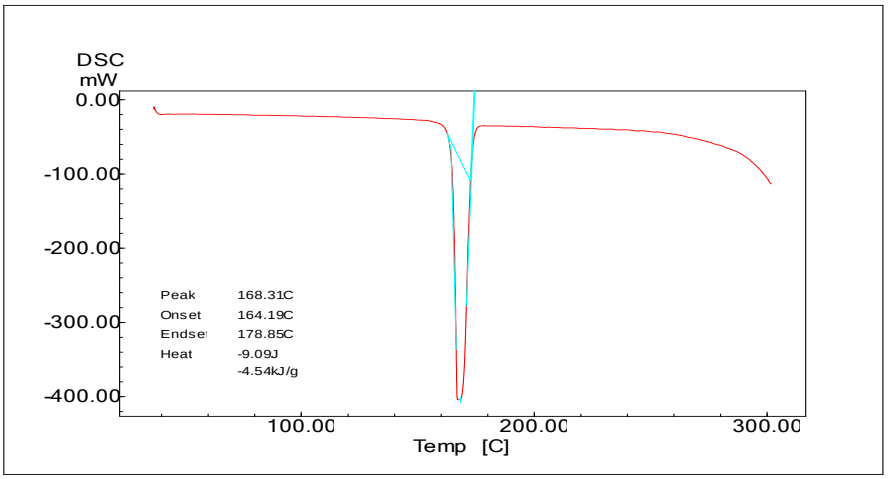


Figure 6b: DSC thermogram of Mannitol.

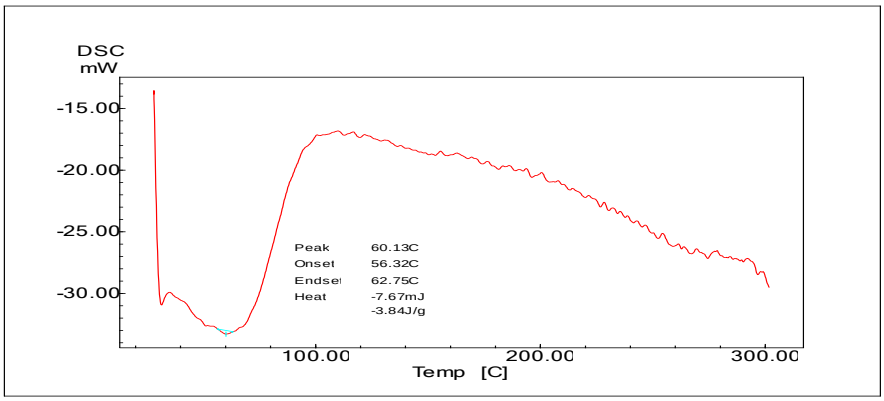


Figure 6c: DSC thermogram of HPMC (Methocel E5).

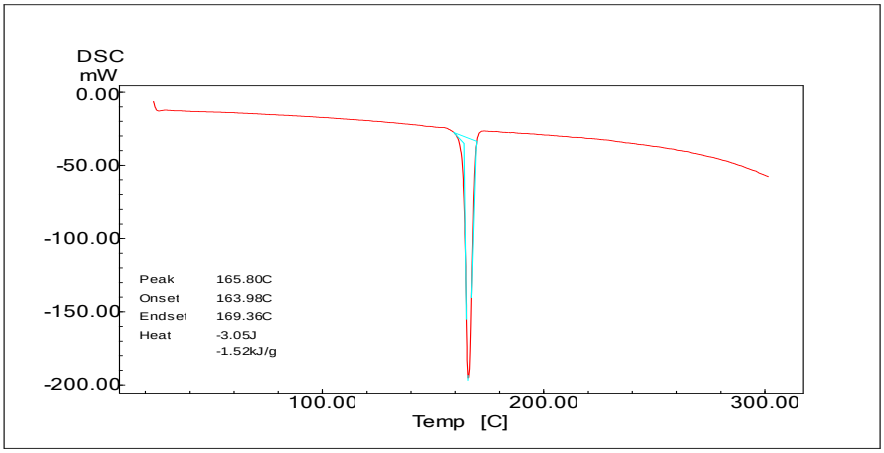


Figure 6d: DSC thermogram of batch-HPO.

Ex-vivo drug diffusion study[9]

Comparative diffusion study was carried out of plain drug and dry nasal powder of desmopressin acetate using diffusion cell for a period of 360 min.

Table 7: % Drug Diffusion at different time point.

Time (min)	Ex vivo drug release study (% drug diffused) (Mean±SEM)	
	Plain drug	Batch-HPO
0	0.00	0.00
15	2.1±1.927	2.9±0.190
30	6.6±0.321	7.3±2.718
45	13.9±2.856	17.6±1.391
60	20.8±0.672	26.4±1.021
90	24.1±1.624	39.0±1.308
120	32.7±0.662	47.9±0.928
180	38.6±1.892	54.1±3.109
240	45.2±2.561	68.7±1.206
300	51.8±1.824	76.5±1.046
360	59.2±2.912	84.9±1.265

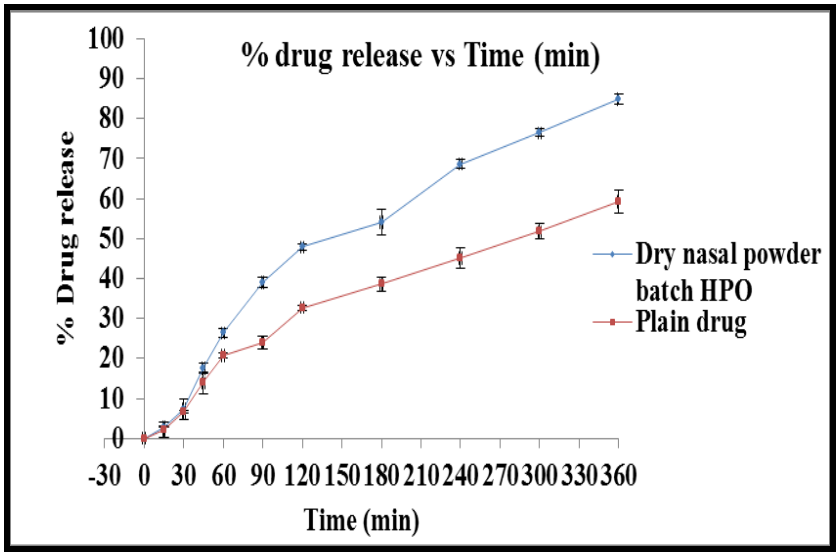
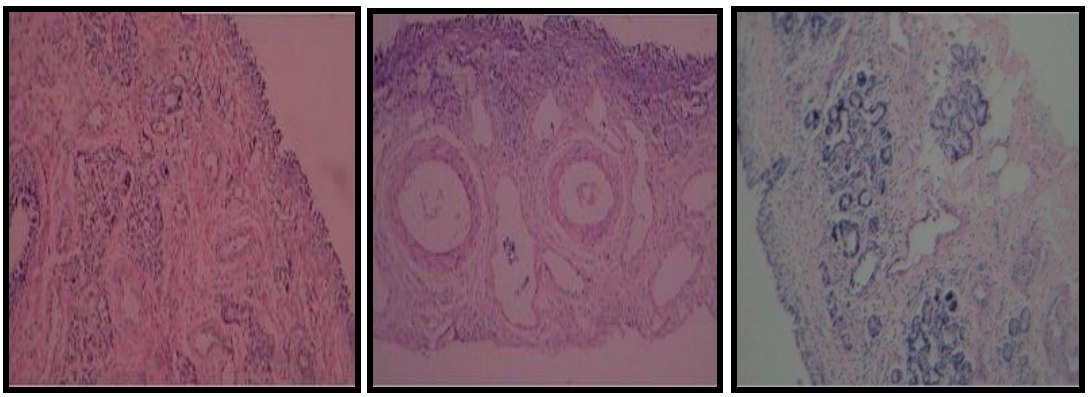


Figure 7: Comparative ex vivo drug diffusion of plain drug and dry nasal powder of desmopressin acetate batch HPO.

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 HPO showed reversible contraction of epithelial layer after 1 hr washing and no damage to the other parts of mucosa were observed.[10]



I **II** **III**

Figure 8: I) PBS pH 7.0, II) IPA, III) Dry nasal powder batch HPO.

***In-vivo* study for antidiuretic activity**

Effect of dry nasal powder of desmopressin acetate on urine volume, serum sodium and potassium concentration shown in Table 8.[11]

Table 8: Effect of dry nasal powder 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: Dry nasal powder of DA batch HPO + HCTZ	1.3 ± 0.87	140.2 ± 0.43	3.8 ± 0.17

Results demonstrated that after intranasal administration of desmopressin acetate dry nasal powder 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 9.[3]

Table 9: Effect of storage condition on dry nasal powder of desmopressin acetate.

Sampling time (days)	Dry nasal powder of desmopressin acetate-% Drug retain (% Assay) (n=3)	
	Room condition (30 ± 2 °C with 60 ± 5 % RH) Batch HPO	Accelerated temp.(40°C ± 2°C/75% RH ± 5% RH) Batch HPO
0	99.60 ± 0.027	99.60 ± 0.027
15	99.59 ± 0.021	97.39 ± 0.029
30	99.52 ± 0.087	93.54 ± 0.016
45	99.49 ± 0.035	89.27 ± 0.014
60	99.46 ± 0.026	82.33 ± 0.026
75	99.30 ± 0.075	76.03 ± 0.011
90	99.21 ± 0.011	71.47 ± 0.056
105	99.03 ± 0.065	69.38 ± 0.059
120	98.97 ± 0.048	63.39 ± 0.024

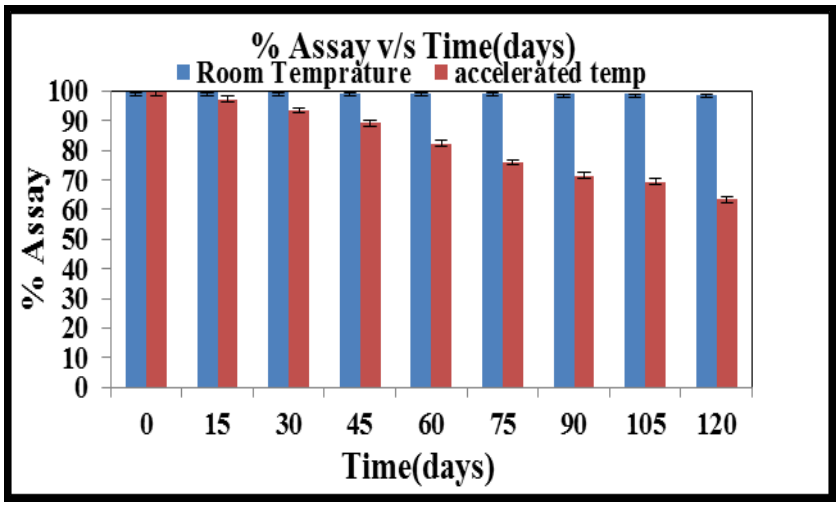


Figure 9: Stability profile of batch HPO: % Assay vs. Time (Days).

Table 10: Effect of storage condition on Particle size.

Sampling time (days)	Dry nasal powder of desmopressin acetate - Particle size* (µm) (n=3)	
	Room condition (30 ± 2 °C with 60 ± 5 % RH) Batch HPO	Accelerated temp.(40°C ± 2°C/75% RH ± 5% RH) Batch HPO
0	13.23±2.091	13.23±2.091
15	13.65±1.321	14.75±2.338
30	13.78±1.452	17.16±1.287
45	13.89±2.874	19.90±1.382
60	13.98±1.937	27.10±1.617
75	14.32±1.673	36.49±0.489
90	14.54±2.618	49.20±1.728
105	14.72±2.169	53.78±2.826
120	14.83±2.371	58.19±2.190

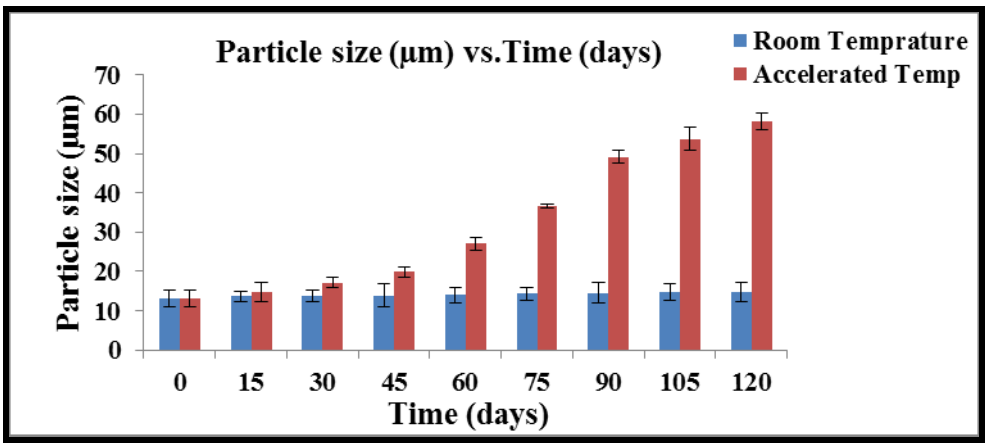


Figure 10: Stability profile of batch HPO: Particle size vs. Time (Days).

There was no significant decrease in % of drug when stored at room temperature. There was slight increase in the particle size after 4 month storage at room temperature. The dry powder were stable at 30°C ± 2°C/65% RH ± 5% RH for period of 4 months so there is no need of refrigerated storage and it increases the stability of peptide hormone (Desmopressin acetate).

CONCLUSION

The advantages of the nasal powder dosage form are the absence of preservative and superior stability of the formulation. The present study was aimed to explore dry nasal powder using mucoadhesive polymer for formulation development using 3^2 factorial design for bioavailability and stability improvement of desmopressin acetate. The 3^2 factorial design was employed using concentration of HPMC (Methocel E5) and feed rate as independent variables and particle size (PS) and % yield 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 particle size, mucoadhesive strength, % yield, % drug content, scanning electron microscopy, DSC study, nasal toxicity study, ex vivo drug release study, stability study and in vivo study. The optimized formulation was found stable at room temperature.

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