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FORMULATION DEVELOPMENT AND EVALUATION OF *IN SITU* NASAL GEL

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ABSTRACT

The aim of current study was to design pH dependent in situ nasal gel of Beclomethasone Dipropionate to optimize the drug release profile and mucoadhesive strength by response surface methodology. Gel was prepared by cold method using Carbopol 934 as pH dependent and mucoadhesive polymer and HPMC K4M as release controlling polymer. Designed based on 3^2 full factorial design containing 2 factors evaluated at two levels and the experimental trials were performed at all possible combinations. Carbopol 934 (X1), HPMC K4M (X2) was taken as independent variable. The dependent variable selected were amount of drug release in 8 h and mucoadhesive strength. Polynomial mathematical model, generated for various response variable using multiple linear regression analysis, were found to be statistically significant. Contour plots and response surface plots were drawn and optimum formulation were selected by feasibility and desirability function. The experimental values obtained from the optimized formulation highly agreed with the predicted values. The results of in vitro diffusion studies and mucoadhesive strength studies indicated that formulation S6 is the most successful formulation of the study, exhibited drug release 87.19% in 8 h and mucoadhesive strength of 90.28 dyne/cm^2. The results demonstrated the reliability of the model in the preparation of in situ nasal gels with predictable drug release profiles. Optimized formulation of Beclomethasone dipropionate showed no change in physical appearance, drug content, or in release pattern after storage at 40°C for 60 days.

KEY WORDS: *Beclomethasone Dipropionate, in situ nasal gel, Carbopol.*

INTRODUCTION

"Gel" is the state between the liquid and solid which consists of physically cross linked networks of long polymer molecules with liquid molecules trapped within a three dimensional polymeric network swollen by a solvent. If the solvent is water, the gel is termed a "hydrogel". In situ gels are one of the types of hydrogels. The term in-situ gelling polymers also describes a stimulus induced gelation response but is generally used more narrowly to denote formulations that gels upon contact with mucosa, that is , they gels once in position.[1] Recent advances in polymer chemistry and hydrogel engineering have promoted the development of in situ forming hydrogels for drug delivery applications. Though intelligent design of monomers/macromers with desired functionalities, hydrogel precursor solutions can be injected and subsequently polymerized in situ. This in situ sol–gel transition enables the surgery or implantation procedure to be performed in a minimally invasive manner. There are specific uses of gelling polymers in which it is greatly preferred that the gelation response occur only when the polymer solution is exposed to multiple stimuli. As used herein, the stimuli may be any environmental stimulus that, in combination with

at least one additional stimulus, causes the gelation of a polymer solution. The stimuli that induce various responses in the hydrogel systems include physical (temperature, electric fields, light, pressure, sound, and magnetic field), chemical (pH, ions) or biological/ biochemical (biomolecules). "Smart" hydrogels, or stimuli-sensitive hydrogels, are very different from inert hydrogels in that they can "sense" changes in environmental properties such as pH and temperature and respond by increasing or decreasing their degree of swelling. These sensing capabilities are attractive in many biomedical applications.[2]

Statistical experimental design has been recognized as a useful technique to find optimal parameters and conditions for various processes, particularly when multiple factors are involved. In particular optimization by means of statistical experimental design methodologies has been successfully applied in developing and optimizing extended-release dosage forms. Single response optimization, even though widely used, could lead to misleading results, since different release curves could show the same percent of drug released at a single reference time. Therefore, in our study a multiple response optimization approach was considered more useful and suitable for optimizing Beclomethasone Dipropionate release profile from *in situ* nasal gel. Finally, the desirability function was used to simultaneously optimize the considered response variables, each having a different target, and to find the optimum formulation conditions in the studied experimental domain.[3]

MATERIALS AND METHODS

MATERIALS

Beclomethasone Dipropionate was obtained as gift sample from Unijules Lifesciences Ltd., Nagpur. Carbopol 934, HPMC K4M was obtained as gift sample from Colorcon Asia Ltd., Goa. All other ingredients used throughout the study were of analytical grade and were used as received. Design of Experiments® 8.5.0.7 (Stat Ease, USA) software was used for generation and evaluation of the statistical experimental design.

Method of preparation of *in situ* nasal gel

The formulations were prepared by dispersing Carbopol 934 in distilled water with continuous stirring (Thermostatic Hot plate with Magnetic Stirrer) until completely dissolved and allowed to hydrate overnight. For the preparation of the solution first HPMC K4M was dissolved in distilled water using magnetic stirrer and allowed to hydrate. Then the Carbopol was sprinkled over this solution and allowed to hydrate overnight. After the complete hydration of polymer, a separate solution of Beclomethasone Dipropionate in ethanol with propylene glycol was added to the polymeric solution. The resultant solution was thoroughly mixed, benzalkonium chloride was then added and mixing was confirmed until a uniform and clear solutions were formed. Final volume was made by adding required amount of distilled water. All the formulations were adjusted to pH 4.5 to 5.5 by using freshly prepared 0.5 M sodium hydroxide solution.

Evaluation of *in situ* nasal gel

In situ nasal gels were evaluated for its parameters like pH, gelation studies, gelation temperature, drug content, viscosity, gel strength and other specific evaluation tests mucoadhesive strength and release rate of drug.

Evaluation of the Mucoadhesive Strength

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified method. [4]

In brief, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after separation. A section of the sheep nasal mucosa was securely placed mucosal side upward within water containing buffer of pH 6.6. Another mucosa was attached to the rubber cork placed in inverted position on height adjustable pan. Formulation was placed in contact between the two mucosal surfaces, for 2 min to ensure intimate contact between them. Then the weight was kept rising in second pan until mucosa get detached from each other. The mucoadhesive force expressed as the detachment stress in dyne/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

$$\text{Mucoadhesive Strength} = \frac{mg}{A}$$

Where,
m is the weight added to the balance in grams;
g is the acceleration due to gravity taken as 980 cm/s^2 and
A is surface area of sheep nasal mucosa.

***In vitro* diffusion studies**

In vitro diffusion study of formulated *in situ* gels was carried out on Franz diffusion cell having 2.0 cm diameter and 16 ml capacity. Dialysis membrane (Himedia) having molecular weight cut off range 12000–14000 kDa was used as diffusion membrane. Pieces of dialysis membrane were soaked in phosphate buffer (PB) pH 6.6 for 24 h prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.6; dialysis membrane was mounted on cell. The temperature was maintained at 37^o. After a pre-incubation time of 20 minutes, formulation equivalent to 2.5 mg of Beclomethasone Dipropionate was placed in the donor chamber. Gelation was induced using SNF.

At predetermined time points, 0.5 ml samples were withdrawn from the acceptor compartment, replacing the sampled volume with PB pH 6.6 after each sampling, for a period of 8 h. The samples withdrawn were suitably diluted and measured spectrophotometrically 240.5 nm. The concentration of drug was determined from previously calculated calibration curve. [5]

Table 1: Parameters and levels selected for statistical analysis

Parameter/Level	Level 1 (%w/v)	Level 2 (%w/v)	Level 3 (%w/v)
Carbopol 934 Conc.	0.2	0.4	0.6
HPMC K4M Conc.	0.2	0.4	0.6

RESULT AND DISCUSSION

The *in situ* nasal gels of Beclomethasone Dipropionate were formulated by using HPMC K4M and Carbopol 934 by cold method. Carbopol 934 was used as mucoadhesive as well as pH dependent polymer. All the prepared batches were found to be clear in appearance. The drug content was uniform (96.84% to 99.48%) and well within the accepted limits with low values of standard deviation indicating uniform distribution of drug within the tablets of same batch.

From the gelation studies it shows that all formulation shows instantaneous gelation. Formulation S1, S2 showed weakest gelation while S8, S9 showed very stiff gelation hence excluded from further studies. Formulations S5 and S6 showing good gelation were further considered. The viscosity of formulations both in sol and gel state; was found to be proportionate with the increasing polymer concentration. In the liquid state all the formulations were observed to be exhibiting Newtonian flow in solution state while in the gel state they exhibits the pseudo plastic flow. Gel strength of the formulations were found to be affected by the concentration of gelling and bioadhesive polymer. In formulations Carbopol was found to increase the gel strength with increasing concentration. Combination of Carbopol with HPMC K4M was found to show better mucoadhesion. Mucoadhesion force increases correspondingly with increase in concentration of Carbopol and HPMC K4M. Carbopol with 0.4% in combination with HPMC K4M (0.6%) shows significant mucoadhesion.

The *in vitro* diffusion profiles of all the prepared *in situ* gels of Beclomethasone Dipropionate were found to control the drug release over a period of 8 h and the drug release decreased with increase in polymer concentration. The runs or formulations, which are designed based on 3^2 full factorial designs, are evaluated for the response variables. The response values are subjected to multiple regression analysis to find out the relationship between the factors used and the response values obtained. The response values subjected for this analysis are;

1. **Mucoadhesive study.**
2. **Drug Release study.**

Graphic analysis of effects allowed the different effect of factor levels to be evaluated. The response surface study allows prediction of the response in all experimental domains studied. In this way, through an analysis of the response surfaces, it is possible to select the best combination of factor levels in order to optimize the considered response. Analysis of variance (ANOVA) indicated that the assumed regression model was significant and valid for each considered response. Starting from these graphs it was possible to select the best conditions to optimize each response and to point out possible interactions between factors. However, it must be taken into account that, for optimizing a sustained-release dosage form, the goal was to obtain specific concentration values of released drug for the responses. Thus having to optimize two responses with different targets, a multicriteria decision approach like desirability function was used. Each response was associated with its own partial desirability function *di.* This varied from 0 to 1, according to the closeness of the response to its target value.

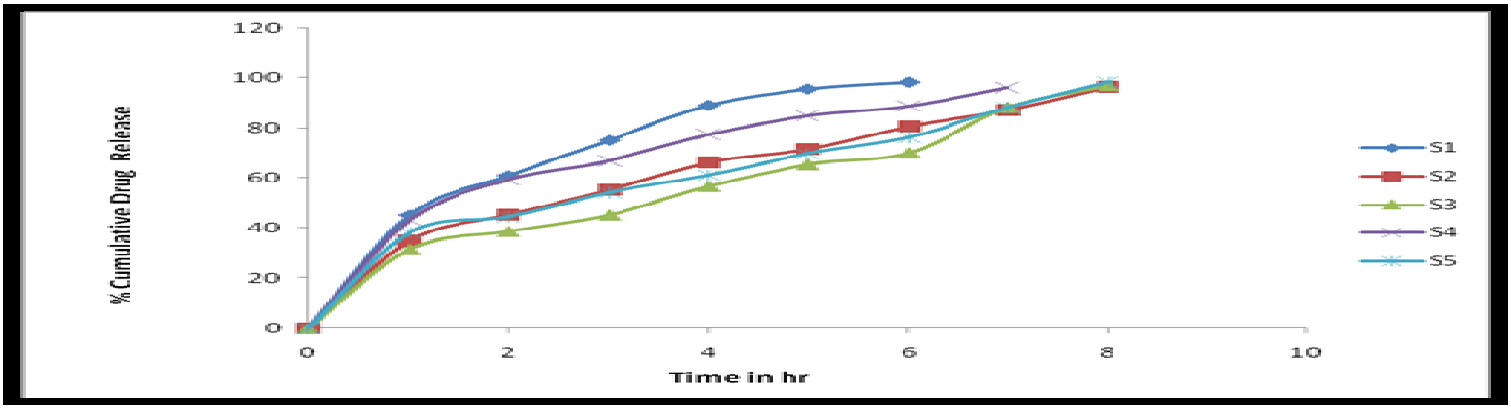

Figure 1: *In vitro* cumulative percentage drug release of formulation from S1 to S5

Table 2: Experimental design

Batch Code	Drug (mg)	Carbopol 934 (%w/v)	HPMC K4M (%w/v)	Propylene Glycol (ml)	Benzalkonium Chloride (%w/v)
S1	250	0.2	0.2	5	0.01
S2	250	0.2	0.4	5	0.01
S3	250	0.2	0.6	5	0.01
S4	250	0.4	0.2	5	0.01
S5	250	0.4	0.4	5	0.01
S6	250	0.4	0.6	5	0.01
S7	250	0.6	0.2	5	0.01
S8	250	0.6	0.4	5	0.01
S9	250	0.6	0.6	5	0.01

Table 3: Evaluation parameter for *in situ* nasal gel

Batch Code	Gelation Study	Gelation Temperature (^{0}C)	Viscosity (cP)		Gel (sec) Strength	Muco-adhesive Strength (dyne/cm^2)	% Drug Release
			Solution	Gel			
S1	+	118±0.45	240	1137	10.4±0.53	71.19±0.15	98.33±1.45
S2	+ +	106±0.68	264	1365	18.5±2.61	74.28±1.23	96.49±0.55
S3	+ +	101±0.52	289	1470	23.38±1.64	77.30±0.79	97.13±1.23
S4	+ + +	108±0.78	312	1510	37.72±0.43	85.12±0.96	96.25±1.24
S5	+ + +	98±0.98	347	1587	41.07±1.42	86.67±0.75	98.48±1.32
S6	+ + +	92±0.36	361	1645	45.14±1.29	90.28±0.84	87.19±1.98
S7	+ + +	104±1.2	389	1701	52.8 ± 2.14	91.82±0.66	97.09±0.59
S8	+ + +	93±0.87	402	1779	54.61±1.28	94.40±0.45	87.49±0.92
S9	+ + ++	87±0.63	428	1802	55.82 ± 0.73	95.95±0.36	79.64±0.67

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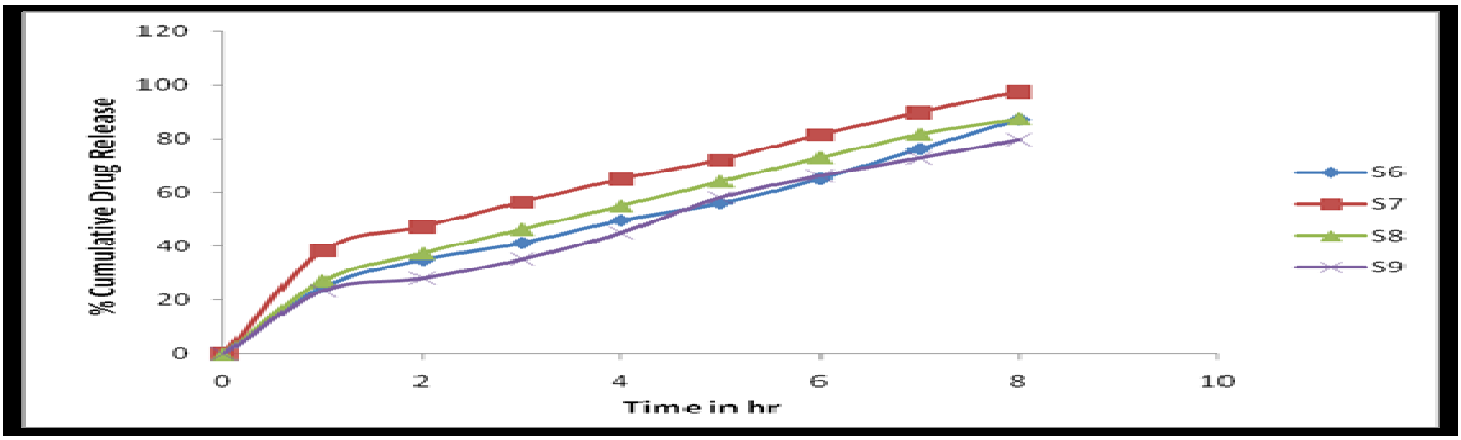

Figure 2: ***In vitro*** **cumulative percentage drug release of formulation from S6 to S9**

Table 4: Anova for selected factorial model % drug release

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	256.13	2	128.07	7.58	0.0228
A-Carbopol 934	128.25	1	128.05	7.59	0.0331
B-HPMC K4M	127.88	1	127.88	7.56	0.0333
Residual	101.43	6	16.90		
Cor Total	357.56	8			

Table 5: Anova for selected factorial model mucoadhesive strength

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	622.59	2	313.79	84.26	<0.0001
A-Carbopol 934	588.06	1	588.06	157.09	<0.0001
B-HPMC K4MC	39.53	1	39.53	10.61	0.0173
Residual	22.35	6	3.72		
Cor Total	649.93	8			

Table 6: Diagnostics case statistics for % drug release

Standard Order	Actual Value	Predicted Value	Internally Studentized Residual	Leverage	Externally Studentized Residual
1	98.32	102.36	-1.318	0.444	-1.427
2	96.49	97.74	-0.328	0.278	-0.330
3	97.13	93.12	1.307	0.444	1.411
4	96.25	97.73	-0.425	0.278	-0.394
5	98.48	93.12	1.383	0.111	1.530
6	87.19	88.50	-0.375	0.278	-0.347
7	97.09	93.11	1.289	0.444	1.398
8	87.47	88.49	-0.293	0.278	-0.270
9	79.64	83.88	-1.318	0.444	-1.529

Since, the predicted Response is in good agreement with actual practically obtained response, model is acceptable.

Table 7: Diagnostics case statistics for % mucoadhesive strength

Standard Order	Actual Value	Predicted Value	Internally Studentized Residual	Leverage	Externally Studentized Residual
1	71.19	72.75	-1.317	0.444	-1.426
2	74.28	75.32	-0.358	0.277	-0.330
3	77.3	77.89	1.307	0.444	1.410
4	85.12	82.65	-0.424	0.277	-0.393
5	86.67	85.22	1.383	0.111	1.530
6	90.28	87.79	-0.375	0.277	-0.346
7	91.82	92.55	1.298	0.444	1.397
8	94.4	95.12	-0.293	0.277	-0.269
9	95.95	97.69	-1.382	0.444	-1.529

Since, the predicted Response is in good agreement with actual practically obtained response, model is acceptable.

TABLE 8: SOLUTIONS OF % DRUG RELEASE FOR OPTIMIZATION

Number	Carbopol 934 (% w/v)	HPMC K4M (%w/v)	Predicted Drug Release (%)	Desirability
1	0.4	0.6	88.57	1
2	0.4	0.65	87.42	0.940

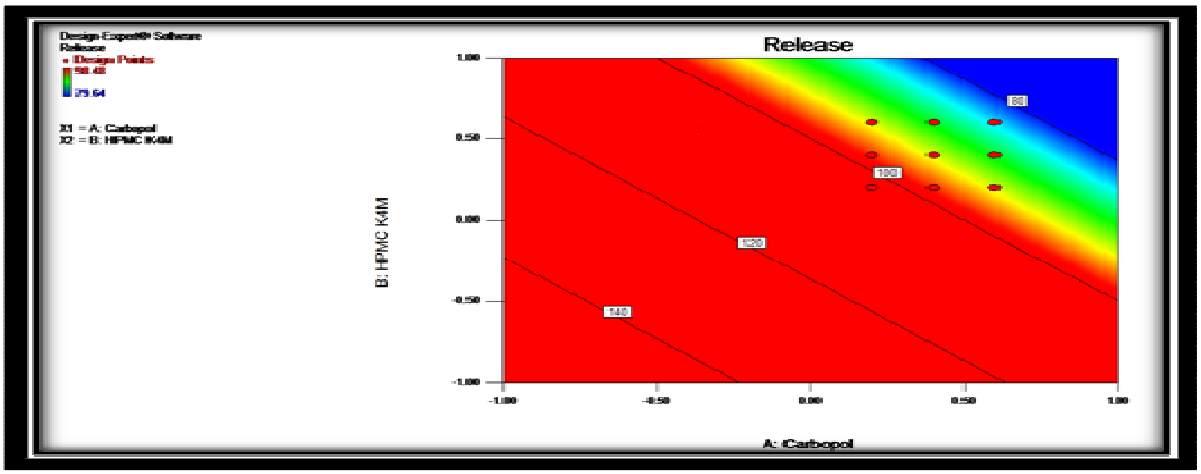

Figure 3: Contour plot of Carbopol 934 Vs HPMC K4M for % drug release (Numerical Optimization Graphs for % drug release)

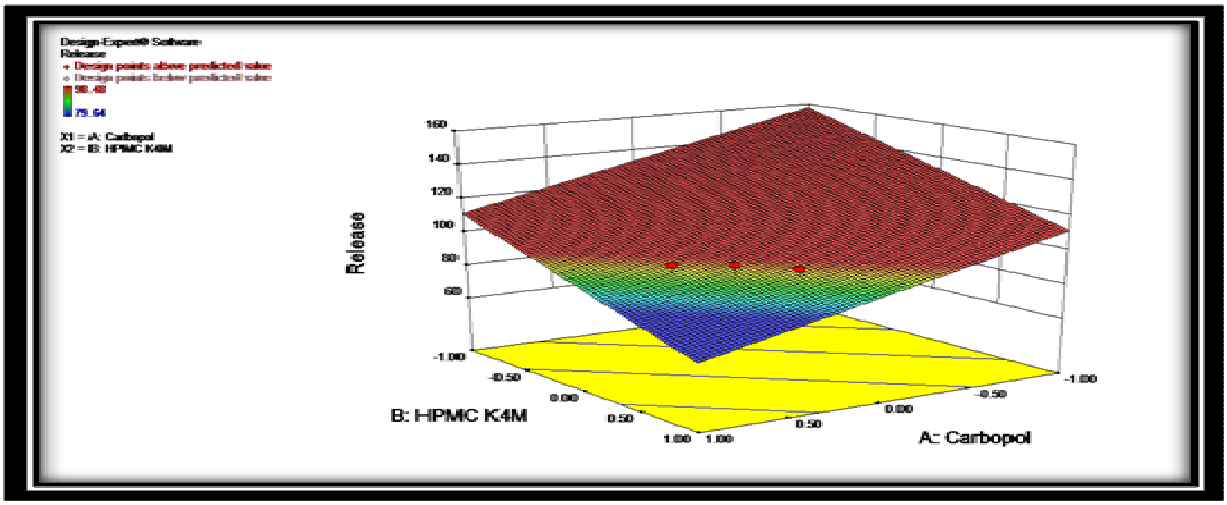

Figure 4: Response surface plot of Carbopol 934 Vs HPMC K4M for % drug release (Numerical Optimization Graphs for mucoadhesive strength)

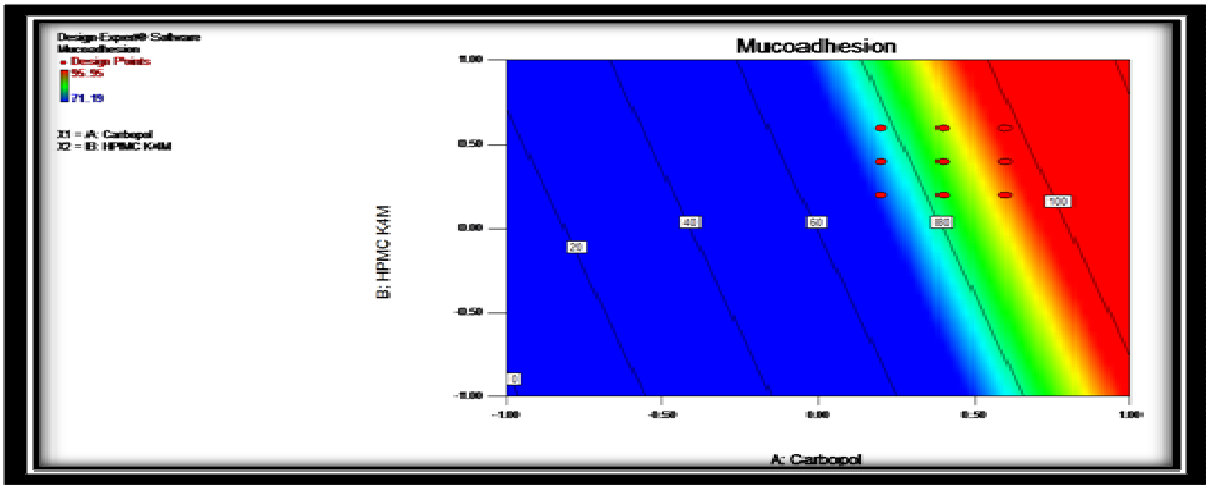

Figure 5: Contour plot of Carbopol 934 Vs HPMC K4M for mucoadhesive strength

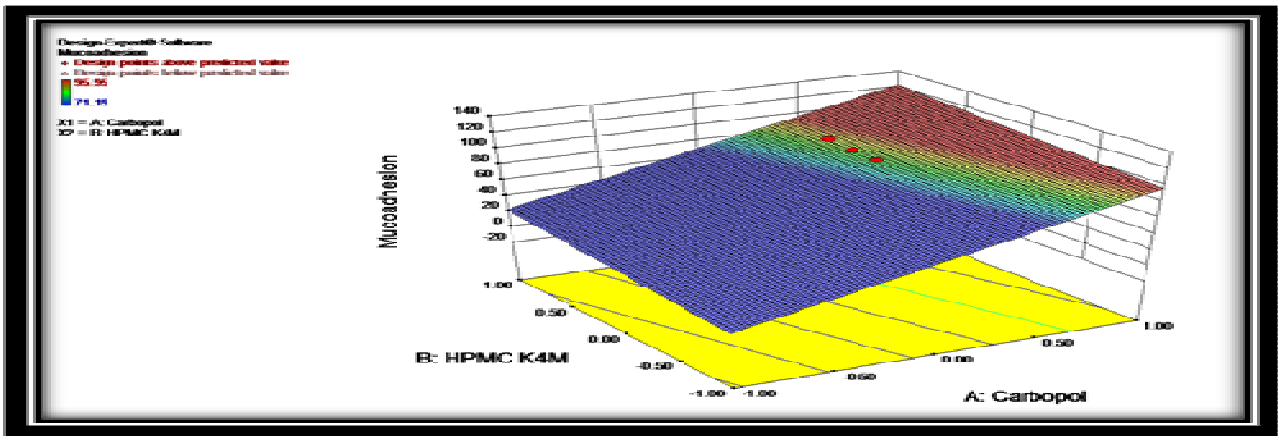

Figure 6: Response surface plot of Carbopol 934 Vs HPMC K4M for mucoadhesive strength

Solutions obtained from design expert software were used for optimization of % dissolution. Solution 1 was choosing for validation of optimized formulation on basis of desirability.

Two different batches of formulation were prepared and studied for evaluation *in vitro* drug release.

Table 9: *in vitro* drug release data from various optimizied formulations

Time (h)	% Cumulative drug release	
	S6 A	S6 B
0	0	0
1	25.17±0.98	23.87±0.42
2	35.65±1.17	34.25±0.56
3	42.33±0.67	41.93±0.58
4	50.57±0.569	49.27±1.36
5	57.43±0.27	56.93±0.79
6	66.88±0.19	64.18±0.87
7	77.11±0.36	75.21±0.69
8	89.19±0.48	87.42±1.25

* All values are expressed as mean ± SD (n=3)

Table 10: Solutions of mucoadhesive strength for optimization

Number	Carbopol 934 (% w/v)	HPMC K4M (%w/v)	Predicted Mucoadhesive Strength (dyne/cm^2)	Desirability
1	0.4	0.6	90.93	1
2	0.4	0.65	88.39	0.943

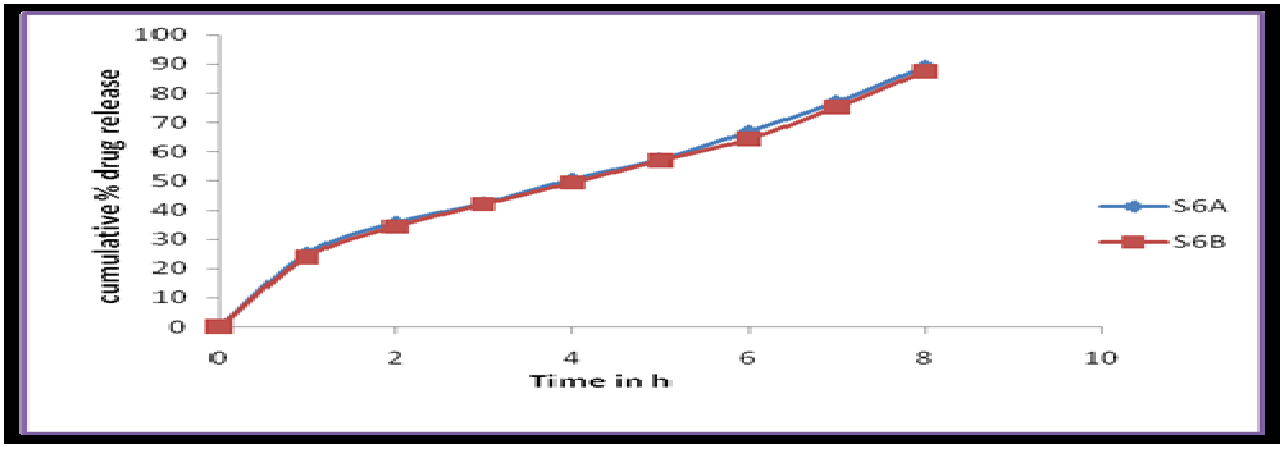

Figure 7: *In vitro* drug release of optimized formulation

Solutions obtained from design expert software were used for optimization of % swelling. Solution 1 was choosing for validation of optimized formulation on basis of desirability.
Two different batches of formulation were prepared and studied for evaluation.

Table 11: Mucoadhesive strength data for optimized formulation

Sr. No.	Formulation code	Mucoadhesive strength	
		Weight in gram (g)	Mucoadhesive Strength (Dyne/cm^2)
1.	**S6A**	8.82+0.418	91.19±0.004
2.	**S6B**	8.71+0.503	88.28±0.004

CONCLUSION

The present study was aimed towards formulating the nasal pH sensitive in situ gels of Beclomethasone Dipropionate using polymer Carbopol 934 and HPMC K4M in different ratios. The in situ gels so prepared were characterized for its gelation properties, viscosity, gel strength, mucoadhesion, drug content, drug release rate. The results of a 3^2 full factorial design revealed that the amount of HPMC K4M and Carbopol 934 significantly affect the dependent variables such as % cumulative drug release, mucoadhesive strength. Thus, it can be concluded that, by adopting a systematic formulation approach, an optimum point can be reached in the shortest time with minimum efforts.

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