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FORMULATION AND EVALUATION OF IN-SITU NASAL GEL OF BUDESONIDE

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Nasal In-Situ, HPMC K4M,Polaxomer 407, Budesonide

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ABSTRACT

Almost 40% of active pharmaceutical ingredients have low oral bioavailability, high hepatic pre-systemic metabolism and also less efficient in crossing the blood brain barrier for brain targeting via oral delivery. So to bypass these problems the nasal drug delivery system has been studied as the nasal drug delivery system comprises of targeting a drug via nasal epithelium. This type of drug delivery has drastic absorptive potential of the nasal mucosa owing to its high permeability because of high perfusion rate.To overcome the limitations of oral and parenteral routes of administration, attempts have been made to employ partial therapy through nasal route. Nasal route offers many advantages mainly avoidance of first pass metabolism, direct transport in to systemic circulation and CNS, rapid absorption, lack of pancreatic and gastric enzymatic activity, and less dilution by gastrointestinal contents. Low permeability and rapid mucociliary clearance of the nasal mucosa to drugs tend to counteract these advantages. Large surface area for drug absorption, rapid achievement of target drug levels.Nasal route is easily suitable for self-medication. The feasibility of drug delivery via the nasal route has received increasing attention from pharmaceutical scientists and clinicians. Budesonide. (16,17-(butylidinebis(oxy))-11,21-dihydroxy-,(11-β,16-□pregna-1,4-diene-3,20-dione)is used to prevent wheezing, shortness of breath, and troubled breathing caused by severe asthma and other lung diseases. It belongs to a class of drugs called corticosteroids.Itexhibits wide range of inhibitory activities against multiple cell types and mediators involved in allergic-mediated inflammation. It is available as tablet,capsule, inhaler and nebulizer.It is readily absorbed from the gastro intestinal tract; the plasma half-life is 2-3.6h and bioavailability of 10-30%. It is 85-90% protein bound.time taken to reach plasma concentration is 1-2h. It is subjected to first pass metabolism in the liver through CYP3A4. It also acts as an anti-inflammatory agent.The prescribed dose of the drug is low (200-400mcg)twice daily, from the above points it is clear that Budesonide is a suitable drug .

INTRODUCTION:[1-5]

Drug are administered traditionally by oral and parentral routes for systemic delivery. The gastrointestinal tract(GI) is the major route of drug entry to the circulation . However for some drugs this route presents problem. the gastrointestinal tract presents a hostile environment .it contains enzymes a wide range of pH conditions and varies in its composition depending upon the presence or absence of food.[1] For many years, drugs have been administered intranasally for their local effect on the mucosa. In more recent years many drugs have been shown to achieve a better systemic bioavailability by self medication through the nasal route than by oral administration.[2]Transmucosal nasal delivery is a promising drug delivery option where common drug administrations, such as intravenous , intramuscular oral are inapplicable. Recently, it has been shown that many drugs have better bioavailability by nasal route than the oral route. This has been attributed to rich vasculature vand highly permeable structure of the nasal mucosa coupled with avoidance of hepatic first pass elimination, gut wall metabolism in the gastrointestinal tract. The physiology of the nose presents obstacles, but offers a g route for non-invasive systemic delivery of numerous therapies and debatably drug delivery route to the brain. Intranasal microspheres, micro emulsions , gels have gained increased intreast in recent years as a delivery system for protein and peptides through nasal route.[3] Today nasal route delivery system is receiving much attention from the pharmaceutical industry. About 2% of the overall drug delivery is administered via the nasal route. The administration of systemically acting products via nasal route began in 1980s. The peptide oxytocin which stimulates uterine contraction and lactation was one of the first nasally administered peptide harmone. Nasal drug delivery is useful delivery method for drugs that are active in low doses and show no minimal bioavailability. The nasal route circumvents hepatic first pass elimination associated with oral delivery, it is easily accessible and suitable for self-medication. Currently, two classes of nasally delivered therapeutics are on the market. The first one comprises low molecular weight and hydrophobic drugs for the treatment of the nasal mucosa and sinus, including decongestants, topical steroids, antibiotics and other products.The second class encompasses a few drugs, which have sufficient nasal absorption fordisplaying systemic effects. Important candidates are the compounds, generally administered by injection and hardly absorbed after oral administration, due to their instability in gastrointestinal tract, poor absorption properties, and their rapid extensive biotransformation.Nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption because it is

permeable to more com-pounds than the gastrointestinal tract due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucus and less dilution by gastrointestinal contents .In recent years many drugs have been shown to achieve better systemic bioavailability through nasal route than by oral administration. Nasal therapy, has been recognized form of treatment in the Ayurvedic systems of Indian medicine, it is also called "NASAYA KARMA".Intranasal drug delivery – which has been practiced for thousands of years, has been given a new lease of life. It is a useful delivery method for drugs that are active in low doses and show no minimal oral bioavailability such as proteins and peptides . One of the reasons for the low degree of absorption of peptides and proteins via the nasal route is rapid movement away from the absorption site in the nasal cavity due to the mucociliary clearance mechanism. The nasal route circumvents hepatic first pass elimination associated with the oral delivery: it is easily accessible and suitable for self-medication. The large surface area of the nasal mucosa affords a rapid onset of therapeutic effect, potential for direct-to central nervous system delivery, no first-pass metabolism, and non-invasiveness; all of which may maximize patient convenience, comfort, and compliance .

MATERIALS AND METHODS:

Balaji chemical gujarat generously gifted the Budesonide, polaxomer 407. were the gift sample from BASF Corporation, Mumbai. hydroxypropylmethyl cellulose of extra pure grade . Benzalkonium chloride was procured from Loba Chemicals, Mumbai, India. All other chemicals were of research grade.

METHOD OF PREPARATION OF NASAL IN-SITU GEL:[6]

The quantities of drug and other ingredients were weighed as per table 7 and formulations were prepared in following manner:

- **Cleaning of glassware and container:** All the glassware's were washed with distilled water and then sterilized by drying at 160-165°C for 1 hr in hot air oven.
- **Preparation of solution 'A':** Accurately weighed quantity (0.1gm) of the Budesonide was dissolved in 10 mL methanol.
- **Preparation of polymer dispersion 'B':** The solutions of Polaxomer 407 and HPMC K4M were prepared using cold method. A certain volume of distilledwater was cooled down to 4°C. Poloxomerand HPMC K4M was sprinkled over 50 mL of deionised cold water separately and was allowed to hydrate for 12 hours to produce a clear solution. Then both the polymer solutions were mix properly with continuous stirring. The

Benzalchonium chloride was added to the above polymer dispersion. Then stored in the refrigerator.

- **Mixing of nasal formulation**: The dispersions were then stored in a refrigerator until clear solutions were obtained and polymer dispersion was slowly added to the drug solution under aseptic condition.

Aseptic filling to container: The formulation was aseptically transferred to previously to previously sterilized glass vials and sealed.

Solubility study of Budesonide: The solubility of Budesonide was checked in different solvents like, methanol, ethanol, chloroform, buffer etc.

Characterization of Drug.

Ultraviolet-visible spectroscopy: [8]

Determination of λ_{max}:

Preparation of Stock Solution

The UV spectrum of Budesonide was obtained using UV jasco V 630.The stock solution of budesonide is prepared by dissolving 100 mg of drug in 100 ml methanol in volumetric flaskwith continuous shaking. 1 ml of sample was withdrawn and diluted to 100 ml of phosphate buffer of ph 6.8 to get 10µg / ml of solution. The solution was than scanned in UV range between 200–400 nm.

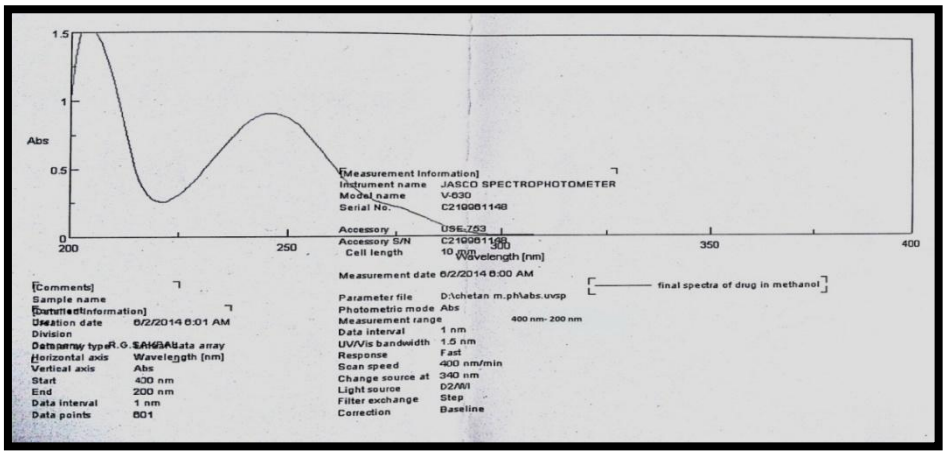

Fig-1 λ_{max} of Budesonide

Preparation of calibration curve:

The prepared stock solution was subsequently diluted to get 2 g/ml, 4µg/ml, 6µg/ml 8µg/ml,10µg/ml. The resulting solutions absorbance was measured at wavelength of 246.0 nm using V using UV jasco 630 spectrophotometric against blank of pH 6.8 buffer. The results obtained were tabulated and plotted a calibration curve of absorbance versus concentration. The slope of the calibration curve is determined by regression equation.

The calibration curve (Fig.2.) was found to be linear in the concentration range of 2-10 ug/ml (Table no .1) having coefficient of regression value R^2=0.999, Y=0.045X+ 0.016.

Table no.1: Absorbance's of different concentration of Budesonide in phosphate buffer.

Sr.no.	Concentration (µg/ml)	Absorbance
1	2	0.1098
2	4	0.1956
3	6	0.2859
4	8	0.3875
5	10	0.4698

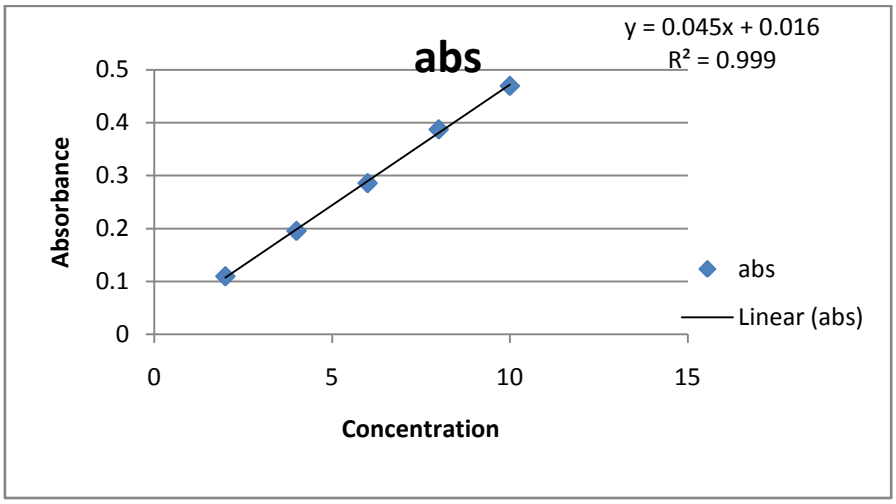

Figure No-2.Calibration curve of Budesonide

Characterization of Polymers[8,9,10]

Description:

The small quantities of each of the excipients were evaluated for its colour, odour and texture.

Table No-2.-Characterization of polymers

Name of excipients	Observation
Poloxomer (407)	White coloured, fluffy, hygroscopic,
HPMC K4M	It is white, yellowish white or grayish white, practically odourless, fibrous powder or granules.
Benzalkonium chloride	Thick gel. It is hygroscopic, soapy to the touch, and has a mild aromatic odour.
Triethanolamine	Triethanolamine is a clear, colorless to pale yellow-colored viscous liquid having a slight ammoniacalodour.
Propylene Glycol	Clear, Colorless Viscous with sweet, slightly acridic taste.

pH:

The pH values of solutions of excipients prepared in specified strength were determined using calibrated (pH 4 and pH 7) digital pH meter.

Gelling property:

Gelling property of polymers were checked by preparing 14-18% w/v aqueous dispersion of polymers. By visual observations fluidity was check to find out concentration of polymers without alkali.

Table no.3: The pH of aqueous solutions of the polymers

Sr. No	Name of excipients	Strength of solution (% w/v)	Observed pH	Reported pH
1	Poloxomer 407	1	4.5	5-7.4
2	HPMC K4M	1	7.5	6.5-8.5

Evaluation of Nasal In-situ

Clarity

On careful visual inspection against dark and white background, all the prepared ophthalmic in-situ gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be clear.

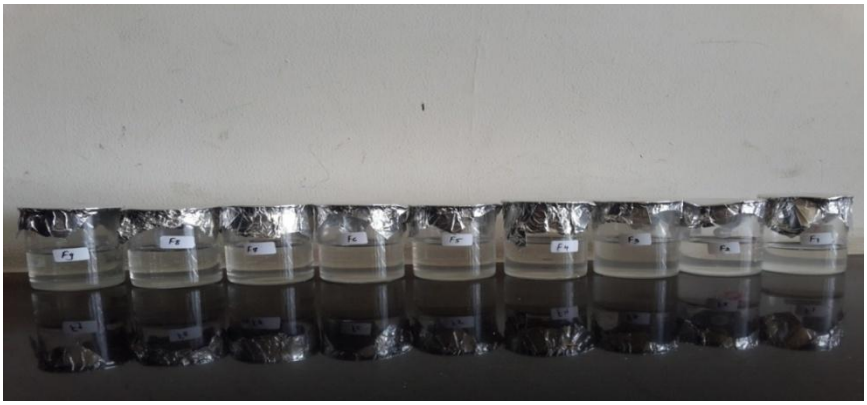

Fig. 3: Formulation Batches

pH of the formulation

The pH of all the formulation batches are shown in Table 4.

Lysozyme is formed in the nasal secretions, which is responsible for destroying certain microbes at acidic pH. Under alkaline pH, lysozyme is inactive and nasal tissue is susceptible to microbial infection. It is therefore advisable to keep the pH of formulation in the range of 4.5-6.5. pH of all the formulation batches were found to be in the range.

Table no 4: pH of the formulation

Sr no	Batch	Reported pH (±S.D.)
1	F 1	6.1 ± 0.01
2	F 2	6.0 ± 0.14
3	F 3	6.3 ± 0.12
4	F 4	5.9 ± 0.2
5	F 5	6.1 ± 0.1
6	F 6	5.8± 0.07
7	F 7	6.0 ± 0.15
8	F 8	6.1± 0.17
9	F 9	6.1 ±0.17

Rheological study [11,12,13]

Viscosity

The rheological properties ofgels were determined by the Brookfield viscometer; type LV 3+ PRO . Viscosity of the formulations were taken at two different temperature that is at 25^0C and the 37^0C with varying shear rate.The viscosities of formulation batches at room temperature are shown in Table 5.

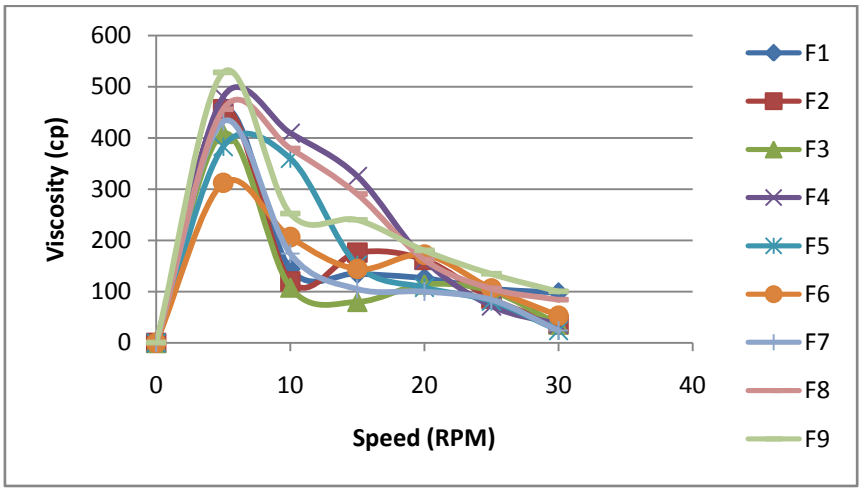

Figure 4: Viscosity profile of formulation batches at room temperature

Table 5.Viscosity of formulation batches at room temperatures

rpm	Viscosity (cps) at Room Temperature								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
5	455.59	455.9	407.9	479.9	383	311.9	431.9	455.9	527.9
10	144	120	108	409.2	359.9	206.2	174	379.2	251.9
15	135.2	175.99	79.98	325	152	144	105	290	239.9
20	126	162	114	162	108	171.98	100	162	180
25	105.6	86.38	100.8	71.98	81.53	105.98	83.98	105.6	134.4
30	87.99	85.25	72.22	84.36	86.23	79.2	89.2	91.8	89.23

Table 6.Viscosity of formulation batchesat 37ºC temperature

rpm	Viscosity (cps) at Room Temperature								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
5	537.2	529.	410.2	487.5	497.9	372	372	507	541.2
10	207.1	290.7	120.9	435.1	401.9	290	290	455.9	327.1
15	121.9	209.2	102	209.7	175	147.2	147.2	175.5	249.9
20	137.2	162	172.3	210	97.2	197.2	197.1	166.7	196.1
25	102.5	86.38	110.2	82.98	100.2	100.5	100.5	207.1	145.2
30	97.6	35.99	47.5	75.2	57.1	59.1	59	97	207

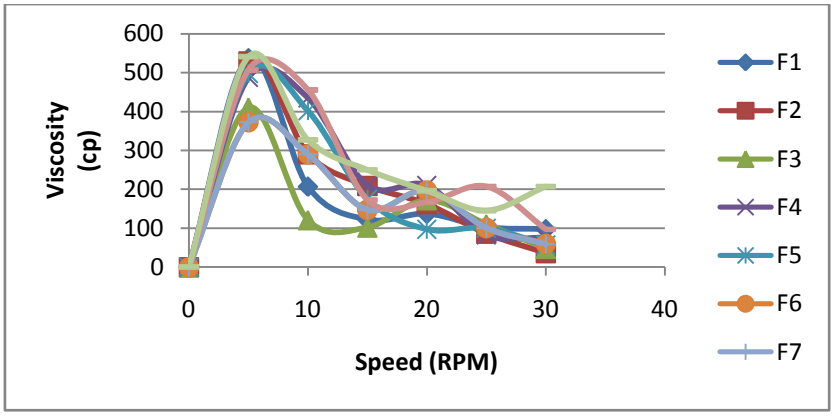

Figure 5: Viscosity profile of formulation batches at 37 c.

Measurement of the Gel Strength

In the development of nasal mucoadhesive gel, the gel strength is important in finding the condition, which can delay the post nasal drip or anterior leakage. The gel strength was found to be affected by concentrations of gelling and mucoadhesive polymers. Optimal mucoadhesive gel must have suitable gel strength so as to be administered easily and can be retained at nasal mucosa without leakage after administration. Gel strength of all formulations showed comparable results as that of viscosity results.

The gel strength at room temperature of the formulation batches is shown in Table 7.

Sr No	Formulation Code	Gel Strength
1	F1	0.55±0.007
2	F2	0.66 ±0.01
3	F3	0.87±0.01
4	F4	0.65±0.01
5	F5	0.75±0.01
6	F6	0.7±0.1
7	F7	0.66±0.1
8	F8	0.65± 0.1
9	F9	0.95±0.1

Table No.8: Gel Strength at 37.4ºC

Sr. No	Formulation code	Gel strength (sec)(±S.D.)
1	F1	0.65±0.01
2	F2	0.75±0.01
3	F3	1.20±0.18
4	F4	0.70±0.01
5	F5	0.54±0.07
6	F6	1.10±0.17
7	F7	0.78±0.007
8	F8	0.76±0.02
9	F9	1.43±0.07

Bioadhesive Strength

The detachment stress of formulation batches is shown in Table 9.

Table 9 Detachment stress of formulation batches

Formulation Code	Detachment Force
F1	0.0850±0.005
F2	0.0617±0.02
F3	0.0850±0.005
F4	0.0850±0.005
F5	0.1079±0.007
F6	0.0948±0.0056
F7	0.3858±0.3
F8	0.1079±0.007
F9	0.1111±0.006

Bioadhesive force means the force with which gels bind to nasal mucosa. Greater bioadhesion is indicative of prolonged residence time of a gel and thus prevents its drainage from nasal cavity. The Bioadhesion force increased significantly as the concentration of

bioadhesion polymers increased. The Detachment Stress was determined for nasal gels. Results of this test indicate that the variable Polaxomer 407 and HPMC K4M both are having effect on bioadhesive strength. It shows that bioadhesive force was increased with the increasing concentration of Polaxomer 407.

Drug content[24]

1ml of each formulation was taken in 10ml volumetric flask, diluted with distilled water and volume adjusted to 10ml. 1ml quantity from these solutions was again diluted with 10ml of distilled water. Finally the absorbance of prepared solution was measured at 246 nm by using UV visible spectrophotometer.The percentage drug content of all prepared nasal formulations was found to be in the range of 71-100%. Therefore uniformity of content was maintained in all formulations.

Table 10: Percent drug content of all formulations. (n=3)

Formulation Code	Drug content (%) (±S.D.)
F1	71.64±0.001
F2	82.50±0.002
F3	86.32±0.003
F4	90.26±0.002
F5	86.78±0.001
F6	91.62±0.001
F7	93.60±0.003
F8	99.53±0.002
F9	95.18±0.001

In-vitro drug release study

The *In-vitro* drug release study of formulation is shown in Table no11

A] Preparation of Simulated Nasal Solution-Weigh accurately 7.45mg/mL NaCl, 1.29mg/mL KCl and 0.32mg/mL $CaCl_2.2H_2O$and dissolve in 1000 mL of distilled water to produce simulated nasal solution; finally adjusted the pH with phosphoric acid to 6.75.

B]*In- vitro* release study of the formulation was carried out using laboratory designed diffusion cell through egg membrane. 0.5 ml of gel was placed in donor compartment and freshly prepared simulated nasal solution in receptor compartment (100ml). Egg membrane was mounted between donor and receptor compartment. Temperature of receiver compartment was maintained at 37 ± 2^0C during experiment and content of the receiver compartment was stirred using magnetic stirrer. The position of donor compartment was adjusted so that egg membrane just touches the diffusion fluid. An aliquot of 1 ml was withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7, and 8 hr. and same volume of fresh medium was replaced. Aliquots so withdrawn were suitably diluted and analyzed using UV visible spectrophotometer at 246nm.

Table 11: Cumulative drug release of all formulations. (n=3)

Cumulative Drug Release (%) (±S.D.)									
Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	14.97±0.05	17.67±0.048	18.81±0.036	18.62±0.025	17.07±0.036	16.4±0.054	16.9±0.01	22.74±0.06	20.54±0.051
2	21.68±0.07	25.83±0.046	26.64±0.024	29.07±0.024	25.5±0.024	24.46±0.058	26.40±0.066	32.60±0.078	29.97±0.052
3	27.92±0.01	31.68±0.046	35.85±0.026	36.32±0.09	32.10±0.016	32.60±0.039	36.82±0.065	43.56±0.054	38.83±0.061
4	34.02±0.05	41.72±0.056	45.96±0.037	46.68±0.062	41.76±0.05	40.46±0.032	41.34±0.01	54.32±0.058	48.85±0.054
5	43.72±0.04	48.43±0.035	55.68±0.052	56.10±0.065	49.92±0.046	48.93±0.021	52.69±0.021	65.25±0.069	60.53±0.058
6	50.35±0.07	56.46±0.027	64.62±0.059	65.35±0.056	57.31±0.01	57.14±0.026	58.54±0.021	77.10±0.096	70.64±0.041
7	58.25±0.01	66.70±0.025	74.27±0.054	74.75±0.054	69.07±0.02	65.59±0.026	69.70±0.058	88.08±0.065	79.56±0.074
8	62.84±0.07	80.02±0.02	81.41±0.063	84.10±0.052	73.82±0.018	73.97±0.021	76.24±0.064	97.84±0.051	80.02±0.056

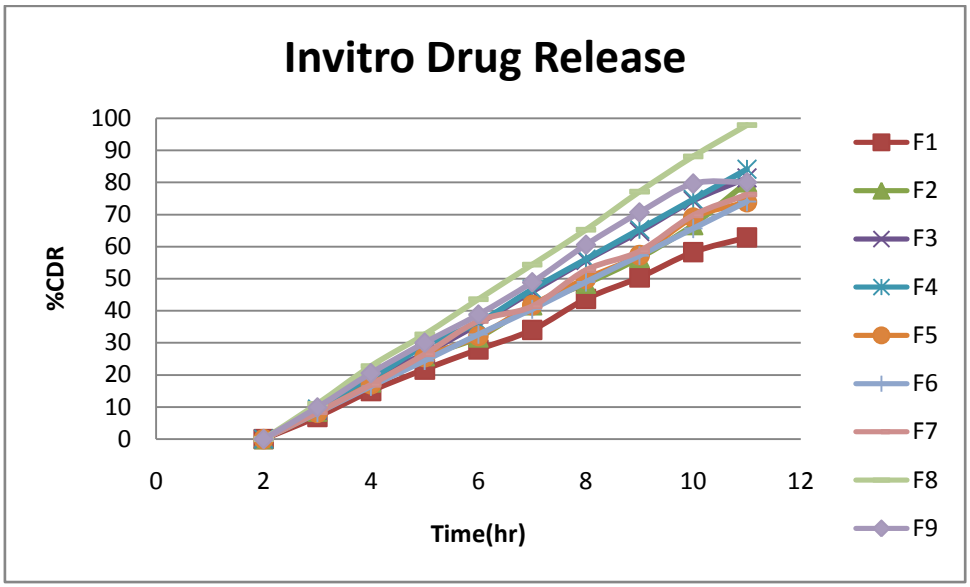

Figure no:6: In-Vitro Drug release

4.7 Optimization

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of polaxomer 407 (X_1) and HPMC K4M (X_2) were selected as independent variables and the dependent variable was % drug release, viscosity and mucoadhesive strength. The data obtained were treated using design expert version 9.0.2.0 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to study the effect of Polaxomer 407 (X_1) and HPMC K4M (X_2) on dependent variable. Table no 8.24 Shows other statistical parameters for the dependent variable % drug release,8.25 for viscosity and 8.26 for mucoadhesive strength.

The values of X_1 and X_2 were found to be significant at $p < 0.05$, hence confirmed the significant effect of both the variables on the selected responses. From this data optimum concentration of polaxomer407 0.1% w/v and HPMC K4M 0.1% w/v was found in F4.

Multiple regression analysis of 3^2 full factorial design batches for in vitro drug release, viscosity and mucoadhesive strength are shown in table 8.21, 8.22 and 8.23 rspectively.

Final Equation in Terms of Actual Factors:

Y1 % CDR=+31.62778+2.08667*polaxomer407+87.75000*HPMC K4M

Table12: Multiple regression analysis for in vitro drug release

Source	Sum of squares	df	Mean squares	Fvalue	Pvalue prob≥ F	Significant/ not significant
Model	566.50	2	283.254	6.45	0.0320	Significant
A- carbopol 940	104.50	1	104.50	2.38	0.1739	
B- xanthan gum	462.00	1	462.00	10.52	0.0176	
Residual	263.59	6	43.93			
Core total	830.10	8				

Final Equation in Terms of Actual Factors:

Y2(Viscosity)=(-80.25)+(+26.77)A+(-382.00)B

Table 13Multiple regression analysis for viscosity

Source	Sum of squares	df	Mean squares	F value	Pvalue prob≥ F	Significant/ not significant
Model	284.62	5	56.62	35.81	0.0071	Significant
A- carbopol 940	72.73	1	72.73	45.76	0.0066	
B- xanthan gum	102.18	1	102.18	64.28	0.0041	
Residual	4.77	3	1.59			
Core total	289.38	8				

Final Equation in Terms of Actual Factors:

Y3(Mucoadhesive Streangth)=(-0.22)+(+0.055)A+(+0.30)B

Table 14: Multiple regression analysis for mucoadhesive strength

Source	Sum of squares	df	Mean squares	Fvalue	Pvalue prob≥ F	Significant/ not significant
Model	0.078	2	0.039	5.27	0.0478	Significant
A- carbopol 940	0.073	1	0.073	9.81	0.0203	
B- xanthan gum	0.031	1	0.03	0.73	0.4259	
AB						
Residual	0.044	6	0.03			
Core total	0.12	8				

Table 15: Other statistical parameters for % drug release.

standard Deviation	R-Squared	% CV	Mean	PRESS	Adequate precision
3.42	0.8876	4.01	85.19	171.72	11.49

Table 16: Other statistical parameters for viscosity

standard Deviation	R-Squared	% CV	Mean	PRESS	Adequate precision
1.26	0.9835	1.48	85.05	57.40	19.177

Table 17: Other statistical parameters for mucoadhesive strength

standard Deviation	R-Squared	% CV	Mean	PRESS	Adequate precision
0.086	0.6371	12.02	0.72	0.11	5.636

The Variance Inflation Factor (VIF) measured how much the variance of that model coefficient was inflated by the lack of orthogonality in the design and was calculated for % drug release, viscosity and mucoadhesive strength. It was found to be near one indicating good estimation of the coefficient. Similarly R-squared was near to zero which led to good model. The values of Prob>F were less than 0.05, which indicated model terms were significant. The linear model obtained from the regression analysis used to build a 3-D graph's in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots. The response surface plot was generated using Design Expert 9.0.2.0 software presented in Fig. 8.16, 8.17, 8.18 to observe the effects of independent variables on the response studied % drug release, viscosity and mucoadhesive strength. From response surface 3 level factorial design was chosen using linear design mode. The range was set from minimum 62.84 to maximum 97.84% for in vitro drug release, 311.9 to 527.9cp for viscosity and 7 to 11gm for mucoadhesive strength. The 9 runs were performed for the response % drug release, viscosity and mucoadhesive strength and model was found to be linear.

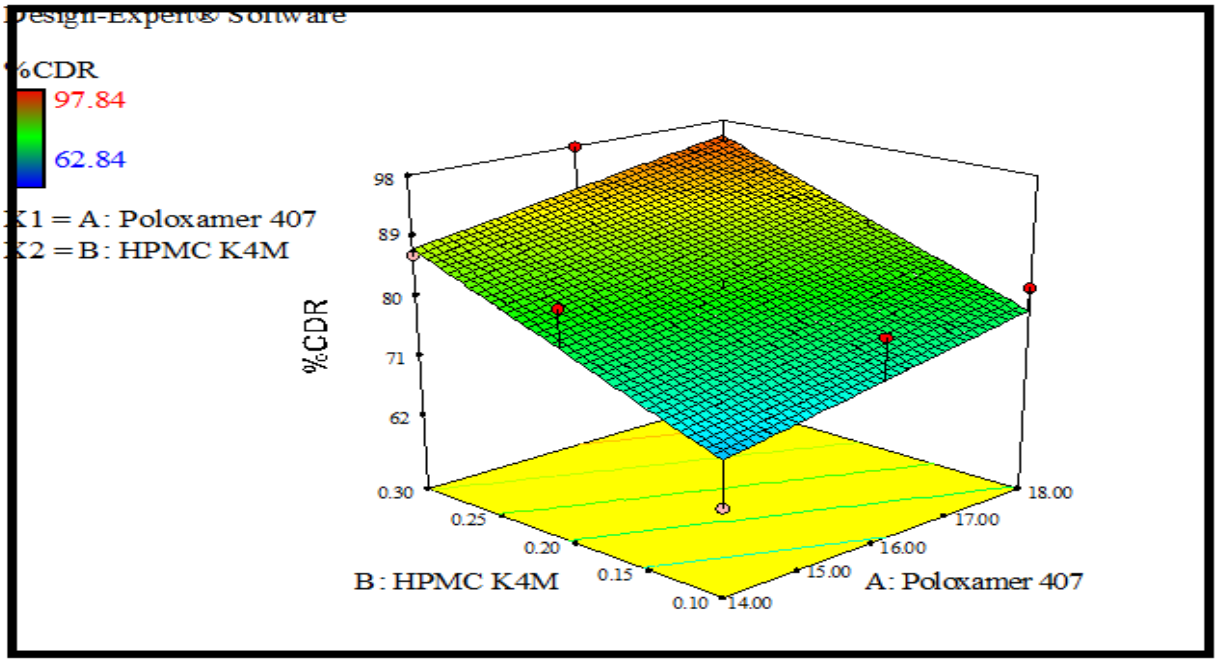

fig.7: Surface response plot showing effect of Polaxomer 407 and HPMCK4M on drug

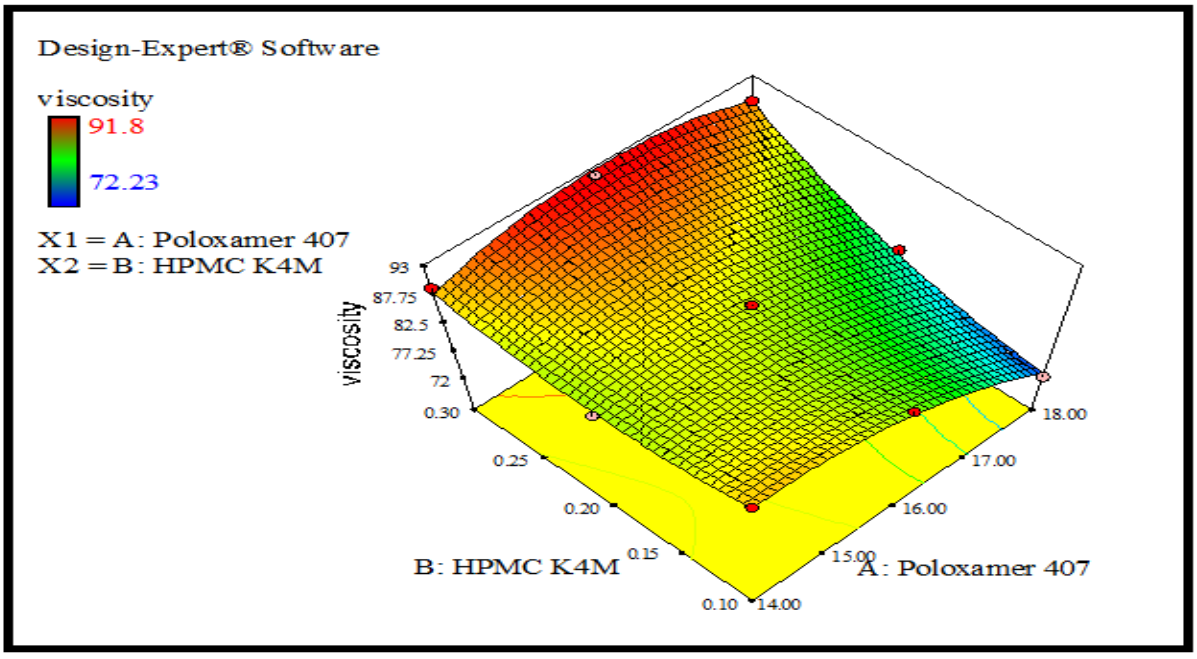

Fig.8: Surface response plot showing effect of Polaxomer 407 and HPMCK4M on viscosity

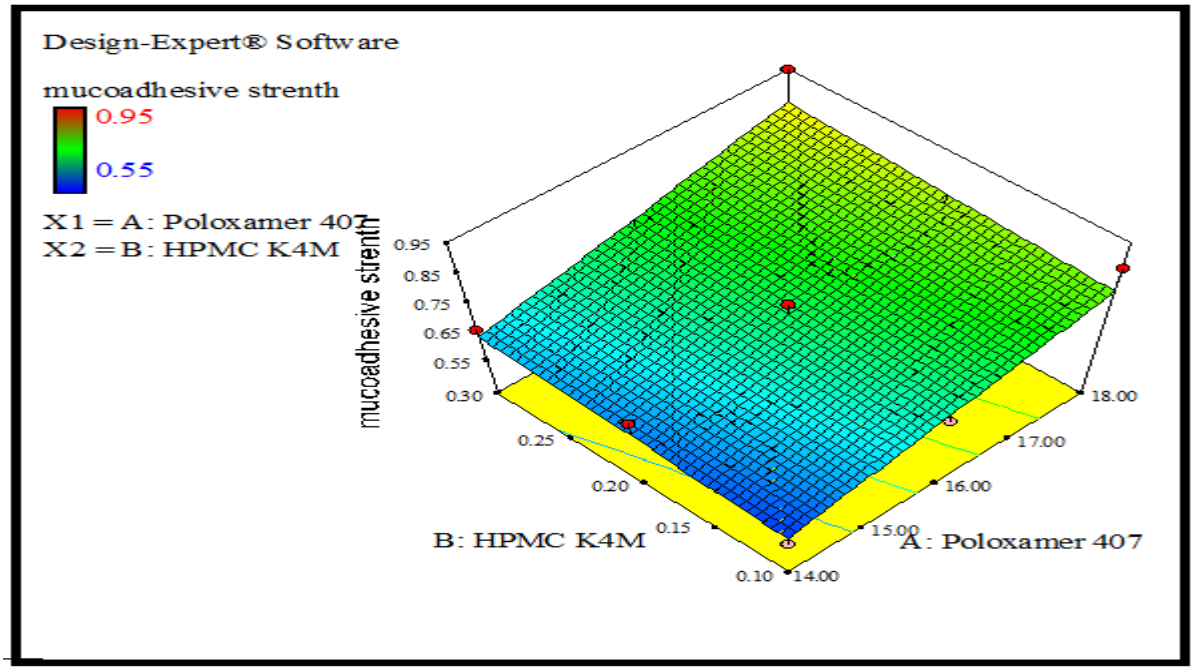

Fig.9: Surface response plot showing effect of Polaxomer 407 and HPMCK4M on mucoadhesive strength.

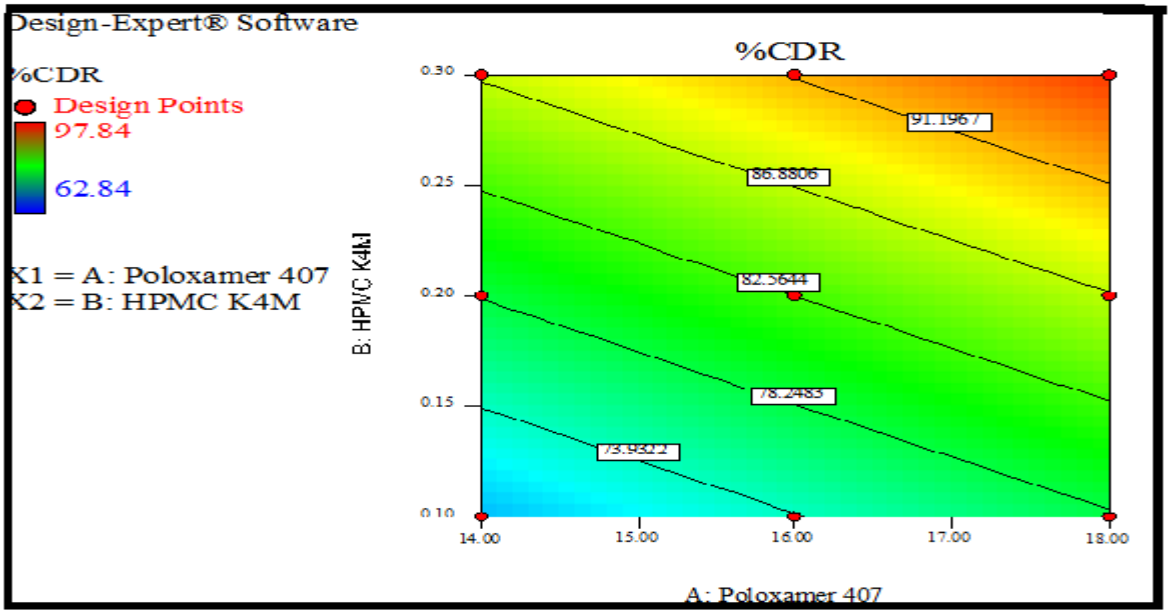

Fig 10: Contour plot showing effect of Polaxomer 407 and HPMC K4M on drug release.

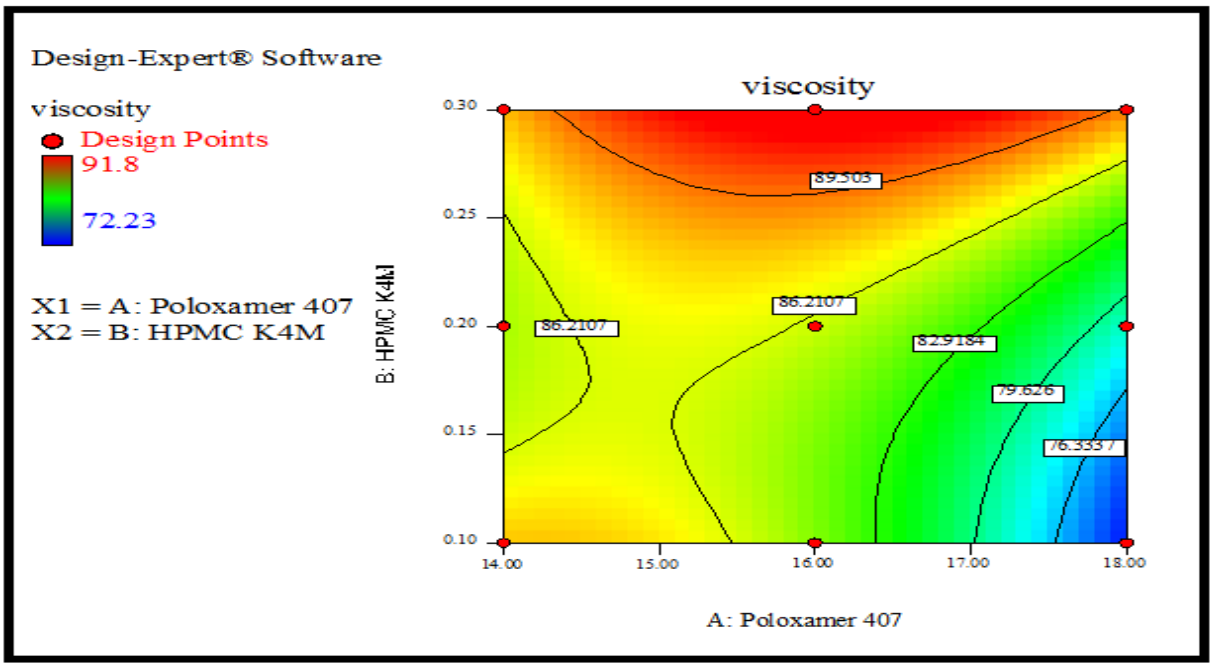

Fig.11: Contour plot showing effect of Polaxomer 407 and HPMC K4M on viscosity

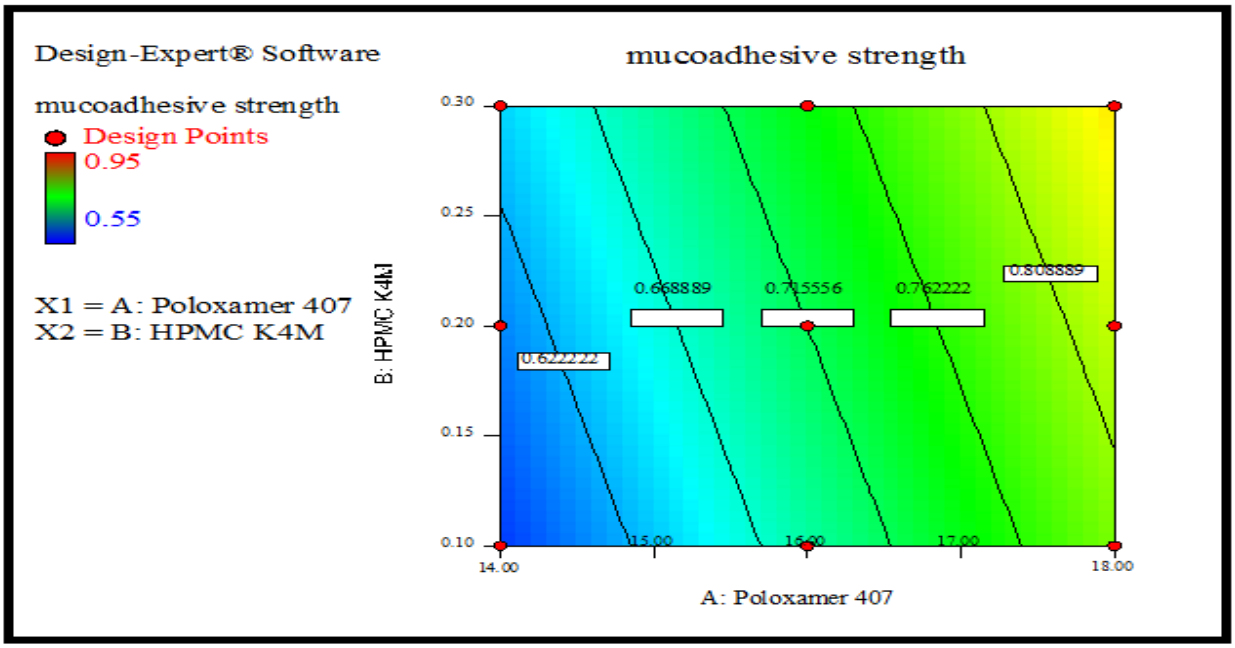

Fig.12: Contour plot showing effect of Polaxomer 407 and HPMC K4M on mucoadhesive strength.

Table No.18 Design Summary

Factor	Name	Units	Type	Min.	Max.	-1 actual	+1 actual	Mean	Std. Dev.
A	Poloxamer407	%w/v	Numeric	14	18	14	18	16	2.82
B	HPMC K4M	%w/v	Numeric	0.1	0.2	0.1	0.2	0.15	0.07

Table No.19: Response summary for drug release

Response	Name	Units	Obs.	Analysis	Minimum
Y1	release	% drug release	9	Polynomial	62.84
Maximum	**Mean**	**Std. Dev.**	**Ratio**	**Trans**	**Model**
97.84	82.56	9.60	1.55	None	Linear

Table No.20: Response summary for mucoadhesive strength

Response	Name	Units	Obs.	Analysis	Minimum
Y2	Mucoadhesive strength	gm	9	Polynomial	0.55
Maximum	**Mean**	**Std. Dev.**	**Ratio**	**Trans**	**Model**
11	0.95	0.117	1.727	None	Linear

Table No.21: Response summary for viscosity

Response	Name	Units	Obs.	Analysis	Minimum
Y3	viscosity	cps	9	Polynomial	72.20
Maximum	**Mean**	**Std. Dev.**	**Ratio**	**Trans**	**Model**
91.80	85.05	5.67	1.271	None	Linear

From design expert version 7.0.0 thirty nine solutions were found in which optimum batch Poloxamer18% w/v and HPMC K4M0.0.2% w/v with desirability 1 was found to be optimum. From this data F8 batch was selected as optimum formulation.

8.8.8 Kinetic Data:[26]

In the present study, the drug release was analyzed to study the kinetics of drug release mechanism. The results showed that the factorial design batches followed first order model kinetics, Higuchi model kinetics and korsemeyer' speppas model, kinetics.

Release Kinetics:

In the present study, the drug release was analyzed by PCP Disso version v3 software to study the kinetics of drug release mechanism. The results showed that the factorial design batches followed Korsmeyer Peppas model kinetics. The R^2 value of Korsmeyer Peppas model was found close to one.

Zero order comparative evaluation model kinetics

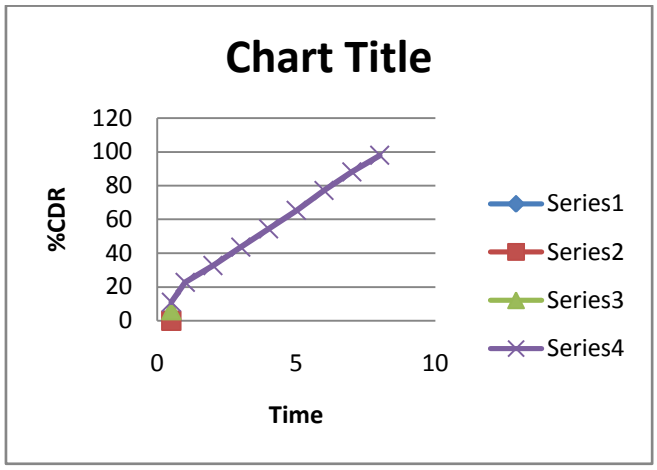

figure No.13: Model graph for comparative evaluation of Zero order release kinetics

Batch	F8
R^2 Value	0.9967

First order comparative evaluation model kinetics

Figure No.14: Model graph for comparative evaluation of First order release kinetics

Batch	F8
R^2 Value	0.8127

Higuchi and Connor's model release kinetics

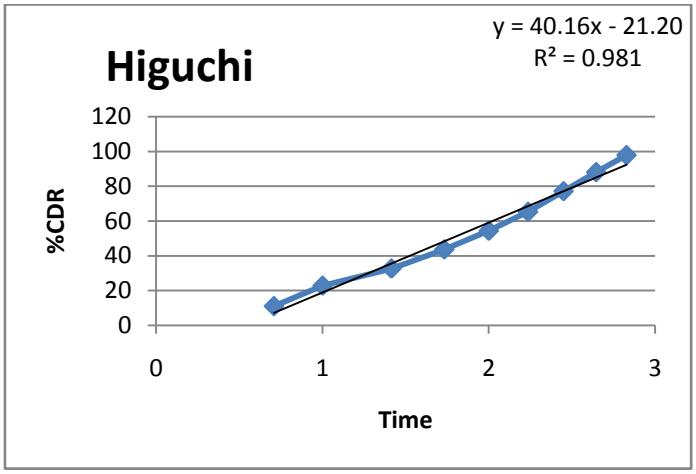

Figure No.15: Model graph for comparative evaluation of Higuchi and Connor's release kinetics

Batch	F8
R^2 Value	0.981

Korsemeyer's Peppas model release kinetics

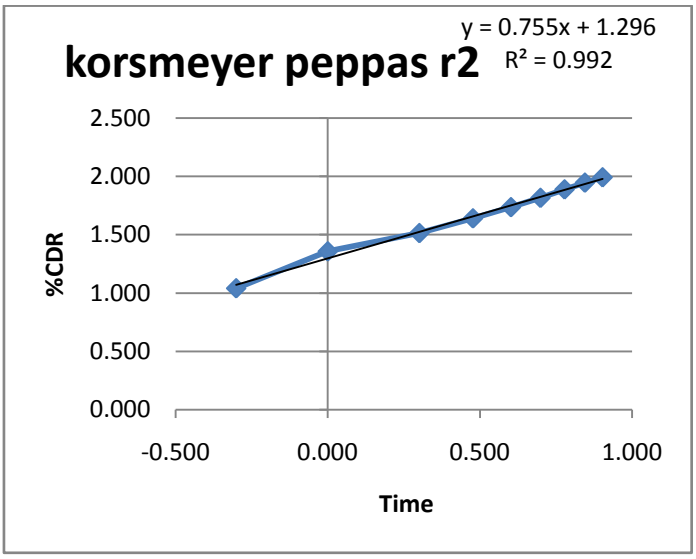

Figure No.16: Model graph for comparative evaluation Korsemeyer's peppas of model release kinetics.

Table no.24: Drug release kinetics for optimized batch

Sr. No.	Model Fitting	R^2 Value	N
1.	Korsmeyer-Peppas	0.992	0.7558

In-vitro permeation study of optimized batch F8

The permeation study of optimized batch F8 was carried out by using laboratory designed diffusion cell in which goat nasal mucosa was use as a diffusion membrane and simulated nasal fluid was used as a diffusion medium. Drug release profile was obtained by plotting percent drug permeation against time (Figure 17) and result of permeation study is given in Table no 25

Table 25: In-vitro permeation study for optimized batch F8

Sr. No.	Time (hrs)	Drug permeation rate (mg/cm/hr) (± S.D.)	% Cumulative drug permeation (±S.D.)
1	30 min	0.0391±0.002	11.95±0.049
2	1	1.8138±0.001	19.65±0.049
3	2	1.0930±0.001	25.29±0.064
4	3	0.9938±0.001	36.61±0.010
5	4	0.9533±0.002	47.11±0.049
6	5	0.9393±0.024	59.98±0.049
7	6	0.8692±0.001	66.16±0.082
8	7	0.7067±0.001	72.62±0.051
9	8	0.6988±0.001	84.21±0.057

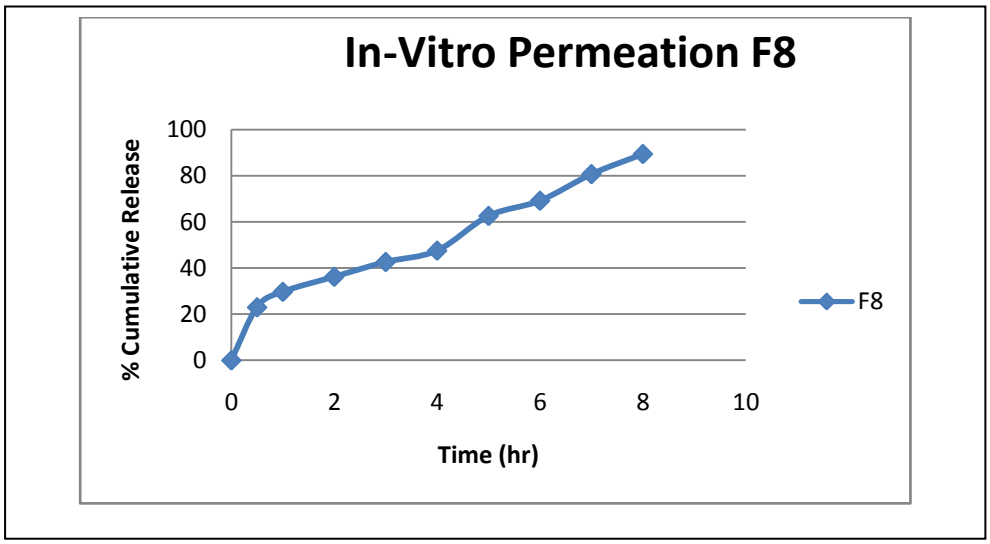

Figure 17: In-vitro permeation release of optimized batch F8

Stability Study[28,98]

Stability study of optimized F8 formulation at room temperature shown in Table 26.

Table 26: Stability study data for F8 batch

Sr. No.	Observation		Before stability testing		During study					
					30 Days		60Days		90 Days	
1.	**Clarity**		Clear		Clear		Clear		Clear	
2.	**Visual appearance**		Transparent		Transparent		Transparent		Transparent	
3.	**pH**		6.1±0.17		6.1±0.15		6.1±0.17		6.1±0.19	
4.	**Viscosity (rpm)**		Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat
		5	455.9	507	455.7	506.8	455.7	506.5	455.6	506.4
		10	379.2	455.9	350.7	455.7	350.5	455	350.4	455
		15	290	175.5	289	175.3	289	175	289	175.5
		20	162	166.7	162	166.4	162	166.6	162	166.4
		25	105.6	207.1	105	207	105.5	207	105.5	106.5
		30	91.8	97	91.8	96.8	91.4	96.4	91.	96.5
5.	**Drug content**		99.53±0.002		99.53±0.0015		99.53±0.019		99.53±0.01	

Stability study of formulation which gave maximum dissolution rate was carried out to point out any visual physical or chemical changes made in the formulation after storing it at elevated temperature and humidity conditions. The optimized formulation was wrapped in vials and stored at room temperature upto three months. Gel was analyzed for the appearance, pH, viscosity, drug content.

Formulations at room temperature were found to be stable upto 3 months. There is no change in drug content, pH, clarity and viscosity.

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