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PREPARATION AND EVALUATION OF IN SITU GEL CONTAINING ACYCLOVIR

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ABSTRACT

In situ gel dosage forms were successfully used as drug delivery systems to prolong the residence time and to reduce the frequency and amount of administration. The main objective was to prepare and evaluate oral mucoadhesive in situ gel containing acyclovir based on the concept of pH triggered system. The system utilizes polymers that exhibit sol to gel phase transition due to change in specific physico-chemical parameters. A pH triggered system consisting of carbopol 934P along with hydroxypropylmethylcellulose was used to prolong the release of acyclovir, Formulations were evaluated for sol-gel temperature, gelling capacity, pH, viscosity, spreadability, gel strength Mucoadhesive force, drug content and in vitro release. The use of carbopol as in situ gel forming system was sustained by the property to transform into stiff gels when the pH was raised. The viscosity was found to be in the range 82-199 centipoise for the sol, where for the gel was up to 1594 centipoise. The maximum gel Strength and mucoadhesion was found to be up to 50.8dynes/cdynes/c2 and 6219 dynes/cm2 respectively. The optimized formulations were able to release the drug up to 97.2%. In situ gel formulation of Acyclovir with mucoadhesive properties was found to be promising for prolonging buccal residence time and thereby better therapeutic effects.

KEYWORDS: Acyclovir, In Situ Gel, Mucoadhesive, Carbopol, HPMC.

1. INTRODUCTION

Acyclovir (ACY), a widely used antiviral agent, is a synthetic purine nucleoside analog derived from guanine, the first agent to be licensed for the treatment of HSV infection, also the most widely used drug for infections such as cutaneous herpes, chickenpox, vericellazoster infection. Herpes simplex virus is a member of family of herpes viridae, a DNA virus, there are 2 types of HSV, HSV type 1 and type 2. Type 1 is herpes viruses that usually responsible for cold sores of mouth so called fever blisters and type 2 is the one that most commonly cause genital herpes the infection cause painful sores on the genital herpes in both men and women.[1]

The presently available conventional therapy is associated with a number of drawbacks such as highly variable absorption and low bioavailability (10-20%) after oral administration.[2] furthermore, with increase in dose, there is decrease in bioavailability. more over, because the mean plasma half lIfe of the drug is 25 hours, 5 times a day administration is required. In order to make oral therapy of acyclovir more patient complaint there is a need to change the formulation as novel drug delivery system – one of the best as in situ gel formulation.[3]

In situ gelling systems can be classified as ion-activated systems (eg. Gellan gum and sodium alginate), temperature dependent systems (eg. Pluronics, tetronics and polymethacrylates) and pH-triggered systems (eg. Carbopol and cellulose acetate phthalate).[4] The principal advantage of in situ gels are ease of administration, lower dose and frequency, improved local bioavailability, patient compliance and comfort. The composition is also less complex and manufacturing coast.[5]

The present research was devised as an attempt to formulate and evaluate an in situ gel containing acyclovir by using various concentration of Carbopol and Hydroxy propyl methyl cellulose. The study thus aimed to increase absorption of the drug leading to an improvement in its bioavailability to reduce its dosing frequency and to achieve sustained release effects.

2. MATERIALS AND METHODS

2.1 Materials

Acyclovir was purchased from YarrowChem, Mumbai. Carbopol 934 was purchased from Kemphasol, Mumbai. HPMC was purchased from. Tween 80 was obtained from Burogyne Burdidges & Co, Mumbai. Ethanol was procured from Medilise, Kannur.

2.2 Methods

2.2.1 Preparation of in situ gel of acyclovir

In situ formulations were prepared using carbopol in combination with HPMC using cold method

- Slowly added HPMC in cold water with continuous agitation. The formed mixture in a beaker were stored overnight at 4^0C
- Carbopol 934 was kept overnight to swell sufficiently in phosphate buffer to adjust the pH
- Carbopol which was allowed to swell was slowly added to HPMC solution with continuous stirring on the magnetic stirrer
- Acyclovir was dissolved in a mixture of Ethanol and tween 80
- The drug solution was then added to polymer solution with constant stirring using magnetic stirrer until a uniform solution was obtained and the volume completed with distilled water.[6][7]

Design Expert Stat Ease Software was used to design formulations. 9 formulations with mucoadhesive polymer HPMC and gelling polymer Carbopol in different concentration were suggested by the software. The formulation is shown in table no.1

Table no.1: Formulation of Acyclovir in situ gel.

INGREDIENTS (in g or ml)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Acyclovir	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol	0.1	0.3	0.3	0.75	0.75	0.75	1.2	1.2	1.4
HPMC	0.75	0.3	1.2	0.1	0.75	1.4	0.3	1.2	0.75
Ethanol + tween 80 (1 : 2 ratio)	15	15	15	15	15	15	15	15	15
Distilled water	50	50	50	50	50	50	50	50	50

3. EVALUATION METHODOLOGY

3.1 pH

The pH is the one of the most important parameter for in situ gel formulation was directly measured using digital pH meter. Determination was carried out in triplicate and an average of these determinations was taken as the pH of gel.[8][9][10]

3.2 Viscosity

Viscosity was measured at 25^0 C and 35^0 C using Brookfield viscometer and spindle number 63 at 100 rpm. First the viscosity of gel solution was measured this solution was allowed to convert to gel by increasing the pH and temperature of the solution with the help of phosphate buffer pH 6.8 on water bath whose temperature was maintained at 37 ± 1°C. Determination was carried out in triplicate and an average of these determinations was taken as the viscosity of gel.[11][12][13][14]

3.3 Sol to gel temperature

The formulation was taken in test tube containing phosphate buffer pH 6.8. This mixture thoroughly mixed and dipped into water bath whose temperature was maintained at 37 ± 1°C.for 2min. The temperature at which solution was converted to gel was noted down by placing the thermometer in the test tube. The maximum limit for gelation was checked upto 60^0C. The gel was said to have formed when there was no flow of the formulation when the container was over turned.[15][16][17][18]

3.4 Spreadability

Spreadability of formulation was determined by using an apparatus designed and developed in laboratory especially for project. Two rectangular glass plates of same dimension were selected. 500mg of sample was placed over the other one to sandwich sample between plates. A 20g weight was placed on top of upper plate to provide a uniform thin film of sample between the plates. Weight was removed. Excess of the in situ gel sample was scrapped off from edges. The top plate was then subjected to pull by using string to which 50mg weight was added. The time required by upper plate to travel a distance of 6cm and separate from lower plate was noted. This was repeated for 3 times. A shorter in travel indicates better spreadability. experiment was repeated and average of 3 attempts were evaluated for each formulation using following formulation.[19][20][21][22]

$$\text{spreadability} = \frac{M.L}{T}$$

M= weight tied to upper slide, L= Length of glass slide and T= Time in seconds

3.5 Determination of gelling capacity

To study in vitro gelling capacity of prepared formulation, simulated saliva is used. 2ml of simulated saliva was placed in a 15ml of borosilicate glass test tube and maintained at 37 ± 2°C. 1ml of formulation to be evaluated was added with a 1mo pipette. The formulation was added in such a way that places the pipette at the surface of fluid in test tube and was released slowly. As the formulations comes in contact with the simulated saliva it was immediately converted on the basis of stiffness of formed gel and time it remains such as visual assessment of the gel as it forms time for gelation as well as time taken for the gel formed to dissolve was monitored during this test.[23][24][25][26]

3.6 Gel strength

30g of the gel was taken in a 50ml beaker and a 50g weight was placed on the surface of the gel and allowed to penetrate through the gel. The time taken by the 50g

weight to penetrate 5cm down through the gel was noted for the all the formulation. The same method was followed for 3 times for each fresh formulation and average time was noted.[27][28][29][30]

3.7 Drug content analysis

The drug content in each Unit dosage form was determined by UV Spectroscopy. The weighted amount of gel equivalent to 2mg of drug was accurately taken and dissolved in phosphate pH 6.8. The UV absorbance of the sample was determined at a wavelength of 231nm. The drug content for batch was measured in triplicate and the average value were recorded.[31][32][33][34]

3.8 Mucoadhesive force

The mucoadhesive forces of the formulas determined using modified physical balance method. This equipment compromised a two-arm balance, one side of which contained two glass plates and the other side contained a beaker.

The membrane used for mucoadhesive testing was fresh sheep buccal mucosa. Fresh sheep buccal mucosa was sprinkled by phosphate buffer (pH 6.8), then fixed using rubber band or glue to the upper slide of the lower plate and another was glued to lower side of the upper plate using rubber band. The in situ gel was placed on the mucosal membrane fixed to the upper slide of the lower plate. Then, the upper plate was placed over the lower plate and 5g preload force (contact pressure) was applied for 2min (preload time). After removal of the preload force, the water was added slowly to previously weighted beaker placed on the right hand pan until vial gets detach, The mucoadhesion force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detaches the tissue from the surface of each formula using the following equation [35][36][37].

Detachments stress dynes/cm^2 = m.g/A

Where, m = The weight added to the balance in g,

G = The acceleration gravity 980 (cm/s^2) and A = Area of tissue exposed,

3.9 In vitro drug release study

The study of Acyclovir in vitro drug release from the in situ gel formulation was conducted using cellophane membrane for a period of phosphate buffer was the dissolution medium of pH 6.8. Cellophane membrane was tied to one end of the glass cylinder. previously soaked overnight in the dissolution medium. Then 1ml of the formulation was wrapped in cellophane membrane and placed in phosphate buffer. The dissolution medium was stirred with magnetic stirrer at 50 rpm. The sample was collected at regular intervals and replaced by a receptor medium volume similar to that. At the time interval predetermined 1ml of the sample was taken and spectrophotometrically analyzed at 252nm,[38][39][40]

3.10 Optimization Study

Optimization Of the Formulations Was Studied By Box-Benken design. The amount of Carbopol (A) and HPMC (B) were selected as independent variables and the dependent variables were viscosity and in vitro drug release. The data obtained were treated using design expert version --- software and analyzed statistically using analysis of variance (ANOVA).[41] The data were also subjected to 3-D response surface methodology to study the effect of carbopol and HPMC on the dependent variable.

3.11. Analysis of release mechanism

To examine the drug release kinetics, the release data of optimized formulation was fitted to models representing Zero order, First order, Higuchi model, Hixson crowell and Kosmeyer's peppas model.

3.12. Stability study

The stability studies was carried out to determine the physical and chemical stabilities of prepared formulation were kept in air tight container covered with aluminum foil at refrigerated temperature, 4^{0}C for a period of 3 months. The formulation was evaluated visually and for its gelation behavior, viscosity, drug content and in vitro drug release.

4 RESULTS AND DISCUSSIONS

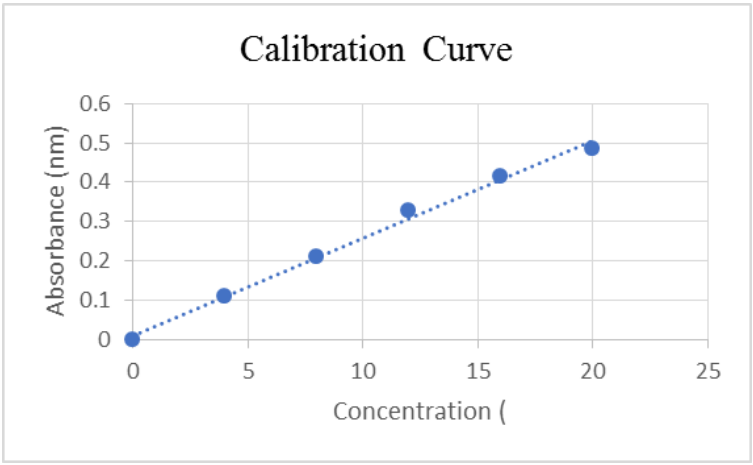

Figure no. 1: Standard graph of Acyclovir in Phosphate buffer pH 6.8.

4.1 Fourier Transforms Infra-Red (FTIR) Spectrophotometric Analysis

FTIR spectroscopy was used to ensure that no chemical interaction between the drug and polymers had occurred. From the FTIR spectral figures 2&3 interpretations the following peaks was obtained. (Table no.2) and Typical peaks of drug and polymers while no new bands or point changes existed suggesting no interaction between drug and polymers.

Table no. 2: Characteristic frequencies in FTIR spectrum of Acyclovir.

Sl.No.	Wave number (cm^{-1})	Inference
1	3441.02	N-H stretching
2	3308.74	O-H stretching
3	3099.14	C-H stretching
4	1717.21	C=O stretching
5	1541.50	C=C and C=N stretching
6	1308.75	$-CH_2$ wagging and twisting
7	1216.74	Aryl alkyl ether
8	1105.79	C-O stretching
9	1083.93	C-O-C stretching

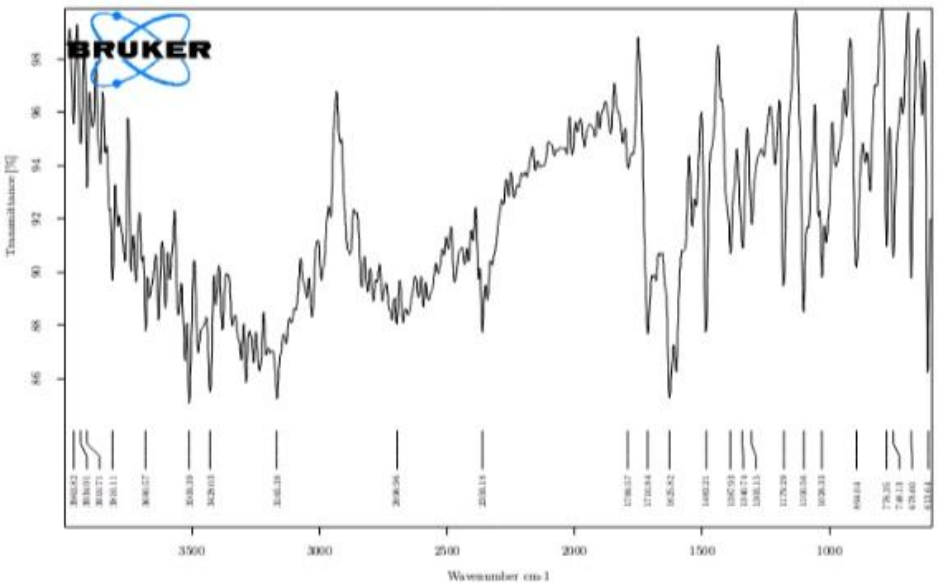

Figure no. 2: FTIR spectrum of pure drug (Acyclovir).

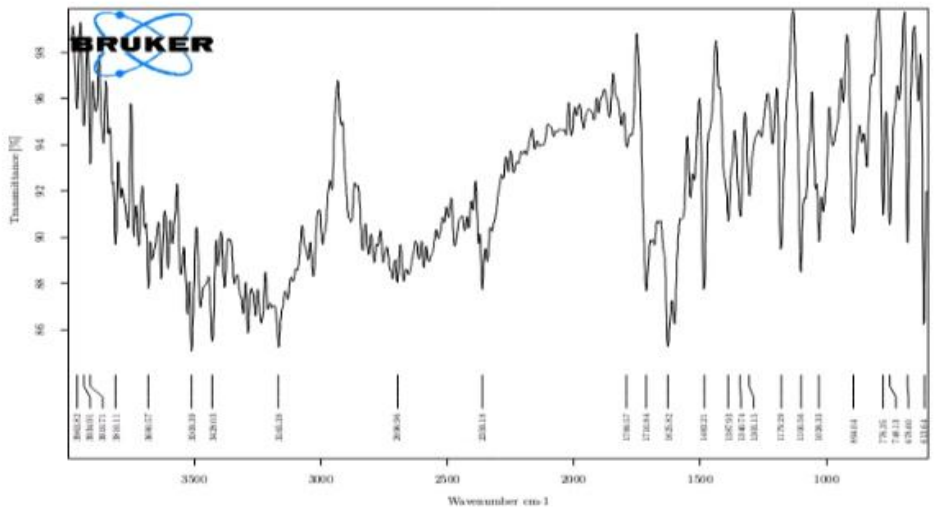

Figure no. 3: FTIR spectrum of Drug + Polymer (Carbopol + HPMC).

4.2 pH

The pH of the formulations was found to be in the range of 6.1-6.3. This indicated the non-irritancy of the formulation in oral cavity. Which suggests the prepared in situ gel formulations as an optimal dosage type to be delivered in a oral cavity without any possible inflammation and improved patients compliance.

4.3 Viscosity

Viscosity of formulation at solution state and gel state was found to be in the range of 82.The viscosity was proportional to the concentration of the mucoadhesive polymer in the formulation. All the formulations exhibited quite low viscosity at low temperature. However, upon increasing the temperature and pH, a gel was formed in well-defined temperature and viscosity of the formulation was increased.

Table no. 3: viscosity of formulations.

Formulation Code	Viscosity (cps)	
	Sol	Gel
F1	98 ± 0.36	1481 ± 0.24
F2	85 ± 0.35	1385 ± 0.83
F3	159 ± 0.70	1564 ± 0.46
F4	82 ± 0.42	1350 ± 0.80
F5	104 ± 0.44	1544 ± 0.80
F6	199 ± 0.60	1594 ± 0.32
F7	93 ± 0.48	1454 ± 0.80
F8	172 ± 0.30	1583 ± 0.60
F9	149 ± 0.68	1560 ± 0.24

All values are expressed as mean ± SD, n=3

4.4 Sol to gel temperature

The gelation temperature of all the formulations was in the range of 33^0C to 35^0C, While the transition temperature of the formulations containing tween 80-ethanol was slightly higher because ethanol might have increased the transition temperature

Table no. 4: Sol-gel temperature of formulations.

Formulation Code	Sol -gel temperature (^{0}C)
F1	35 ± 0.56
F2	35 ± 0.72
F3	34 ± 0.51
F4	34 ± 0.45
F5	34 ± 0.65
F6	35 ± 0.85
F7	35 ± 0.53
F8	33 ± 0.98
F9	33 ± 0.87

All values are expressed as mean ± SD,n=3

4.5 Spreadability

With increase in the concentration of the polymeric component, viscosity of solution was increased. At the same time spreadability of the formulation was reduced. This can be observed from the evaluation test data compiled in table no.5

Table no.5: Spreadability of in situ formulations.

Formulation Code	Spreadability
F1	23 ± 0.58
F2	25 ± 0.22
F3	20 ± 0.18
F4	25 ± 0.56
F5	22 ± 0.49
F6	18 ± 0.46
F7	24 ± 0.72
F8	19 ± 0.64
F9	21 ± 0.85

All values are expressed as mean ± SD, n=3

4.6 Determination of gelling capacity

Concentrations of gelling and bioadhesive polymers were found to impact the gel composition with appropriate tolerance to water is crucial. Table no.8 shows the measurement of the gel strength data. After 15 minute, formulation F2 containing lower polymer concentration displayed the poorest gelation and dispersed rapidly while shaking. Formulation F6 containing higher polymer concentration demonstrated immediate gelation effect and the gels formed were rigid and continued to be stable for a prolonged period. This study showed that the fluid intensity of the formulation of the in situ gel was 33-35^{0}C, Which increased as the HPMC concentration increased.

Table no. 6: Gelling capacity of in situ formulations.

Formulation Code	Gelling Capacity
F1	++
F2	+
F3	++
F4	+
F5	++
F6	+++
F7	+
F8	++
F9	++

All values are expressed as mean ± SD, n=3

4.7 Gel strength

The results obtained for strength test of all the formulations are mentioned in table no.7. It has been observed that gel strength increased with the increase in the concentration of mucoadhesive polymer in the formulation.

Table no. 7: Gel strength of in situ formulations.

Formulation Code	gel strength
F1	35.6 ± 0.23
F2	25.4 ± 0.20
F3	42.6 ± 0.23
F4	19.7 ± 0.15
F5	32.3 ± 0.08
F6	50.8 ± 0.11
F7	28.5 ± 0.14
F8	46.3 ± 0.11
F9	38.2 ± 0.07

All values are expressed as mean ± SD, n=3

4.8 Drug content analysis

All the formulations reflected fairy uniform drug content ensuring adequacy in the method of preparation of the in situ gel. Drug content was found to be within the range of 98.06 – 99.71%.

Table no. 8: Drug content of in situ formulations.

Formulation Code	Drug content
F1	98.24 ± 0.07
F2	98.06 ± 0.11
F3	98.92 ± 0.13
F4	98.11 ± 0.31
F5	98.40 ± 0.33
F6	99.28 ± 0.44
F7	98.24 ± 0.57
F8	99.71 ± 0.26
F9	98.95 ± 0.31

All values are expressed as mean ± SD, n=3

4.9 Mucoadhesive force

The mucoadhesion strength is one of the most important physicochemical parameters for prolonging mucoadhesive retention time and thereby better therapeutic effects of the mucoadhesive polymer. The degree of mucoadhesion depends on type and concentration of polymer, excipients used in the dosage form, degree of hydration, polymer chain length, and molecular weight of the polymer. The mucoadhesion properties of the formulations of varying ratio of polymers are shown in table no.11.

It was seen that formula F6 which contains Carbopol 0.75 and 1.4 HPMC gave best result among the other formula, this may be attributed to the effect of hydrophilic properties of carbopol, that resulted in a hydration of polymeric chains which involve glycoprotein chain of mucin in the oral mucous membranes in addition the HPMC, appeared to have maximum mucoadhesive force compared with other viscosity enhancer used. The latter result is reffered to in an increasing the number of penetrating hydrophilic chain to glycoprotein with concentrations of polymer increase.

Table no.09: Mucoadhesive force of in situ formulations.

Formulation Code	Mucoadhesive force (dyne/cm^2)
F1	5013 ± 0.38
F2	4360 ± 0.95
F3	5561 ± 0.91
F4	4274 ± 0.12
F5	5047 ± 0.60
F6	6219 ± 0.68
F7	4692 ± 0.11
F8	5843 ± 0.80
F9	5321 ± 0.84

All values are expressed as mean ± SD, n=3

4.10 In vitro drug release study

Drug loaded in situ gel Formulations were conducted for in vitro release experiments was carried out using pH 6.8 phosphate buffer in the diffusion medium for 8 h. The formulations F3 released 92.8% of the drug within 8 h. The immediate release of the drug that was observed in formulations was due to the low concentration of HPMC. These observations indicated that the drug's immediate release is attributed to the weak gelling capacity at low concentration of HPMC. Due to higher amount of HPMC than other formulations, the formulation containing 1.4g HPMC showed about 97.2% of the drug release within 8 h. Study of the release of drugs in vitro revealed that the release rate was dependent on concentration of HPMC. The greater the concentration of HPMC, the lower the drug release rate.

Table no. 10: In vitro drug release of in situ formulations.

Time (hrs)	Cumulative Percentage Drug Release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	1.62	1.28	1.92	1.31	1.45	1.83	1.24	1.71	1.95
2	11.84	11.56	12.01	11.65	11.66	12.60	11.45	11.92	12.14
3	13.92	13.11	14.62	13.60	13.81	13.78	12.92	13.81	14.11
4	30.41	29.98	30.98	30.11	30.22	35.98	29.65	30.98	31.25
5	42.20	41.81	43.06	42.01	41.98	48.85	45.62	43.67	43.18
6	68.28	66.50	69.37	67.21	67.82	72.59	65.23	71.61	69.21
7	74.93	74.01	77.71	74.83	76.52	83.59	74.36	80.61	75.64
8	85.3	82.1	92.80	82.9	90.6	97.2	84.2	94.2	87.9

4.13 Optimizaton

The formulation is optimizd by Design expert Software version 13.0.7.0. Box-Behnken design was used to find the optimized formulation.9 formulations were suggested by the software and after optimization of the analyzed data, 12 solutions were obtained. From the 12 solutions, one was selected by considering the Viscosity and *in vitro* drug release. The batch with carbopol- 0.75g, HPMC- 1.4g with desirability 1 was found to be optimum. From this data, formulation F6 was selected as the optimized formulation.

Out of 9 formulations prepared, F6 containing 0.75g carbopol as gelling agent and 1.4g HPMC as mucoadhesive agent was selected as the best formulation after optimization as it showed the highest values for viscosity and *in vitro* drug release.Further studies including kinetic study and stability study were conducted on selected formulation as per ICH guidelines.

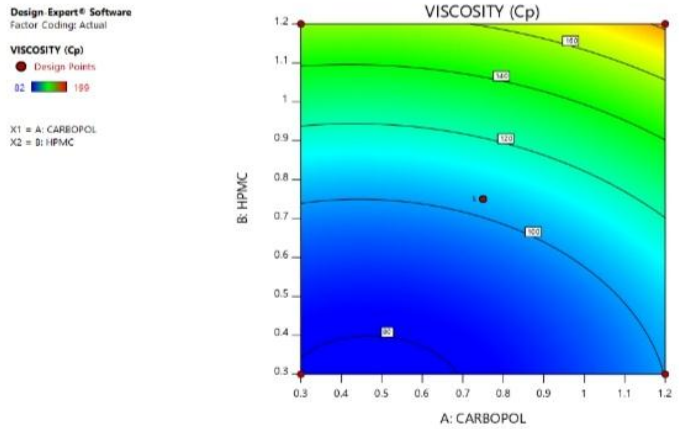

Figure no. 4: Countour plot showing the effect of carbopol and HPMC on Viscosity.

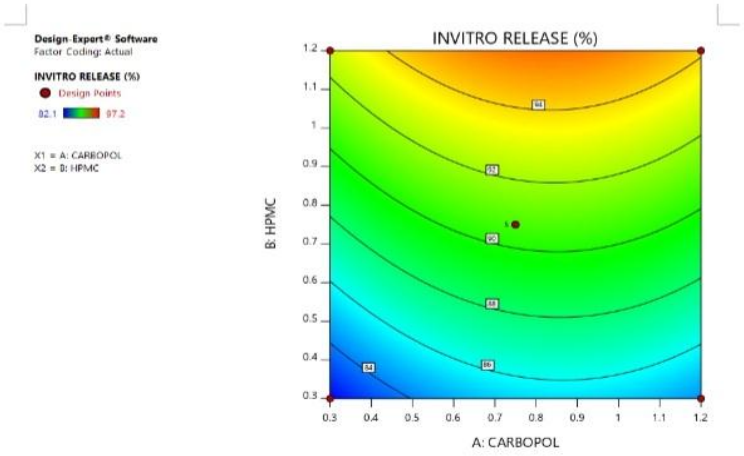

Figure no.5: Countour plot showing the effect of carbopol and HPMC on *IN VITRO* Drug release.

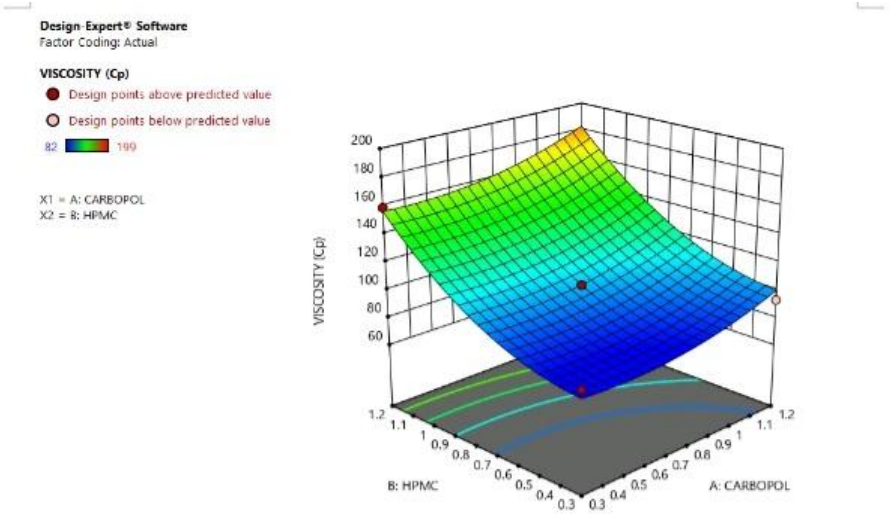

Figure no.6: 3-D Surface response plot showing the effect of carbopol and HPMC on Viscosity.

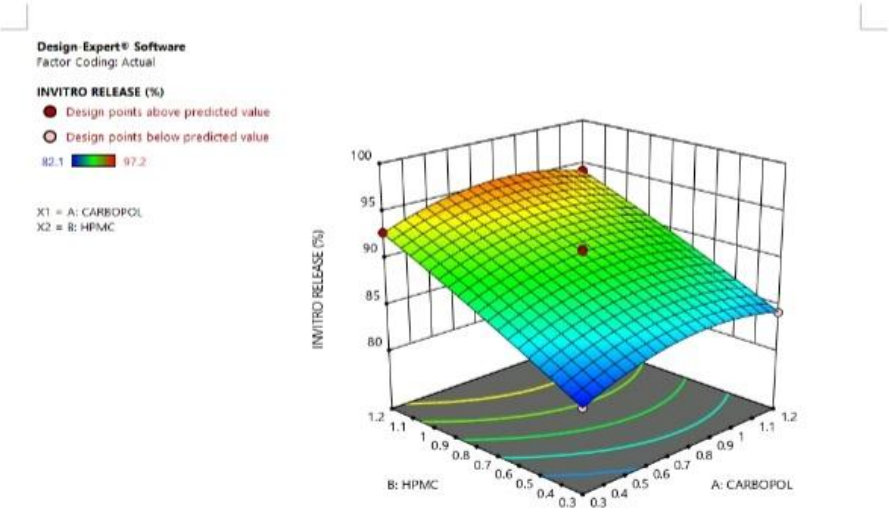

Figure no. 7: 3-D Surface response plot showing the effect of carbopol and HPMC on *IN VITRO* Drug release.

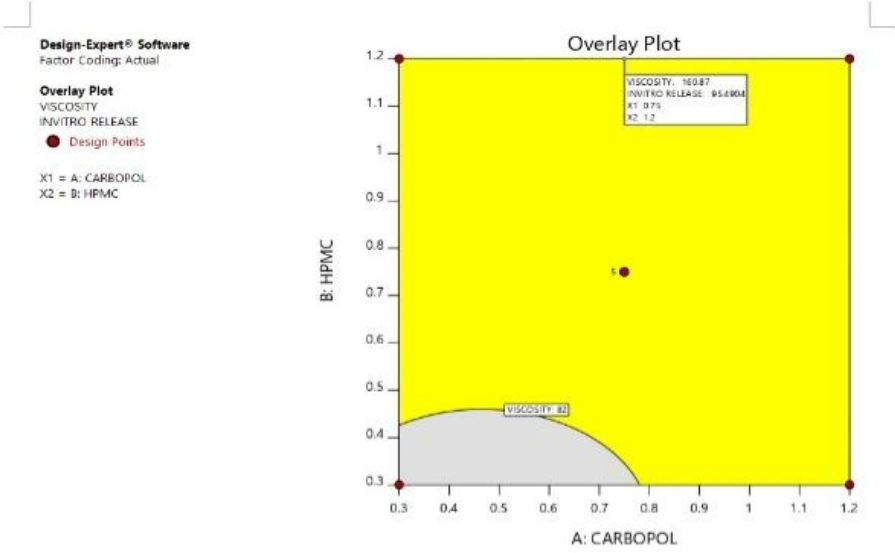

Figure no. 7: Overlay plot.

4.11Analysis of release mechanism

The resulting data were fitted into the following mathematical models (table no. 11) to determine the release pattern and release mechanism. The in vitro drug release data from the in situ gel was plotted using various kinetic models such as zero order, first order, Higuchi and kosmeyer peppas models.

Table no.11: Release kinetics of in situ formulation.

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI	KORSMEYER-PEPPAS	
	R^2	R^2	R^2	R^2	N
F6	0.9225	0.8811	0.8149	0.9366	2.4367

4.12 Stability studies

The optimized formulation F6 was selected for stability studies. The study was carried out at refrigerated temperature (4^0C) for a period of 3 months. The parameters analyzed includes appearance, gelation behavior, viscosity, drug content and in vitro drug release.

Table no.12: Stability Studies.

Parameter	Initial	After 3 months
Appearance	Viscous liquid	Viscous liquid
Viscosity (cp)	199 ± 0.60	200 ± 0.23
Gelation temperature (^{0}C)	35 ± 0.85	35 ± 0.93
Gelation strength	50.8 ± 0.11	51.2 ± 0.06
Mucoadhesive force	6219 ± 0.68	6224 ± 0.28
Drug content (%)	99.28 ±0.44	99.22 ±0.28
Cumulative percentage drug release (%)	97.20	98.11

5 CONCLUSION

pH sensitive in situ gel of Acyclovir was successfully prepared for controlled release of drug that provide a number of advantages over conventional dosage forms. Formula F6 with 0.75 Gcarbopol and 1.4 GHPMC showed excellent physical property, pH-triggered in situ gelling capacity (means it will give longest resident time in the oral cavity), good viscosity and mucoadhesive force and sustain the release of Acyclovir in test time period. In situ gel formulation of Acyclovir with mucoadhesive properties was found to be promising for prolonging buccal residence time and thereby better therapeutic effects. In addition, they provide intimate contact between a dosage form and the absorbing tissue which may result in high drug concentration in local area. The in situ formation may improve the patient compliance, as the formulation is applied in the form of sols, which on contact forms the corresponding gels causing less irritation or pain.

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