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Development and Evaluation of *In-Situ* Gel Formation for Treatment of Mouth Ulcer

Short Title: *In-Situ* Gel Formulatio

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ABSTRACT
INTRODUCTION: Mouth ulcers are one of the most prevalent conditions that can be caused by a range of circumstances. Many formulations, such as solution, suspension, and ointments, are available commercially. However, because there is no long-term effect, no medication can be regarded to be totally effective in the treatment of mouth ulcers. The use of a bio adhesive method can boost the therapy's efficacy. Because it is easier to administer than prepared gel formulations, the phenomena of sol to gel conversion can be beneficial.
The major goal of this study is to develop and test in situ gels for treating mouth ulcers utilizing choline salicylate and borax as model medicines.
METHODS: Because a thermosensitive polymer was employed in this formulation, the Sol-to-gel change was thermally reversible, and the frequency of administration was reduced by using the mucoadhesive polymer Carbopol. Gelation temperature, pH, gel strength, Spreadability, in vitro mucoadhesion, and in vitro drug release were all measured in the formulations.
RESULTS: The experimental section indicated that viscosity of sols and gel strength was increased with increase in temperature, i. e. gel can be created at site of application owing to body temperature. When Poloxamer 407 was used at a concentration of 14 to 16 percent w/v, the gelling temperature was close to the body temperature (35-38°C), but when here Carbopol 934P was added, the gelling temperature was raised. All formulations had a pH between 5.5 and 6.8, All formulations had viscosities of less than 1000 cps, allowing for simple administration of the formulation to a mouth ulcer.
DISCUSSION AND CONCLUSION: As a result, a correctly developed in-situ gel for oral ulcers can extend the duration spent at the application site and minimize the frequency of administration. These findings show that the created technology is a viable alternative to traditional drug delivery systems and can help patients comply.

Keywords: In-situ gel, Thermo reversible, Mucoadhesive, Choline salicylate, Mouth ulcer, 22 factorial design

INTRODUCTION:

The numerous routes of administration employed so far in new drug delivery systems, localized drug delivery to oral cavity tissues, has been examined for the treatment of periodontal disease, bacterial and fungal infection, aphthous ulcer, and other disorders.[1] The oral mucosa is the 'skin' that covers the majority of the mouth cavity, aside from the teeth. It can be used for a multitude of things. Its main purpose is to serve as a deterrence.[2] It protects deeper tissues such as fat, muscle, nerves, and blood vessels from mechanical trauma such as chewing. Oral mucosal disease is the most common disease that affects people. Mouth ulcers are painful, round or oval sores that develop in the mouth, usually on the inside of the cheeks or lips.

Mouth ulcers also called Recurrent Aphthous Stomatitis (RAS), aphthae, aphthosis, and canker sores. The word aphthous is derived from the Greek word aphtha, which signifies ulcer. Despite the redundancy, these oral sores are still referred to as Aphthous ulcers in medical literature.[3] RAS has an etiology that is either unknown or unclear.[4] Idiopathic RAS, rather than being a singular entity, may be the presentation of a number of illnesses with quite distinct etiologies. Nutritional deficiencies, such as iron and vitamins, especially B12 and C, poor dental hygiene, infections, stress, indigestion, mechanical injury, food allergies, hormonal imbalance, and skin illness are all common causes of mouth ulcers. Hematinic deficit and blood disorders, Gastro intestinal disorders, immune deficiencies such as people with Human immunodeficiency virus (HIV), neutropenia, and other conditions may predispose to RAS such as microbial illness, chronic prescription of non-steroidal anti-inflammatory drugs, alendronate, nicorandil, and other cytotoxic drugs. In some circumstances, quitting smoking might trigger or worsen RAS.[4, 5]

Various topical therapy techniques can be used to effectively treat a mouth ulcer. However, there are a number of problems that emerge from the drug's short retention duration, which could be the cause of limited therapeutic efficacy and should be addressed.[5,6]

The advantages of in-situ forming polymeric drug delivery systems, such as ease of administration and better patient comfort, have piqued interest. Increases the amount of time spent at the application site. Deformable dosage forms have less adverse effects than other dosage forms because they can conform to the contour of the surface on which they are placed. In situ forming polymeric formulations are drug delivery systems that are in sol form before being distributed in the body but gel in situ to create a gel after being delivered. Recent advances in polymer chemistry and hydrogel engineering have facilitated the development of in situ forming hydrogels for drug delivery applications. In situ gels have the properties of linear polymer solutions outside of the body [allowing for easy injection/administration].but they gel in situ within the body, resulting in prolonged drug release patterns. In order to accomplish in situ gelation, both physical and chemical cross-linking techniques have been used. Hydrogel precursor solutions can be injected and then polymerized in situ using intelligent design of monomers/ macromers with desired functionalities. The surgery and implantation technique can be completed with a minimum of invasiveness thanks to the in situ sol-gel transition.[7]

Choline salicylate (ChS), the medication employed in this study, is an analgesic. By acting locally on oral mucosal cells, it reduces pain severity.[8] ChS gel, which is commercially available, gives pain relief but only for a short time since it can be washed away from the site by salivation and tongue movement, accidental engulfing cause adverse effects such as stomach ulcers and increased blood concentration. This is required in order to examine the formulation that enhances the drug's residence time and availability at the application location. Borax is a homoeopathic

medication with antibacterial properties that has been used to cure mouth ulcers since ancient times. It also keeps the oral mucosa dry, allowing the mouth ulcer to heal more quickly. As a result, it can be used for both to treat mouth ulcer and as a preservative to the formulation.[9]
An attempt was made to develop a thermo reversible in-situ gel containing ChS and borax to treat mouth ulcers, to evaluate the formulation for various parameters, and to investigate the effect of the formulation on residence time, gelling temperature, and polymer muco adhesive properties. Poloxamer 407 and carbopol P 934 were employed as the polymers. Poloxamer 407 acts as a gelling agent and is temperature sensitive, while carbopol P 934 is a pH sensitive mucoadhesive polymer.[10]

OBJECTIVE:

The main goal of this research is to develop and evaluate a thermoreversible *in-situ* gel for treating mouth ulcers in order to find the best formula for improving patient compliance.

MATERIAL AND METHODS

ChS solution BP was obtained from Shreenath Chemicals, Bhoisar, Mumbai. Poloxamer 407(PF127) purchased from Sahyadri chemicals, Islampur, Maharashtra, and Carbopol 934P was provided as gift sample by Corel Pharma Chem. Ahmadabad. Borax obtained from Raj Chemicals, Mumbai and sodium hydroxide, Methanol, Ferric chloride, Hydrochloric acid, Acetic acid was obtained from S.D. Fine- Chem limited, Mumbai. All the other materials used were of analytical grade**.**

Instruments required for the work:

Franz- Diffusion cell, SFDC6 Model(manufactured by Logan); UV-Visible double beam Spectrophotometer (manufactured by Jasco, Japan); Fourier transform infrared spectroscopy (FTIR), FTIR-410 Model (manufactured by Jasco, Japan); Stability Chamber (Tempo instruments pvt. ltd); Electronic Balance, AUX 220 Model (Shimadzu, Japan)

Software required for Research work:

Design Expert software (Star Ease, Inc. producer) used for research work.

Analytical UV-Visible method development and validation: A simple UV-visible spectroscopic method was developed for ChS by following the procedure given below.

Preparation of stock solution I: Since the ChS solution BP contains 50% of ChS; 2 ml (1000mg) of ChS solution BP was mixed in 100 ml Phosphate buffered saline (PBS) of pH 6.8 to get 10mg/ml. Further diluted to get 100µg/ml concentration of drug.

1 ml, 2 ml, 3ml, 4ml and 5ml aliquots were withdrawn from stock solution I (100µg/ml) and diluted it up to 10 ml with PBS 0.6 pH in 10 ml volumetric flasks in order to get 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml concentration of drug. The absorbance was measured at 238 nm by using PBS of pH 6.8 as blank.

The method were validated using various parameters as per ICH guideline such as accuracy, precision, Limit of quantification (LOQ), Limit of detection (LOD), % RSD.

Formulation of in situ gel:

Preparation & optimization of thermo reversible PF127 aqueous solution: [11, 12]

The gel is prepared using the cold technique. Poloxamer concentrations ranging from 10% w/v to 20% w/w were generated by dissolving the polymer in distilled water at temperatures below 5°C in 50 ml. To guarantee complete polymer disintegration, the solutions were stored in the refrigerator for 24 hours. The temperature of gelation was then determined by visually inspecting each concentration. In a water bath, a beaker holding 20 ml of cold poloxamer solution was stored. A magnetic bead was placed in the beaker, and a calibrated thermometer was hung in the beaker so that the tip of the thermometer was in the solution, but it did not touch the beaker's floor and did not disturb the magnetic bead's spin. The system was agitated at 100 rpm with the

help of a magnetic stirrer, and the temperature was allowed to rise at a rate of 2°C/min. The temperature of gelation was measured when the magnetic bead stopped rotating due to the production of gel. Concentrations that gelled close to body temperature (35-37°C) were chosen for further optimization with other components.

Optimization of other ingredients with PF 127 concentration:

The effect of other ingredients on gelling temperature of Poloxamer solution was studied

Effect of Carbopol 934P on gelling temperature:

Carbopol 934P was prepared in various concentrations ranging from 0.1 percent to 0.5 percent w/v. For this, a weighed amount of polymer was combined with a little amount of water and allowed to swell overnight. With the use of a magnetic stirrer, these concentrations and Poloxamer solution were mixed together, and the gelation temperature was recorded.

a) **Effect of other ingredients on gelation temperature of solution Poloxamer 407 and Carbopol 934P mixture:** The weighed quantity of drug and other ingredients were mixed in the solution containing Poloxamer 407 and Carbopol 934P. Changes in gelation temperature were noted down.

b) **Formulation of batches based on design of experiment**: Depending on gelation temperature at or near to the body temperature concentrations were optimized and designed the experiment by. 2^2 factorial design.

I. **Selection of independent variables:** Gelation temperature of the in-situ gel at body temperature depends upon concentration of both polymers. Thus independent variables of both polymers were selected based on gelation temperature and mucoadhesive properties and coded low level as -1 and high level +1.

II. Experiment design 2^2 full factorial design :

Table no. 1 Coded values for levels of factors

Formulations / Variables	F1	F2	F3	F4
X1	+1	-1	-1	+1
X2	+1	-1	+1	-1

Evaluation of formulation:

Prepared batches of formulation were evaluated for following parameters:

Appearance: The prepared gel were visually inspected under light against white and black background for its clarity.

pH of the gel: Digital glass electrode pH meter was used to measure pH of the gel by placing the electrode directly in to the gel.[13]

Gelation temperature: In a water bath, a beaker holding 20 ml of the formulation's cold solution form was preserved. A magnetic bead was placed in the beaker, and a calibrated thermometer was hung in the beaker so that the tip of the thermometer was in the solution, but it did not touch the beaker's floor and did not disturb the magnetic bead's spin. Temperature was allowed to rise at a rate of 2°C/min while the systems were agitated at 100 rpm. Temperatures of gelation were measured at the point where the magnetic bead ceased to rotate due to the formation of gel.[14,18]

Thermoreversible study: Using a constant temperature bath, a thermoreversible investigation was carried out. The in-situ gel compositions were kept in a temperature bath at a constant temperature. The instrument was adjusted at a temperature of 4-5°C. Temperature was allowed to rise at a rate of 2°C per minute, and the shift from sol to gel phase was observed, as well as changes in viscosity as point rose to the gelling temperature.[16 17,18]

Similarly, the temperature was allowed to decline until the gel transformed into a sol, and the viscosity was recorded as a function of temperature.
Viscosity of all prepared formulation was measured by using Brookfield viscometer (Brookfield viscometer RTV) with spindle no.62 at speed of 10 rpm. The rheological properties were also studied by measuring viscosity of all formulation at speed of 10, 50 and 100 rpm with spindle no.62.
Shear rate (sec^{1}) was calculated by using following formula:
Shear rate (sec^{1}) = $2\omega \times R_c^2 R_b^2 \div X^2 \times [Rc^2 - R_b^2]$
Where,

R_c = Radius of the container (in centimeters)

R_b = Radius of the spindle (in Centimeters)

X = Radius at which shear rate is to be calculated (normally the same value as Rb; in centimeters)

ω = Angular velocity of the spindle (Rad / Sec)

ω = 2 ÷ 60 x N

N = Spindle speed in RPM

Observed values:
R_c =1.5cm R_b = 1.25cm

Shear stress (dynes / cm^2) was calculated by using following formula:
Shear Stress = Shear Rate (sec^{-1}) ÷ Viscosity (cps)
Drug Content:
Percentage ChS BP content was determined by dissolving 0.5 gm of the gel in 100 ml of pH 6.8 PBS and scanning the resultant solution with a UV-Visible spectrophotometer set to 238 nm. A calibration curve was used to calculate the drug content.[12,17,18]
Determination of mucoadhesive force:
The mucoadhesive force was determined according to Desai V, Shirsand S description (2018)[20]. The assembly, which involved two glass vials, was completed in-house. One is hung in a downward position while the other is placed on the floor in an upward position. The upper vial is fastened to one end of the thread, and a pan is tied to the other end of the thread.[14,18]
A piece of goat buccal tissue was glued to both glass vials with the mucosal side facing out. Before performing the test, these vials were kept at 37°C for 10-15 minutes. On the lower vial, around 1 gm of gel was applied before the upper vial was inserted. 1gm of weight was added to the pan. The weight was gradually increased until the two vials were still connected. The mucoadhesive force (gm) was calculated using the smallest weights that could separate the two vials. The bioadhesive force was determined using the equation below.
Bioadhesive force = Bioadhesive Strength x 9.81//100
In vitro drug release study:
The Franz Diffusion cell was used to conduct an in-vitro drug (ChS BP) release study of an in-situ gel. In the donor compartment, 1 ml of formulation (F3) (equal to 1 gram of gel) was deposited, and in the receptor compartment, freshly produced PBS (pH 6.8) was poured. A cellophane membrane was fitted between the chambers. One cell as blank was filled with only

filled PBS solution. The units then were placed on a magnetic stirrer with a thermostat. The medium was kept at a constant temperature of 37°C ±0.5. After each 1 hr interval, 1 ml of sample was withdrawn & same amount of PBS solution from blank were transferred into the sample cell for maintaining sink condition. Then the withdrawal amount diluted to 10 ml in PBS pH 6.8, then concentration of ChS BP was measured using a UV-Visible spectrophotometer at 238 nm with PBS pH 6.8 as a blank. The calibration curve was plotted and used to determine the percent cumulative ChS BP release, and the best fit model was tested for Korsmeyers, Peppas, and Fickinian diffusion mechanism for their kinetics.[15,18]

Drug diffusion kinetic study:

The formulations' in-vitro release data was evaluated kinetically to determine drug kinetics. Microsoft Excel 2013 was used to fit the models. The models of zero order, first order, Higuchi, and Korsemeyer Peppas were investigated. The model with the best fit was chosen because of its comparatively high co-relation coefficient value.[18]

Statistical optimization of in situ gel formulation:

Gelatin temperature, viscosity of gel, diffusion of drug at 1 hr, time required for 90% drug diffusion are major variables for prepared in-situ gel formulation performance. Formation of gel at oral temperature is fundamental of prepared in situ gel. Drug release from gel is indirectly proportional to viscosity of gel. Thus viscosity of gel is major variable to be consider during designing of in-situ gel formulation. Salivation in oral cavity restrict sustain release gel formulations since gel may gel washout with saliva. Thus drug release at 1hr and time required for 90% drug release must be considered. Both the factors helps to decide dosing frequency of the formulation. For the statistical optimization of in-situ gel following criteria for selection of suitable feasible region was decided.

Table no 2. Desirable values of dependent variables for optimization:

Sr. No.	Response variable	Desirable value
1	Gelatin temperature (Y1)	37° C
2	Viscosity	<1000cps
3	Diffusion at 1 hr (Y3)	40%
4	Time required for 90% drug diffusion	4hrs

Antimicrobial test:

An antimicrobial study was conducted to assess the medication borax's antibacterial activity and to determine whether the formulation had enough antimicrobial properties. The test was carried out utilizing the well diffusion method against Gram positive (*Escherichia coli*) and Gram negative bacteria (*Staphylococcus aureus*).

5% w/v of Mac Conkey's agar for E.coli and 11.1% w/v Mannitol agar for Staph aureus were prepared and sterilized. The liquid was then put into a sterile glass plate and allowed to set. The bacterial strains were aseptically dispersed over the agar after solidification. Each agar plate had three wells: one for the test (F3), one for the standard (ZYTEE), and one for the plane borax solution. The samples were placed in the well and placed in the refrigerator for 15-20 minutes to allow the materials to diffuse into the agar. The plates were then incubated in an incubator at 37°C for 24 hours. The zone of inhibition was assessed after the incubation period.[13,15,16]

Animal model study:

The goal of the study was to see how a produced formulation affected the healing of an oral ulcer in rats. In this study, 15 healthy female wistar albino rats (weight 130-150 grammes) were chosen and separated into three groups, each with five animals. Before anaesthesia, a 5 mm diameter filter paper soaked in 50% acetic acid was placed on the tongue of rats for 60 seconds to form a

circular ulcer. The test group received an optimized formulation (F3), the standard group received ZYTEE gel (a commercialized ChS product), and the control group received no treatment. For 7 to 10 days, the ulcer's healing progress was examined.[19,21]

Results:

Analytical UV-Visible method development and validation:

The λ_{max} of ChS in PBS 6.8 was found to be 238 nm. The drug follows linearity in the concentration range 10-50 µg/ml with a correlation coefficient value of 0.9903. The accuracy of the method was checked by recovery experiment performed at three different levels, i.e., 80%, 100%, and 120%. The % recovery was found to be in the range of 98.54– 99.98%. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intraday; inter-day variations, and repeatability. The % RSD value < 2 indicates that the method is precise (Table no 3). Ruggedness of the proposed method was studied with the help of two analysts.

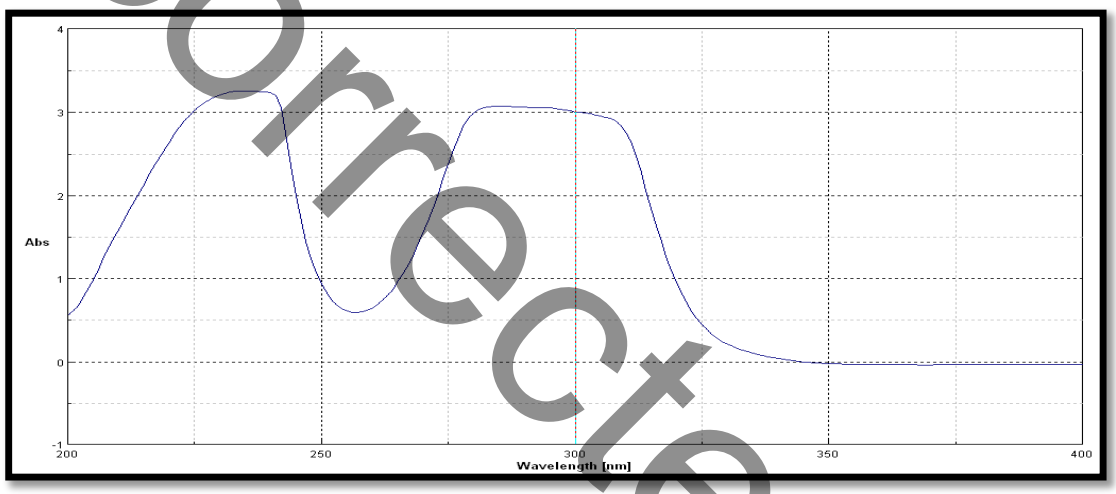


Fig. no.1 UV spectra of ChS BP

Table no.3 Results for analytical UV-visible method development and validation

mcg/ml	Observation			average	SD ±	%RSD	LOD mcg	LOQ mcg
	1	2	3					
10	0.1692	0.1752	0.1632	0.1692	0.006	3.546099	0.112692	0.341491
20	0.3838	0.3888	0.3788	0.3838	0.005	1.302762	0.09391	0.284576
30	0.4951	0.4971	0.4931	0.4951	0.002	0.403959	0.037564	0.11383
40	0.7089	0.7129	0.7049	0.7089	0.004	0.564254	0.075128	0.227661
50	0.8343	0.8457	0.8229	0.8343	0.0114	1.366415	0.214115	0.648833

Formulation of in situ gel:

Preparation and optimization of thermo reversible PF127 aqueous solution: The solution of poloxamer 407 with concentration of 10% w/v to 20% w/v was prepared in distilled water. Gelation temperature of the solutions were found as per depicted in table no 4.

Table no. 4 Gelation temperature of Poloxamer 407

Concentration of Poloxamer 407(%w/v)	Gelation temperature (^{O}C)
11	46
12	42

13	39
14	38
15	37
16	35
17	34
18	30
19	28
20	25

The concentrations of 15 % w/v to 20% w/v were considered as optimum for formulation.

B. Optimization of other ingredients with PF 127 concentration:

Effect of carbopol 934P on gelling temperature: The optimum poloxamer concentration solutions were mixed with 0.1 %w/v Carbopol solution and gelling temperatures were observed as shown in table no. 5

Table no. 5 Gelation temperature of poloxamer 407 and carbopol 934P mixture

Concentration of poloxamer 407 (% w/v)	Concentration of carbopol 934P (%w/v)	Gelling temperature (°C)
15	0.1	41.4
16	0.1	41
17	0.1	40.5
18	0.1	39.1
19	0.1	38
20	0.1	37.5

It was observed that there was an increase in gelling temperature on addition of Carbopol 934P. Thus concentration of Poloxamer was increased order to form the gel near to body temperature. Gelation temperature were observed as given in table no 6.

Table no.6 Gelation temperature of 407 and 934P mixture

Conc. Of carbopol 943P (%w/v) →	**0.1**	**0.4**	**0.6**
Conc. Of poloxamer 407 (%w/v) ↓			
20	37.2°C	37.8°C	40.3 °C
21	35.2 °C	36.5 °C	39.7 °C
22	34.7 °C	35.8 °C	38.7 °C
23	34.1 °C	34.9 °C	37.8 °C
24	32.9 °C	32.8 °C	33.6 °C
25	30 °C	31.3 °C	32.5 °C

Effect of other ingredients gelation temperature of solution poloxamer and carbopol 934P mixture: Other ingredients such as drug ChS (8 %), borax (1%) and propylene glycol were

added to the poloxamer 407 and carbopol 943P solution and gelling temperature were observed (Table no 7). It was observed that there is no significant difference upon addition of the other ingredients.

Table no. 7 Gelling temperature of mixture of ChS, borax, Carbopol 934P and poloxamer 407 at different concentrations

Ingredients	**Concentration (%w/v)**							
Poloxamer 407	20	21	22	23	20	21	22	23
Carbopol 934P	0.1	0.1	0.1	0.1	0.4	0.4	0.4	0.4
Choline salicylate	8	8	8	8	8	8	8	8
Borax	1	1	1	1	1	1	1	1
Gelation temperature (°C)	**36.5**	**35.5**	**35**	**33.9**	**37.5**	**37**	**35**	**34.6**

C. Formulation of batches based on design of experiment. Different formulation batches F1 to F4 were prepared based on design of experiment by 2^2 factorial design.

I. Selection of independent variables:

Table no. 8 Selected Independent Variables

Level	**Variable**	**X1 (concentration of poloxamer 407)**	**X2 (concentration of carbopol 934P)**
Low	-1	20	0.1
High	+1	23	0.4

Table no. 9 Composition of in-situ gel formulation as per coded values in Experiment design 2^2 full factorial design

Sr. No.	**Formulation Ingredients**	**F1 %w/v**	**F2 %w/v**	**F3 %w/v**	**F4 %w/v**
1	Poloxamer 407	23	20	23	20
2	Carbopol 934P	0.1	0.1	0.4	0.4
3	ChS BP	8	8	8	8
4	Borax	1	1	1	1

Evaluation of formulation:

1. Appearance:

In both solution and gel form, all of the formulations were determined to be clear and transparent. A clear translucent gel creation on a mouth ulcer will increase patient compliance because it will mimic natural oral mucosa, allowing for daytime application.

pH of the gel:

The pH of all formulations was found to be between 5.5 and 6.8. (Table no. 10). To avoid irritation of the mucosa and further damage to the ulcer, the pH of the formulation produced to treat mucus ulcers must be close to neutral. In general, any formulation utilised for the mucosa should have a pH of 4.5 to 7.

Gelation temperature:

The temperature at which the formulation's solution form transforms entirely into semisolid form is known as the gelation temperature. The gelling temperature is the most important requirement

for the in-situ gel formulation. At close to body temperature, the in-situ gel formulation for the oral ulcer should quickly change from sol to gel. (37°C 5°C), and the resulting gel should not erode or dissolve. The gelling temperature of the produced mixture was determined to be between 34 and 38 degrees Celsius (Table no. 10).

The gelling temperature and integrity, on the other hand, are mostly determined by the polymer content. At 38°C, Formulation F2 formed the weakest gel, whereas Formulation F1 generated a strong gel at 35°C. It could be because the F2 formulation has a lower concentration of both polymers while the F3 formulation has a larger concentration of both polymers.

As a result of the observed gelling temperature, it can be concluded that the concentration of poloxamer 407 has a proportional effect on gelling temperature, whereas the gelling temperature increases when the carbopol 943P is added, and it is also directly proportional to the carbopol 934P concentration.

Thermoreversible study:

In the same way that an increase in temperature causes the sol to gel phase transition in in-situ gel formulation, a decrease in temperature causes the gel to sol phase transition. The procedure is the polar opposite of the sol-gel mechanism. As the temperature rises, the micelles generated at CMC come into touch with one another, resulting in polymerization and thus gel formation. As the temperature drops, micelle packing and micelle entanglement diminish, and the network breaks down. The formulation's gel form begins to transform into a solution form, and at a certain point, the gel is totally transformed into a solution. The temperature difference between gel and sol is known as gel to sol temperature.

The gelation phenomenon will be aided by a mechanism based on micelle packing and entanglements, as well as conformational changes in the orientation of the methyl group in the side chain of the poly(oxy propylene) polymer chain constituting the micelle's core, and the expulsion of the hydrating water from the micelle.

It was discovered from the phase diagram in Fig. no. 1 that when the polymer concentration increased, the gelation temperature decreased while the sol temperature increased.

In comparison to previous formulations, formulation F1 comprises a larger concentration of polymers, resulting in lower gelation and solution temperatures. Similarly, formulation F2 has the lowest polymer concentration, thus it takes more heat to create a gel, but it converts to a sol form fast and at high temperatures when compared to other formulations.

As can be seen from the phase diagram, (Fig. no. 2) the smallest concentration of the polymer has the highest gelation temperature & low sol temperature. The micelle created from the smallest amount of polymer was unstable, and breaking the hydrogen bond formed during temperature aggregation needed the least amount of energy. The energy required to break the bond is provided by external heat.

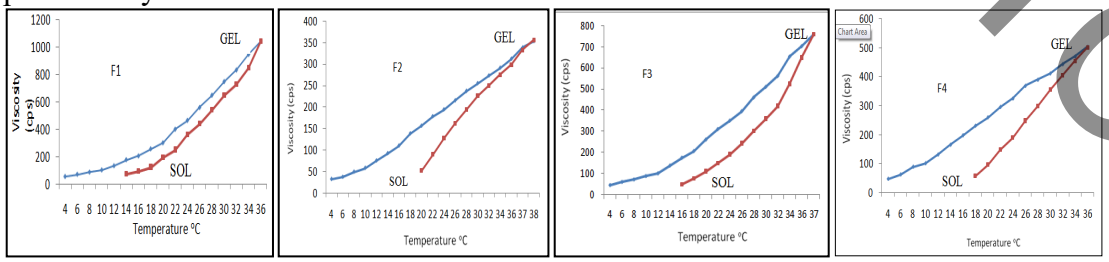


Fig. no. 2 Thermoreversible Gel to sol phase diagram of prepared in-situ gel formulations

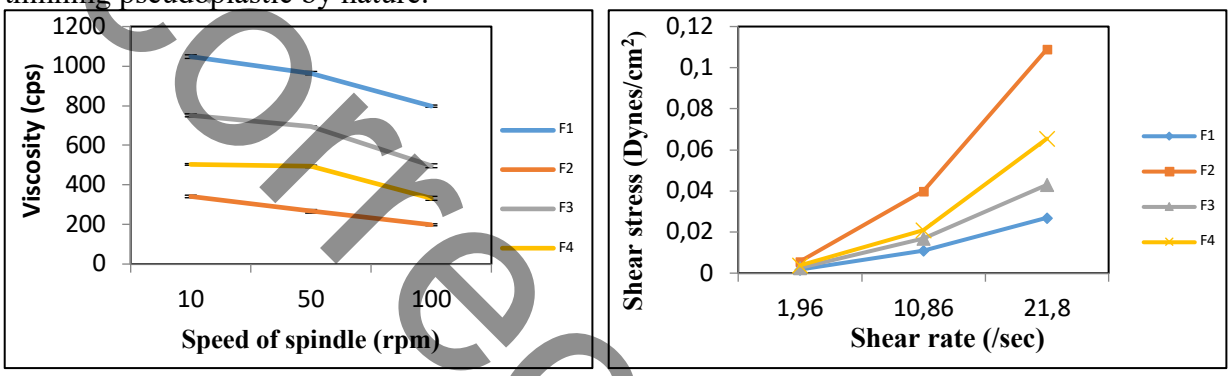
Study of viscosity and rheological properties:

This is one of the most significant requirements for in-situ gel formulation. In order to remain for a long time at the site of application, the in-situ gel formulation should have a viscosity of more

than 100 cps when it is applied and less than 1000 cps when it converts to the gel after administration.

The viscosity of all formulations F1, F2, F3, and F4 was found to be polymer concentration dependent. Viscosity increased in the order F1>F3>F4>F2 as the concentrations of the polymers Poloxamer 407 and Carbopol 934P increased. Table 10 provides the viscosity (centipoises) of the prepared formulations, and Figures 3(a) and 3(b) show the shear rate (sec) and shear stress (dyne/cm2) of all batches.

It was discovered that the viscosity varied depending on the shearing rate. In other words, the ratio of shear stress to shear rate was not constant, and viscosity dropped as the shear rate increased. As a result, the prepared in-situ gel was found to be Non-Newtonian fluid. As the shear rate increased, the viscosity of the gel dropped. This demonstrated that the in-situ gel was shear thinning pseudoplastic by nature.



(a) **(b)**

Fig. No. 3(a) Viscosity (cps) v/s speed of spindle (rpm) graph, 3(b) Shear stress v/s shear rate graph showing Non-Newtonian fluid

Drug content:

As stated in Table No.10, the percent ChS BP of all formulations were determined to be in the range of 98 to 100 %. It's possible that the discrepancy in medication content is attributable to human mistake during dilution or to production loss during formulation preparation.

Determination of mucoadhesive force:

Mucoadhesion is an interfacial phenomena that involves two materials, one of which is the mucus layer of mucosal tissue, to which the medication is held together for a long time by interfacial forces. The longer the retention duration, the stronger the mucoadhesive force. Various studies have shown that the presence of polyoxyethylene groups in poloxamer 407 is responsible for their mucoadhesion via H-bonding, but when it forms a gel, the cross linkage between Poloxamer 407 increases, rendering the polyoxyethylene groups unavailable for mucoadhesion. According to the diffusion interlocking hypothesis, when cross-link density rises, chain mobility falls, and therefore the effective chain length that may penetrate the mucus layer falls, lowering mucoadhesive strength. Thus addition of carbopol 934P leads to increase in mucoadhesion Carbopol is synthetic mucoadhesive agent. It adhere to the mucosa by COOH bond. The formulations F3 and F4 containing higher concentration of the Carbopol was showed strong bioadhesion as compare to other formulations.

Table no. 10 Observations of various evaluations tests

Batch	Appearance	pH	Gelling temperature (°C)	Viscosity (cps)	% drug content	Bioadhesive strength (gm)	Mucoadhesive force (gm)

F1	Clear	5.8 ±0.05	35 ±0.2	936.9 ±7.76	100 ±1.2	10	0.981
F2	Clear	6.2 ±0.05	38 ±0.2	936.9 ±7.76	99.01 ±0.9	6	0.588
F3	Clear	5.5 ±0.05	37 ±0.1	627.5 ±6.7	99.86 ±0.9	18	1.765
F4	Clear	6.8 ± 0.05	36 ±0.3	443.36 ±6.84	98.75 ±0.6	20	1.962

In-vitro diffusion study: An in-vitro diffusion study was conducted utilizing a Franz diffusion cell with a pore size of 40 um and a cellophane membrane. In Fig. no. 4, the percent cumulative ChS BP diffusion obtained from all formulations is shown. Formulation F2 had the fastest diffusion compared to the other formulations, while Formulation FI had the slowest diffusion from the gel. In the case of F2, 90% of the drug was diffused up to 3.5 hours, however in the case of F1, only 80% of the drug was diffused by the fifth hour. It could be because F2 has a lower concentration of both polymers while F1 has a higher concentration of both polymers.
In general, the drug diffusion rate reduces as the cross-linking of the polymer in the formulation, such as gel, increases. Based on the findings, it can be concluded that as the polymer concentration grew, the drug diffusion rate decreased. Diffusion of drugs is thus a polymer concentration-dependent process. Since I'm tempted to create an in-situ gel that exhibits 40% drug release after 1 hour and 90% drug release after 4 hours. The F1 formulation was not determined to be optimum. (Fig.no. 4)

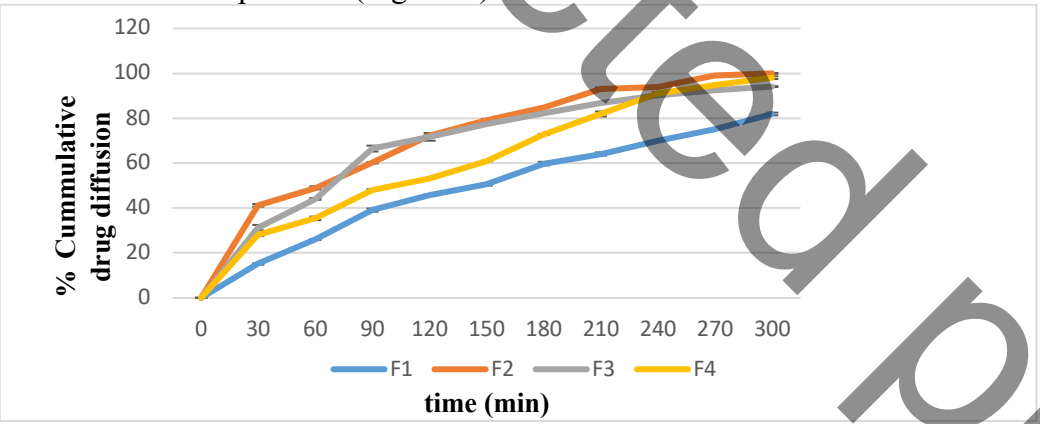


Fig. no. 4 In-vitro drug diffusion study of prepared in-situ gel formulations

Drug diffusion kinetic study:

According to data from diffusion studies, the generated in-situ gel had a significant initial drug release (burst effect) and then decreased as gelation progressed. This is a bi-phasic pattern, which is a common feature of matrix diffusion kinetics. As the concentration of polymer grew, the first burst effect decreased, as in the case of F1, which contains high concentrations of both polymers. The Korsmeyer Peppas model is commonly used to confirm the drug's release process from the matrix. The 'n' value (Korsmeyer Peppas model release exponential) was used to characterise the various release mechanisms in the following way:

n<0.5 Quasi Fickian diffusion

n-0.5: Diffusion Mechanism

0.5<n>1 Anomalous non-fickian diffusion (both diffusion and erosion)

n = 1 case 2 transport (zero order)
n>1 supercase 2 transport relaxation
For each formulation, a graph of log CDR v/s log was plotted to determine the diffusion mechanism of the created in-situ gel according to the Karsmeyer Peppas model. For all formulations, the correlation of co-efficients of all straight lines was determined to be in the range of 0.954 to 0.992.
The n value was recorded for all formulations and was utilized to modify the diffusion mechanism from the formulations. Since n values of 0.7 and 0.57 were reported, the formulations F1 and F4 follow an atypical non-fickian diffusion mechanism. Due to n=0.43 and 0.48, respectively, F2 and F3 followed a quasi-fickian diffusion mechanism.
The dissolution data for the Higuchi model was investigated to see if the drug release was diffusion regulated or not. For all formulations, a graph of percent CDR vs. square root of time was drawn. All straight line correlation coefficients were determined to be in the range of 0.943 to 0.996. As a result, all of the formulations followed Higuchi's diffusion model.

Table. No. 10 Results of Drug diffusion kinetic study

Formulation	Zero order	First order	Higuchi	Korsmeyer Peppas	n
F1	0.996	0.966	0.996	0.992	0.7
F2	0.882	0.882	0.989	0.986	0.43
F3	0.83	0.865	0.943	0.943	0.48
F4	0.96	0.96	0.981	0.982	0.57

Statistical optimization of in situ gel formulation:
Primary process parameter analyses revealed that components such as Poloxamer 407 (X1) and Carbopol 934P (X2) had a substantial impact on gelation temperature, viscosity, and drug diffusion, as well as the time required for 90% drug diffusion. As a result, these two variables were used in subsequent statistical optimization research. For all four formulation batches, all dependent variables revealed a wide range of data.
The software Stat Ease: Design Expert 10 was used to derive conclusions based on the amount of the co-efficient and the mathematical sign (positive or negative) they carried.

Table no. 11 Results of experimental design batches of variables

Formulation code	Diffusion at 1 hour Y1 (%)	Time required for 90% drug diffusion Y2 (hrs)	Gelation temperature Y3 (^{O}C)	ViscosityY4 (cps)
F1	26.01	6	34	1042
F2	49.01	3.5	38	342.1
F3	44.1	4	37	751.5
F4	35.42	4	35	503.8

Optimization of polymer concentrations for gelation temperature:

Concerning Y1 (gelation temperature) the data clearly indicated, it is strongly dependent on the selected variables X1 and X2

YI 36.56-0.98X1-49X2 +0,042X1X2

The findings of multiple linear analysis revealed that both co-efficients, B1 (-0.98) and 3 (-0.49), had a negative sign, indicating that when individual concentrations of poloxamer 407 or carbopol 934 increase, the gelation temperature decreases. The combination of the two polymers, on the other hand, has a positive effect on gelation temperature and micellar aggregation. Only when the concentration of Poloxamer 407 exceeds the micellar concentration, resulting in micelle production, can the gel phase occur. The hydrophobic sections of the pluronic are kept apart by hydrogen bonding between the POP chains and the water when the material is immersed in cold water. The hydrogen bonding is broken as the temperature is elevated, and hydrophobic interactions cause a gel to form. Carbopol 934P was added in escalating quantities to lower the gelation temperature even more. As the concentration of mucoadhesive polymers (Carbopol 934P) grew, the gel's gelation temperature decreased. It's probable that the ability of mucoadhesive polymers to reduce gelation temperature is linked to increased viscosity following polymer disintegration. The ability of mucoadhesive polymers to adhere to the polyoxyethylene. chains contained in Poloxamer 407 molecules could explain their capacity to lower gelation temperature. This would encourage dehydration, resulting in increased entanglement of neighboring molecules and increased intermolecular hydrogen bonding, lowering the gelation temperature. When bioadhesive agents and Poloxamer 407 were combined, the effect on gelation temperature revealed that adding Carbopol 934P increased micelle packing and tangling, resulting in a drop in gelation temperature. Using a response surface, the relationship between formulation variables (X and X2) and Y1 was further clarified. Fig.no.5 (c) shows the effects of X1 and X2 on Y. The gelation temperature was reduced as the amount of Poloxamer 407 and Carbopol 934P was increased.

Optimization of polymer concentrations for viscosity:

According dependent to results of multiple linear regression analysis the viscosity is strongly on the X_1 and X_2. The fitted equation for full model relating the viscosity to selected factors can be explained by following polynomial equation:

Y2 661.33 +114.41X_1 +238.39X_2+ 33.51 X_1X_2

The results revealed that both X1 and X2 have positive co-efficients. As a result of rising X1 and X2 values, viscosity is projected to rise. Both elements have a favourable effect on viscosity when used separately and in combination. The fact that X2 has a higher coefficient value than X shows that X2 is more effective in terms of viscosity than X1. Surface plot Fig no.5 (d) can be used to explain the relationship between selected parameters and response viscosity.

Optimization of polymer concentrations for drug diffusion at 1 hr:

The data clearly showed that drug diffusion values at 1 hour are substantially reliant on the specified independent variables, namely Poloxamer 407 concentration and carbopol 934P concentration. The transformed factor is related to the response (release at 1 hour) by the fitted equation (for full model).

Y_1+39.15 -7.89 X_1 -3.83X_2-1.12X_1X_2

The coefficients 1 and 2 to the prediction of release at 1 hour were found to be significant at p0.05. The coefficients 1 (-7.89) and 2 (-3.83) have a negative sign, according to the results of multiple linear regression analysis. It appears that increasing the amount of poloxamer 407 or carbopol 934P in the formulation reduces the release levels after one hour. The coefficient of

poloxamer 407 is larger than that of carbopol 934P, indicating that poloxamer 407 is more effective than carbopol 934p in terms of 1 hour release. Using a response surface plot (Fig.no.5 (a)) the link between formulation variables poloxamer 407 (X1) and carbopol 934P (X2) was further explored.

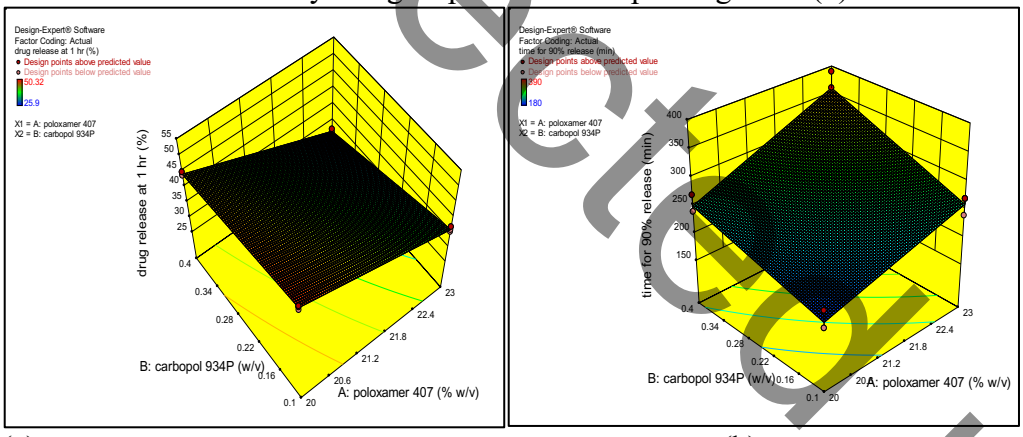
Optimization of polymer concentrations for time required for 90 % drug diffusion:

In case of Y_2 the result of multiple regression analysis showed that the coefficient diffusion: (+45) and P_2 (+40) bears positive sign. The positive sign of both X1 and X2 coefficient indicates that as concentration of both poloxamer 407 or carbopol 934P creased the time required for 90% drug diffusion increased. The summary of regression analysis can be explained by the following polynomial equation

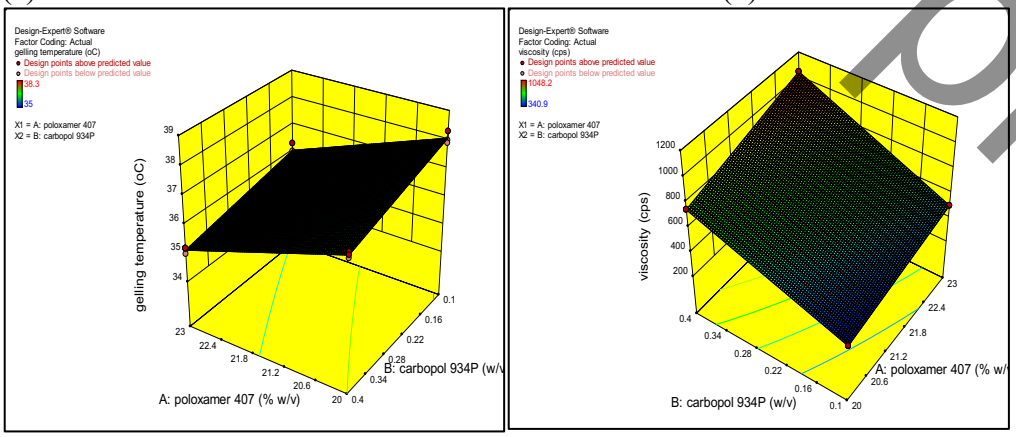
$Y4 = 265 + 45X_1 + 40X_2 + 10X_1 X_2$

The Y2 exhibited a good correlation co-efficient of 1.000 for all batches F1 to F4. XI had a p value of 0.0001 and X2 had a p value of 0.0001. Both p values are less than 0.05, indicating that the independent factors have a substantial impact on the time necessary for 90% drug diffusion. The time required for 90 percent drug diffusion increased as the concentrations of poloxamer 407 and carbopol 934P rose. It could be attributed to an increase in cross-linkage as a result of higher polymer concentrations, resulting in lower drug diffusion from the in-situ gel's polymeric network.

The relationship between formulation variables i.e poloxamer 407 (X1) and Carbopol 934P (X2) was further elucidated by using response surface plot Fig. no.5 (b)



(a) (b)



(c) (d)

Fig. No. 5 Response surface plot of Optimization of polymer concentrations for (a) drug diffusion at 1 hr (b) time required for 90 % drug diffusion (c) Gelation temperature (°C) (d) viscosity (cps)

Analysis of variance:

The R2 values for gelation temperature (Y), viscosity (Y2), CPR at 1 hour (Y1), and time required for 90% drug release (Y) are 0.9822, 1.000, 0.9959, and 0.9255, respectively, suggesting that dependent and independent variables are well correlated.

Antimicrobial test:

Antimicrobial medicines are also used to treat mouth ulcers: These inhibit microbial growth on the ulcer, allowing it to heal more quickly. Borax has been shown to have antibacterial, antifungal, and anti-allergy properties in studies. As a result, borax can be used as both an anti-ulcer and a preservative. The zone of inhibition obtained by improved formulation (F3) in sol form, conventional ZYTEE gel, and glycerol-borax as per shown in Fig no.6 can act on both Gram positive (Escherichia coli) and Gram negative bacteria (Staphylococcus aureus).

Table no.12 Zone of inhibition (mm) shown by prepared formulation

Micro-organisms	Formulation	Standard	Glycero-borax
E.coli	22mm	25mm	14mm
Staph. Aureus	25mm	28mm	17mm

(a) (b)

Fig no. 6 Zone of inhibition of prepared in-situ gel formation batch F3 (sol form) (a) *Escherichia coli* and (b) *Staphylococcus aureus*

There is a negligible difference between the zone of inhibition of standard and the formulation in gel form which shows the formulation has preservative property resemble to standard.

Animal model study:

In most cases, an oral ulcer heals on its own within 7 to 10 days. The formulations produced to treat mouth ulcers speed up the healing process, requiring less time than natural healing and reducing the pain associated with ulcers. As a result, the patient's comfort with an oral ulcer will improve.

The wistar albino rats were used as an animal model in this investigation. In comparison to conventional ChS gel (ZYTEE), the effect of a developed formulation (F3) on the healing of an

oral ulcer in rats. The ulcer healing properties of the formulation were found to be comparable to those of the standard. (Fig. 7) The observation was made based on the ulcer's everyday ocular observations.
Within 5 days, all animals in the test group that were given the formulation were free of ulcers. Similarly, all animals in the standard treated group were cured on the fifth day after therapy began. However, on the fifth day, three out of five animals in the control group, i.e. those who were not treated, developed an ulcer, and it took them eight days to completely recover. As a result, the developed formulation of in-situ gel containing ChS is effective in the treatment of mouth ulcers.

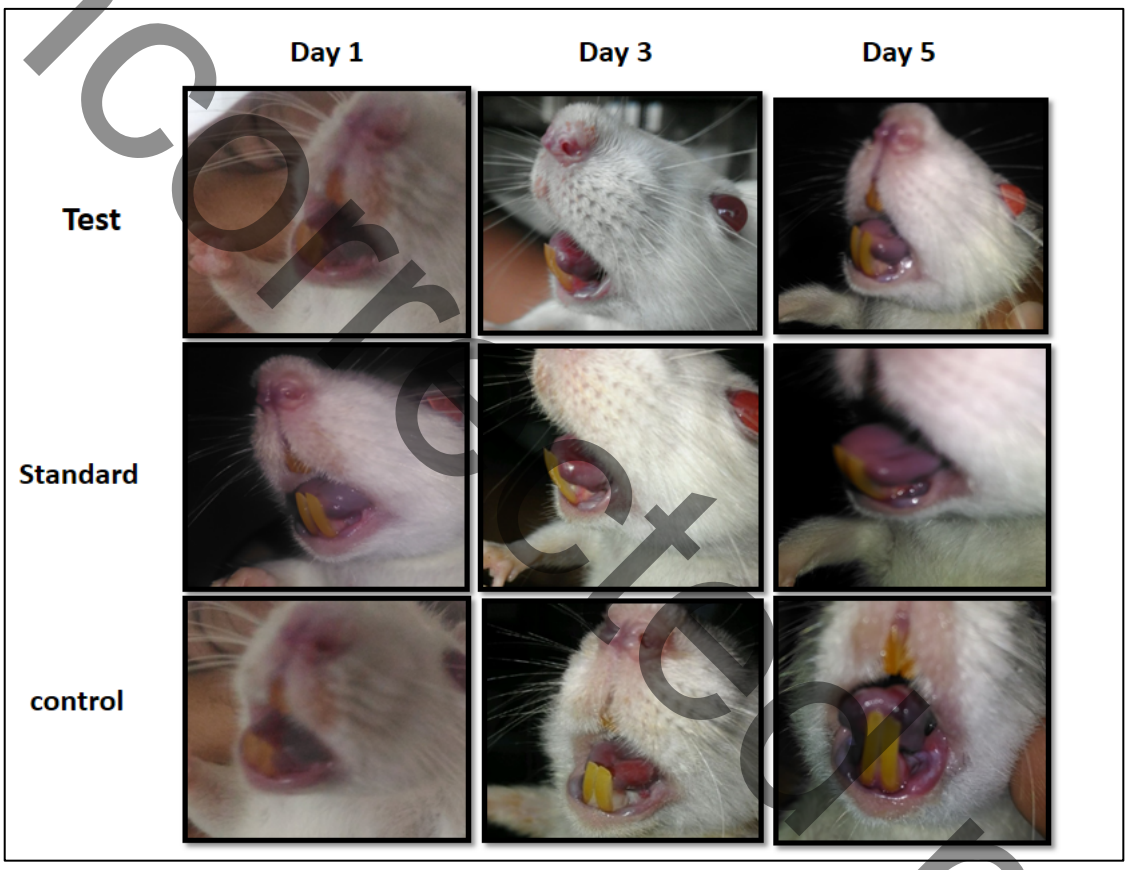


Fig. No. 7 Animal Model Study

Conclusion:
Using the thermoreversible polymer Poloxamer 407 and the mucoadhesive polymer Carbopol 934P, a thermoreversible in-situ gel containing ChS and borax for the treatment of mouth ulcers was successfully created.
It has been determined through compatibility studies that medications and polymers are compatible. When Poloxamer 407 was used at a concentration of 14 to 16 percent w/v, the gelling temperature was close to the body temperature (35-38°C), but when Here carbopol 934P was added, the gelling temperature was raised. Carbopol may cause micelle aggregation, size, and entanglement to decrease, resulting in an increase in gelation temperature. The addition of ChS and borax to the gelation temperature had no effect. The in-situ gel was thus created based on the gelation temperature, pH, thermoreversibility, viscosity, mucoadhesion study, drug

content, in-vitro drug diffusion, drug diffusion kinetics, statistical formulation optimization, antimicrobial, and animal model study of optimized formulations were all examined.
The thermoreversibility of the formulations was discovered. All formulations had a pH between 5.5 and 6.8, which is considered a safe range for mucosal drug delivery. All formulations had viscosities of less than 1000 cps, allowing for simple administration of the formulation to a mouth ulcer. Theological tests revealed that the in situ gel had a Non-Newtonian flow and was shear thinning pseudo-plastic. It is thought to be a beneficial characteristic for in situ gel. The content homogeneity of all of the formulations was excellent. The insignificant discrepancy between them could be attributable to human error or a loss of output. Mucoadhesion was good in all of the formulations. The formulations F3 and F4 with higher carbopol concentrations have better mucoadhesive properties than the other formulations F1 and F2. According to an in-vitro drug diffusion research, F4 had the lowest diffusion rate and F2 had the greatest. It can be argued that when viscosity rises, drug diffusion decreases, and the concentration of both polymers is proportional to viscosity. The Higuchi model of drug diffusion was seen in all of the formulations. The formulations Fl and F4 revealed non-fickian diffusion mechanisms, while F2 and F3 showed quasi-fickian diffusion mechanisms, according to Korsemeyer Peppas model. The formulation including 04 percent w/v Carbopl 934P and 20 percent w/v Poloxamer 407, i.e F3, was found to be the most desirable. Antimicrobial testing of the improved sol formulation of F3 revealed a satisfactory zone of inhibition for Gram negative and Gram positive microorganisms. As a result, the formulation can be concluded to have good preservation properties. However, it revealed a smaller zone of inhibition in gel form, implying that the borax diffusion is reduced in the gel phase of the formulation. It has antibacterial properties and can be used to treat mouth ulcers. In an animal model research, the formulation (F3) was found to be as effective as standard (ZYTEE) in the healing of mouth ulcers. The formulation was found to be stable under accelerated temperature and humidity conditions in a stability investigation.
As a result, a correctly developed in-situ gel for oral ulcers can extend the duration spent at the application site and minimize the frequency of administration.

Future prospect:

In situ gelling systems have garnered a lot of interest in the previous decade. The in situ gel meets the key requirement of a successful controlled release product: increasing patient compliance. The steady and prolonged release of drug from the in situ gel, as well as its good stability and biocompatibility, make it a very reliable dosage form. The use of mucoadhesive compounds and polymers that can both gel in situ and interface with the mucosa and/or mucus improves formulation performance even more. This system gels at the place of action when given as a solution. Finally, in situ gets are simple to use and reduce the size, pain, and colour of lesions. Although, more research on its stability and storage conditions statements must be carried out. The above successfully researched formulation look forward to develop in-situ gel spray form for ease of administration in oral cavity.

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