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ORIGINAL ARTICLE

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

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ABSTRACT

Objectives: Mouth ulcers are one of the most prevalent conditions that can be caused by a range of circumstances. Many formulations, such as solutions, suspensions, and ointments are available commercially. However, because there is no long-term effect, no medication can be regarded as totally effective for treating mouth ulcers. The use of bioadhesive methods can boost the therapy efficacy. Because it is easier to administer than prepared gel formulations, the phenomenon of the sol-to-gel conversion can be beneficial. The major goal of this study was to develop and test *in situ* gels for treating mouth ulcers using choline salicylate and borax as model medicines.

Materials and 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 increased with increasing temperature, *i.e.*, gel can be created at the 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 carbopol 934P was added, the gelling temperature was raised. All formulations had 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.

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 developed technology is a viable alternative to traditional drug delivery systems and can help patients comply.

Key words: *In situ* gel, thermo reversible, mucoadhesive, choline salicylate, mouth ulcer, 2^2 factorial designs

INTRODUCTION

Numerous routes of administration employed so far in new drug delivery systems, localized drug delivery to oral cavity tissues, have been examined for the treatment of periodontal diseases, bacterial and fungal infections, aphthous ulcers, and other disorders.[1] The oral mucosa is the "skin" that covers most of the mouth cavity, besides the teeth. It can be used for 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 are also called recurrent aphthous stomatitis (RAS), aphthae, aphthosis, and canker sores. The word aphthous is derived from the Greek word "aphtha", which signifies the 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 several illnesses with quite distinct etiologies. Nutritional deficiencies such as iron and vitamins, especially B12 and C, poor dental hygiene, infections, stress, indigestion,

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mechanical injury, food allergies, hormonal imbalance, and skin illness are all common causes of mouth ulcers. Hematinic deficits and blood disorders, gastrointestinal disorders, immune deficiencies such as in people with human immunodeficiency virus, 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 mouth ulcers. However, there are some 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]

Advantages of *in situ* forming polymeric drug delivery systems, such as ease of administration and better patient comfort, have piqued interest. They increase 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 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. To accomplish *in situ* gelation, both physical and chemical crosslinking 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 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 causes adverse effects such as stomach ulcers and increased blood concentration. This is required to examine the formulation that enhances the drug residence time and availability at the application location. Borax is a homeopathic 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 ulcers 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 mucoadhesive properties. Poloxamer 407 and carbopol P 934 were employed as 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 to find the best formula for improving patient compliance.

MATERIALS AND METHODS

This study certify that the project title "Development and evaluation of *in situ* gel formation for treatment of mouth ulcer" has been approved by IAEC at Appasaheb Birnale College of Pharmacy, Sangli (reference no: IAEC/ABCP/13/2015-16) issued on 07-11-2015.

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 a gift samples by Corel Pharma Chem Ahmadabad. Borax was 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 other materials used were of analytical grade.

Instruments required for the work

Franz-diffusion cell (SFDC6 model, manufactured by Logan); ultraviolet (UV)-visible double beam spectrophotometer (manufactured by Jasco, Japan); fourier transform infrared spectroscopy (FTIR-410 model, manufactured by Jasco, Japan), stability chamber (Tempo instruments PVT, Ltd.), and electronic balance, AUX 220 Model (Shimadzu, Japan) were used as the instruments.

Software required for research work

Design Expert Software (Star Ease, Inc.) was 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 (1000 mg) of ChS solution BP was mixed in 100 mL phosphate buffered saline (PBS) of pH 6.8 to get 10 mg/mL. Further diluted to get 100 µg/mL concentration of drug.

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

The method was validated using various parameters as *per* International Council for Harmonisation (ICH) guidelines such as accuracy, precision, limit of quantification (LOQ), limit of detection (LOD), and % relative standard deviation (RSD).

Formulation of in situ gel

Preparation and optimization of thermo-reversible PF 127 aqueous solution[11, 12]

The gel was prepared using the cold technique. Poloxamer concentrations ranging from 10% to 20% (*w/v*) 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 refrigerator for 24 h. 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, while temperature was allowed to rise at a rate of 2 ˚C/min. 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 the 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 to 0.5% (*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 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 the body temperature, concentrations were optimized and the experiment was designed by 2^2 factorial design.

Selection of independent variables

Gelation temperature of *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 (Table 1).

Experiment design 2^2 full factorial design

Table 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 the following parameters:

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

pH of the gel: Digital glass electrode pHmeter was used to measure pH of the gel by placing the electrode directly into 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 magnetic bead ceased to rotate due to the formation of gel.[14-18]

Thermoreversible study: Using a constant temperature bath, thermoreversible investigation was conducted. *In situ* gel compositions were kept in a temperature bath at 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 a shift from sol to gel phase was observed as well as changes in viscosity as point rose to the gelling temperature.[16-18]

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 formulations was measured using a Brookfield viscometer (Brookfield viscometer RTV) with spindle no: 62 at the speed of 10 rpm. The rheological properties were also studied by measuring viscosity of all formulations at speeds of 10, 50 and 100 rpm with spindle no: 62.

Shear rate (sec^{-1}) was calculated using the 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)

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

R_b= Radius of the spindle (in centimeters)

ω = $2 \div 60 \times N$

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

N = Spindle speed in RPM

Observed values:

R_c=1.5 cm; R_b = 1.25 cm

Shear stress (dynes/cm²) was calculated using the following formula:

Shear stress = Shear rate (sec^{-1}) ÷ Viscosity (cps)

Drug content

Percentage ChS BP content was determined by dissolving 0.5 g of the gel in 100 mL of pH 6.8 PBS and scanning the resultant solution with UV-visible spectrophotometer set to 238 nm. Calibration curve was used to calculate the drug content.[12,17,18]

Determination of mucoadhesive force

The mucoadhesive force was determined according to Desai and Shirsand[20] description (2018). 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 min. On the lower vial, around 1 g of gel was applied before the upper vial was inserted and 1 g 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

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 g 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 were then placed on a magnetic stirrer with thermostat. The medium was maintained at a constant temperature of 37 °C ± 0.5. After each 1 h interval, 1 mL of sample was withdrawn and same amount of PBS solution from blank was transferred into the sample cell for maintaining sink condition. Then, withdrawal amount was diluted to 10 mL in PBS pH 6.8, and 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. The best fit model was tested for Korsmeyers, Peppas, and Fickinian diffusion mechanism for their kinetics.[15,18]

Drug diffusion kinetic study

In vitro release data of the formulations 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. Model with best fit was chosen because of its comparatively high correlation coefficient value.[18]

Statistical optimization of in situ gel formulation

Gelatin temperature, viscosity of gel, diffusion of drug at 1 h, and time required for 90% drug diffusion are major variables for performance of the prepared *in situ* gel formulation. Formation of gel at oral temperature is fundamental to the prepared *in situ* gel. Drug release from gel is indirectly proportional to viscosity of the gel. Thus, viscosity of gel is a major variable to consider during design of *in situ* gel formulations. Salivation in the oral cavity restricts sustained release of gel formulations since gel may wash out with saliva. Thus, drug release at 1 h and the time required for 90% drug release must be considered. Both factors help to decide dosing frequency of the formulation. For statistical optimization of *in situ* gel, following criteria for selection of a suitable feasible region were decided (Table 2).

Antimicrobial test

An antimicrobial study was conducted to assess the medication borax antibacterial activity and to determine whether the formulation had enough antimicrobial properties. The test was conducted using 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 *S. aureus* was prepared and sterilized. The liquid was then put into sterile glass plate and allowed to set. The bacterial strains were dispersed aseptically over 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 wells and kept in the refrigerator for 15-20 minutes to allow the materials to diffuse into agar. The plates were then incubated in an incubator at 37 °C for 24 h. Zone of inhibition was assessed after incubation period.[13,15,16]

Animal model study

The study indicated how the produced formulation affected the healing of an oral ulcer in rats. In this study, 15 healthy female Wistar albino rats (weighed 130-150 g) 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 s to form a circular ulcer. The test group received an optimized formulation (F3), the standard group received ZYTEE gel (a commercialized ChS product), while the control group received no treatment. For 7 to 10 days, the ulcer healing progress was examined.[19-21]

Table 2. Desirable values of dependent variables for optimization

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

RESULTS

Analytical UV-visible method development and validation

λ_{max} of ChS in PBS 6.8 was found to be 238 nm. The drug follows linearity in the concentration range 10-50 g/mL with a correlation coefficient value of 0.9903. (Table 3). The accuracy of the method was checked by recovery experiments performed at three different levels, *i.e.* 80%, 100%, and 120%. Percentage recovery was found to be in the range of 98.54-99.98%. The low values of %RSD indicate accuracy and reproducibility of the method. Precision of the method was studied as intraday, interday variations, and repeatability. %RSD value <2 indicates that the method is precise (Table 3). Ruggedness of the proposed method was studied with the help of two analysts.

Formulation of in situ gel

Preparation and optimization of thermo-reversible PF 127 aqueous solution: The solution of poloxamer 407 with concentration of 10% *w/v* to 20% *w/v* was prepared in distilled water. Gelation temperatures of the solutions were found as depicted in Table 4.

Concentrations of 15% (*w/v*) to 20% (*w/v*) were considered as optimum for formulation.

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 5.

It was observed that there was an increase in gelling temperature on addition of carbopol 934P. Thus, concentration of poloxamer was increased to form the gel near body temperature. Gelation temperatures were observed as given in Table 6.

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 poloxamer 407 and carbopol 943P solutions and gelling temperature were observed (Table 7), where there was no significant difference upon the addition of other ingredients.

The formulation of batches based on the design of experiment

Different formulation batches F1 to F4 were prepared based on the design of experiment by 2^2 factorial design (Table 7).

Selection of independent variables (Tables 8, 9)

Evaluation of formulation

Appearance: In both solution and gel forms, all the formulations were determined to be clear and transparent. A clear translucent gel created on a mouth ulcer will increase patient compliance because it mimics natural oral mucosa, allowing for daytime application.

Table 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

SD: Standard deviation, LOD: Limit of detection, LOQ: Limit of quantification, UV: Ultraviolet

Table 4. Gelation temperature of poloxamer 407

Concentration of poloxamer 407 (% *w/v*)	Gelation temperature (°C)
11	46
12	42
13	39
14	38
15	37
16	35
17	34
18	30
19	28
20	25

Table 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

pH of the gel: pH of all formulations was found to be between 5.5 and 6.8 (Table 10). To avoid irritation of the mucosa and further damage to the ulcer, pH of the formulation produced to treat mucus ulcers must be close to neutral. In general, any formulation utilized for the mucosa should have a pH of 4.5 to 7.

Gelation temperature: 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 *in situ* gel formulation. At close to body temperature, *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 ˚C (Table 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 F2 formulation had lower concentration of both polymers, while the F3 formulation had larger concentration of both polymers.

Because of the observed gelling temperature, it can be concluded that concentration of poloxamer 407 had a proportional effect

Table 6. Gelation temperature of 407 and 934P mixture

Conc. of carbopol 943P (% *w/v*) → / Conc. of poloxamer 407 (% *w/v*) ↓	0.1	0.4	0.6
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

Table 7. Gelling temperature of the 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**

Table 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 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

ChS: Choline salicylate

on gelling temperature, whereas the gelling temperature increased, when the carbopol 943P was 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 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 pack 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. 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 Figure 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 (Figure 2), the smallest concentration of the polymer has the highest gelation

Table 10. Observations of various evaluation 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

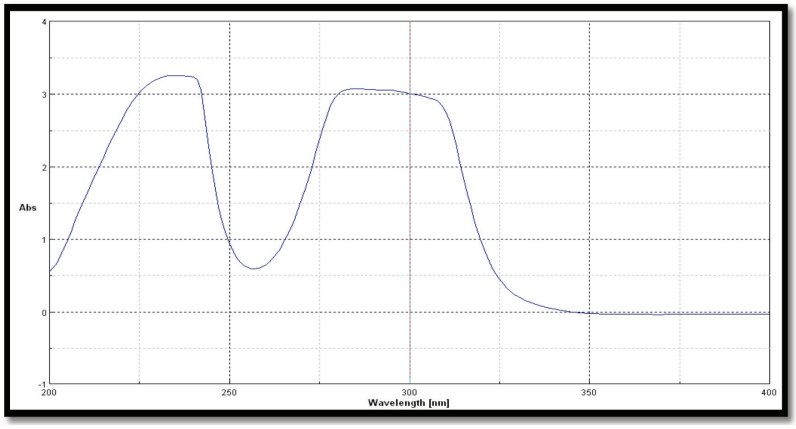

Figure 1. UV spectra of ChS BP
UV: Ultraviolet, ChS: Choline salicylate

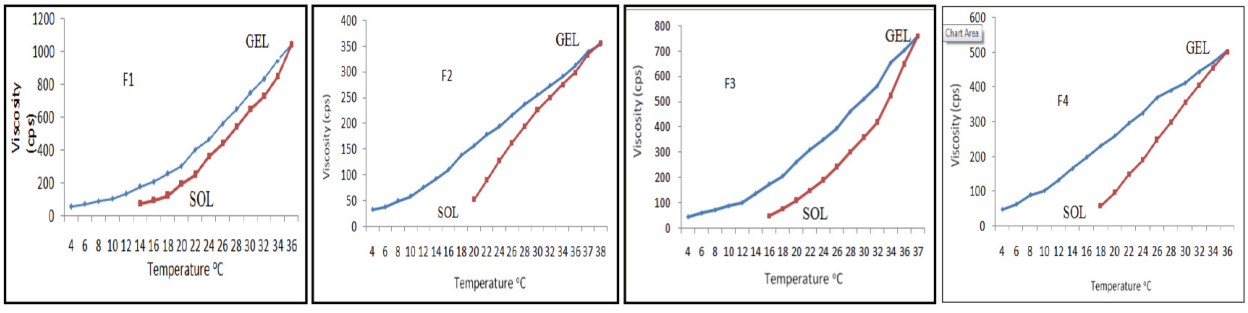

Figure 2. Thermoreversible gel to sol phase diagram of prepared *in situ* gel formulations

temperature and 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.

Viscosity and rheological properties: This is one of the most significant requirements for *in situ* gel formulation. To remain for a long time at the site of application, *in situ* gel formulation should have a viscosity of more than 100 cps, when it is applied and less than 1000 cps and when it converts to the gel after administration.

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 polymers poloxamer 407 and carbopol 934P increased. Table 10 provides viscosity (centipoises) of the prepared formulations, and Figures 3a and 3b display the shear rate (sec) and shear stress (dyne/cm^2) of all batches.

It was discovered that viscosity varied depending on the shearing rate. In other words, the ratio of shear stress to the 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 a non-Newtonian fluid. As the shear rate increased, viscosity of the gel dropped. This demonstrated that *in situ* gel was shear thinning pseudoplastic by nature.

Drug content: As stated in Table 10, percent ChS BP of all formulations was determined to be in the range of 98 to 100%. It is possible that discrepancy in medication content is attributable to human mistake during dilution or to production loss during the formulation preparation.

Determination of mucoadhesive force: Mucoadhesion is an interfacial phenomenon 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 gel, the cross linkage between poloxamer 407 increases rendering the polyoxyethylene groups unavailable for mucoadhesion. According to the diffusion interlocking hypothesis, when crosslink 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 an increase in mucoadhesion. Carbopol is a synthetic mucoadhesive agent. It adheres to the mucosa by a -COOH bond. Formulations F3 and F4 contain higher concentrations of carbopol and indicate strong bioadhesion as compared to other formulations (Table 10).

In vitro diffusion study: An *in vitro* diffusion study was conducted using Franz diffusion cell with pore size of 40 µm and cellophane membrane. In Figure 4, the percentage cumulative ChS BP diffusion obtained from all formulations is displayed. 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 5th hour. It could be because F2 had lower concentration of both polymers, while F1 had higher concentration of both polymers.

In general, the drug diffusion rate reduces as the crosslinking 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. The diffusion of drugs is thus a polymer concentration-dependent process. An *in situ* gel that exhibits 40% drug release after 1 hour and 90% drug release after 4 h was tempted to prepare. F1 formulation was not determined to be optimum (Figure 4).

Diffusion kinetic study: According to data from diffusion studies, the generated *in situ* gel had significant initial drug release (burst effect) and then decreased as gelation progressed. This is a biphasic 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 (Table 11).

Korsmeyer-Peppas model is commonly used to confirm the drug release process from the matrix. The "n" value (Korsmeyer-Peppas model release exponential) was used to characterize the various release mechanisms in the following way:

n<0.5 Quasi Fickian diffusion

n-0.5: Diffusion mechanism

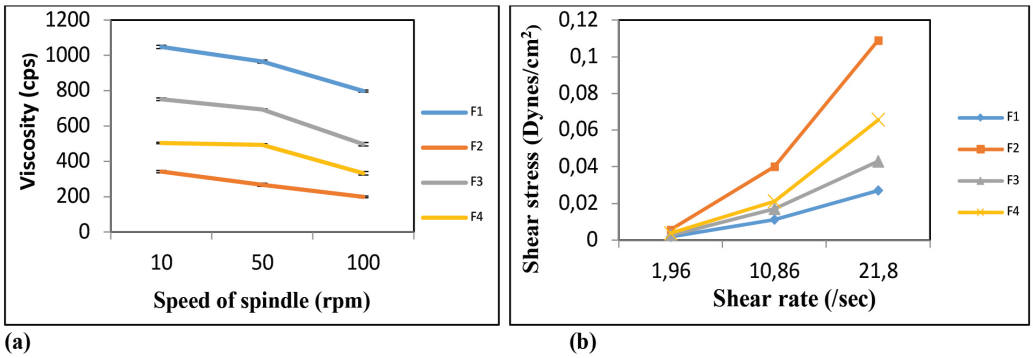

Figure 3. (a) Viscosity (cps) v/s speed of spindle (rpm) graph, (b) shear stress v/s shear rate graph showing non-Newtonian fluid

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 Korsmeyer-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 utilized to modify the diffusion mechanism from formulations. Since n values of 0.7 and 0.57 were reported, 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 (Table 11).

The dissolution data for Higuchi model was investigated to see, if the drug release was diffusion regulated or not. For all formulations, a graph of percentage CDR *vs.* square root of time was drawn. All straight line correlation coefficients were determined in the range of 0.943 to 0.996. As a result, all formulations followed Higuchi's diffusion model (Table 11).

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 several data.

Software stat ease: Design Expert 10 was used to derive conclusions based on the amount of the coefficient and the mathematical sign (positive or negative) they carried.

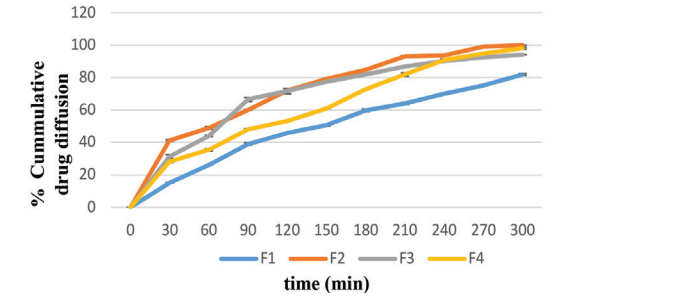

Figure 4. *In vitro* drug diffusion study of prepared *in situ* gel formulations

Optimization of polymer concentrations for gelation temperature: Concerning Y1 (gelation temperature) the data clearly indicated that it is strongly dependent on the selected variables X1 and X2

YI 36.56-0.98 X1-49X2 + 0.042 X1X2

The findings of multiple linear analysis revealed that both coefficients 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. Combination of two polymers, on the other hand, had positive effect on gelation temperature and micellar aggregation. Only when the concentration of poloxamer 407 exceeds the micellar concentration, resulting in the micelle production, gel phase can occur. The hydrophobic sections of the pluronic are kept apart by hydrogen bonding between the POP chains and water, when the material is immersed in cold water. 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) increased, gelation temperature decreased. It is probable that the ability of mucoadhesive polymers to reduce gelation temperature is linked to increased viscosity the following polymer disintegration and the ability of mucoadhesive polymers to adhere to 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. Figure 5c displays the effects of X1 and X2 on Y. The gelation temperature was reduced as the amount of poloxamer 407 and carbopol 934P was increased (Table 12).

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

$Y2\ 661.33 + 114.41X_1 + 238.39X_2 + 33.51\ X_1X_2$

The results revealed that both X_1 and X_2 have positive coefficients. Because of rising X_1 and X_2 values, viscosity is projected to rise. Both elements have favourable effect on

Table 11. Results of drug diffusion a kinetic study

Formulation	Zero order	The 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

viscosity, when used separately and in combination. The fact that X_2 has a higher coefficient value than X shows that X_2 is more effective in terms of viscosity than X_1. Surface plot Figure 5d can be used to explain the relationship between selected parameters and response viscosity (Table 12).

Optimization of polymer concentrations for drug diffusion at 1 h: The data clearly indicated that drug diffusion values at 1 h are substantially reliant on the specified independent variables, namely poloxamer 407 concentration and carbopol 934P concentration. Transformed factor is related to the response (release at 1 hour) by the fitted equation (for full model).

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

Coefficients 1 and 2 for the prediction of release at 1 h were found to be significant at p=0.05. 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. 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 h release (Table 12). Using a response surface plot (Figure 5a), the link between formulation variables poloxamer 407 (X_1) and carbopol 934P (X_2) was further explored.

Optimization of polymer concentrations for the time required for 90% drug diffusion: In the case of Y2, the result of multiple regression analysis showed that the coefficient diffusion (+45) and P_2 (+40) bear positive signs. The positive sign of both X_1 and X_2 coefficients indicates that as concentration of both poloxamer 407 and carbopol 934P increased the time required for 90% drug diffusion increased. Summary of regression analysis can be explained by the following polynomial equation:

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

Y2 exhibited a good correlation coefficient of 1.000 for all batches F1 to F4. XI had a p value of 0.0001 and X2 had a p

Table 12. Results of experimental design batches of variables

Formulation code	Diffusion at 1 hY1 (%)	Time required for 90% drug diffusion Y2 (hrs)	Gelation temperature Y3 (°C)	Viscosity Y4 (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

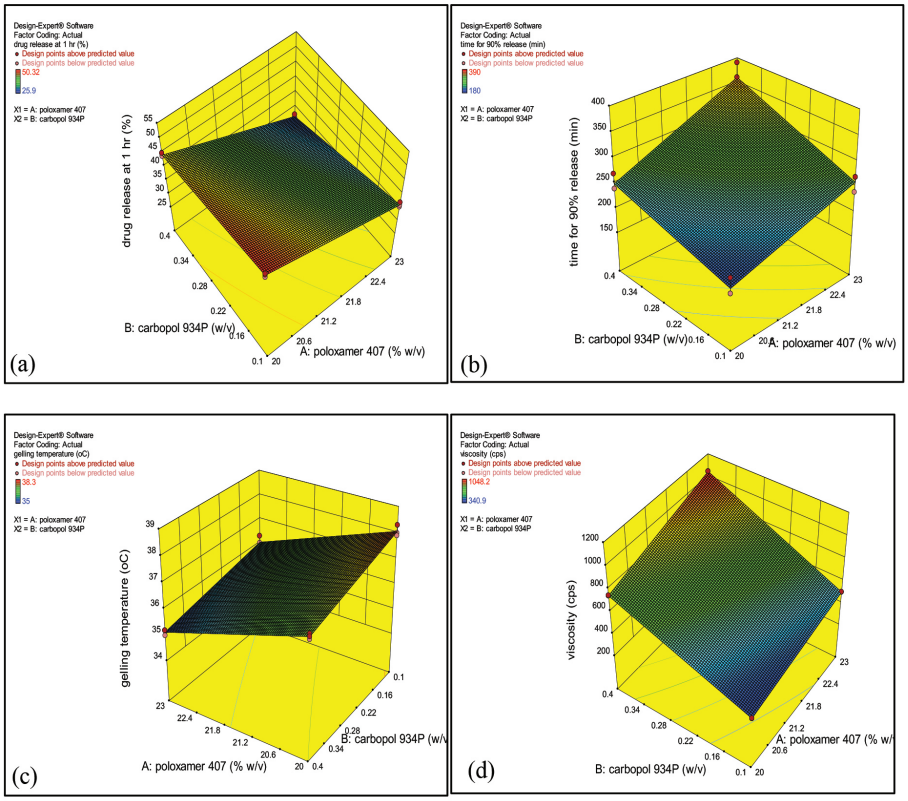

Figure 5. Response surface plot of optimization of polymer concentrations for (a), drug diffusion at 1 hr (b), time required for 90% drug diffusion (c), gelation temperature (°C) (d) viscosity (cps)

value of 0.0001. Both *p* values were less than 0.05, indicating that the independent factors have substantial impact on the time necessary for 90% drug diffusion. The time required for 90 % drug diffusion increased as the concentrations of poloxamer 407 and carbopol 934P rose (Table 12). It could be attributed to an increase in cross-linkage because of higher polymer concentrations resulting in lower drug diffusion from *in situ* gels polymeric network.

The relationship between formulation variables, *i.e.* poloxamer 407 (X_1) and carbopol 934P (X_2), was further elucidated using the response surface plot Figure 5b.

Analysis of variance

The R^2 values for gelation temperature (Y), viscosity (Y2), CPR at 1 h (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 antibacterial, antifungal, and antiallergic properties. As a result, borax can be used as both an antiulcer and a preservative. Zone of inhibition obtained by improved formulation (F3) in sol form, conventional ZYTEE gel, and glycerol-borax as shown in Figure 6 and Table 13 can act on both Gram-positive (*E. coli*) and Gram-negative bacteria (*S. aureus*).

There is a negligible difference between zones of inhibition of the standard and the formulation in gel form, which shows that the formulation has preservative properties similar to those of the 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.

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. Ulcer healing properties of the formulation were found to be comparable to those of the reference (Figure 7). The observation was made based on the ulcer's every day 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 for treating mouth ulcers.

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% (*w/v*), the gelling temperature was close to the body temperature (35-38 °C), however, when carbopol 934P was added, the

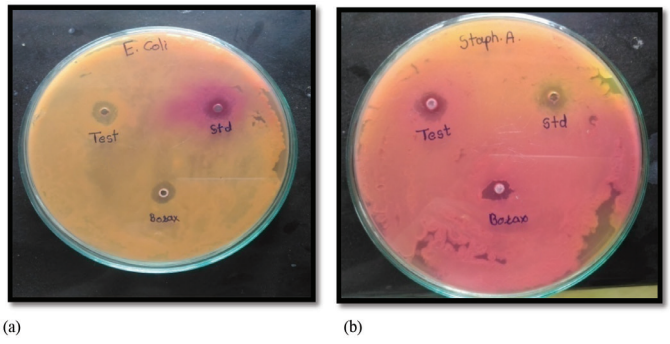

Figure 6. Zones of inhibition of prepared *in situ* gel formation batch F3 (sol form) (a) *Escherichia coli* and (b) *Staphylococcus aureus*

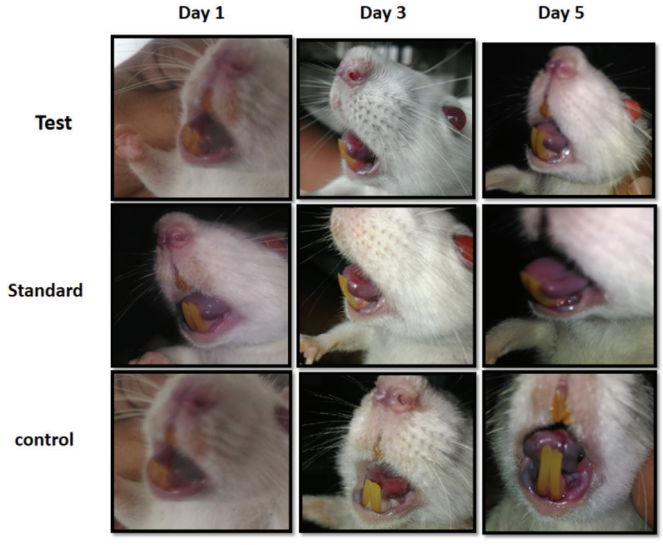

Figure 7. Animal model study

Table 13. Zone of inhibition (mm) shown by prepared formulation

Microorganisms	Formulation	Standard	Glycero-borax
Escherichia coli	22 mm	25 mm	14 mm
Staphylococcus aureus	25 mm	28 mm	17 mm

gelling temperature was raised. Carbopol may cause micelle aggregation, size, and entanglement to decrease, resulting in an increase in gelation temperature. 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.

Thermoreversibility of the formulations was discovered. All formulations had 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 simple administration of the formulation to a mouth ulcer. Rheological tests revealed that the *in situ* gel had a non-Newtonian flow and was a shear-thinning pseudo-plastic. It is thought to be a beneficial characteristic for *in situ* gel. Content homogeneity of all the formulations was excellent. The insignificant discrepancy between them could be attributable to human error or loss of output. Mucoadhesion was good in all formulations. The formulations F3 and F4 with higher carbopol concentrations have better mucoadhesive properties than the other formulations F1 and F2. According to *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. Higuchi model of drug diffusion was seen in all formulations. Formulations Fl and F4 revealed non-Fickian diffusion mechanisms, while F2 and F3 showed quasi-Fickian diffusion mechanisms according to Korsmeyer-Peppas model. The formulation including 0.4% (*w/v*) carbopol 934P and 20% (*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. Nevertheless, it revealed a smaller zone of inhibition in gel form, implying that borax diffusion is reduced in gel phase of the formulation. It has antibacterial properties and can be used to treat mouth ulcers. In animal model research, 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 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 prospects

In situ gelling systems have garnered a lot of interest in the past decade. *In situ* gel meets the key requirement of a successful controlled release product, increasing patient compliance. The steady and prolonged release of drug from *in situ* gel and 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 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* treatments are simple to use and reduce the size, pain, and the colour of lesions. However, more research on its stability and storage conditions statements must be carried out. The above successfully researched formulation looks forward to developing an *in situ* gel spray form for ease of administration in the oral cavity.

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Ethics

Ethics Committee Approval: Ethical approval of this study was obtained from the Institutional Animal Ethics Committee, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India, (reference no: IAEC/ABCP/13/2015-16).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.H.G., Concept: N.H.G., Design: N.H.G., Data Collection or Processing: N.H.G., Analysis or Interpretation: N.H.G., P.S.H., Literature Search: N.H.G., P.S.H., Writing: N.H.G., P.S.H.

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