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Original Article

Design and Optimization of Brimonidine-Embedded Hydrogel: *In vitro* and *in vivo* Characterization

Dipti Gohil*, Dixit Patel, Nirmal Shah, Chintan Aundhia, Sunil Kardani, Rahul Trivedi

Department of Pharmacy, Sumandeep Vidyapeeth (Deemed to be University), Vadodara, Gujarat, INDIA.

ABSTRACT

Background: Topical alpha agonists approved by the FDA are used to treat rosacea. Because of its potent vasoconstrictor properties, brimonidine tartrate topical gel (0.33%) efficiently reduces erythema. The aim of this investigation was to develop and optimize a hydrogel containing brimonidine for topical application. **Materials and Methods:** Different gelling agents were used in variable amounts to generate gel formulations loaded with brimonidine tartrate. Following the evaluation of the preliminary batches, the One Factor at a Time (OFAT) design was used for optimization. An optimized batch of Gel was evaluated for pH, washability, homogeneity, viscosity, %Drug release and skin irritation study. **Results and Discussion:** Among the diverse polymers considered, the obtained results highlight Carbopol 934 was chosen for its stability, gel strength and consistent drug content in the dispersion medium. An optimization study revealed that the concentration of Carbopol 934 played a crucial role in formulation development, viscosity and *in vitro* drug release. **Conclusion:** Carbopol was chosen as a gelling agent for optimisation based on the preliminary investigation. The optimized batch exhibited desirable characteristics in terms of both drug release and viscosity. Further, the evaluation of the prepared gel confirmed the desirable properties. Additionally, a skin irritation study confirmed the suitability of the application of the gel.

Keywords: Rosacea, Brimonidine tartrate, Hydrogel formulation, Design of Experiment, Topical delivery.

Correspondence:
Dr. Dipti Gohil
Department of Pharmacy, Sumandeep Vidyapeeth (Deemed to be University), Piparia-391760, Vadodara, Gujarat, INDIA.
Email: diptigohil2014@gmail.com

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INTRODUCTION

Rosacea is identified by a diverse range of signs and symptoms, with persistent facial erythema being the most prominent[1-4] This erythema is marked by recurrent episodes of exacerbation and remission. It is estimated that at least 10% of individuals with a Caucasian background are affected by this condition and it is more prevalent in women.[2] Although the precise cause of rosacea remains elusive, it is believed to stem from a complex process, possibly linked to hereditary susceptibility. Various factors, such as microbes, exposure to UV radiation and disruption of the epidermal barrier, can trigger an augmented innate immune response and/or neurovascular dysregulation.[3] Rosacea predominantly impacts the skin on the face, leading to adverse effects on emotional and psychological health.[4,5]

A highly selective α2-adrenoreceptor agonist, Brimonidine Tartrate, causes constriction of subcutaneous blood capillaries. Additionally, animal studies have demonstrated its anti-inflammatory properties.[6] Food and Drug Administration approved brimonidine as the first topical medication specifically prescribed for rosacea-related facial erythema.[7-9]

Brimonidine tartrate, a class I biopharmaceutical medication, possesses notable characteristics such as high permeability and solubility.[10] Prolonged administration of the medication in gel form circumvents initial metabolic effects. The gel, containing brimonidine tartrate, exhibits efficient drug release, offering rapid relief from erythema. Its consistent dispersion, attributed to a strong affinity between the dispersed phase and the dispersion medium, prevents settling and ensures uniform distribution. Notably, its high permeability and minimal toxicity contribute to enhancing patient compliance and safety.[11]

This study aimed to formulate a gel containing brimonidine tartrate with various gelling agents at different concentrations. The optimization was carried out using an appropriate design of experiments to achieve the optimal gel properties.

MATERIALS AND METHODS

Brimonidine was purchased from Indoco remedies, Carbopol 934, Triethanolamine was purchased from Loba chemie. Other excipients used were of analytical grade.

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Preparation of Hydrogel

The accurately weighed drug was dissolved in a measured volume of distilled water. The solution was stirred by a glass rod. The gelling agent was added to the mixture. The mixture was kept a side for a while. The neutraliser- Triethanolamine was added drop by drop to obtain the required pH.[12]

Compatibility Study of Drug with Excipients to be Used for Formulation Development

The compatibility study was performed using Fourier Transform IR spectroscopic analysis. Spectra were obtained for the API and in combination with other excipients, forming a physical mixture used in the formulation development, within the wavelength range of 4000-400 cm^{-1}.[13]

Experimental Design

Various gelling agents, including HPMC K4M, HPMC K15M, HPMC K100M, HPMC E5, HPMC E10, Sodium Alginate, Pectin, Xanthan Gum and Carbopol were utilized in different concentrations ranging from 1-3% w/w to prepare preliminary batches. The polymers were examined both individually and in combination. Based on the preliminary study findings, Carbopol 934 was identified as the optimal choice for further optimization.

A factorial design methodology was utilized to determine the optimal percentage of Carbopol 934. A 1-level, 1-factor design incorporating a centre point optimization process was employed to assess the impact of brimonidine tartrate. Seven potential pairings were systematically examined through a series of experiments.[14] Throughout the trials, the concentration of the Carbopol (X1), the consistent parameters of the primary trial batch, including distilled water, triethanolamine and brimonidine tartrate, remained unchanged while manipulating the independent variables.[15] Viscosity (measured in cps) and %Drug Release over a 5 hr period were selected as the dependent variables (Table 1). The study focused on individual criteria at a single level for the independent variables.

The experiments were carried out employing Design Expert software (Version 11.0.1.0, Stat-Ease Inc., Minneapolis, USA) for the generation of experimental trials. DoE program was employed to explore potential interactions among the selected independent variable and the dependent variables. Furthermore, it furnished essential information regarding the critical values required to achieve the desired response. A one factorial design, encompassing four model points, five lacks of fit and five central points, was selected for the study.

Evaluation of Gel

pH Determination

A digital pH meter, previously calibrated with standard buffer solutions, was utilized to assess the pH of the gel preparation. After calibration, the electrode was immersed in an aqueous gel solution (1 g in 10 mL water) at a temperature consistently maintained at 25°±5°C for pH measurement.[16]

Homogeneity Test

To ensure an even distribution of components within the topical gel, a homogeneity test is conducted. This evaluation involves pressing 0.1 g of the gel between the thumb and index finger to assess its uniformity and detect any coarse particles adhering to or being removed from the finger.

Grittiness

The optimized batch of gel was subjected to examination to detect the presence of aggregates or particle debris. Visual examination of the gel was performed for the presence of visible particles or uneven texture for determination of potential grittiness.

Washability Test

This study of gel formulation was performed to assess the washability of the gel formulation post-application. A quantity of 0.1 g of gel was applied and rubbed onto the back of the hand. Subsequently, the gel-coated area was allowed to dry before attempting to clean it with tap water.

Spreadability Test

A Spreadability apparatus was utilized, consisting of a wooden board equipped with a scale and two glass slides affixed with pans on one side through a pulley system. The sample was placed between the glass slides and compressed to a consistent thickness using a 100-gram weight. The time taken to move the slides 5 cm determined Spreadability.[17]

The formula used to calculate Spreadability (S) might be represented as follows:

$$S=M.\ L/T$$

Where, M=Weight fastened to the top slide, L is the Length of the glass slide, 'T' refers to the Time (sec) required to achieve the separation of the two slides.

Drug content

Drug content is the percentage of Brimonidine tartrate added to the gel after measurement. The optimized gel batch equivalent to 0.33% w/w was weighed, and dissolved in methanol. Subsequent dilutions were prepared and assessed for drug content using a UV spectrometer.

Viscosity

For Viscosity measurement, the sample was positioned beneath the Helipath T-F 95 spindle on the Brookfield Viscometer (DV-III). The spindle was set at 0.1 rpm and the motor was turned off. The sample was allowed to rest for 5-7 min to ensure

consistent and reliable outcomes.[18] To get stable and constant results, the torque and viscosity of the samples were observed at 25±5°C temperature.

% Drug Release

The dialysis membrane had been pre-soaked in 100 mL of water for 24 hr and subsequently cut to the desired length. For *in vitro* assessment of drug release, a Franz diffusion cell was utilized, with cellophane paper serving as the semipermeable membrane. The diffusion study was carried out by placing a 5 mL dispersion of gel in the donor compartment and 100 mL of PBS (pH 5.5) serving as the receptor compartment, maintaining a constant temperature of 37±0.5°C.[19] Readings were recorded at 1 hr intervals over a span of 5 hr. The quantity of drug released was measured using UV spectroscopy.

Skin irritation study

The Animal Ethical Committee granted consent for the animal study conducted in this research and ethical clearance was obtained with the clearance number SVU/DP/IAEC/2022/06/55 at the Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara, Gujarat.

A skin irritation study was conducted on the shaved dorsal skin of New Zealand albino rabbits. The dorsal skin was shaved and the area was monitored for 24 hr to detect any signs of irritation resulting from the shaving procedure. After confirming the absence of injury, the animals were randomly divided into three groups: Group I served as the normal control (0.9% w/v saline), Group II was the disease control (2% NaOH as an irritant) and Group III received the gel formulation (0.33% w/w). The rabbits were housed in a climate-controlled environment with a 12 hr light/dark cycle, maintained at 25±2°C and 45% relative humidity. A single application of the formulation, equivalent to 0.33% w/w gel, was applied to the shaved region using a cotton swab. Skin irritation was assessed based on visual observations of redness or erythema, with assigned values as follows: no erythema=0, slight erythema (light pink)=1, moderate erythema (dark pink)=2, moderate to severe erythema (light red)=3 and severe erythema (intense redness)=4.

Stability Study

The stability assessment focused on the optimized formulation. Glass vials were utilized to store the gel as per the ICH-recommended conditions[20] (25±2°C/60%±5% RH, 40±2°C/75%±5% RH) over a 30-day duration. Subsequently, the samples were analysed to measure the percentage of drug release after a 30-day period, within a 5-hour timeframe.

RESULTS

Compatibility Study of Drug with Excipients to be used for Formulation Development

All spectra were compared with the Brimonidine Tartrate spectrum to detect any potential interactions between the API and excipients.

Optimization of Formulation

Utilizing statistical design expert® software, a correlation between the factors and responses was established through a rigorous analytical process.

The first step entailed fitting the investigational data into an appropriate model. Several factors, including the *p*-value, regression co-efficient were taken into account to determine the most suitable model. ANOVA at a significance level of 5% was carried out to establish the model significance. In instances,

Table 1: Variables and responses used in one factorial design.

Batch code	Conc. of Carbopol (%)	% Drug Release (5 hr)	Viscosity (cps)
1	2.10787	84.65	10945
2	1.89	90.1	10234
3	2	87.24	10384
4	1.78419	93.17	9214
5	1.64	96.24	8453
6	2.215	81.95	11487
7	2.36	79.34	12697
8	2.215	82.45	11245
9	1.78419	92.14	9459
10	1.5	97.14	7214
11	2.5	76.19	13647
12	2.5	75.53	13892
13	1.5	96.54	7539
14	2.215	83.67	11584

where multiple models demonstrated significance ($p<0.05$), the choice of the optimal mathematical model for a specific response was based on the correlation between adjusted R^2 and Predicted Residual Sum of Square (PRESS) values. A smaller PRESS value indicated a more satisfactory fit for the model. Simultaneous fitting of observed responses from 14 formulated variations was conducted across linear, 2FI, quadratic and cubic models. Among these models, the Cubic response surface model demonstrated the strongest fit.

The effect of the Concentration of Carbopol on drug release and viscosity was studied using the One Factor at a Time (OFAT) design. Drug release was observed in the range of 75.53- 97.14% and Viscosity in the range of 7214-13892 cps.

Evaluation of optimized formulation

pH Determination

The pH of the gel was observed 5.55±0.85; which fell within the normal range, suggesting that the gel formulation is suitable for application on the skin.

Homogeneity Test

Gel (0.1 g) was assessed to notice the consistency of the gel. It was observed that the Gel had a smooth texture and homogenous appearance with no signs of phase separation.

Grittiness

Addressing grittiness in topical formulations is crucial for enhancing user satisfaction and ensuring the product's effectiveness without causing discomfort during application. The formulated hydrogel was noted to be smooth and devoid of any grittiness.

Washability test

Washability test can help to determine the effectiveness of the removal of gel from the skin. Applied layer of gel was easily washed off from the skin which is crucial in evaluating the product's user-friendliness and its ability to be easily removed after application.

Spreadability test

A Spreadability test ensures that the product spreads evenly over the surface, preventing localized concentrations that might affect its efficacy. The prepared hydrogel demonstrated a notable impact on its Spreadability, as per below the calculation.

$$S=M.\ L/T$$

Where, S=Spreadability, M=Weight tied to upper slide, L=Length of glass slide, T=Time taken to travel 5 cm.

$$Spreadability=500*3/41=36.5\ g\bullet cm/sec$$

Drug content

The drug content analysis of brimonidine-loaded gel revealed a high concentration, measuring 96.43±1.25%. This indicates a robust and efficient loading of brimonidine within the gel matrix. The percentage suggests a successful incorporation and distribution of the drug. The obtained drug content aligns with the anticipated levels, signifying the accuracy in accommodating the intended drug concentration in the hydrogel. Such high drug content signifies the potential efficacy of gel for delivering the specified dose of brimonidine.

Viscosity

The viscosity value of gel indicates the resistance to flow at a low shear rate. The recorded viscosity measurement at the specified rotational speed provides valuable information about the gel's physical properties, aiding in its characterization and potential application in controlled drug release or topical delivery systems. The Gel viscosity of 10384 cps signifies a substantial thickness, which could impact the Spreadability and adherence to the targeted area upon application.

Table 2: Skin Irritation test of Gel.

Time	Score (Control Group)		Score (Treatment Group)	
	Erythema	Oedema	Erythema	Oedema
1 hr	0	0	0	0
24 hr	0	0	0	0
2nd day	0	0	0	0
3rd day	0	0	0	0
4th day	0	0	0	0
5th day	0	0	0	0
6th day	0	0	0	0
7th day	0	0	0	0

%Drug release

At the initial time point, no significant drug release was observed, indicating minimal leakage or immediate release. Subsequently, brimonidine release increased gradually, demonstrating a progressive and sustained drug release profile. The release data (Figure 1), suggesting a substantial and consistent release of brimonidine from the gel formulation.

Skin Irritation study

The dermal irritation results are outlined in Table 2. No visible signs of skin irritation, including oedema and erythema, or inflammation were observed throughout the study period, in comparison to the control (Figure 2). One hour after the removal of the test substance and subsequently, all rabbits exhibited a score of "0" for both oedema and erythema.

Stability Study

The optimized formulation was subjected to a stability study in accordance with ICH guidelines. The gel from the optimized formulation was dispensed into glass vials and subjected to storage conditions (Table 3). After this duration, the samples were assessed for % Drug Release (5 hr) and Viscosity, which revealed no notable changes.

DISCUSSION

From the result of the IR spectrum (Figure 3), it was concluded that no significant interaction occurred between the drug and the chosen excipients, affirming their suitability for formulation development.

The outcome of washability, grittiness, homogeneity and Spreadability tests validate the appropriateness of the formulated gel. The elevated viscosity indicates a notably thick consistency, potentially enhancing adherence and prolonging retention on the targeted surface. The release of brimonidine from the gel follows a time-dependent pattern during the specified durations.

The skin irritation study affirms the suitability of the dermal application for the developed dosage form, while the stability study provides data supporting the stability of the formulated dosage form.

Optimization of Formulation

As depicted in the graph (Figure 4), the drug release was highest when the gelling agent concentration was low and decreased progressively as the concentration of the gelling agent increased. Reduced drug release can be attributed to the notably high binding characteristics of the gelling agent at higher concentrations.

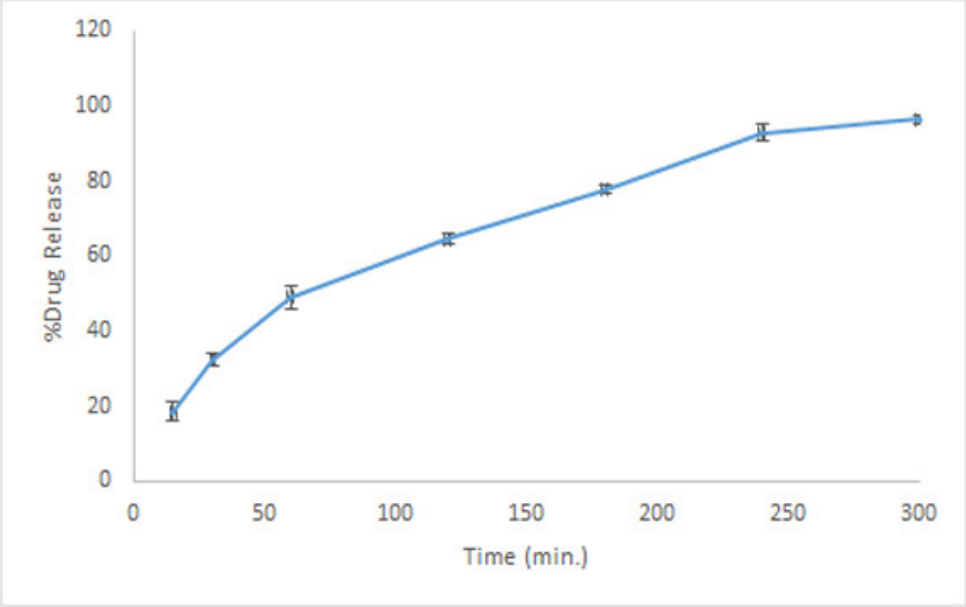

Figure 1: Drug Release of Optimized Batch.

Table 3: Stability study of Optimized formulation.

Test parameter	Initial	25°C/60 % RH (1 month)	40°C/75 % RH (1 month)
% Drug Release (5 hr)	92.28	91.56	90.84
Viscosity (cps)	10384	10256	9895

Table 4: **Formula for optimized batch of gel.**

Sl. No.	Ingredients	Quantity (%)
1	Drug	0.33
2	Carbopol 934	2
3	Triethanolamine	q.s.
4	Distilled water	Up to 100 mL

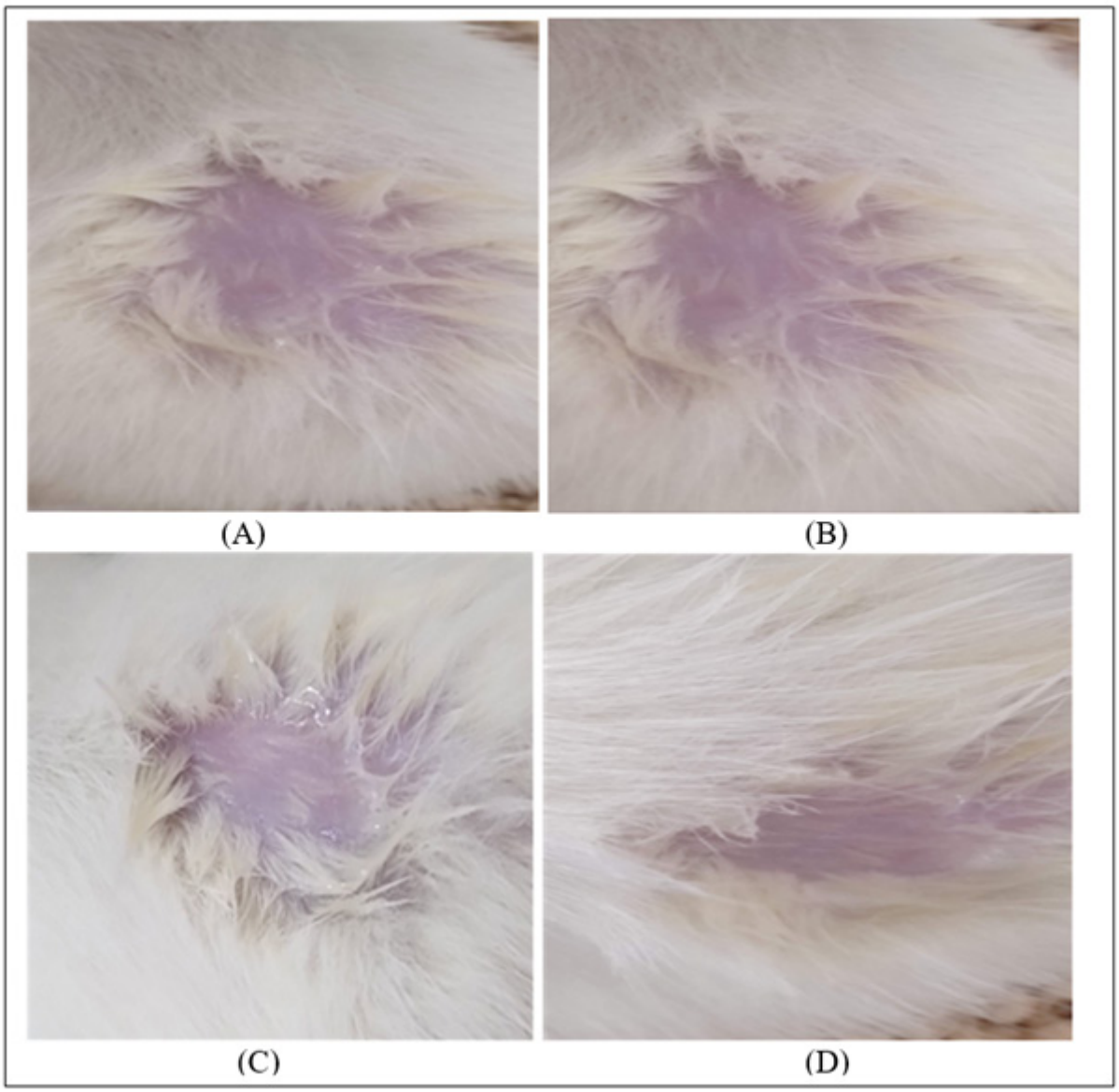

Figure 2: Skin irritation study (A) Control Group, (B) Treatment group after 1 hr, (C) Treatment group after 24 hr, (D) Treatment group after 7 days.

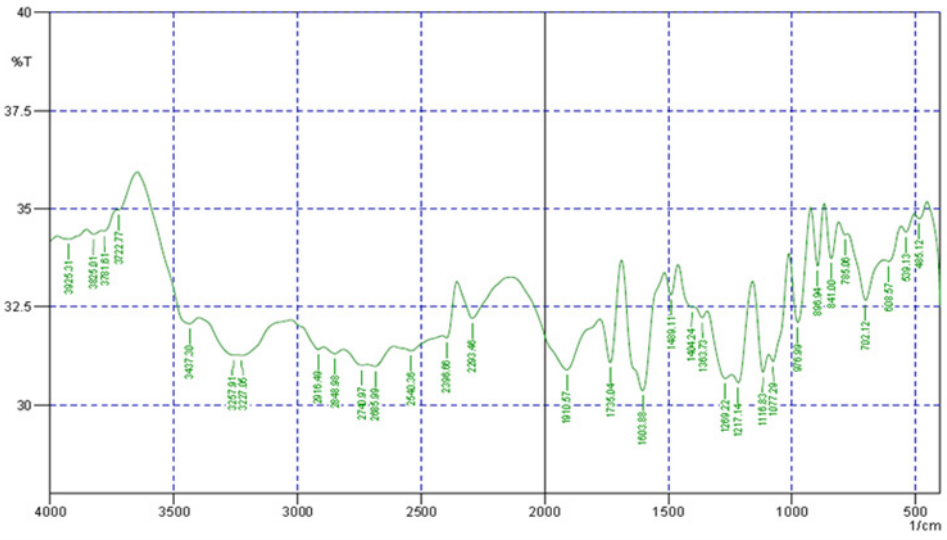

Figure 3: FT-IR Spectrum of pure drug.

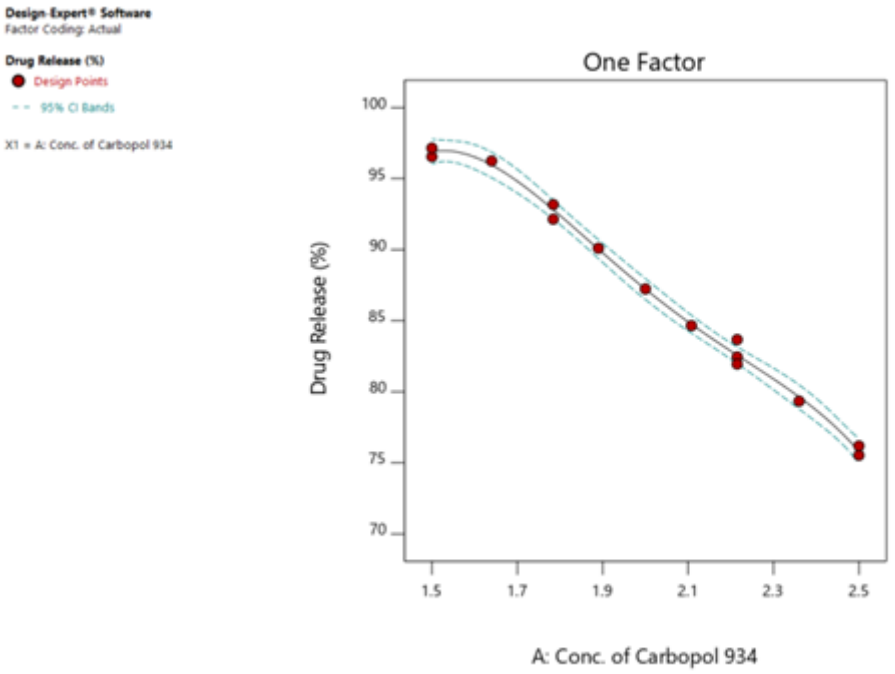

Figure 4: Effect of the independent variable on %Drug Release.

The graphical representation (Figure 5) illustrated the relationship between the concentration of the Carbopol and viscosity. As depicted in the graph, viscosity exhibited a trend of being lower at lower gelling agent concentrations and higher at higher concentrations. This change occurred due to the effective thickening properties inherent in the gelling agent. With increasing concentrations of the gelling agent, there was a consistent and continuous rise in viscosity.

The selection of the optimized batch (Table 4) of Gel involved assessing the proximity of the desirability factor to 1. Consequently, based on the selected criteria, i.e., viscosity <10000 cps and maximum % Drug release; software suggested an optimal concentration of 2% for the gelling agent might be considered ideal for facilitating medication release.

The experimental values for the responses of the optimized formulation closely matched the predicted values, affirming the validity of the Response Surface Methodology (RSM) model.

The analysis of R^2 values indicated a superior fit of the release data to the Higuchi model (0.9934), suggesting that the model is suitable for explaining the behaviour of drug release from the

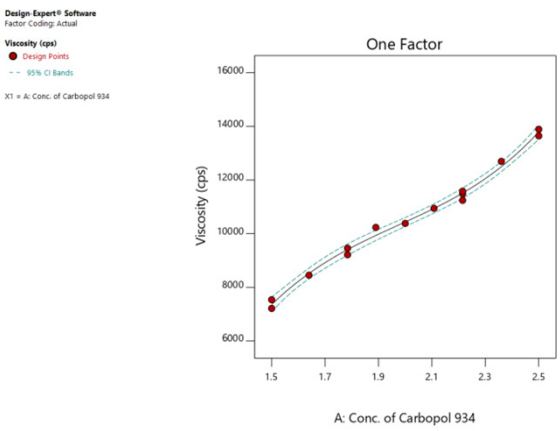

Figure 5: Effect of the independent variable on Viscosity.

formulation. This finding implies that the drug release mechanism follows a diffusion-controlled process.

CONCLUSION

Brimonidine tartrate-loaded Carbopol gel was developed for rosacea with the aim of topical application and bypassing the systemic side effects. For the preparation of gel, various types of gelling agents were used for gel strength, optimum viscosity and uniform content. The concentration of Carbopol used to prepare the gel was optimised using the OFAT design, which had a substantial impact on viscosity and drug release percentage. The optimized gel was evaluated for various parameters and its acceptability for application based on skin irritation was verified in an animal study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL

The study was conducted in accordance with the CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC) (SVU/DP/IAEC/2022/06/55).

ABBREVIATIONS

FDA: Food and Drug Administration; **OFAT:** One Factor at a time; **IR:** Infrared; **HPMC:** Hydroxy Propyl Methyl Cellulose; **DoE:** Design of Experiment; **UV:** Ultraviolet; **PBS:** Phosphate Buffer Saline; **ICH:** International Conference on Harmonization; **API:** Active Pharmaceutical Ingredient; **ANOVA:** Analysis of Variance.

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