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Original Article

Factorial Design-Based Development and Evaluation of a Thermosensitive *in situ* Gel of *Tagetes erecta* for Otitis Media

Sonia Paliwal[1,3,*], Himanshu Joshi[2], Amrita Pargaien[1], Kanak Pandey[1]

[1]College of Pharmacy, Graphic Era Hill University, Bhimtal, Uttarakhand, INDIA.
[2]Devsthali Vidyapeeth Institute of Pharmacy, Lalpur, Rudrapur, Uttarakhand, INDIA.
[3]Centre for Promotion of Research, Graphic Era (Deemed to be) University, Dehradun, Uttarakhand, INDIA.

ABSTRACT

Background: Otitis Media (OM) is a common middle ear infection that has traditionally been treated with systemic antibiotics or invasive procedures because it is hard to cross the dense, lipophilic barrier of the intact tympanic membrane. The conventional otic drops have short duration of action and not good contact time. **Objectives:** The purpose of this study was to develop, optimise, and assess an *in situ* otic drug delivery system based on an ethanolic leaf extract of *Tagetes erecta* (TE), with sustained *in vitro* drug release, supporting the formulation's potential for prolonged retention and release in the ear canal. **Materials and Methods:** *TE* extract was mixed into a thermoresponsive polymer system of Poloxamer 407 and Carbopol 934, using a cold method for preparation. A 3^2 factorial design was run for the optimisation of the polymer concentration to the properties like viscosity, gelation temperature and gelation time. The formulations assessed for their pH, gelling ability, spreadability, drug content, *in vitro* release through Franz-diffusion cells and stability. **Results:** The formulations turned out to be liquefying at room temperature and transforming to stable gels at physiologic temperature (30-37°C) in 104 to 144 s. The pH (4.1 to 5.3) matched with ear physiological health. The best formulation (F4) showed high drug content (98.85±0.04%) and *in vitro* release profile with total 98.09% in 6 h and numerical optimisation using the desirability function (D=0.995) confirmed F4 as the optimal formulation, closely matching experimental values. Stability testing showed good gelling capacity and maintenance of the matrix after 90 days. **Conclusion:** *In situ* gel system is an effective patient-friendly drug delivery approach for otic administration with great potential for localised treatment of OM.

Keywords: *In situ* gel, Optimization, Otitis media treatment, Poloxamer, Response surface methodology, TE, Thermoreversible gel.

Correspondence:
Dr. Sonia Paliwal
College of Pharmacy, Graphic Era Hill University, Bhimtal, Uttarakhand, INDIA.
Email: soniapaliwal49@gmail.com

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INTRODUCTION

Middle ear infection, OM is widespread in every age group.[1] It is a condition that arises when Eustachian tube that links middle ear with throat back gets obstructed or infected. Bacterial or viral infections can cause OM, which is more common in children but also can occur in adults.[2] Symptoms of the condition usually include ear pain, loss of hearing, ear fluid, and even fever.[3] Early diagnosis and proper management are critical in relieving symptoms, avoiding complications and maintaining hearing capacity.[4] Although there are several treatment modalities, the formulation of new drug technology is promising to expand the therapeutic usefulness and adherence to therapy.[5]

This article deals with ear infections by developing a new otic *in situ* gel of TE. The goal is to realise effective and patient compliance drug delivery increasing the sustained localised drug release and residence time in the middle ear.[6] This novel gel system is transformed into gel state when it comes in contact with the body temperature and it allows a long duration of drug release and an increase in residence time at the infection site (Figure 1). Tympanic membrane integrity is important to aid in the drug delivery from middle to inner ear.[7] The therapeutic effectiveness of otic drug delivery is governed by the diffusion of the drug across the intact tympanic membrane into the middle and inner ear. This permeation can be significantly enhanced by incorporating suitable permeation enhancers into the formulation. Studies have reported that hydrogel-based systems containing permeation enhancers facilitate the direct transport of antibiotics to the middle ear, providing a promising non-invasive strategy for the management of OM.[8,9]

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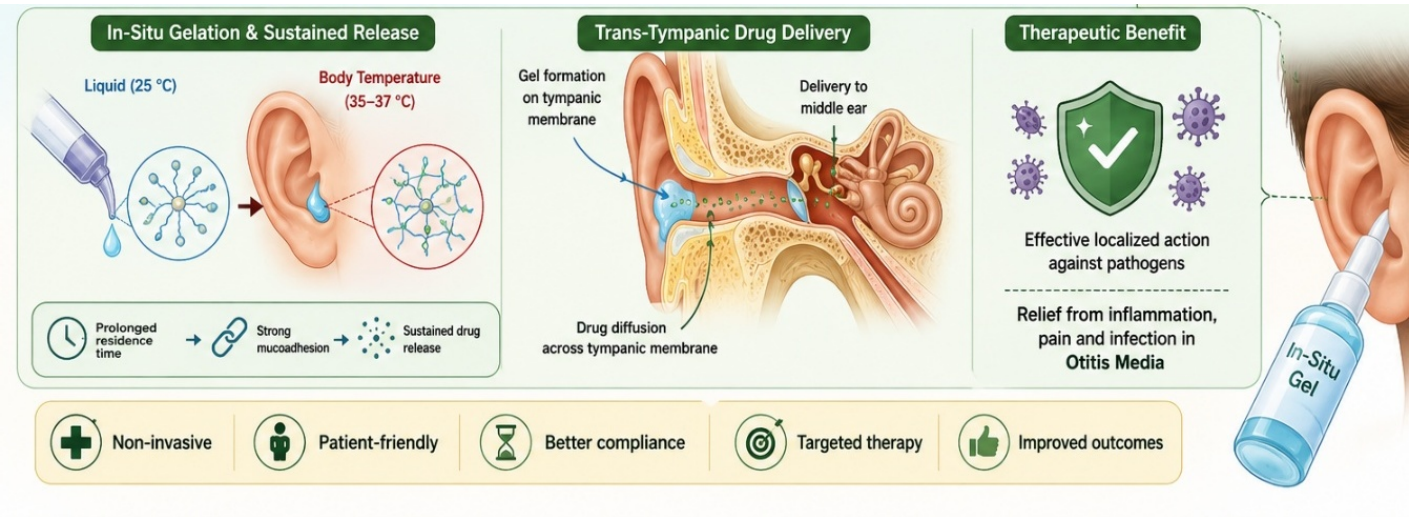


Figure 1: Administration of *in situ* gel.

In situ gels are liquid solutions that are highly viscous and mucoadhesive, made up of polymers.[10] They are able to change from sol to gel form in the ear canal based on variations in temperature and pH.[11] The amount to be delivered can be easily regulated by modulating the duration of the time that the drug remains in the ear, thus avoiding ear disorders.[12] As *in situ* gels are temperature and pH sensitive and prepared either with natural or synthetic polymers.[13] The *in situ*-forming gel systems can find numerous medicinal uses, such as drug delivery, tissue healing and cell encapsulation.[14,15] Reverse gelation and gel formation can be done in a number of different ways including solvent exchange, UV irradiation, ionic cross-linking, pH variation, temperature variation.[16–20]

In situ gels offer a number of benefits when prepared by the reverse gelation method. They are increased length of action that enhances patient adherence and comfort, decreased dosing of drugs, decreased frequency of drug intake, and can be administered in regular droppers and containers utilised with solutions.[21,22] The article seeks to add to the existing literature in this area giving a holistic insight into the process of developing and evaluation of otic *in situ* gel of ethanol extract of TE as a possible treatment option of ear infections.

The novelty of the present study is the formulation of *in situ* thermosensitive loaded gel with ethanolic leaf extract of *TE* for targeted delivered in the ear. In contrast to previously reported thermosensitive otic gels which are mostly made up of synthetic antimicrobial agents, but in the present formulation is based on the herbal extract and formulate a thermosensitive polymeric matrix of Poloxamer 407 and Carbopol 934. In addition, a 3^2 full-factorial design of the formulation variables was conducted to obtain the desired viscosity, gelation temperature and gelation time for using the formulation in otic administration. This formulation is a free flow liquid at room temperature which can easily be administered, but will rapidly transform into a gel once contacted with body temperature. This localised gel stays in place on the ear drum for much longer than standard ear drops, which drain quickly and don't form a good seal, and doesn't leak out the formulation, while providing a sustained, controlled release of the antimicrobial extract directly benefits the patient as it directly helps in formulation adherence to the ear drum. All of these findings indicate the potential of a thermosensitive herbal *in situ* gel as a promising platform for localised drug delivery into the ear.

MATERIALS AND METHODS

Materials

Poloxamer 407, Carbopol 934, Methyl paraben, Transcutol were purchased from BS & BSR CHEMICAL Uttarakhand, India. Plant leaves were collected from the Ramnagar Uttarakhand, India nursery and dried 5-6days over the shed and send it for authentication. The plant identity was verified by Botanical Survey of India (BSI) Dehradun, Uttarakhand, India.

Preparation of crude extracts

The dried *T. erecta* leaves were finely powdered and exposed to extraction using the maceration technique. The powdered material was placed in a glass beaker and soaked in 96% ethanol, followed by continuous stirring at 200 rpm for 24 h to ensure efficient extraction. After extraction, the mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator. The resulting concentrated extract was then stored at 4-8°C until further use shown in Figure 2.[23]

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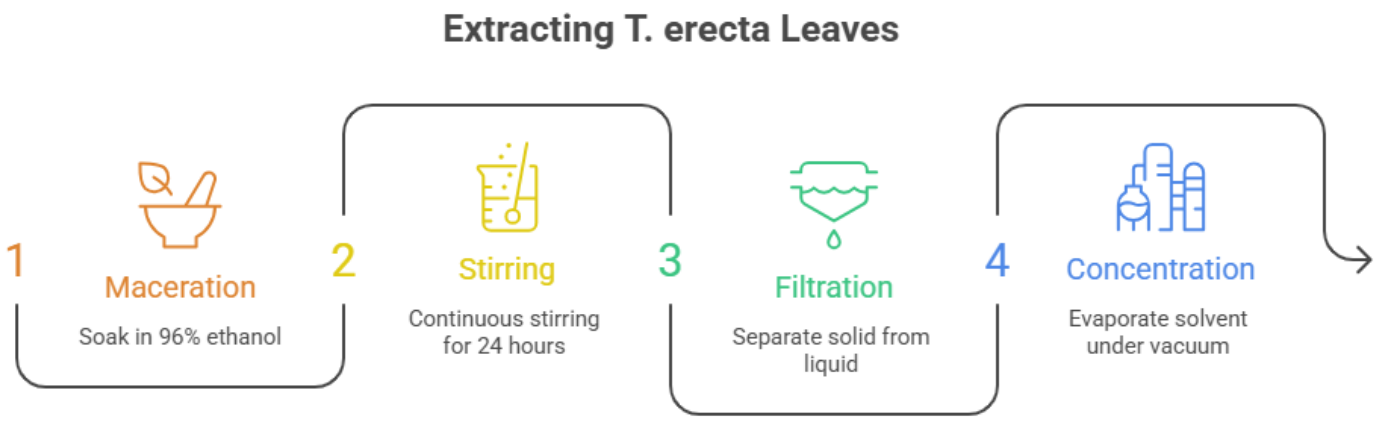


Figure 2: Extract method.

Preformulation study

Preformulation studies were carried out to determine compatibility of extract, poloxamer 407 and final formulation. The functional groups present in extract, poloxamer 407 and their physical mixture were qualitatively estimated and identified using an FTIR spectrophotometer (Spectral Range: -350 to 8,000 cm^{-1}, Alpha-II, Bruker, Germany).[24]

Spectroscopy Study

To determine the absorption maximum of quercetin, scan a 10 µg/mL sample and standard solution over 200.0 nm to 400.0 nm against a phosphate buffer pH 7.4 (PBS) blank, measuring the maximum absorbance. For the calibration curve, dilute 2.5 mL of standard stock solution to 50 mL with PBS pH 7.4 to obtain a 50 µg/mL working solution, then filter through Whatman No. 41 paper. Transfer aliquots of 0.4, 0.8, 1.2, 1.6, and 2.0 mL into separate 10 mL volumetric flasks and dilute to volume with PBS pH 7.4 to yield concentrations of 2, 4, 6, 8, and 10 µg/mL, respectively. Finally, measure the absorbance of each dilution at 256 nm against the PBS blank and plot mean absorbance versus concentration to construct the standard curve.[25]

Preparation of *in situ* gel

The *in situ* gel was prepared as follows:

1. Distilled water (15 mL) was placed in a beaker on a magnetic stirrer, Poloxamer 407 was added, and the mixture was stirred for 2 h at 42°C; the dispersion was then refrigerated overnight.
2. Carbopol 934, Transcutol, and methyl paraben (0.05%) were added to the poloxamer dispersion, and the mixture was stirred constantly.
3. The preservative solution was prepared separately by dissolving methyl paraben in hot water; it was then cooled and added to the above dispersion.
4. The extract solution (1 g extract in distilled water) was then added to the poloxamer dispersion.
5. The pH of the final formulation was adjusted (Table 1).[26]

Optimisation of *in situ* gel

A 3^2 factorial design was used to optimise the formulation process for poloxamer 407 and carbopol 934. X1, poloxamer 407 and X2, carbopol 934 were considered as independent factors in this experimental design at 3 different level coded as -1, 0, +1. The run at the centre point was performed in triplicate to validate the design and prevent the experimental error and Y1, viscosity; Y2, gelation temperature and Y3, gelation time were considered as dependent factors. Poloxamer 407 (X1) was given three levels: 5% (low) and 15% (high), with 10% as the centre point. The lower and higher concentrations were 0.2% 0.4% and 0.6% for carbopol 934 (X2), respectively. To better understand how each formulation factor, as well as their combined interaction, influenced the response variables, seven experimental formulations were developed using a 3-level factorial design. This design enabled a systematic evaluation and optimisation of the formulation variables, and the resulting experimental runs are presented in Table 2.[27,28]

Evaluation Parameter

Clarity and pH

The clarity of *in situ* gels was examined by visual observation after and before gelation on white, black background. The pH was measured with a calibrated pH meter at 25°C.

Sol-Gel Transition Temperature

Approximately 2-3 mL of each sol formulation was transferred into separate glass test tubes. The samples were gradually heated from 32°C to 38°C, with a 10-min interval at each temperature to allow equilibration. The temperature at which the formulation transformed from a free-flowing sol into a semisolid gel was recorded.

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Table 1: Composition of *in situ* gel.

Formulation	Extract (%)	Poloxamer 407 (%)	Carbopol 934 (%)	Methyl paraben (%)	Transcutol (mL)
F1	1	5	0.2	0.05	2
F2	1	5	0.6	0.05	2
F3	1	15	0.2	0.05	2
F4	1	15	0.6	0.05	2
F5*	1	10	0.4	0.05	2
F6	1	10	0.2	0.05	2
F7	1	10	0.6	0.05	2
F8	1	5	0.4	0.05	2
F9	1	15	0.4	0.05	2

*Center point formulation, prepared and evaluated in triplicate.

Table 2: Variables in factorial design.

	Low (-1)	Middle (0)	High (+1)
X_1-Poloxamer 407	5	10	15
X_2- Carbopol 934	0.2	0.4	0.6

Note: Dependent variables Y_1-Viscosity cps, Y_2=Gelation temperature, Y_3=Gelation time.

Gelling Time

It was determined using a glass chamber inclined at an angle of 45°C and maintained at 37±0.5°C. An aliquot of 100-200 µL of each formulation was carefully placed on the outer surface of the chamber. The time required for the formulation to undergo phase transition from a liquid sol to a viscous gel was monitored visually and recorded as the gelling time.

Gelation Capacity

Gelling capacity of the prepared formulations was estimated by dropping 1 mL sample into 5 mL of room temperature pH 7.4 phosphate buffer in a watch glass.[29]

Drug content uniformity

Accurate weight of each formulation (n=3) was diluted with phosphate buffer 7.4 and the absorbance was measured at 256 nm by using UV visible spectrophotometer (Shimadzu, USA).

Viscosity

The rheological behaviour of the optimised thermosensitive *in situ* gel was evaluated at 25°C and 37°C using a rheometer. The formulation was subjected to shear rates ranging from 10 to 500 s^{-1}, and the corresponding shear stress and apparent viscosity were recorded. The relationship between shear stress and shear rate was used to obtain the flow curves.[30]

Spreadability

It was estimated using the glass slide method. A measured quantity of gel was placed at the center of a 5 × 5 cm glass slide, and a second glass slide of the same dimensions was carefully placed over it from a height of 3 cm. The time required for the two slides to separate completely under a specified weight was recorded. It was calculated using the following equation:

$$S = \frac{m * l}{t}$$

Data analysis

Data analysis was done by minitab software, on the basis of the optimisation results, which showed three-level factorial design. For formulation optimisation, viscosity, gelation temperature, and gelling time were selected as the critical response variables. These parameters were chosen because they are quantitative measurements that directly influence the performance and applicability of the *in situ* gel formulation. The experimental data obtained for these responses were subjected to statistical analysis and optimisation.[31] In contrast, organoleptic characteristics, such as appearance and clarity, were evaluated qualitatively and optimised based on descriptive observations.

In vitro drug release study

Drug release from F2, F4, F6 and marketed conventional ear drop were determined using a Franz diffusion cell. The cellophane membrane was placed on the receptor cell. 1 g of *in situ* gel formulation was filled in the donor cell. During the experiments, the receptor compartment was filled with phosphate buffer pH 5.4. A pH 5.4 buffer was selected as the release medium, rather than a standard pH 7.4 buffer, to better approximate the mildly acidic environment of the healthy and diseased ear canal (reported pH range 4.1-5.3), so that the *in vitro* release data would more closely reflect the conditions the formulation would encounter *in*

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situ. To ensure homogeneity it was constantly stirred by magnetic bar with stirring speed of 200 to 250 rpm and temperature was maintained at 37±1°C by circulating hot water. The samples were withdrawn at regular intervals of time (0, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min) and changed with the same quantity of a pH 5.4 phosphate buffer to help maintain the sink condition. UV-visible spectrophotometer was used to analyse the samples at 256 nm and record the concentration.[32]

Drug release kinetic Analysis

The release kinetics of the optimised thermosensitive *in situ* gel were evaluated by fitting the *in vitro* drug release data to zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Baker-Lonsdale mathematical models. Linear regression analysis was performed using Microsoft Excel and the kinetic parameters together with the coefficients of determination (R^2) were determined. The model exhibiting the highest R^2 value was considered to best represent the release behaviour and was used to elucidate the predominant drug release mechanism.

Stability Testing

During stability drug decomposition or degradation may occur either through chemical change of the active ingredients or due to product instability, which reduces the concentration of the drug in the dosage form. Formulations F4 was stored at 2-8°C in clean, dry, airtight container and stored out of light for 90 days.[33]

Statistical Analysis

It was performed using Minitab software. A 3^2 factorial design was used to evaluate the effects of Poloxamer 407 (X_1) and Carbopol 934 (X_2) on the 3 dependent variables are: viscosity (Y_1), gelation temperature (Y_2), and gelation time (Y_3). Experimental data were fitted to a second-order polynomial (quadratic) model, and the significance of the regression model and individual model terms was assessed using Analysis of Variance (ANOVA). The suitability of the fitted models was evaluated by the *F*-value, *p*-value, coefficient of determination (R^2), adjusted R^2, and predicted R^2. Response surface plots and contour plots were generated to investigate the effects of the formulation variables on the responses and to identify the formulation exhibiting the most desirable characteristics. Differences were measured statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Appearance and Percentage Yield

Colour of the extract was dark green with semisolid consistency. Percentage yield of hydroalcoholic of dry leaves was 25.5%.

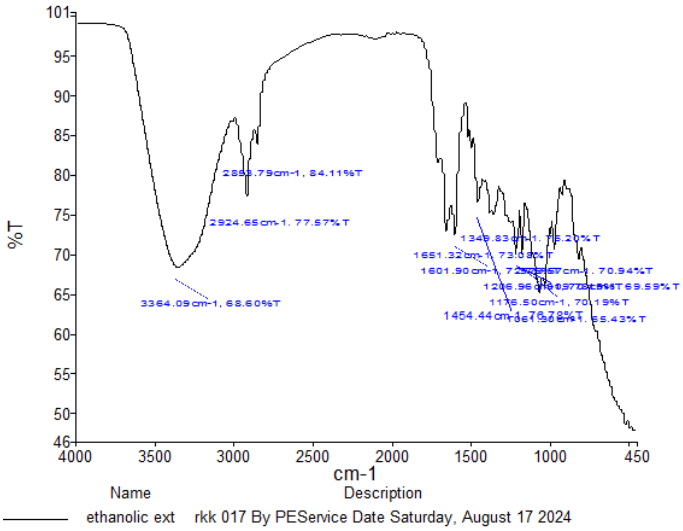


Figure 3: IR spectra of extract.

Preformulation Study

The FTIR spectrum of the pure ethanolic extract (Figure 3) showed a broad -OH stretching band at 3320 cm^{-1} (phenolics/flavonoids), aliphatic C-H stretching at 2923 cm^{-1} and 2850 cm^{-1}, and a flavonoid C=O band at 1623 cm^{-1}. The characteristic bands of the poloxamer 407 were quite strong, such as symmetric C-H stretching at 2880 cm^{-1} and the strong C-O-C ether linkage stretching at 1099 cm^{-1}, as shown in Figure 4). The preservation of main functional peaks virtually without changes is indicative of weak physical interactions (e.g., hydrogen bonding) that take place during the formation of the gel network. There are no new bands, or bands with no change in intensity, seen as a result of the extract being absorbed by the polymeric matrix, indicating excellent chemical compatibility and structural stability of the extract within the polymeric matrix.

Spectroscopy Study

The observed absorbance maxima were found to be 256 nm. The calibration curve of was determined in the con. range of 2-10 µg/mL. From the Figure 5 it is clearly shows that it follows the beers lambert law and the regression equation is y=0.09x+0.0397, higher correlation Coefficient is R^2=0.9923, indicating a strong positive correlation between concentration and absorbance.

Quercetin found- (8.7/10) *100=87% i.e., 8.7 µg/mL

Quercetin was selected as the marker compound for standardisation of the TE extract because it is a major flavonoid constituent of *Tagetes erecta* leaves and is readily quantifiable by UV spectroscopy at 256 nm, making it a practical and reproducible index of extract potency across batches. In the present study, extract dose was standardised on a batch basis using this quercetin content (87%, 8.7 µg/mL) together with the percentage yield

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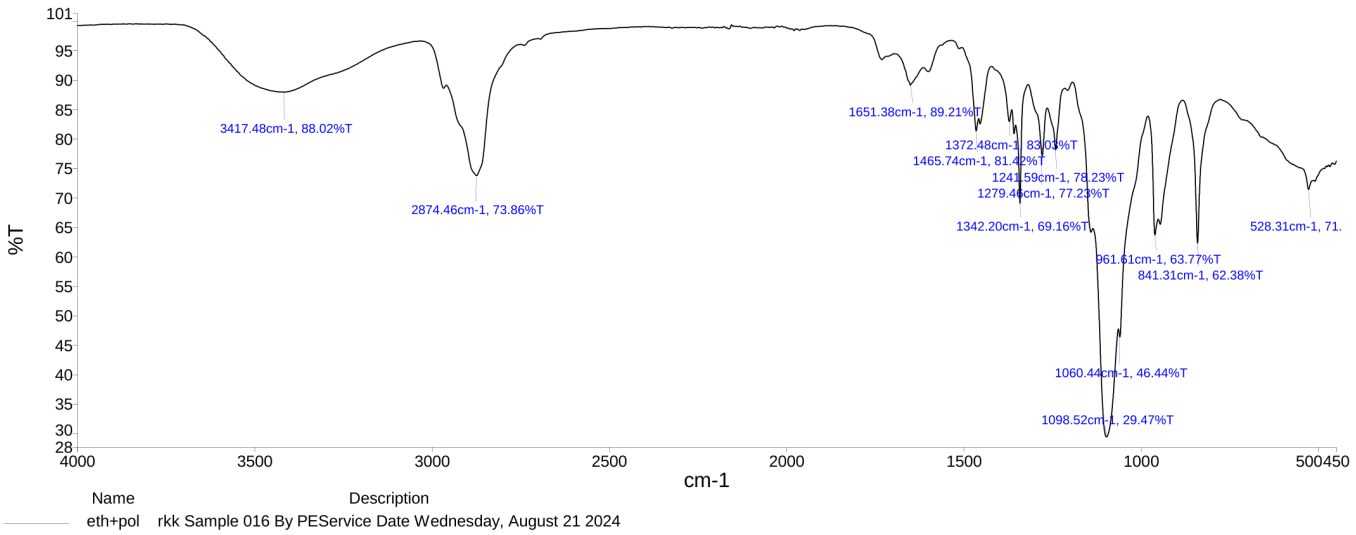


Figure 4: FTIR of physical mixture.

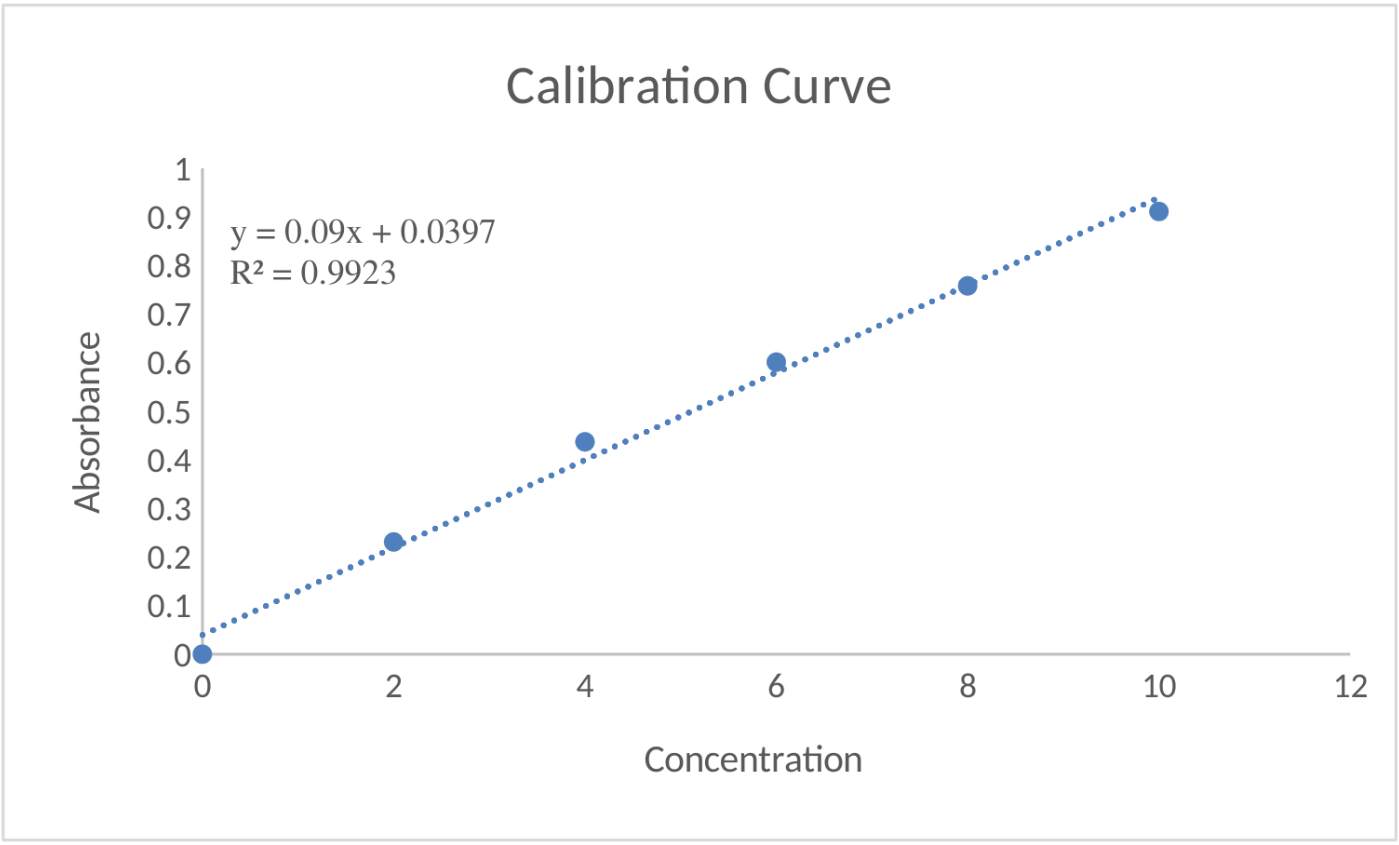


Figure 5: Calibration curve.

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Table 3: Characteristics of *in situ* gel.

Formulation	Clarity	pH in sol phase	Gelation Capacity
F1	Clear	4.8±0.21	++
F2	Clear	5.2±0.03	+++
F3	Light Yellowish	4.6±0.08	+++
F4	Clear	4.7±0.07	+
F5	Clear	5.3±0.18	++
F6	Clear	4.1±0.17	++
F7	Clear	4.9±0.01	+++
F8	Clear	5.1±0.21	++
F9	Clear	4.9±0.27	+++

Note-+ immediately gelling and change in solution phase in few minutes, ++ gelation and change in solution phase in few hours, +++ Stable gelation, no changes in solution phase.

of the hydroalcoholic extraction (25.5%), with a fixed weight-equivalent of dried extract incorporated per formulation batch; no additional secondary marker compound was quantified. As with other plant-derived extracts, batch-to-batch variability arising from differences in plant harvest season, growing conditions, drying time, and extraction efficiency is an inherent limitation of herbal formulations. This was partly mitigated in the present work by sourcing leaves from a single, authenticated plant source (BSI, Dehradun) and by following a standardised extraction and formulation protocol; however, only a single extraction batch was evaluated in this study. Future work should quantify quercetin content (and ideally a second marker compound) across multiple independently prepared extraction batches to formally establish reproducibility and to define acceptable batch-to-batch variation limits before scale-up.

Clarity and pH

All the *in situ* gel was homogeneous and clear and pH was found to be 4.1 to 5.3 that is acceptable for otic delivery. Healthy ear pH is 4.1 to 5.3 and reacidification of ear canal may lead to resolution of OM (Table 3).

Sol-to-Gel Temperature and pH

The temperature-induced gelation of a poloxamer solution is represented by a temperature dependent change in micellar properties. The micellar aggregation number of poloxamer solution increases and the critical micelle concentration decreases with the heating, which facilitates the formation of a denser and more viscous gel. Furthermore, the gelation process is attributed to conformational rearrangements of the methyl groups in the poly(oxypropylene) side chains forming the micellar core. This rearrangement results in the release of hydrated water from the micelles, thereby promoting gel formation.[15] The gelation temperature varied from 30 to 39°C. Formulations F1, F2, F5, F6 and F8 (37°C) exhibited gelation temperatures at or below physiological ear temperature (30-37°C), indicating their suitability for prompt *in situ* gel formation following administration. In contrast, formulations F3, F4 and F9 exhibited gelation temperatures slightly above the physiological range, which may modestly delay gel formation under physiological conditions (Table 4).

Gelling Time

The gelation time of the formulations ranged from 104 to 144 s. F3 demonstrated the shortest gelation time followed by F1, F8, F6 and F5, indicating rapid conversion of the liquid formulation into a gel (Table 4). Conversely, F7, F2, F9 exhibited a longer gelation time followed by F4. A shorter gelation time is considered advantageous for otic delivery, as it minimises formulation leakage from the ear canal and improves residence time. This time range allows convenient dropwise administration in the sol state, followed by rapid sol-gel transition under physiological temperature. Such gelation helps minimise formulation leakage from the ear canal and enhances residence time at the site of action.

Viscosity

The viscosity of the formulations ranged from 907 to 952 cP, indicating that all formulations possessed adequate flow properties for administration as liquid drops before undergoing sol-to-gel transition. The highest viscosity (952 cP) was observed in F4 containing 15% Poloxamer and 0.6% Carbopol 934, while the lowest viscosity (907 cP) was recorded for F1 containing 5% Poloxamer and 0.2% Carbopol 934 (Table 4). The increase in polymer concentration generally resulted in an increase in viscosity, thereby enhancing the potential of the formulation to remain in the external auditory canal after gelation. Appropriate viscosity is a critical parameter in the development of otic *in situ* gel formulations, as it directly influences both ease of administration and residence time within the ear canal. The formulation should remain sufficiently fluid during instillation and subsequently undergo

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Table 4: Gelation Temperature and Time of all formulation.

Formulation	Viscosity at room temperature (°C)	Viscosity at 37°C (cps)	Gelation Temp (°C)	gelation time (sec)
F1	304	907	30	108
F2	309	921	33	138
F3	311	930	38	104
F4	312	952	39	143
F5	308	926	37	127
F6	312	907	30	125
F7	311	911	37	137
F8	306	910	32	117
F9	313	930	36	140

a sol-to-gel transition under physiological temperature and pH conditions. These systems are expected to exhibit shear-thinning behaviour, characterised by a decrease in viscosity with increasing shear rate, thereby facilitating administration. The *in situ* gels developed in this study displayed typical pseudoplastic behaviour, supporting their potential as effective otic drug delivery systems. These observations indicated that the formulation exhibited non-Newtonian flow behaviour (Table 4).

Interpretation of Results

The ANOVA results confirm that the quadratic/factorial model was significant for all three response variables, indicating that the fitted equations adequately describe the influence of poloxamer 407 (X1) and carbopol 934 (X2) on gel performance shown in Table 5. For viscosity, the model F-value of 277.15 with a *p*-value of 0.0004 indicates a highly significant model, and the non-significant lack of fit (p=1.000) confirms that the model fits the experimental data well. The very high R^2 (99.6%) and Adjusted R^2 (99.3%) show excellent correlation between predicted and observed values, while the low coefficient of variation (0.12%) reflects high precision and reproducibility of the runs. An Adequate Precision ratio of 52.5, far above the desirable threshold of 4, indicates a strong signal-to-noise ratio, confirming the model can reliably be used to navigate the design space for viscosity optimisation. At the individual factor level, both poloxamer 407 (F=566.92, p=0.0002) and carbopol 934 (F=251.86, p=0.0005) were highly significant contributors to viscosity, with poloxamer exerting the larger effect. The poloxamer-carbopol interaction was also significant (F=12.46, p=0.0387), though its contribution was comparatively minor, indicating that while the two polymers act mainly independently, a small synergistic effect on viscosity exists between them.

For gelation temperature, the model F-value of 9.95 (p=0.0456) is significant at the 5% level, though closer to the threshold than the other two responses, suggesting that this response is comparatively more sensitive to experimental variability. The non-significant lack of fit (p=0.0634) still supports the adequacy of the model. The R^2 of 90.9% and Adjusted R^2 of 81.7% indicate a reasonably good fit, and the Predicted R^2 (80.3%) is in reasonable agreement with the Adjusted R^2, since the difference between them is less than 0.2. The CV of 3.75% and Adequate Precision of 8.9 (above 4) confirm that the model, while less robust than the viscosity model, is still statistically valid for predicting gelation temperature trends. Individually, only poloxamer 407 was a significant driver of gelation temperature (F=27.04, p=0.0138); neither carbopol 934 (F=2.22, p=0.2338) nor the poloxamer-carbopol interaction (F=0.55, p=0.5112) reached significance. This indicates that gelation temperature is governed almost entirely by the poloxamer concentration, consistent with poloxamer's known role in thermoreversible sol-gel transition.

For gelation time, the model F-value of 50.22 (p=0.0046) is highly significant, and the non-significant lack of fit (p=1.000) again confirms good model fit to the experimental data. The R^2 (98.0%) and Adjusted R^2 (96.1%) values are high, and the Predicted R^2 (94.2%) is close to the Adjusted R^2, indicating good predictive capability. The low CV (2.27%) and high Adequate Precision (18.2) further validate the reliability and reproducibility of the model. Individually, carbopol 934 was the dominant and highly significant driver of gelation time (F=148.11, p=0.0012), whereas poloxamer 407 (F=0.03, p=0.8712) and the poloxamer-carbopol interaction (F=2.53, p=0.2106) were not significant. This suggests that gelation time is controlled primarily by carbopol 934 contribution to the gel network's mucoadhesive and cross-linking behaviour, largely independent of the poloxamer concentration. Taken together, all three models exhibited non-significant lack of fit and adequate precision values well above 4, confirming that the 3^2 factorial design equations for viscosity, gelation temperature, and gelation time can be used with confidence to navigate the design space and identify the optimised *in situ* gel formulation.

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Table 5: ANOVA for response X1, X2, X3.

Source	Sum of Square	d_f	Mean Square	F	*p*-value
Viscosity					
Model	1069.000	3	356.333	277.15	0.0004
X1	729.05	1	729.05	566.92	0.0002
X2	323.89	1	323.89	251.86	0.0005
X1X2	16.02	1	16.02	12.46	0.0387
Residual	3.857	3	1.286	-	-
Lack of fit	3.857	1	3.857	0	1
Pure error	0.00	2	0	-	-
Total	1072.857				
Gelation Temperature					
Model	54.000	3	18.000	9.95	0.0456
X1	48.94	1	48.94	27.04	0.0138
X2	4.02	1	4.02	2.22	0.2338
X1X2	0.99	1	0.99	0.55	0.5112
Residual	5.429	3	1.810	-	-
Lack of Fit	4.762	1	4.762	14.29	0.0634
Pure Error	0.667	2	0.333	-	-
Total	59.429	6			
Gelation Time					
Model	1210.750	3	403.583	50.22	0.0046
X1	0.26	1	0.26	0.03	0.8712
X2	1190.35	1	1190.35	148.11	0.0012
X1X2	20.32	1	20.32	2.53	0.2106
Residual	24.107	3	8.036	-	-
Lack of fit	24.107	1	24.107	0.00	1.0000
Pure error	0.000	2	0.000	-	-
Total	1234.857	6			

Response Surface and Contour Plot Analysis

Analysis of the response surfaces revealed that viscosity, gelation temperature, and gelation time are governed by distinct polymer-specific mechanisms. Viscosity was influenced significantly by both polymers, with poloxamer 407 exerting the dominant effect through concentration-dependent micellar packing into an ordered cubic array, while carbopol 934 contributed independently via chain swelling and hydrogen-bonded network formation; the significant positive interaction term further indicated a synergistic effect, attributable to hydrogen bonding between carbopol carboxylic acid groups and the ether oxygen of poloxamer's PEO blocks, which reinforced the packed micellar lattice beyond the additive contribution of either polymer alone. Gelation temperature, in contrast, was governed almost exclusively by poloxamer concentration, consistent with its intrinsic thermoresponsive sol-to-gel transition driven by progressive dehydration of the PPO blocks; notably, gelation temperature increased rather than decreased with rising poloxamer content, a reversal of the trend typically reported for poloxamer-only systems, which was attributed to steric interference from the pre-existing carbopol network delaying micelle-micelle percolation. Gelation time, conversely, was governed almost exclusively by carbopol 934 concentration, reflecting the comparatively slow, chain-relaxation-controlled kinetics of carbopol 934 swelling, uncoiling, and entanglement relative to the faster, diffusion-limited micellization of poloxamer, such that increasing carbopol 934 content raised local micro viscosity and delayed network percolation. Collectively, these findings indicate that poloxamer 407 primarily dictates the thermal responsiveness of the system, while carbopol 934

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Table 6: Numerical optimisation and validation of predicted responses.

Response	Predicted	Desirability (d)	Observed (F4)	% Error
Viscosity (cps)	951.36	0.986	952	0.07%
Gelation Temp (°C)	39.71	1.000	39	1.79%
Gelation Time (s)	144.61	1.000	143	1.11%
Overall D	0.995			

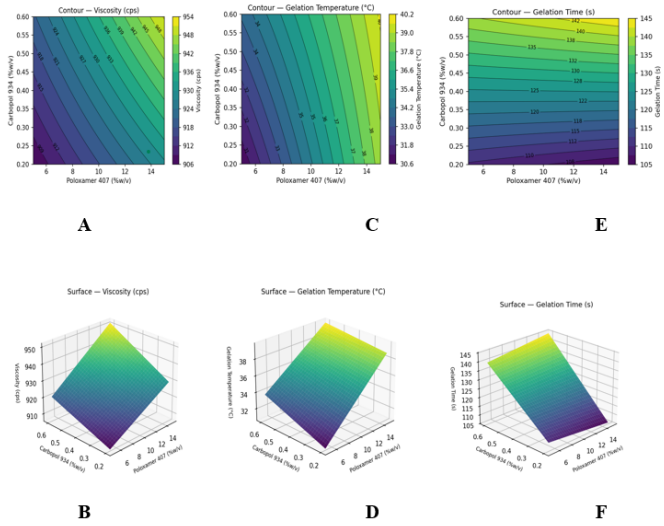


Figure 6: Response surface and contour plot showing the effect of independent variables on viscosity (A,B), Gelation temperature (C,D) and gelation time (E,F).

primarily governs the gelation kinetics, with both polymers jointly and synergistically determining the final gel viscosity (Figure 6).

To identify the formulation offering the best overall balance among viscosity, gelation temperature, and gelation time, numerical optimisation was performed using the desirability function approach. Target criteria were set to maximise viscosity for prolonged ear-canal retention, while constraining gelation temperature and gelation time within the physiologically relevant ranges observed. The individual desirability values obtained were d=0.986 for viscosity (951.36 cps), d=1.000 for gelation temperature (39.71°C), and d=1.000 for gelation time (144.61 s), yielding an overall desirability of D=0.995 (geometric mean of the individual desirability). A D value this close to 1 indicates that the predicted optimum simultaneously satisfies all three response criteria with minimal compromise.

The composition predicted to achieve this optimum (15% Poloxamer 407, 0.6% Carbopol 934) corresponds to formulation F4. Comparison of the model-predicted values against the experimentally observed values for F4 (viscosity: 952 cps; gelation temperature: 39°C; gelation time: 143 s) showed close agreement, with prediction errors of 0.07%, 1.8%, and 1.1%, respectively, confirming the validity and predictive reliability of the factorial design model (Table 6).

Table 7: Drug content and spreadability of formulation.

Formulation	Drug Content (%)	Spreadability (g·cm/s)
F1	87.03±0.18	5.0±0.21
F2	98.15±0.03	5.1±0.02
F3	86.01±0.07	4.5±0.21
F4	98.85±0.04	5.2±0.04
F5	89.65±0.14	4.1±0.71
F6	97.72±0.02	4.2±0.05
F7	91.75±0.16	4.8±0.02
F8	88.92±0.01	4.9±0.75
F9	90.56±0.02	5.0±0.22

Beyond the numerical agreement between predicted and observed values, F4 is also the mechanistically consistent optimum. F4 corresponds to the highest tested levels of both polymers (15% Poloxamer 407, 0.6% Carbopol 934), which, as discussed above, are the composition conditions under which poloxamer-driven micellar packing and carbopol-driven chain entanglement are both maximised. Because viscosity was shown to depend significantly on both polymers (with a positive interaction term), while gelation temperature and gelation time are governed predominantly by poloxamer and carbopol, respectively, the formulation that maximises both polymer concentrations simultaneously maximises viscosity without pushing gelation temperature or gelation time outside the physiologically acceptable window. The numerical optimum identified by the desirability function is therefore not merely a statistical curve-fitting outcome but reflects a composition where the underlying polymer-specific mechanisms are jointly optimised.

Drug Content

Results of the drug content of the prepared *in situ* gel formulations showed a range of 86.01±0.07% (F3) to 98.85±0.07% (F4). Formulations F2 (98.15±0.03%) F4(98.85±0.07%) and F6 (97.72±0.02%) exhibited exceptionally high drug incorporation uniformity (>95%). (Table 7).

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Table 8: Release profile of 3 formulation.

Time(min)/Formulation	Percentage Release Rate			
	F4	F2	F6	Marketed ear drop
0	0	0	0	0
15	27.92	19.92	11.83	29.02
30	39.84	28.09	27.86	38.32
45	48.73	38.97	35.07	49.71
60	57.03	49.43	46.92	53.01
90	61.59	57.90	51.62	59.04
120	69.92	67.82	59.76	64.21
180	74.47	74.87	68.07	69.04
240	80.24	84.73	72.57	75.13
300	85.83	89.02	80.92	88.21
360	98.09	94.82	87.63	99.02

Spreadability

Spreadability is an important operational parameter for topical or mucosal *in situ* gels which will determine the ease of application and consumer compliance. The spreadability values for the formulations ranged from 4.1 ±0.71 g·cm/s (F4) to 5.2± 0.04 g·cm/s (F2). Formulations with a higher spreadability index, such as F4 (5.2±0.04) and F2 (5.1 ±0.02), indicate a fluidic behaviour at room temperature that allows for effortless distribution over the biological membrane before triggering thermoreversible gelation (Table 7).

In vitro Release

The drug release profiles of the selected *in situ* gels (F2, F4, and F6) and marketed conventional ear drop were obtained *in vitro* over a period of 6 h and showed a unique biphasic sustained drug release profile with an initial burst release followed by a controlled diffusion (Table 8). The initial burst release for F4 was 27.92%, which was highest among the three formulations, whereas the initial burst release for F2 and F6 were 19.92% and 11.83%, respectively, which is due to the dissolution of the drug close the surface of matrix before polymer fully turns into a dense gel network. After the first hour, the release rate gradually decreased in all the formulation, which was attributed to the increased tortuosity and reduced diffusion pathway due to the cross-linked polymeric matrix of Poloxamer-407 and Carbopol-934. Nearly 98.09% and 94.82% of the drugs were released after 6 h for F4 and F2, respectively, while a peak release of 87.63% was obtained after 6 h for F6. This slower diffusion rate in F6 confirms a more rigid and compact gel structure, which causes the internal micro-channels to be narrower, making F4 the optimal formulation with the best release profile. The marketed conventional ear drop shows 99.02% release rate after 6 h as comparison to our optimal formulation F4 show the sustained release pattern with 98.09% in same time.

Table 9: Drug release kinetics analysis of F4.

Kinetic Model	Linear Equation	R^2
Zero Order	y=0.0023x + 0.2643	0.7673
First Order	y=-0.0024x + 1.9554	0.8619
Korsmeyer-Peppas	y=0.052x - 0.2912	0.0502
Higuchi release	y=0.049x + 0.0895	0.9534
Baker-Lonsdale	y=0.0009x + 0.0015	0.9365

Drug release kinetic Analysis

To understand exactly how the *in situ* gel releases its drug, formulation tested the data against several standard kinetic models shown in Table 9. The Higuchi model emerged as the clear standout (R^2=0.9534), with the Baker-Lonsdale model following closely behind (R^2=0.9365). This tells a straight forward physical story: the system relies on classic matrix diffusion. Once the gel sets, surrounding fluids seep into its 3D network, dissolve the drug, and carry it outward through the gel's microscopic channels. We also saw a much better fit for the First-Order model than the Zero-Order one (R^2=0.8619 vs. 0.7673), which means the release rate is directly tied to concentration. Simply put, as the drug supply inside the gel depletes and has further to travel to escape the matrix, the overall release rate naturally slows down over time.

Stability Studies

The stability study results are presented in Table 10. Formulation F4 remained physically stable throughout the 90-day storage

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Table 10: Stability studies on formulation F4.

Parameter	30 Days	60 Days	90 Days
Appearance	Clear	Clear	Clear
pH	5.2±0.05	5.2±0.08	5.2±0.12
Gelling capacity	+++	+++	+++
Sol viscosity	125 ±4.2	129±5.1	134±6.8
Gel viscosity	926 ±21	926 ±33	929 ±53
In vitro drug release (%)	98.09%	98.08%	98.08%

period under the specified storage conditions shown in Table 10. No significant changes were observed in the appearance, pH, gelling capacity, or viscosity of the formulation during the study. The pH remained within the acceptable range (5.0-6.5), indicating that the formulation maintained its physicochemical integrity over time. Furthermore, the formulation consistently exhibited a gelling capacity of +++ at all evaluation time points, demonstrating the preservation of its gel-forming ability.

A sol phase viscosity slight increases during storage, rising from 125±4.2 cP at 30 days to 129±5.1 cP at 45 days and 134±6.8 cP at 90 days. In contrast, the viscosity of the gel phase remained essentially unchanged at approximately 925 cP throughout the study period. The observed increase in sol-phase viscosity was minimal and did not adversely affect the gelation behaviour or overall performance of the formulation.

Overall, these findings indicate that formulation F4 possesses satisfactory physical stability for at least 90 days under the tested storage conditions. The minor increase in sol-phase viscosity may be attributed to slight physicochemical changes within the polymeric system during storage, such as polymer chain rearrangement or enhanced intermolecular interactions, rather than excipient degradation alone. Nevertheless, these changes were not statistically or pharmaceutically significant and are unlikely to affect the formulation's quality, efficacy, or safety. Extended stability studies, conducted for 3 to 6 months under ICH-recommended conditions, are recommended to further confirm the formulation's shelf life and evaluate its long-term physicochemical stability.

CONCLUSION

In conclusion, the development of the novel *in situ* gel of TE leaf extract was successfully done for the treatment of OM. A 3^2 factorial design was used to optimism the thermoresponsive polymer matrix (Poloxamer 407 and Carbopol 934) so that it could undergo sol-to-gel transition at physiological temperatures, thus overcoming the problems of conventional otic drops that include formulation leakage and short duration of drug activity. F4 exhibited an optimal physiological pH, excellent content uniformity of the drug, and a highly desirable biphasic sustained release profile with the nearly complete delivery of the drug in 6 h in controlled manner. Best formulation confirmed both by desirability-based numerical optimisation and experimental validation. In addition, stability testing for 90 days showed that the formulation maintains its key physicochemical characteristics, gelling ability and structural integrity. By providing prolonged local drug release at the site of infection, the *in situ* gel may reduce the need for repeated systemic antibiotic administration in otitis media. This localised approach may help minimise systemic exposure and the risk of adverse effects, although clinical studies are required to confirm this potential benefit.

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None.

ABBREVIATIONS

OM: Otitis Media; **TE:** *Tagetes erecta*; **BSI:** Botanical Survey of India; **FTIR:** Fourier Transform Infrared Spectroscopy; **RSM:** Response Surface Analysis; **ANOVA:** Analysis of Variance; **ICH:** International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use; **QbD:** Quality by Design; **UV:** Ultraviolet.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

SP: Conceptualization, Methodology, Investigation and Writing-Original Draft Preparation. **HJ:** Supervision, Review. **AP:** Validation. **KP:** Statistical Analysis.

ETHICAL APPROVAL AND CONSENT

Not Applicable.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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