Int J Adv Life Sci Res. Volume 9(3) 25-42
https://doi.org/10.31632/ijalsr.2026.v09i03.003

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

Enhancement of Topical Delivery of Aceclofenac Using Limonene: A Quality by Design Approach

Sangeetha Govindarajan*, Sushmitha Narayanappa, Makeshwar Periya Samy, Sakshi Satish Patil, Tejashwini Keshava Reddy

Department of Quality Assurance, Krupanidhi College of Pharmacy, Varthur, Bengaluru, Karnataka 560035, India

Corresponding Author's Email: sange2008@gmail.com

Abstract

Aceclofenac is a non-steroidal anti-inflammatory drug used to treat pain and inflammation caused by musculoskeletal disorders. Oral administration of Aceclofenac is associated with various gastrointestinal side effects like dyspepsia, abdominal pain, or discomfort. To reduce systemic side effects and to improve patient compliance, a topical gel was formulated using the Quality by Design approach. The Aceclofenac gel was prepared by using Carbopol 974P as a polymer and optimized by custom design. The design suggested 8 runs for 3 factors (Carbopol 974P, limonene and PEG 400) and 2 responses (viscosity, *in vitro* drug release). The 8 different formulations were prepared and evaluated. The optimized topical gel was characterized for homogeneity, pH, spreadability, viscosity, drug content, *in vitro* drug release, *ex vivo* permeation, and stability studies. The optimized Aceclofenac gel formulation predicted global desirability was 0.9980; *in-vitro a*nd *ex vivo* studies were found to be 84.64% and 77.42%. The drug release kinetics were analyzed using zero-order drug kinetics, the highest correlation of 0.999. A stability study was performed, and the shelf life was predicted to be 23.8 months using JMP statistical software. The optimized Aceclofenac gel avoids the first pass metabolism, and it showed a significantly enhanced permeability through the porcine skin and good stability.

Keywords: *Aceclofenac; Custom Design; Limonene; Quality by Design; Shelf Life*

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder associated with a chronic inflammatory process that affects the synovial joints, causing swelling and joint damage, and also affects the lungs, heart, and nervous system. According to the National Health Interview Survey (NHIS) in 2022, the percentage of adults with arthritis was 18.9%. Arthritis prevalence was lowest among adults 18-44 years; women were the only group more likely than men to have arthritis (Conforti *et al.,* 2021; Elgaddal*et al.,* 2024). NSAIDs are used to treat rheumatoid arthritis and related conditions, for example, Diclofenac, Ibuprofen, Naproxen, and Meloxicam (Salloum *et al.,* 2024). Aceclofenac, also an NSAID is derived from diclofenac, and it showed good results in the treatment of RA compared to Diclofenac (Moretti *et al.,* 2024). Aceclofenac is chemically [(2-{2, 6-dichlorophenyl} amino} phenyl] aceto-oxyacetic acid with a molecular weight of 354.2. It inhibits the cyclo-oxygenase (COX2) enzyme involved in the formation of prostaglandins (PG), which results in fever, inflammation, and pain. Aceclofenac is a BCS class II drug (low bioavailability, high permeability), practically insoluble in water with good permeability (Korzeniowska & Malesza, 2023). Aceclofenac is available as tablets and film-coated tablets with oral bioavailability of 14% and a half-life of 4 hours. When the drug is administered orally at 100 mg twice daily, it causes some adverse effects, such as gastrointestinal ulcers of the

Received on :8th October 2025; Revised version received on :11th May 2026; Accepted: 15th May 2026

mucosal surface, suppression of bone marrow, stomatitis, and hepatotoxicity, such as cirrhosis and fibrosis (Moretti *et al.,* 2024; Ali *et al.,* 2025). To overcome these oral adverse effects, aceclofenac gel was prepared to enhance the permeability through the skin using a suitable permeation enhancer, limonene (Ali *et al.,* 2025). Campos EV*et al.* showed that limonene significantly improves the permeation of benzocaine by disrupting the lipid layer of the stratum corneum and enhancing drug delivery through the skin. Limonene does not affect the pH or stability of the gel (Fytory *et al.,* 2026). Topical drug delivery systems provide an attractive alternative to oral administration in numerous instances. It circumvents the disadvantages of oral dosing, such as gastrointestinal discomfort and first-pass metabolism in the liver. Drugs administered topically bypass the first-pass metabolism, avoiding potential degradation or inactivation that can occur when medications pass through the digestive tract (Anaya-Mancipe *et al.,* 2025; Gavinet *et al.*, 2024).

Aceclofenac gel was developed using Quality by Design (QbD), a structured, scientific, and risk-based approach that ensures that manufacturing processes reliably meet product specifications. The primary goal is to understand how both the process and formulation factors influence product quality attributes using statistical JMP software (Benetti & Benetti, 2024).QbD involves a comprehensive evaluation of all potential factors affecting the quality of the product.

Materials and Methods

Materials

Aceclofenac was obtained as a gift sample from Global Pharma (Chennai, India); Carbopol (Lubrizol, Maharashtra, India); Limonene (Himalaya Terpenes, Maharashtra, India); Tri-ethanolamine (Merck Specialties Ltd., Maharashtra, India); Propylene glycol and Polyethylene glycol (The Dow Chemical, Chennai, India); Methanol (Swati Methanol and Allied Chemicals Ltd., Uttar Pradesh, India); Propyl paraben, Sodium Propyl paraben, Ethanol, Potassium dihydrogen phosphate (SD Fine Chemicals, Maharashtra, India); and Dialysis membrane (Sigma Aldrich, Bangalore, India).

Methods

Preformulation Studies

Solubility Analysis

An excess amount of aceclofenac was added to the measured quantity of water, ethanol, and phosphate buffer (pH 7.4) in a stoppered flask to form a saturated solution. The solution was then stirred continuously until undissolved drug particles remained. After 24 h, the drug solution was filtered, diluted with the respective solvents and the concentration was determined using a UV spectrophotometer. The average of the three readings was recorded (Ahirrao *et al.,* 2022).

Determination of Melting Point

The melting point of aceclofenac was determined using Theil's melting point apparatus. The small amount of the drug was taken in a capillary tube closed at one end and placed in Theile's tube. The temperature was noted when the drug started melting, and the average of three readings was recorded (Hanif *et al.,* 2023).

Calibration Curve of Aceclofenac in Phosphate Buffer pH 7.4

A stock solution of 100 mg of aceclofenac was dissolved in a phosphate buffer, pH 7.4, in a 100 ml volumetric flask, and the solution was made up to volume with the buffer solution. Aceclofenac stock solutions were diluted with a pH 7.4 buffer solution to obtain a series of dilutions of 5-50 µg/ml. The absorption of the dilution was measured by a UV spectrophotometer at 276 nm using buffer as a blank (Chaturvedi *et al.,* 2021).

Drug-Excipient Compatibility Studies

Aceclofenac alone and a mixture of drugs and physical mixtures consisting of other excipients, such as carbopol 974 P and limonene, were mixed in a 1:1 ratio and kept at room temperature for one

month under open and closed conditions. The samples were analysed for drug-excipient compatibility by FTIR techniques (Schlich *et al*., 2021).

Quality by Design

Quality by Design (QbD) is an approach used in the development of aceclofenac topical gel to improve safety, quality, and efficacy and decrease product variability. Using Quality by Design (QbD) to design the formulation can reduce future failures and enhance the understanding of the product and process, particularly regarding the Critical Quality Attributes (CQAs), their critical Process Parameters (CPPs), and how to control their interactions through a well-structured control strategy (Karamkar *et al*., 2023).

Quality Target Product Profile (QTPP)

For aceclofenac topical dosage forms, the QTPP defines the quality attributes of the drug product that will ensure acceptability to the patient's therapeutic response and assure its identity, strength, quality, and purity. It also helps identify CQAs to ensure product performance (Gavinet *et al.,* 2024).Table 1 summarizes the QTPP elements for the topical gel formulations.

Table 1: *QTPP Framework for The Formulation of Aceclofenac Topical Gel*

QTPP Elements	Target	Justification
Dosage form	Topical gel	Ease of administration.
Dosage strength	20 mg	To obtain the therapeutic effects of the drug.
Dosage type	Gel	Improves patient compliance.
Route of administration	Topical	To avoid first-pass metabolism
Stability	9 months shelf life at room temperature	To assess the degradation pattern of the formulation by monitoring product quality.
pH	Between 5 and 8	Compatible with skin.
Drug release	*In vitro and ex vivo* skin permeation.	To understand the drug release and permeation pattern
Microbial limits	Within pharmacopoeia limits	Ensures product safety and stability
Impurities	Below toxicological limits	No contaminants are present.
Container closure system	Collapsible tube	Product preservation during its shelf life.

Critical Quality Attributes (CQAs)

The CQAs of the aceclofenac topical gels do not directly impact the overall quality but play a significant role in the performance of the product and patient acceptability. CQAs, such as viscosity, pH, spreadability, and *in vitro* drug release, directly influence the quality and performance of the final product. These attributes must be carefully controlled throughout all stages of formulation development to ensure the quality, efficacy, and therapeutic effects of the gel (Kovács *et al.,* 2024). Table 2 lists the identified CQAs and their justifications.

Table 2: *CQAs of Aceclofenac Topical Gel*

Quality Attributes of a Drug Product		Target	CQA	Justification
Physical attributes	Color	Acceptable to patients	No	Physical attributes are not considered critical attributes as they are not linked with patient safety and efficacy.
	Odor			
	Appearance			
Drug content		100%	Yes	Ensures the correct amount of drug is present in each unit dose for efficacy
pH		Between 5 and 8	Yes	It affects the product's solubility, stability, and compatibility with the skin surface. It should be within range to avoid skin irritation.
Viscosity		15,000-20,000 cP	Yes	Determines the spreadability, ease of administration, and retention on the skin. It should be over time and under different storage conditions.
Spreadability		20-40 gm.cm/sec	Yes	Determine ease of application and patient acceptability. Poor spreadability may lead to inconsistent dosing.
Gel strength		Medium	Yes	The polymer concentration determines gel strength.

Risk Assessment

Risk management is a strategic framework aimed at minimizing product-related risks using various tools. Risk assessment identifies process parameters and critical material attributes that affect critical quality attributes. Quality Risk Management provides a systematic approach for the assessment, control, and ongoing review of risks throughout the development of a drug product, ensuring product quality and patient safety. The Ishikawa diagram and risk estimation matrix are two primary qualitative tools used for risk assessment of aceclofenac topical gel (Ali *et al.,* 2025).

Ishikawa Diagram

The Ishikawa or fishbone diagram shows the main causes that affect the performance of a product. Key categories include material attributes, process parameters, instrumentation, and product-related factors (Ali *et al.,* 2025). The causes and sub-cause parameters affecting the effect on aceclofenac gel were depicted in the fishbone diagram shown in Figure 1.

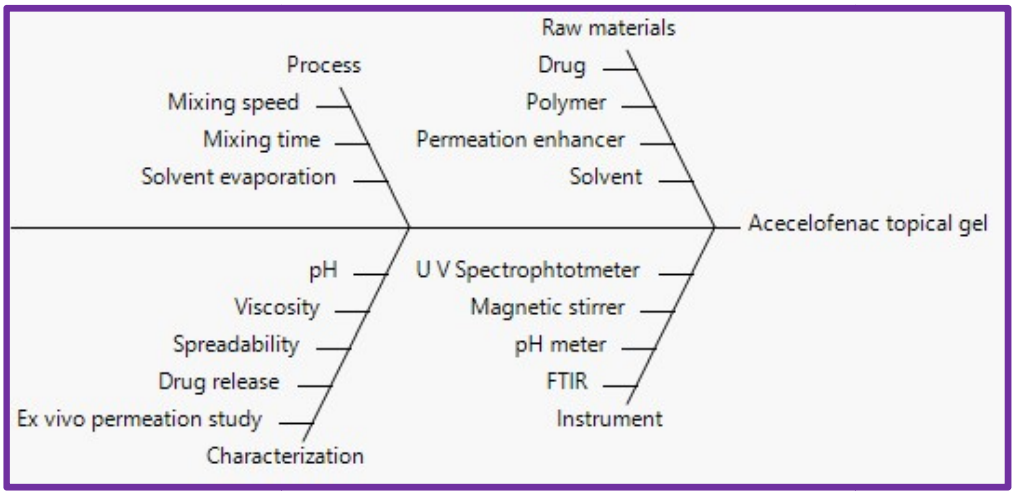

Figure 1: *Fishbone Diagram Depicting the Causes and Sub-Cause Parameters Affecting the Effect on Aceclofenac Gel*

Risk Estimation Matrix

The risk estimation matrix (REM) was analyzed to determine the relationship between the selected variables and assess the risk relationship between CQAs and CMAs/CPPs. Risk level was studied for CMAs and CPPs against the CQAs. A three-level scale was employed to categorize the risk level as low, medium, and high and colored as green, yellow, and red, respectively (Kovács *et al.,* 2024). Based on the risk estimation matrix table, the factors and responses were screened for the construction of a design of experiment. The REM results are summarized in Table 3.

Table 3: *Risk Estimation Matrix for the Formulation of Aceclofenac Topical Gel*

Cma/Cpp / Cqas	Aceclofenac	Polymer	Solvent	Limonene	Stirring Speed	Stirring Time
Viscosity	Medium	High	Medium	Medium	High	High
Ph	High	Medium	Medium	Low	Low	Low
Gelling Time	Medium	High	Medium	Medium	Low	Low
Drug Content	High	High	High	Low	Medium	Medium
Permeability	High	Medium	High	High	Low	Low
In Vitro Drug Release	High	High	Medium	Medium	Low	Low

Design of Experiment

Aceclofenac gel was formulated and optimized using the Design of Experiments (DoE). The custom design was constructed using JMP 14.0 software (trial version). Custom design enables the specification of essential and desirable effects to estimate within a limited number of runs (Sanap *et al.,* 2022). The custom design was constructed with three independent variables, including Carbopol 974P, limonene, and PEG 400 in two levels, and two dependent variables were selected as viscosity (cP) and *in-vitro* drug release, as shown in Tables 4 and 5. These factors and their levels were fixed based on the risk levels and the preliminary trials of the formulation. The experimental design consisted of eight runs generated using the custom design platform to estimate main effects and selected two-factor interaction effects. The choice of an eight-run design was based on minimizing experimental runs while retaining the ability to evaluate critical factor effects identified during preliminary studies and risk assessment. Experimental runs were randomized to reduce systematic bias. No replicate or center points were included; however, this approach limits the estimation of lack-of-fit. All experimental runs were performed, and the results are presented in Table 6.

Table 4: *Independent Variables and their Ranges*

Factors	Lower Limit	Higher Limit
Carbopol 974P (mg)	300	600
limonene (mg)	600	900
PEG 400 (mg)	15	30

Table 5: *Dependent Variables and their Ranges*

Responses	Goal	Lower Limit	Higher Limit
Viscosity (cP)	Match Target	15000	20000
In-vitro drug release (%)	Maximize	60	85

Table 6: *Custom Design Matrix of Aceclofenac Gel*

Runs of Experiments	Carbopol 974P (mg)	Limonene (mg)	PEG 400 (mg)	Viscosity (cP)*	In Vitro Drug Release (%) *
1	300	600	15	17459±44.7	84.9±0.04
2	600	900	30	18673±65.2	80.7±0.27
3	600	600	15	16895±53.8	82.4±0.09
4	300	900	30	17980±60.1	86.5±0.35
5	600	900	15	17530±37.3	80.1±0.27
6	300	600	30	17489±46.8	87.4±0.20
7	600	600	30	17546±67.2	78.9±0.19
8	300	900	15	18870±42.5	75.8±0.22

**Expressed as Mean ±SD*

Preparation of Aceclofenac Topical Gel

A weighed quantity of carbopol 974P was dispersed in purified water, soaked overnight, and stirred using a Remi stirrer to obtain a uniform dispersion. Weighed quantities of aceclofenac, propyl paraben, methanol, and limonene were dissolved in ethanol with continuous stirring using a magnetic stirrer and dispersed into the carbopol 974P dispersion. The pH of the gel was adjusted using triethanolamine to 6.5, and a weighed quantity of sodium propyl paraben, propylene glycol, and polyethylene glycol 400 was mixed into it with continuous stirring until a homogenous gel was formed (Hanif *et al.,* 2023).

Determination of Viscosity

The viscosity of the prepared gels was measured using a Brookfield viscometer with spindle No. 4 at a controlled temperature of 25±2°C and 50 rpm for the gel. The selection of the spindle and rpm is based on the trial-and-error method. The gel was filled in a jar, and the spindle was lowered perpendicularly while ensuring that the spindle did not touch the bottom of the jar. The viscosity of the gel was noted (Deshmukh *et al*., 2022).

In vitro Drug Release Study

An in vitro drug release study was carried out using a Franz diffusion cell. Aceclofenac gel was weighed equivalent to 20mg and was taken in a donor compartment. A cellulose membrane was placed between the donor and receptor compartments. The optimized gel was placed over a cellulose membrane with a 70-micron pore size. The receptor compartment of the diffusion cell was filled with pH 7.4 phosphate buffer solution and kept at 32±5℃. The receptor compartment was placed in a magnetic stirrer, and the solution was stirred continuously using a magnetic bead at 50 rpm. 1 mL sample was removed from the medium at specified intervals of 0.5, 1, 2, 4, and 6 h and refilled with an equivalent volume of fresh phosphate buffer. The withdrawn samples were evaluated spectrophotometrically at 276 nm, and the percentage of drug released was calculated (Devi *et al.,* 2025).

Design of Evaluation

Design Diagnostics

The efficiency of a design is assessed using design evaluation parameters known as design diagnostics, which are quantified by the design's percentage efficiency. The Evaluate Design platform produces results summarized in the Design Evaluation outlines commonly provided by various DOE software. These diagnostics include a color map on correlations, which visually represents the absolute correlations between effects using an intensity scale and design diagnostics, which offer efficiency metrics to evaluate the quality and robustness of the experimental design.

Statistical Analysis

The responses obtained from all 8 aceclofenac gel formulations were used to assess model fit. A multiple linear regression model was applied, with the intercept set to 0, to analyze both the viscosity and percentage of drug release. Analysis of variance (ANOVA) was conducted to determine the significance of model terms. Regression coefficients, *p*-values, and model fit statistics (R^2 and adjusted R^2) were calculated.

Statistical Optimization by Prediction Profiler

The desirability function approach was used for the simultaneous optimization of aceclofenac gel by numerical methods. This approach uses a prediction profiler to optimize the aceclofenac gel by calculating the individual desirability of the factors (Carbopol 974P, limonene, and PEG 400) and responses (viscosity and *in vitro* drug release). The overall global desirability function value ranges from 0 to 1, with values close to one signifying a greater maximization of desirability. The predicted optimized formulation was prepared and further evaluated for quality testing.

Evaluation of the Optimized Formulation of Aceclofenac Gel

The optimized aceclofenac gel formulation was prepared and evaluated for its physicochemical properties, *in vitro* release, *ex vivo* permeation, and stability.

Physicochemical Parameters

Measurement of pH

5g of gel was weighed and dispersed in 100 mL of purified water. The pH of the dispersions was measured using a digital pH meter. The pH was measured three times, and the average value was calculated (Deshmukh *et al.,* 2022).

Homogeneity

The optimized gel was placed in a container, and a homogeneity test was performed by visual inspection. They were evaluated for appearance and the presence of aggregates (Opatha *et al.,* 2022).

Determination of Viscosity

The viscosity of the prepared gels was measured using a Brookfield viscometer, as described previously.

Spreadability

Spreadability was evaluated by placing 1 g of the optimized gel between two glass slides (20 × 20 cm). The mass of the upper plate was standardized to 200 g for 1 min to obtain a uniform thickness of glass plates. Approximately 700 g of weight was added to the pan. The time required for the upper glass slide to move across the lower plate was measured. The spreadability was calculated by using the following formula (Deshmukh *et al.,* 2022).

$$S = m \times \frac{1}{t}$$

Where, S - spreadability
m – Weight tied to upper plate
l – Length moved on the upper plate
t - Time taken

Drug Content

The 10 mg of optimized Aceclofenac gel was diluted in phosphate buffer pH 7.4 and vortexed for 30 minutes. 1 mL was withdrawn, diluted with phosphate buffer pH 7.4, and the percentage of drug content was measured at 276 nm in a UV spectrophotometer (Devi *et al.,* 2025).

In Vitro Drug Release Study

In vitro drug release studies were carried out using a Franz diffusion cell, as described previously.

Drug Release Kinetics

Various release kinetics models were analyzed to evaluate the drug release mechanism and the release kinetics of the aceclofenac gel. The data were incorporated into zero-order, first-order, Higuchi, and Korsmeyer Peppas release models to analyze the drug release rate kinetics (Shah *et al.,* 2021).

Ex vivo Permeation Study

A Franz diffusion cell was used to study the permeation of aceclofenac gel using a porcine ear skin membrane. The excised porcine ear skin membrane was placed with the stratum corneum in front of the donor compartment and fixed in position between the compartments of the cell. In the receptor compartment, a magnetic stirrer bar was fixed and filled with phosphate buffer saline (pH 7.4) as the medium. The entire system was maintained over a magnetic stirrer at 37±1°C. 1g of the optimized gel was placed on the surface of the excised porcine ear skin membrane. From the receptor compartment, 1 mL of receptor medium was withdrawn at predetermined time intervals, and the same volume of fresh receptor medium was immediately substituted into the receptor compartment. The amount of Aceclofenac permeated through the excised porcine ear skin was assayed by UV-VIS spectrophotometer at a 276 nm wavelength (λ max) against a blank (Devi *et al.,* 2025).

Stability Studies

The optimized aceclofenac gel was tested for 9 months at a relative humidity of 60±5% and a temperature of 25±2°C. The pH and drug content of the gels were evaluated at 3, 6, and 9 months. The drug content results were computed to predict the shelf life of the aceclofenac gel by using JMP statistical software (Bayan *et al.,* 2023).

Results

Solubility Studies

The solubility of Aceclofenac was found to be 9.78±0.28 mg/ml and 4.98±0.23 mg/ml in ethanol and phosphate buffer pH 7.4, respectively and slightly soluble in water.

Melting Point

The melting point of Aceclofenac was observed at 149±0.7°C, which was within the reported range of 149–150°C.

Calibration Curve of Aceclofenac in Phosphate Buffer Saline (PBS) pH 7.4

The calibration curve of Aceclofenac in phosphate buffer pH 7.4 was 5-50 µg/mL, showing good linearity with a regression equation of y=0.0466x with a correlation coefficient (R^2)value of 0.999.

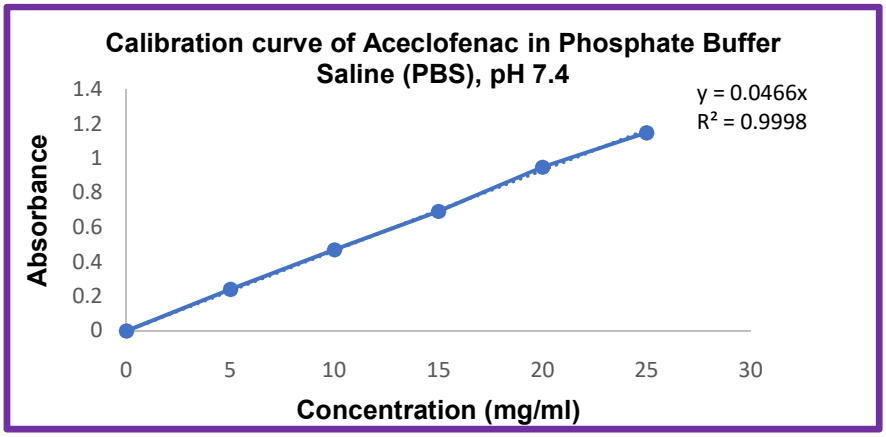

Figure 2:*Calibration Curve of Aceclofenac in Phosphate Buffer Saline (PBS), pH 7.4*

Drug-Excipient Compatibility Studies

The FTIR spectrum of a mixture of aceclofenac and excipients (Carbopol 974P and limonene) was analyzed to evaluate compatibility. Aceclofenac showed characteristic peaks at 3317 cm^{-1} due to O-H stretching, C=C stretching at 1600-1500 cm^{-1}, and C=O at 1770-1700 cm^{-1}.

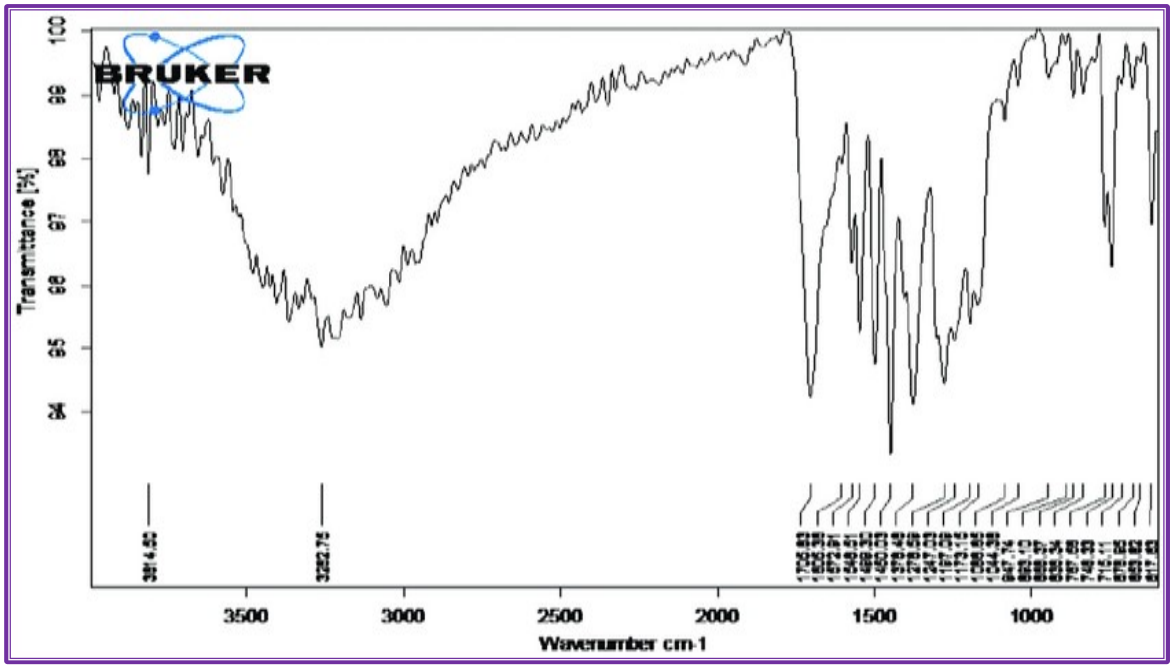

Figure 3: *FTIR Spectrum of Aceclofenac and Carbopol 974P*

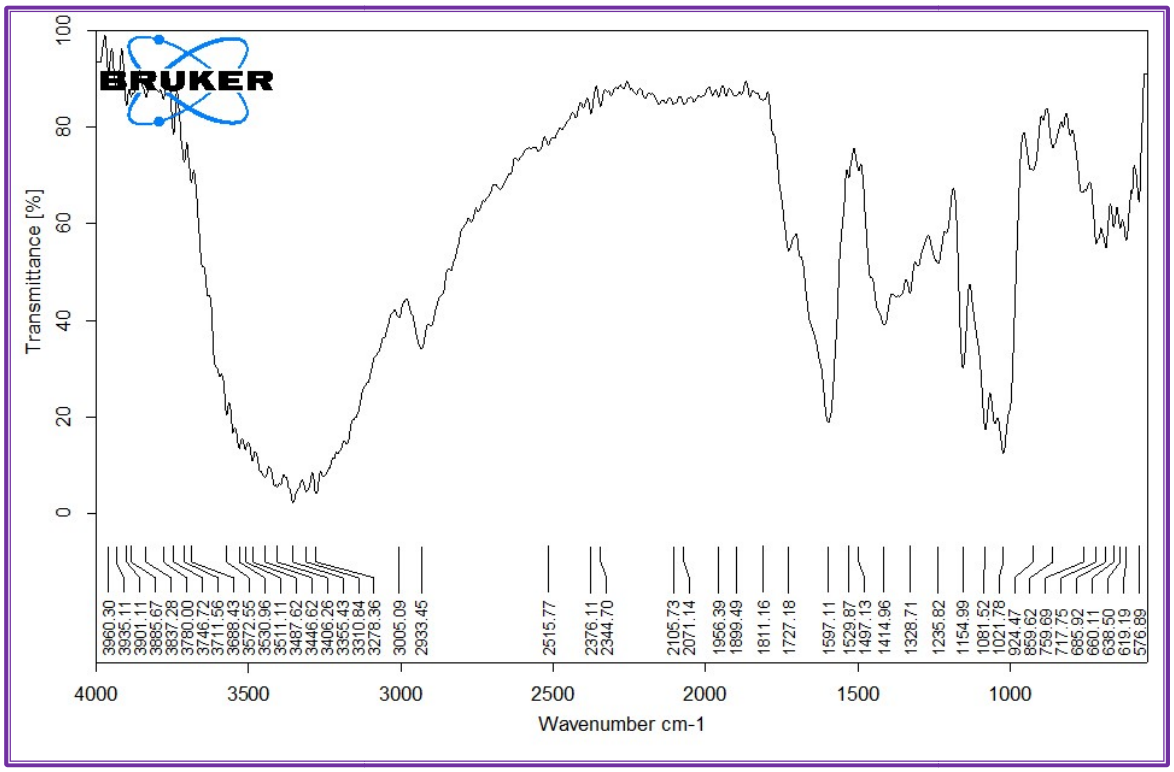

Figure 4: *FTIR Spectrum of Aceclofenac and Limonene*

Formulation and Development of Aceclofenac Topical Gel using Custom Design

Design Diagnostics

The model was evaluated using design diagnostics, which showed the highest efficiency of the design. The D, G, and A efficiencies were 100%. The higher the efficiency, the more the constructed result, as shown in Figure 5. A color map illustrates the effects of each component and their combinations on the responses. Figure 6 shows the color intensity to represent the strength of the relationships; deep red shows strong and effective combinations, while shades of blue represent weaker correlations.

Design Diagnostics

D Efficiency	100
G Efficiency	100
A Efficiency	100
Average Variance of Prediction	0.25
Design Creation Time (seconds)	0

Figure 5: *Design Diagnostics of Custom Design*

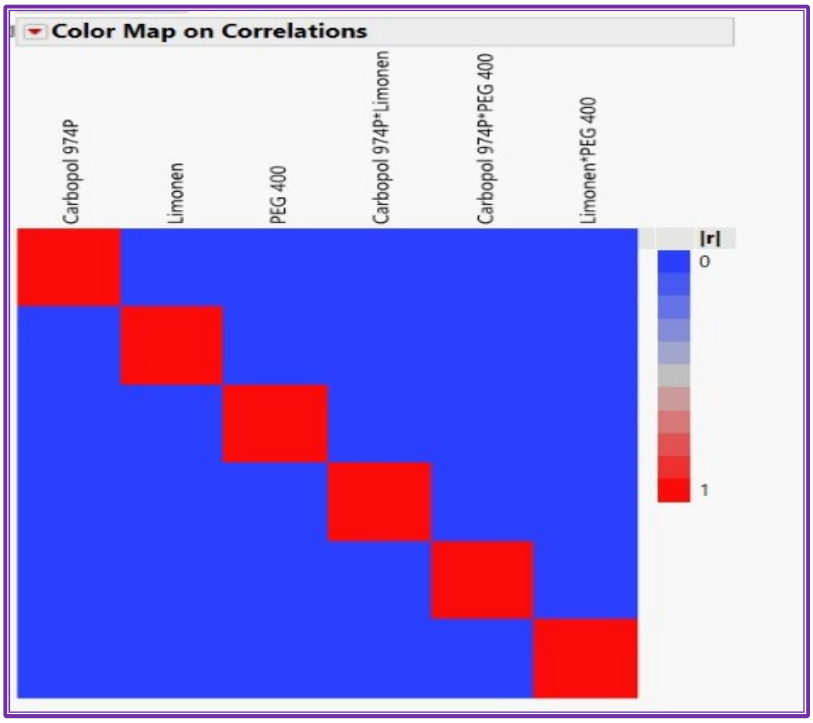

Figure 6: *Colour Map on Correlation of Custom Design*

Effects Summary

Carbopol 974P showed the highest impact with a log worth 2.567, *p*=0.00271. Limonene and PEG 400 had significant effects(*p*<0.05) (Table 7).

Table 7: *Effect Summary of The Selected CMAs*

Source	**Log Worth**		***P* Value**
Carbopol 974P (mg) (300,600)	2.567		0.00271
Limonene (mg) (600,900)	1.769		0.01703
PEG 400 (mg) (15,30)	1.662		0.02178

Statistical Analysis

The actual vs. predicted plot of viscosity (R^2=0.85, *P* Value=0.0486) and *in vitro* drug release (R^2 = 0.95, *P* value=0.0050) are shown in Figures 7 and 8.

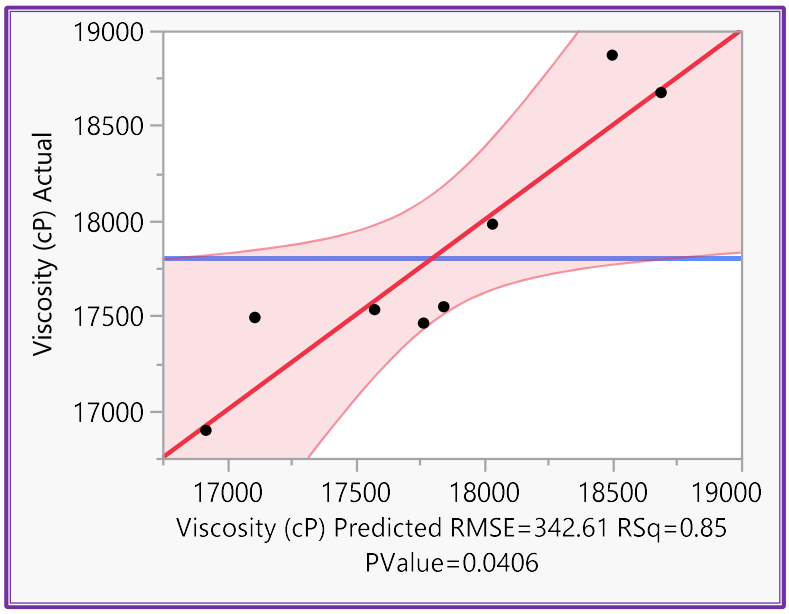

Figure 7: *Actual Versus Predicted Plot of Viscosity*

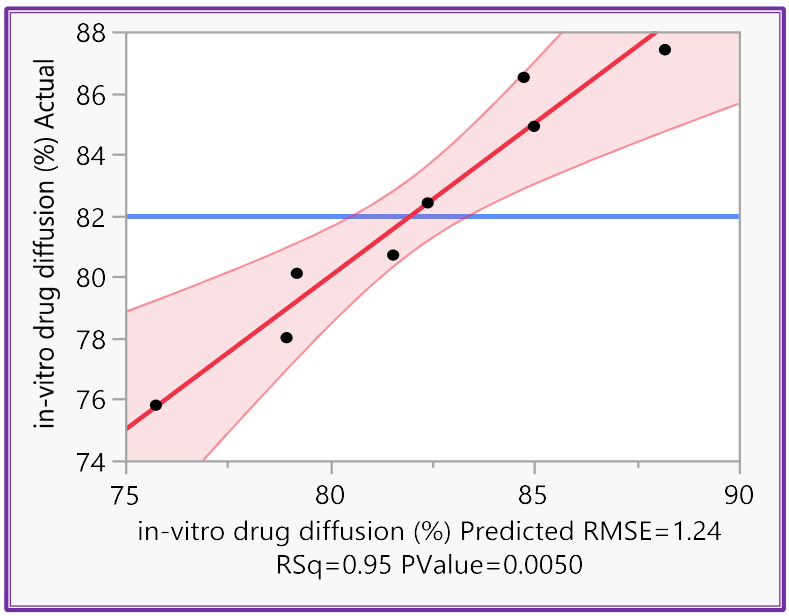

Figure 8: *Actual Versus Predicted Plot of in Vitro Drug Release*

Statistical Optimization of Selected Response

The optimization of viscosity and *in vitro* drug release was performed using the prediction profiler and global desirability. The model predicted an *in vitro* drug release and viscosity of 86.78% and 17493.81 cP, respectively, and the overall global desirability value was 0.998, indicating that the selected factors and responses were statistically significant and reliable for predicting the optimal formulation.

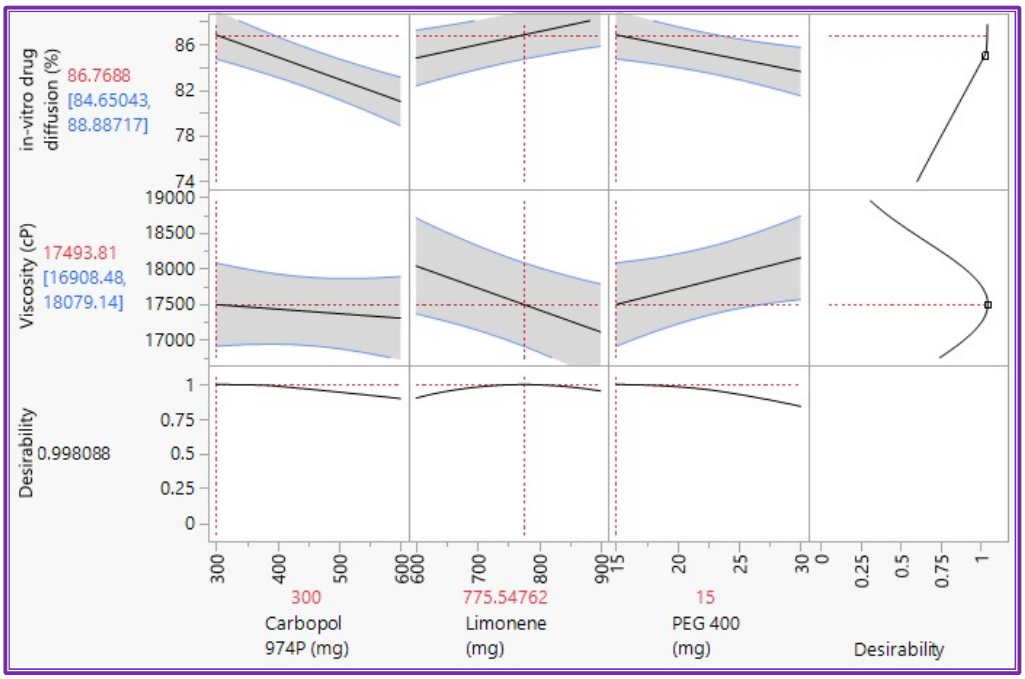

Figure 9: *Optimization of Aceclofenac Topical Gel Using Prediction Profiler*

Evaluation of The Optimized Aceclofenac Gel

Ph Determination

The optimized aceclofenac gel pH was measured using a digital pH meter, and it was found to be 5.96**.**

Viscosity

The viscosity of aceclofenac gel increased upon the addition of Carbopol 974P, as it formed a firm gel. The resulting viscosity was 17631±78.3 cP.

Spreadability

The spreadability of the optimized aceclofenac gel was 24.9±0.38g x cm/s, confirming that the gel exhibited good spreadability.

Drug Content

The drug content ensured that the aceclofenac was uniformly distributed in the gel formulation, and it was found to be 98.11%.

In Vitro Drug Release Study

The *in vitro drug* study of the optimized aceclofenac gel exhibited a sustained drug release for 6 h, with a cumulative drug release of 84.64%, as shown in Table 8 and Figure 10.

Table 8: *In Vitro Drug Release Study of Aceclofenac Gel*

Time (hrs.)	Average % of Cumulative Drug Release
0	0
0.5	7.39±0.0455
1	14.13±0.0332
2	31.21±0.0289
3	42.52±0.226
4	59.97±0.0817
5	74.71±0.012
6	84.64±0.09

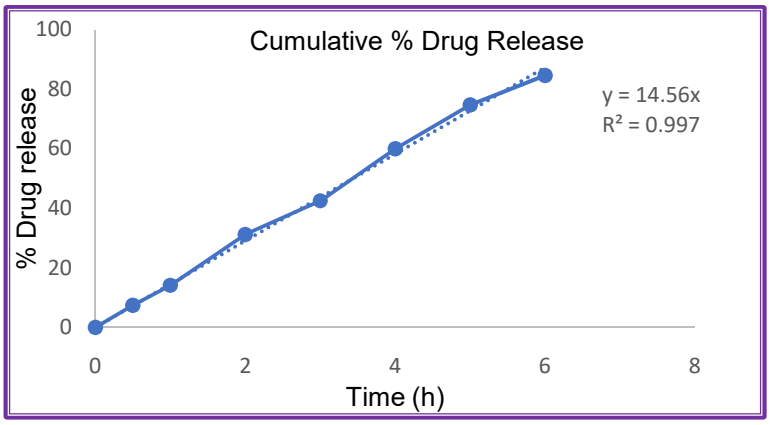

Figure 10: *in vitro Drug Release Study of Aceclofenac Gel*

Drug Release Kinetics

The optimized Aceclofenac gel's *in vitro* drug release data fit the zero-order kinetics, i.e., R^2 = 0.9971. The first order kinetics and Higuchi showed significantly less R^2 = 0.9433 and R^2 = 0.9281, respectively. Korsmeyer Peppas showed an 'n' value of 1.04 indicates non-Fickian release (Figure11).

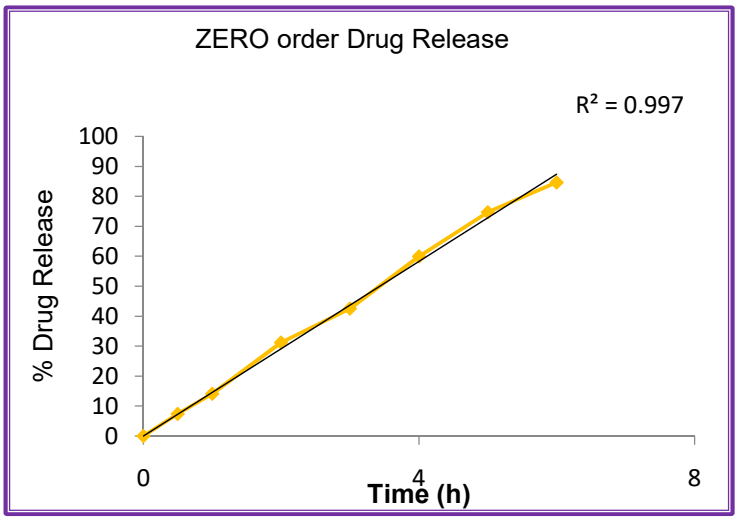

Figure 11: *In Vitro Drug Release Kinetics of Aceclofenac Gel*

Ex Vivo Permeation of Aceclofenac Gel

Ex vivo permeation studies using porcine ear skin for aceclofenac gel showed cumulative drug permeation of 77.421±3.63µg/cm² at the end of 6 h, which was significantly higher than that of the pure drug (control), which showed 40.02±2.776 µg/cm²/h. The steady-state flux of the gel was calculated to be 13.37 µg/cm²/h, as presented in Table 9 and Figure 12.

Table 9: *Ex Vivo Permeation of Aceclofenac Gel and Control*

Time (hr)	**The Average Cumulative Amount of Drug Permeated Per Cm2 (µg/cm²/h)**	
	Aceclofenac (Control)	**Aceclofenac Gel**
0	0	0
1	2.70155±1.0996	11.838±1.835
2	6.108±0.4001	24.725± 1.608
3	17.82± 1.0258	36.419± 3.68
4	24.08± 0.908	51.602± 3.69
5	31.08± 1.2201	69.537± 4.38
6	40.02± 2.776	77.421± 3.65
Flux (µg/cm²/h)	6.956	13.37

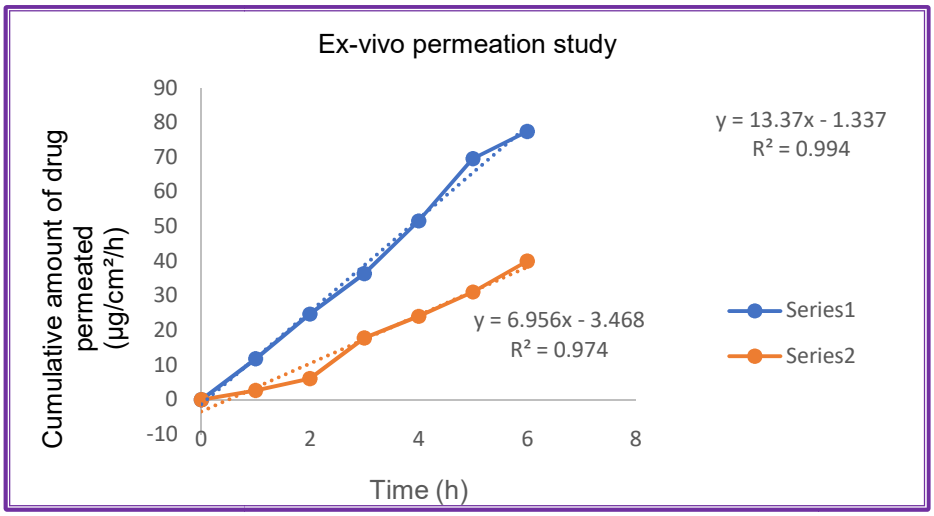

Figure 12: *Ex Vivo Permeation of Aceclofenac Gel and Control*

Prediction of Shelf Life

The shelf life of the aceclofenac gel was predicted using JMP software for three different batches. We estimated the earliest crossing time at 23.873 months with 95% confidence. The estimated shelf-life plot for all batches is represented in Figure 13.

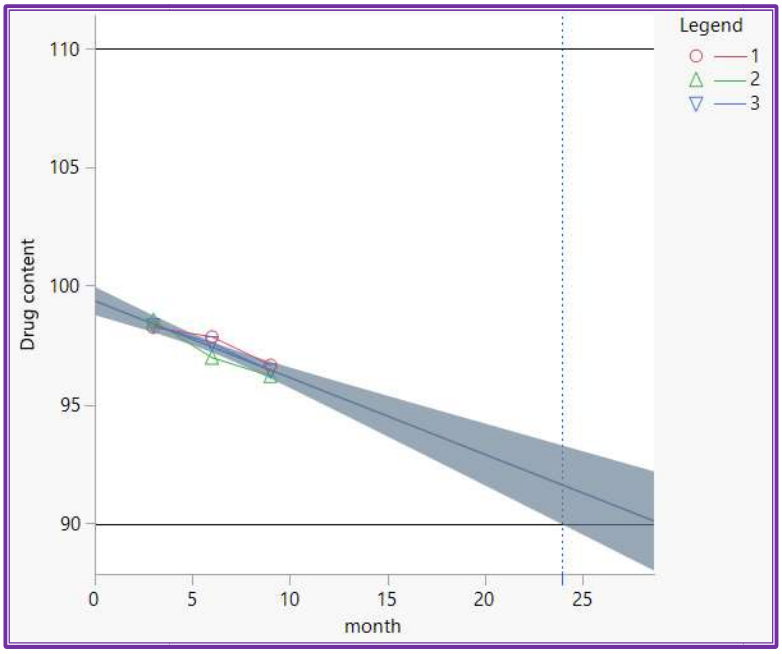

Figure 13*: Predicted Shelf Life of Aceclofenac Topical Gel*

Discussion

The solubility study of Aceclofenac may be attributed to its lipophilic nature and limited affinity for aqueous media. The increased solubility in ethanol suggests its suitability as a cosolvent in topical gel formulations, aiding drug solubilization and potentially enhancing skin permeation. The moderate solubility in buffer indicates pH-dependent solubility due to partial ionization. Overall, these results support the need for cosolvents to improve drug release and effectiveness in topical delivery (Korzeniowska & Malesza, 2023). The melting point of Aceclofenac confirms the purity and identity of the drug. This indicates the absence of polymeric forms in the drug. The calibration curve showed good linearity, indicating that the drug obeys Beer–Lambert law (Figure 2). The curve was further used to determine the concentration of unknown samples and to calculate the limit of detection (LOD) and limit of quantification (LOQ) (Chaturvedi *et al.,* 2021). The characteristic peaks appeared in the

FTIR spectra of the physical mixture, which indicates that there was no interaction between Aceclofenac and Carbopol 974P (Figure 3). In Figure 4, a peak was observed at 2924 cm^{-1} of C-H stretching vibration, which confirms the presence of Limonene. A new peak indicated that no significant interaction occurred between aceclofenac and limonene. The results showed that the aceclofenac drug was compatible and stable with the excipients (Kumar & Kumar, 2025).

The design showed 100% D-, G-, and A-efficiencies, indicating an optimal design for estimating the selected model terms. However, these values reflect design efficiency rather than model validity; therefore, further evaluation was performed using ANOVA and regression analysis. The effects summary was evaluated to identify the statistical significance of each independent variable on the responses of the aceclofenac gel. As mentioned in Table 7, the Carbopol showed the highest impact, with a log worth of 2.567 and a *p*-value of 0.00271, which affects the viscosity of the gel.

Figures 7 & 8 shows that the R^2 values indicate a good agreement between the predicted and experimental responses, with a stronger model fit observed for drug release compared to viscosity. The statistically significant *p*-values ($p < 0.05$) confirm the model's reliability. Overall, the model demonstrates acceptable predictability for viscosity and excellent predictability for drug release. The statistical optimization results demonstrated a significant influence of formulation variables on *in vitro* drug release and viscosity. The prediction profiler, as shown in Figure 9, increasing the concentration of Carbopol results in high viscosity with a slight decrease in *in vitro* drug release, whereas limonene showed a positive effect on drug release and a decrease in viscosity. The PEG 400 prediction plot suggested a slight increase in drug release, as it improved the solubility of the drug and increased viscosity (Hanif *et al*., 2023). The prediction profiler suggested the optimized factors for aceclofenac gel, such as Carbopol 974P, limonene, and PEG400, were 300 mg, 775.54 mg, and 15 mg, respectively; and the responses were 86.76% in vitro drug release and 17493.81 cP of viscosity. Aceclofenac gel pH, as mentioned in Table 8, indicates minimal skin irritation and maintains the stability of aceclofenac gel.

The viscosity of the gel enhances its adherence to the skin and extends the gel's residence time. The higher the concentration of Carbopol 934P, the higher the viscosity of the gel. The result confirmed the effectiveness of carbopol as a gelling agent for a clear, bio-adhesive gel with optimal rheological properties suitable for topical delivery.

The spreadability improves convenience and spreads effectively over the skin with minimal effort and time. The concentration of the polymer helps maintain the gel's consistency.

The optimized gel's drug content confirms that it was efficiently incorporated into the gel matrix to achieve the desired therapeutic effect. The drug release study indicated that increasing the concentration of Carbopol 974P resulted in higher viscosity, leading to a more sustained drug release profile. The release pattern (Table 9 and Figure 10) showed a gradual increase over time, suggesting that the Aceclofenac gel is suitable for topical delivery and may provide prolonged therapeutic action at the site of application (Hanif *et al*., 2023).

The *in vitro* release data of the optimized Aceclofenac gel were fitted to various kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, to elucidate the release mechanism. The correlation coefficient (R^2) was used to evaluate the goodness of fit. Among the models, zero-order kinetics showed the highest correlation ($R^2 = 0.9971$), compared to first-order ($R^2 = 0.9433$) and the Higuchi model ($R^2 = 0.9281$), indicating a concentration-independent release pattern. The release exponent ($n = 1.04$) suggests a super case-II transport mechanism, indicating that drug release is governed by polymer relaxation and erosion (Rani *et al.,* 2022).

The *ex vivo* permeation study demonstrated that the optimized Aceclofenac gel containing limonene enhanced drug permeation across the skin. This effect may be attributed to the penetration-enhancing property of Limonene, which is known to interact with the lipids of the stratum corneum, thereby reducing barrier resistance. Additionally, limonene may improve drug solubility and partitioning into the skin, similar findings have been demonstrated in a previous study where essential oils effectively

improved transdermal drug delivery (Mahanty *et al*., 2023). The results (Table 10 and Figure 12) indicate that the formulated gel significantly enhances the permeation of Aceclofenac compared to the pure drug.

The shelf life was estimated as the time at which the lower confidence limit of the regression line intersects the minimum acceptable specification, with 95% confidence. The model was found to be appropriate, exhibiting common slopes and intercepts at a significance level of 0.25 (Figure 13), indicating no significant batch-to-batch variation. This confirms the stability and predictability of the optimized formulation (Bayan *et al*., 2023).

Limitations

Although Aceclofenac topical gel was optimized using a custom design through JMP software, the study was limited to eight experimental runs with three formulation variables; however, the design does not fully represent the complex factor interactions. The *ex vivo* permeation study was performed using porcine ear skin, which, although structurally similar to human skin, does not entirely replicate *in vivo* human conditions.

Future Scope

Future studies may focus on increasing the number of formulations to better elucidate the interactions between formulation variables and responses. Further, permeation studies using different skin models could reveal more about drug permeation behavior. The present study did not encompass *in vivo* pharmacokinetic, pharmacodynamic, clinical, and detailed dermatological safety evaluations, which future investigations should address.

Conclusion

In this study, a topical gel of Aceclofenac was successfully developed to minimize the adverse effects associated with oral NSAID therapy and to provide a suitable alternative for patients intolerant to oral administration. Being a BCS class II drug, aceclofenac was formulated with Limonene, a GRAS-listed permeation enhancer, to improve its solubility and skin permeation. The custom design enabled systematic optimization, and the optimized formulation exhibited maximum desirability for the selected responses. The developed statistical model was found to be significant for the selected variables, indicating good predictability and robustness of the aceclofenac gel. *In vitro* drug release and *ex vivo* permeation studies demonstrated enhanced drug diffusion and a twofold increase in permeation across porcine skin compared to the control. However, the present study is limited by the absence of *in vivo* pharmacokinetic evaluation and the reliance on a single *ex vivo* model using porcine skin, which may not fully replicate human skin conditions. Therefore, further studies involving pharmacokinetic profiling and validation in human or multiple skin models are warranted to confirm the clinical applicability of the formulation. Overall, the findings suggest that the use of GRAS-listed permeation enhancers in topical delivery systems holds promise for improving the therapeutic efficacy of aceclofenac and may offer a viable alternative to conventional routes, subject to further *in vivo* study.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgement

The authors gratefully acknowledge the management of Krupanidhi College of Pharmacy for providing the necessary facilities and institutional support to carry out this work.

References

Ahirrao, S. P., Sonawane, M. H., Bhambere, D. S., Udavant, P. B., Ahire, E. D., Kanade, R., & Kuber, D. (2022). Cocrystal formulation: A novel approach to enhance solubility and dissolution of etodolac. *Biosciences Biotechnology Research Asia, 19*(1), 111-119. http://dx.doi.org/10.13005/bbra/2971

Ali, S. S., De, P. K., & Maji, H. (2025). Development and assessment of aceclofenac gel-based topical formula. *International Journal of Scientific Research and Technology*, *2*(2), 115 – 124. https://doi.org/10.5281/zenodo.14873940

Anaya-Mancipe, J. M., Carlos, A. L. M., Bastos, J. V. D. D. A., Tovar Ambel, E. M., Velasco-Díez, G., Fialho, R. L., &Thiré, R. M. D. S. M. (2025). Solution blow spun mats with beaded-fiber morphologies as a drug delivery system with potential use for skin wound dressing. *ACS Applied Materials & Interfaces*, *17*(16), 23466-23483. https://doi.org/10.1021/acsami.4c16675

Bayan, M. F., Chandrasekaran, B., & Alyami, M. H. (2023). Development and characterization of econazole topical gel. *Gels*, *9*(12), 929. https://doi.org/10.3390/gels9120929

Benetti, C., & Benetti, A. A. (2024). Quality by Design in Formulation Development. In: Jain, N.K., Bajwa, N. (eds) Introduction to Quality by Design (QbD). Springer, Singapore. https://doi.org/10.1007/978-981-99-8034-5_6

Chaturvedi, S., Ayaz, S., & Shah, K. (2021). Validated UV Spectrophotometric Method for in-vitro Dissolution Studies in Phosphate Buffer pH 7.4. *International Journal of Pharmaceutical Sciences and Research*, *12*(4), 2417-2421. https://doi.org/10.13040/IJPSR.0975-8232.12(4).2417-21

Conforti, A., Di Cola, I., Pavlych, V., Ruscitti, P., Berardicurti, O., Ursini, F., ... & Cipriani, P. (2021). Beyond the joints, the extra-articular manifestations in rheumatoid arthritis. *Autoimmunity Reviews*, *20*(2), 102735. https://doi.org/10.1016/j.autrev.2020.102735

Deshmukh, P. P., Changediya, V., & Rajurkar, V. (2022). Formulation and development of transdermal patches of ibuprofen with novel penetration enhancers. *World Journal of Pharmaceutical Research, 11*(2), 561-577. https://doi.org/10.20959/wjpr202212-25317

Devi, K., Joshi, V. G., Kabra, P., Malviya, N., Bukka, R., Nargund, R., ... & Nargund, S. L. (2025). Transfersomes: A Review for Transdermal Drug Delivery. *World Journal of Research and Review (WJRR), 20*(5), 32-38. https://doi.org/10.31871/WJRR.20.5.25.

Elgaddal, N., Kramarow, E. A., Weeks, J. D., & Reuben, C. (2024). Arthritis in adults age 18 and older: united states, 2022. US Department of Health and Human Services, Centers for Disease Control and Prevention. *National Center for Health Statistics.* https://dx.doi.org/10.15620/cdc:145594

Fytory, M., Alabrahim, O. A. A., Abouzid, A. E., Lababidi, J. M., ……&Azzazy, H. M. E. (2026). β-Cyclodextrin Inclusion Complex of d-Limonene: A Promising Biocompatible Formula for Enhanced Wound Healing—In Vitro and In Vivo Studies. *Journal of Biomedical Materials Research Part A, 114*, e70016. https://doi.org/10.1002/jbma.70016

Gavinet, B., Sigurani, S., Garcia, C., & Roso, A. (2024). Alternatives to Conventional Topical Dosage Forms for Targeted Skin Penetration of Diclofenac Sodium. *International Journal of Molecular Sciences*, *25*(13), 7432. https://doi.org/10.3390/ijms25137432

Hanif, A. M., Bushra, R., Shafiq, Y., Aslam, N., Mughal, M. A., Huma, A., ... & Khan, M. A. (2023). Influence of different gelling polymers on dexibuprofen gel formulation: In-vitro characterization and stability profile. *Pakistan Journal of Pharmaceutical Sciences*, *36*(1 special), 287-293. https://doi.org/10.36721/PJPS.2023.36.1.SP.287293.1

Karamkar, P. G., Agrawal, A., &Chatap, V. K. (2023). A review article: Formulation of topical gel by QbD approach. *Advances in Pharmacology and Pharmacy*, *11*(2), 90-101. https://doi.org/10.13189/app.2023.110202

Korzeniowska, K., &Malesza, K. (2023). Aceclofenac-induced gastrointestinal adverse reactions-a case report and literature review. *Anaesthesiology& Rescue Medicine / Anestezjologia i Ratownictwo*, *17*(1), 19 pages. https://doi.org/10.53139/AIR.20231703

Kovács, A., Falusi, F., Gácsi, A., Budai-Szűcs, M., Csányi, E., Veréb, Z., ... & Berkó, S. (2024). Formulation and investigation of hydrogels containing an increased level of diclofenac sodium using risk assessment tools. *European Journal of Pharmaceutical Sciences*, *193*, 106666. https://doi.org/10.1016/j.ejps.2023.106666

Kumar, A., & Kumar, M. (2025). Design, characterization, and evaluation of an aceclofenac cocrystal for improved solubility and stability. *Journal of Applied Pharmaceutical Research*, *13*(6), 132-145. https://doi.org/10.69857/joapr.v13i6.1699

Mahanty, J., Rasheed, S. H., Kumar, S., Singh, H., & Sharma, A. (2023). Potential of essential oils as alternative permeation enhancers for transdermal delivery. *World Journal of Traditional Chinese Medicine*, *9*(3), 258-269. https://doi.org/10.4103/2311-8571.351508

Moretti, A., Snichelotto, F., Liguori, S., Paoletta, M., Toro, G., Gimigliano, F., &Iolascon, G. (2024). The challenge of pharmacotherapy for musculoskeletal pain: an overview of unmet needs. *Therapeutic Advances in Musculoskeletal Disease*, *16*, 1759720X241253656. https://doi.org/10.1177/1759720X241253656

Opatha, S. A. T., Titapiwatanakun, V., Boonpisutiinant, K., &Chutoprapat, R. (2022). Preparation, characterization and permeation study of topical gel loaded with transfersomes containing asiatic acid. https://doi.org/10.3390/molecules27154865

Rani, K. R. V., Rajan, S., Bhupathyraaj, M., Priya, R. K., Halligudi, N., Al-Ghazali, M. A., ... &Pol, P. D. (2022). *The Effect of Polymers on Drug Release Kinetics in NanoemulsionIn Situ Gel Formulation. Polymers 2022, 14(3), 427*.https://doi.org/10.3390/polym14030427

Salloum, R., Baddour, F., & Abbood, A. (2024). A Questionnaire to Evaluate Undergraduate Students' Consumption and Awareness of Non-Steroidal Anti-Inflammatory Drugs in Syria. *International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR)*, *4*(4), 1-6. http://doi.org/10.54105/ijapsr.C4041.04040624

Sanap, S. N., Bisen, A. C., Mishra, A., Biswas, A., Agrawal, S., Yadav, K. S., ... & Bhatta, R. S. (2022). QbD based antifungal drug-loaded ophthalmic liposomal formulation for the management of fungal keratitis: in vitro, ex vivo and in vivo pharmacokinetic studies. *Journal of Drug Delivery Science and Technology*, *74*, 103517. https://doi.org/10.1016/j.jddst.2022.103517

Schlich, M., Lai, F., Maria Fadda, A., Sinico, C., & Pini, E. (2021). Drug-excipients compatibility studies in proniosomal formulation: A case study with resveratrol. *Journal of Nanoscience and Nanotechnology*, *21*(5), 2917-2921. https://doi.org/10.1166/jnn.2021.19056

Shah, R. S. (2021). Formulation and evaluation of Turmeric emulgel. *Asian Journal of Pharmacy and Technology*, *11*(3), 213-2199. http://dx.doi.org/10.52711/2231-5713.2021.00035