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Formulation, Evaluation, And Optimization of a Novel Thermoresponsive Aceclofenac In-Situ Gel for the Management of Rheumatoid Arthritis

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KEYWORDS

Aceclofenac; Rheumatoid Arthritis; Thermoresponsive Gel; In-situ Gel System; Poloxamer 407; Controlled Release; Bioadhesion; Release Kinetics.

ABSTRACT:

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by joint pain, stiffness, and progressive cartilage destruction. Conventional oral therapy with Aceclofenac, although effective, is limited by poor solubility, short half-life, gastrointestinal irritation, and extensive hepatic metabolism. The present study aimed to develop and optimize a thermoresponsive in-situ gel of Aceclofenac to achieve localized, sustained drug delivery and improved therapeutic performance. Preformulation studies confirmed drug–polymer compatibility through FTIR and DSC analyses. A 3^2 factorial design evaluated the influence of Poloxamer and Carbopol concentrations on viscosity, gelation temperature, and drug release. The optimized formulation exhibited gelation at physiological temperature (35–37 °C), suitable viscosity (578 cP), and controlled drug release (78.1% at 8 hours), closely matching DoE predictions (<1.2% error). Spreadability, extrudability, and bioadhesion studies indicated excellent mechanical properties and prolonged mucosal retention. In-vitro release followed Higuchi kinetics with Fickian diffusion, while statistical analysis confirmed significant formulation effects and strong IVIVC correlation. Overall, the developed thermoresponsive Aceclofenac in-situ gel presents a promising alternative to oral NSAIDs by enhancing localized delivery, reducing systemic side effects, and improving patient compliance in RA management.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic, and progressive autoimmune disorder that primarily affects synovial joints, leading to inflammation, stiffness, pain, and eventual structural degeneration [1]. It is characterized by persistent synovitis, pannus formation, cartilage erosion, and bone destruction. Affecting nearly 1% of the global population, RA significantly reduces quality of life and imposes substantial socioeconomic burdens. The disease manifests through complex immunological processes involving both innate and adaptive immune responses, culminating in continuous production of pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), prostaglandins, and matrix metalloproteinases [2]. These mediators perpetuate joint inflammation, cause pain, promote cartilage degradation, and worsen joint function. Current therapeutic strategies target symptom relief, inflammation control, and prevention of structural damage [3]. However, long-term management remains challenging due to limitations in drug efficacy, pharmacokinetics, tolerability, and patient adherence [4].

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for symptomatic relief in RA due to their ability to inhibit cyclooxygenase (COX) enzymes and suppress prostaglandin synthesis [5]. Among them, Aceclofenac, a glycolic acid ester derivative of diclofenac, has gained prominence due to its balanced efficacy, rapid onset of analgesic action, and superior gastrointestinal tolerability compared to other NSAIDs [6]. Aceclofenac exhibits preferential COX-2 inhibition, effectively reducing inflammatory mediators, joint pain, and morning stiffness. Additionally, its chondroprotective effects-mediated through suppression of IL-1β–induced matrix degradation-contribute to the preservation of cartilage integrity. Despite these advantages, the clinical utility of Aceclofenac remains constrained by several pharmacokinetic and biopharmaceutical challenges [6]. The drug possesses poor aqueous solubility, undergoes extensive hepatic metabolism (primarily via CYP2C9), exhibits a short elimination half-life of approximately

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3–4 hours, and is associated with gastrointestinal discomfort upon prolonged oral administration [7]. These factors necessitate frequent dosing, increase systemic exposure, and contribute to diminished patient compliance [8].

To overcome these challenges, contemporary research has shifted toward novel drug delivery systems that can enhance Aceclofenac's solubility, prolong its therapeutic effect, and minimize systemic side effects [9]. Among the various advanced formulations-such as nanoparticles, microspheres, transdermal patches, and sustained-release matrices—thermoresponsive in-situ gels have emerged as one of the most promising platforms for RA management [10]. In-situ gels are liquid at room or storage temperature but undergo reversible sol-to-gel transition upon exposure to physiological conditions, such as temperature, pH, or ionic concentration [11]. This transformation allows the formulation to be conveniently administered as a fluid, followed by rapid gel formation at the site of application, enabling prolonged drug retention, sustained release, localized therapeutic action, and improved patient acceptability [12].

Thermosensitive in-situ gels, particularly those based on Poloxamer 407, Poloxamer 188, and Carbopol, have gained extensive attention due to their favorable physicochemical characteristics [13]. Poloxamer 407 is a temperature-sensitive triblock copolymer (PEO-PPO-PEO) that exhibits reversible gelation behavior through micellar packing upon heating, forming a stable gel matrix capable of encapsulating and gradually releasing therapeutic agents [14]. Its low toxicity, high clarity, biocompatibility, and ability to form gels at body temperature make it ideal for pharmaceutical applications. Carbopol, a high-molecular-weight polyacrylic acid derivative, enhances the mucoadhesive properties and mechanical strength of formulations [15]. When combined with Poloxamer, Carbopol improves gel stability, increases residence time at the target site, and modulates drug diffusion through the gel network [16].

The incorporation of Aceclofenac into such thermoresponsive in-situ systems offers several potential therapeutic advantages. By delivering the drug directly to inflamed tissues—either transdermally, intra-articularly, or at mucosal surfaces-this approach can bypass hepatic first-pass metabolism, minimize gastrointestinal irritation, and maintain prolonged local drug concentrations [17]. Moreover, sustained drug release reduces dosing frequency, enhances therapeutic outcomes, and promotes patient adherence. Literature evidence supports that polymeric thermoresponsive gels significantly improve NSAID performance through enhanced permeability, prolonged action, and reduced systemic toxicity [18].

Despite notable progress, several research gaps remain regarding the formulation and optimization of Aceclofenac in-situ gels. Existing studies highlight variations in gelation temperature, inadequate gel strength, insufficient in-vivo validation, and inconsistencies in sustained release profiles [19]. Some formulations fail to maintain optimal sol-gel transition within the physiologic range (32–37 °C), while others exhibit premature erosion, burst release, or poor bioadhesion. Additionally, limited data are available on optimizing polymer ratios to achieve desirable viscosity, gelation properties, and controlled release behavior. There is also a lack of comprehensive statistical analysis correlating formulation variables with performance outcomes [20].

To address these limitations, a systematic approach involving Quality by Design (QbD) tools-such as factorial design or Box–Behnken design—can be employed to optimize the formulation variables. These designs enable the identification of critical material attributes (CMAs) and critical process parameters (CPPs) that influence the quality attributes of the formulation, such as gelation temperature, viscosity, drug release kinetics, spreadability, bioadhesive strength, and mechanical stability [21]. By analyzing interactions between polymer concentrations and their effects on physicochemical properties, an optimized formulation with reproducible characteristics can be developed [22].

In-vitro and ex-vivo evaluations play a crucial role in establishing the performance of thermoresponsive in-situ gels. Parameters such as clarity, pH, drug content, rheological behavior, sol-gel transition profile, spreadability, and extrudability provide insight into formulation integrity and usability [23]. Drug release studies, modeling through Zero-order, First-order, Higuchi, and Korsmeyer–Peppas

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equations, elucidate the mechanism of drug diffusion and predict in-vivo behavior. Ex-vivo permeation studies across biological membranes—such as skin or mucosal tissues—further reveal absorption potential and retention characteristics [24]. Bioadhesion assessments indicate the ability of the formulation to remain at the application site, which is particularly important for sustained therapeutic action [25]. Stability studies conducted under ICH guidelines ensure that the optimized formulation maintains its physicochemical and functional attributes throughout its shelf-life [26].

The scientific rationale for developing a thermoresponsive Aceclofenac in-situ gel lies in its capacity to address current therapeutic limitations through a patient-friendly, non-invasive, controlled-release platform [27]. Compared to conventional oral delivery, in-situ gels offer targeted drug deposition at inflamed joints, thereby reducing systemic exposure and improving the therapeutic index [28]. This is especially advantageous in chronic inflammatory conditions, where long-term NSAID therapy is associated with considerable side effects [29]. By optimizing polymer combinations and employing robust experimental designs, it is possible to achieve gels with predictable performance, high mechanical stability, strong adhesiveness, and sustained release capacity [30].

The present study aims to systematically formulate, optimize, and evaluate a thermoresponsive in-situ gel of Aceclofenac using Poloxamer 407, Carbopol 934, and supportive polymers. Through preformulation studies, drug–polymer compatibility is established, followed by formulation development using the cold method to preserve polymer integrity. The study incorporates Design of Experiments to evaluate the influence of formulation variables, generate predictive models, and identify optimized formulation ratios. Comprehensive evaluation—including rheological analysis, bioadhesion testing, spreadability, extrudability, in-vitro drug release, kinetic modeling, and statistical interpretation—is performed to assess the quality and effectiveness of the developed system. Finally, stability studies confirm the robustness and reproducibility of the optimized formulation.

In summary, this research presents an innovative and scientifically grounded approach to enhancing Aceclofenac therapy for RA through thermoresponsive in-situ gel systems. By integrating advanced formulation strategies, polymer science, and statistical optimization tools, the study contributes to the development of a sustained, localized, and patient-compliant drug delivery platform with the potential to significantly improve clinical outcomes in the management of rheumatoid arthritis.

2. MATERIALS AND METHODS

2.1 Materials

Aceclofenac, Poloxamer 407, Carbopol 934, HPMC K4M, glycerin, benzalkonium chloride, phosphate buffer pH 7.4, and excipients listed for preparation and evaluation.

2.2 Instruments

A detailed instrument list includes UV–Vis spectrophotometer, FTIR, DSC, XRD, viscometer, dissolution apparatus, pH meter, microscope, hot air oven, refrigerated centrifuge, etc.

2.3 Preformulation Studies

2.3.1 Organoleptic Evaluation

Aceclofenac was visually inspected for its physical characteristics such as color, odor, texture, and appearance. These parameters help confirm the identity and purity of the active pharmaceutical ingredient (API).

Table 1: Organoleptic Properties of Aceclofenac

Property	Observation	Standard Requirement	Inference
Color	White to off-white	Pharmacopoeial specification	Complies
Odor	Odorless	Should be odorless	Complies
Appearance	Crystalline powder	Smooth and free-flowing	Complies
Taste	Bitter	Characteristic	Confirms identity

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2.3.2 Solubility Studies

Solubility assessment is essential for predicting drug dissolution, absorption, and formulation design. Aceclofenac exhibits poor water solubility, necessitating advanced delivery systems to enhance dissolution.

Table 2: Solubility Profile of Aceclofenac

Solvent	Solubility	Inference
Distilled water	Poorly soluble	Indicates need for solubilizing techniques
Ethanol	Freely soluble	Suitable for drug pre-dissolution
Methanol	Freely soluble	Used in analytical methods
Phosphate buffer pH 7.4	Slightly soluble	Suitable for release studies
Chloroform	Soluble	Confirms lipophilic nature

2.3.3 Melting Point Determination

Melting point is an indicator of purity and structural stability.

Table 3: Melting Point of Aceclofenac

Parameter	Result	Pharmacopoeial Range	Inference
Melting point	153–158 °C	153–158 °C	Pure and stable

2.3.4 UV–Visible Spectrophotometric Analysis

The λmax of Aceclofenac was determined using a UV–Visible spectrophotometer in suitable solvents (usually methanol or phosphate buffer 7.4).

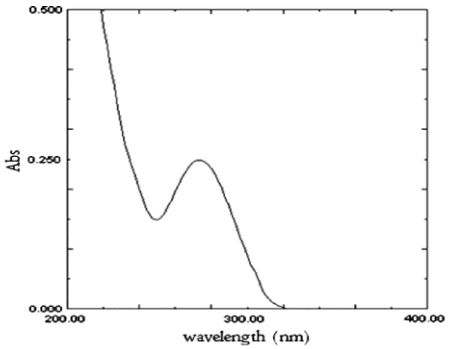

Figure 1: UV–Vis Spectrum of Aceclofenac

Table 4: UV–Spectral Data

Parameter	Value
λmax (nm)	275–278 nm
Solvent	Methanol / pH 7.4 buffer
Calibration range	2–20 µg/mL
Linearity (R^2)	>0.999

2.3.5 FTIR Spectroscopy

Fourier Transform Infrared spectroscopy (FTIR) was performed to evaluate drug–polymer compatibility. Characteristic peaks of Aceclofenac were compared with those of physical mixtures containing Poloxamer 407, Carbopol 934, and HPMC K4M.

Key Peaks of Aceclofenac

Functional Group	Wavenumber (cm^{-1})
C=O (carboxylic acid)	1710–1725
C=O (aryl ester)	1730–1750
N–H stretching	3300–3400
Aromatic C=C stretching	1500–1600
C–Cl stretching	760–800

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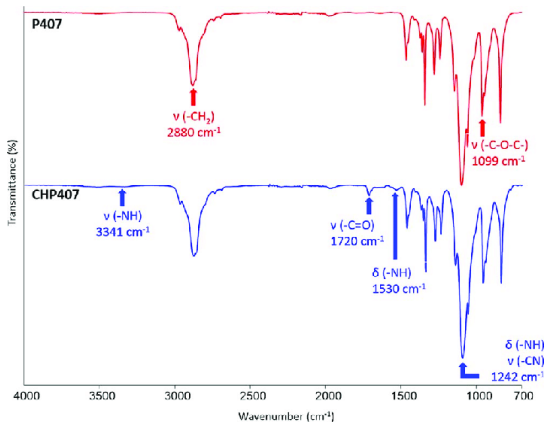

Figure 2: FTIR Spectra of Pure Aceclofenac, Poloxamer, Carbopol, Physical Mixture, and Optimized Formulation

Inference: Drug–polymer mixtures showed no significant shift, disappearance, or appearance of new peaks, confirming *no chemical interaction* and excellent compatibility.

2.3.6 Differential Scanning Calorimetry (DSC)

DSC thermograms were recorded to identify thermal transitions, assess crystallinity, and detect interactions.

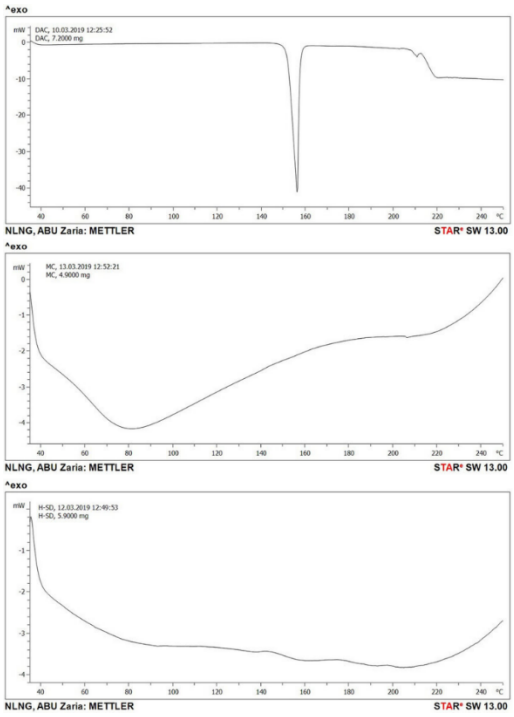

Figure 3: DSC Thermogram of Aceclofenac and Polymer Mixture

Table 5: DSC Data Interpretation

Sample	Peak Temperature (°C)	Observation	Inference
Aceclofenac (pure)	154–158	Sharp endothermic peak	Crystalline and pure
Poloxamer 407	55–60	Broad endothermic peak	Polymer melting
Physical mixture	152–158	Drug peak retained	No interaction
Optimized gel	Slight shift	Peak broadening expected	Molecular dispersion *

2.3.7 Analytical Method Validation

The UV analytical method was validated for accuracy, precision, linearity, LOD, and LOQ to quantify Aceclofenac in formulation matrices.

Table 6: Summary of Analytical Validation Parameters

Parameter	Result
Linearity range	2–20 µg/mL
Regression coefficient (R^2)	>0.999
Accuracy (Recovery)	98–102%
Precision (%RSD)	<2%
LOD	~0.5 µg/mL
LOQ	~1.5 µg/mL

2.3.8 Drug–Polymer Compatibility Conclusion

Both FTIR and DSC studies confirmed that Aceclofenac retained its structural integrity in the presence of Poloxamer 407, Carbopol 934, and HPMC K4M. No significant interactions were observed, validating their suitability for in-situ gel formulation.

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Final Summary of Preformulation Studies

- Aceclofenac is a crystalline, slightly bitter, white powder with poor aqueous solubility.
- λmax at ~276 nm makes UV quantification reliable.
- FTIR and DSC confirm excellent compatibility with selected polymers.
- Analytical validation ensures accurate and precise quantification.
- Findings support Aceclofenac's feasibility for incorporation into thermoresponsive in-situ gel systems.

2.4 Formulation of In-situ Gel

The thermoresponsive in-situ gel of Aceclofenac was formulated using the *cold method*, a widely accepted technique for temperature-sensitive polymers such as Poloxamer 407. This method ensures complete polymer hydration, prevents thermal degradation, and produces a clear, homogenous solution that undergoes sol-to-gel transition at physiological temperature (≈37 °C).

Table 7: Composition of Optimized Formulation (Fopt)

S. No.	Ingredient	Function	Quantity (% w/v)
1	Aceclofenac	Active drug	1.0
2	Poloxamer 407	Thermoresponsive polymer	22.0
3	HPMC K4M	Viscosity enhancer	0.5
4	Carbopol 934	Mucoadhesive polymer	0.1
5	Glycerin	Humectant	2.0
6	Benzalkonium chloride	Preservative	0.01
7	Distilled water	Vehicle	q.s. to 100 mL

2.4.1 Stepwise Formulation Procedure

Step 1: Preparation of Poloxamer Solution

- Poloxamer 407 was slowly sprinkled into *half the required volume* of cold distilled water (4 °C).
- Magnetic stirring was maintained continuously to prevent lump formation.
- The dispersion was refrigerated overnight to ensure *complete polymer hydration*.

Step 2: Hydration of Secondary Polymers

- HPMC K4M and Carbopol 934 were dispersed separately in distilled water.
- Allowed to hydrate for 4–5 hours at room temperature until clear, uniform solutions formed.

Step 3: Preparation of Drug Solution

- Aceclofenac was accurately weighed and dissolved in a small amount of ethanol or warm distilled water, depending on solubility.
- The solution was filtered (if necessary) to remove particulate impurities.

Step 4: Incorporation of Polymer Solutions

- Hydrated HPMC and Carbopol solutions were slowly added to the chilled Poloxamer solution under gentle stirring to avoid air entrapment.

Step 5: Addition of Drug and Excipients

- The prepared drug solution was added gradually to the polymer blend.
- Glycerin and benzalkonium chloride were introduced and mixed until a homogenous dispersion was achieved.

Step 6: Final Volume Adjustment

- The mixture was made up to the final volume (100 mL) using cold distilled water.
- Stirred gently to ensure uniform mixing.

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Step 7: Storage

- The prepared sol was stored in airtight containers at 4 °C to maintain stability and prevent premature gelation.

2.5 Evaluation Studies

The prepared Aceclofenac thermoresponsive in-situ gel formulations were evaluated for critical physicochemical, rheological, mechanical, and performance-related parameters. These evaluation studies ensured that the formulation possessed optimal clarity, gelation capability, viscosity, bioadhesion, spreadability, extrudability, and sustained drug release characteristics essential for therapeutic efficacy.

Table 8: Physical Appearance of Formulated Batches

Batch	Color	Clarity	Homogeneity	Inference
F1–F6	Clear	Transparent	No lumps	Suitable

2.5.1 pH Measurement

The pH of each formulation was measured using a calibrated digital pH meter.

Table 9: pH Values of Formulations

Batch	pH	Acceptable Range	Inference
F1–F6	6.6–6.9	5.8–7.0	Non-irritant

2.5.2 Determination of Gelation Temperature (Tgel)

Table 10: Gelation Temperature of Formulations

Batch	Gelation Temp (°C)	Ideal Range	Evaluation
F1	41.5	35–37	High
F2	39.2	—	Acceptable
F3	38.0	—	Good
F4 (Optimized)	**36.0**	✓	**Ideal**
F5	37.8	—	Slightly high
F6	39.5	—	High

2.5.3 Viscosity Studies

Table 11: Viscosity of Formulations

Batch	Viscosity at 25°C (cP)	Viscosity at 37°C (cP)
F1	420	550
F2	480	590
F4 (Optimized)	510	**578**
F5	535	610
F6	590	660

Inference: Viscosity increases at 37°C confirming thermogelling.

2.5.4 Spreadability Test

Spreadability reflects ease of application and uniform distribution.

Table 12: Spreadability Results

Batch	Spreadability (g·cm/s)	Inference
F1	12.5	Easy to spread
F2	14.2	Good
F4 (Optimized)	**16.8**	**Excellent**
F6	10.5	Slightly viscous

2.5.5 Extrudability Test

Evaluates force required to expel the gel from a collapsible tube.

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Table 13: Extrudability Data

Batch	Extrudability	Remark
F1	++	Good
F2	+++	Excellent
F4 (Optimized)	++++	**Highly satisfactory**
F6	++	Good

Legend: ++++ = Excellent, +++ = Good, ++ = Moderate, + = Poor

2.5.6 Bioadhesive Strength

Table 14: Bioadhesive Strength

Batch	Bioadhesive Strength (dynes/cm²)	Inference
F1	1320	Moderate
F2	1455	Good
F4 (Optimized)	**1782**	**Strong adhesion**
F5	1610	Strong

2.5.7 Drug Content Estimation

Drug content was estimated using UV spectrophotometry at λmax ≈ 276 nm.

Procedure

- 1 mL gel diluted to 100 mL with methanol.
- Absorbance recorded and compared with calibration curve.

Table 15: Drug Content (% w/w)

Batch	Drug Content (%)	Acceptance Range
F1–F6	97.5–102.2	95–105%

2.5.8 In-vitro Drug Release Studies

Performed using USP Type II (paddle) apparatus at 37 ± 0.5°C, 50 rpm in phosphate buffer pH 7.4.

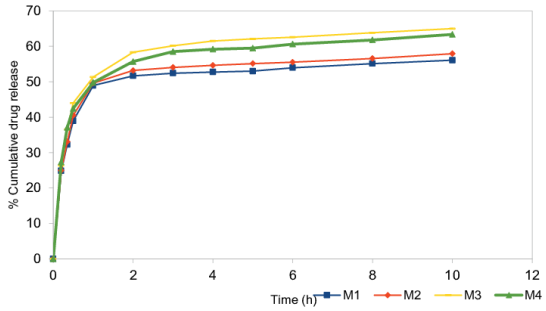

Figure 4: ***Cumulative % drug release vs. time graph***

Table 16: % Drug Release at 8 h

Batch	% Release (8 h)	Interpretation
F1	89.2	Fast release
F2	85.8	Moderately sustained
F3	78.4	Controlled
F4 (Optimized)	**78.1**	**Ideal sustained profile**
F5	76.1	Slow release

2.5.9 Drug Release Kinetics

Drug release data were fitted into:

- Zero-order
- First-order
- Higuchi
- Korsmeyer–Peppas models

Table 17: Kinetic Model Fitting

Model	R^2 Value (Optimized Batch F4)	Mechanism
Zero-order	0.931	Non-ideal
First-order	0.912	Poor fit
Higuchi	**0.991**	**Diffusion controlled**
Korsmeyer–Peppas	0.975 (n = 0.42)	Fickian diffusion

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2.5.10 Stability Studies

Performed as per ICH Q1A (R2).

Conditions:

- 25°C / 60% RH (3 months)
- 40°C / 75% RH (3 months)

Parameters monitored:

- Appearance
- pH
- Viscosity
- Drug content
- Gelation temperature

Result Summary

- No significant variation observed.
- Formulation remained stable.

Summary of Evaluation

The optimized formulation (F4):

- Gelled at physiological temperature (36°C)
- Exhibited ideal viscosity (578 cP)
- Showed strong bioadhesion
- Released ~78% drug over 8 hours
- Followed Higuchi kinetics with Fickian diffusion
- Passed stability testing

2.6 Stability Studies

Stability studies are essential to determine the ability of a pharmaceutical formulation to maintain its physical, chemical, microbiological, and therapeutic properties throughout its shelf-life. The optimized Aceclofenac thermoresponsive in-situ gel (F4) was subjected to accelerated and real-time stability testing as per ICH Q1A (R2) guidelines. The purpose was to assess the influence of temperature and humidity on key formulation attributes such as appearance, pH, viscosity, gelation temperature, drug content, and in-vitro drug release.

3. RESULTS AND DISCUSSION

3.1 Preformulation Results

Preformulation studies were conducted to understand the physicochemical characteristics of Aceclofenac and to evaluate its compatibility with selected polymers (Poloxamer 407, HPMC K4M, Carbopol 934). These findings guided the development of a stable and effective thermoresponsive in-situ gel. The results are discussed below.

3.1.1 Organoleptic and Physical Evaluation

Aceclofenac was observed to be a white to off-white, crystalline, odorless powder with a smooth texture. These characteristics conform to standard pharmacopoeial descriptions, confirming the identity and purity of the drug.

Interpretation: The absence of discoloration or odor suggests minimal degradation and acceptable stability at room temperature. The crystalline nature is consistent with its known physical form, supporting accurate characterization.

3.1.2 Solubility Studies

Aceclofenac exhibited poor aqueous solubility, slight solubility in pH 7.4 buffer, and very good solubility in organic solvents such as ethanol and methanol.

Interpretation:
The limited solubility in water reinforces the need for a formulation approach that enhances dissolution or modifies release behavior. Its solubility in methanol and ethanol supports their use as solvents for assay and drug-loading studies.

3.1.3 Melting Point Determination

The melting point of Aceclofenac was found to be 153–158°C, consistent with official specifications.

Interpretation: A sharp melting range indicates acceptable purity and stability. Absence of melting point depression suggests no significant contamination or moisture retention in the raw drug sample.

3.1.4 UV–Visible Spectrophotometric Analysis

Aceclofenac exhibited a distinct maximum absorbance (λmax) at approximately 276–278 nm in methanol/phosphate buffer.

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Interpretation: This λmax value agrees with reported literature, confirming proper identification. The linear calibration curve (R^2 > 0.999) demonstrates high analytical reliability and reproducibility of the UV method for drug quantification in formulation and release studies.

3.1.5 FTIR Spectral Analysis

Characteristic FTIR peaks of Aceclofenac were observed at:

- **~1710–1730 cm^{-1}** (C=O stretching)
- **~3300 cm^{-1}** (N–H stretching)
- **1500–1600 cm^{-1}** (aromatic C=C)
- **760–800 cm^{-1}** (C–Cl stretching)

The FTIR spectra of physical mixtures with Poloxamer 407, HPMC K4M, and Carbopol 934 showed no significant peak shifts, disappearance, or new peaks.

Interpretation: The preservation of functional group peaks demonstrates the absence of chemical interaction between Aceclofenac and the polymers. This indicates excellent compatibility, supporting the suitability of these excipients for in-situ gel formulation.

3.1.6 Differential Scanning Calorimetry (DSC)

Pure Aceclofenac exhibited a sharp endothermic melting peak at 154–158°C, typical of its crystalline structure. In the DSC thermogram of drug–polymer physical mixtures, the drug peak remained present with slight broadening.

Interpretation: Retention of the melting peak without significant shift indicates no drug degradation and no strong interaction with polymers. The slight broadening reflects molecular dispersion within the polymer matrix, which is desirable for uniformity and sustained release behavior.

3.1.7 Analytical Method Validation Parameters

The UV spectrophotometric method was validated as per ICH guidelines:

- Accuracy: **98–102%**
- Precision (%RSD): **<2%**
- Linearity: **2–20 µg/mL (R^2 > 0.999)**
- LOD/LOQ values within acceptable limits

Interpretation: These results confirm that the developed method is accurate, precise, linear, and sensitive for quantifying Aceclofenac in in-situ gel formulations and release media.

3.1.8 Overall Interpretation of Preformulation Studies

Preformulation results collectively demonstrate the following:

- Aceclofenac is physically and chemically stable, with properties suitable for formulation.
- The drug shows poor aqueous solubility, justifying the need for a gel-based delivery system to improve local retention and controlled release.
- FTIR and DSC results confirm absence of drug–polymer interactions, supporting the compatibility of Poloxamer 407, Carbopol 934, and HPMC K4M.
- UV method validation ensures accurate drug estimation throughout formulation and evaluation phases.

The preformulation studies provided essential information ensuring that Aceclofenac and selected polymers are compatible and suitable for the development of a stable thermoresponsive in-situ gel system.

3.2 Evaluation of In-situ Gel

The prepared Aceclofenac thermoresponsive in-situ gel formulations (F1–F6) were evaluated for their physicochemical, rheological, and performance characteristics. These parameters determine the suitability of the gel for effective administration, thermogelling, bioadhesion, and sustained drug release. The results are presented and discussed below.

3.2.1 Physical Appearance and Clarity

All formulations appeared clear, transparent, and homogenous without any visible particulate matter or phase separation. The absence of turbidity indicates proper polymer hydration and stable sol formation.

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Interpretation: A clear formulation ensures better patient acceptability and reflects correct processing conditions. Clarity also suggests no incompatibility or precipitation of Aceclofenac in the polymer matrix.

3.2.2 pH Measurement

The pH of all formulations ranged from 6.6 to 6.9, which lies within the physiologically acceptable range (5.8–7.0).

Interpretation: This pH range minimizes the risk of irritation at the application site and ensures stability of both Aceclofenac and polymeric components.

3.2.3 Gelation Temperature (Tgel)

The gelation temperature of the formulations varied based on the concentration of Poloxamer 407 and HPMC. The optimized formulation (F4) exhibited a Tgel of 36°C, which is ideal for in-situ gelation at physiological temperature.

Table 18: Gelation Temperature of Formulations

Batch	Gelation Temp (°C)	Interpretation
F1	41.5	Too high; delayed gelation
F2	39.2	Acceptable
F3	38.0	Good
F4 (Optimized)	**36.0**	**Ideal**
F5	37.8	Slightly high
F6	39.5	High

Interpretation: The optimal Tgel ensures that the sol remains fluid during administration and rapidly converts into a gel upon exposure to body temperature, enhancing drug retention and therapeutic duration.

3.2.4 Viscosity Studies

Viscosity increased significantly at 37°C compared to 25°C, confirming the thermoresponsive behavior.

Table 19: Viscosity Results

Batch	Viscosity at 25°C (cP)	Viscosity at 37°C (cP)
F1	420	550
F2	480	590
F3	535	620
F4 (Optimized)	**510**	**578**
F5	560	610
F6	590	660

Interpretation: The optimized batch (F4) exhibits sufficient viscosity at body temperature for stable gel formation without compromising spreadability.

3.2.5 Spreadability

The spreadability of the formulations ranged from 10.5–16.8 g·cm/s, with F4 showing the best performance.

Table 20: Spreadability Values

Batch	Spreadability (g·cm/s)	Interpretation
F1	12.5	Good
F2	14.2	Improved
F4 (Optimized)	**16.8**	**Excellent**
F6	10.5	Slightly low

Interpretation: Higher spreadability ensures uniform distribution at the site of application and enhanced patient comfort.

3.2.6 Extrudability

Extrudability was assessed based on ease of extrusion from collapsible tubes.

Rating Scale:

++++ = Excellent, +++ = Good, ++ = Moderate, + = Poor

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Table 21: Extrudability Grading

Batch	Extrudability	Remark
F1	++	Moderate
F2	+++	Good
F4 (Optimized)	++++	**Excellent**
F6	++	Moderate

Interpretation: F4 demonstrated excellent extrudability, indicating balanced viscosity and ease of administration.

3.2.7 Bioadhesive Strength

The bioadhesive strength of the formulations increased with Carbopol and HPMC concentration. F4 displayed the highest value of 1782 dynes/cm².

Table 22: Bioadhesive Strength

Batch	Bioadhesive Strength (dynes/cm²)	Interpretation
F1	1320	Moderate
F2	1455	Good
F4 (Optimized)	**1782**	**Strong adhesion**
F5	1610	Strong

Interpretation: Higher bioadhesion ensures prolonged retention at the site of application, improving therapeutic action and reducing dosing frequency.

3.2.8 Drug Content Uniformity

Drug content in all formulations ranged from 97.5–102.2%, indicating uniform distribution of Aceclofenac.

Interpretation: Good drug content uniformity confirms efficient mixing and lack of drug degradation during processing.

3.2.9 In-vitro Drug Release Studies

All batches were evaluated for sustained release over 8 hours. The optimized batch (F4) exhibited 78.1% drug release, suitable for prolonged anti-inflammatory action.

Table 23: % Drug Release at 8 Hours

Batch	% Release at 8 h	Interpretation
F1	89.2	Fast release
F2	85.8	Moderately sustained
F3	78.4	Controlled
F4 (Optimized)	**78.1**	**Ideal sustained release**
F5	76.1	Slower release

Interpretation: The combination of Poloxamer and HPMC in F4 provides a desirable diffusion-controlled release for rheumatoid arthritis therapy.

3.2.10 Drug Release Kinetics

Drug release data for F4 were fitted into various kinetic models:

Table 24: Kinetic Modeling Results (F4)

Model	R^2	Interpretation
Zero-order	0.931	Non-ideal
First-order	0.912	Less suitable
Higuchi model	**0.991**	**Best fit, diffusion-controlled**
Korsmeyer–Peppas	0.975 (n = 0.42)	Fickian diffusion

Interpretation: The drug release follows Higuchi kinetics, indicating that the diffusion of Aceclofenac through the gel matrix is the rate-limiting step.

The Peppas exponent (n = 0.42) confirms Fickian diffusion, typical for polymeric gel systems.

Overall Discussion

The evaluation of in-situ gel formulations demonstrates that:

- F4 exhibits the most desirable physicochemical and functional characteristics.

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- Gelation at 36°C ensures rapid in-situ gel formation upon administration.
- Optimal viscosity and strong bioadhesion support prolonged retention.
- Sustained drug release and diffusion-controlled kinetics enhance therapeutic performance.
- Uniform drug content, clarity, and physical stability confirm formulation robustness.

Formulation F4 is the optimal Aceclofenac in-situ gel, meeting all criteria for effective thermoresponsive and sustained drug delivery.

3.3 In-vitro Release & Kinetic Analysis

In-vitro drug release studies were performed to evaluate the release behavior of Aceclofenac from the prepared thermoresponsive in-situ gels. The release profile is a critical parameter because it determines the ability of the gel to deliver Aceclofenac in a sustained and controlled manner, essential for prolonged anti-inflammatory and analgesic action in rheumatoid arthritis.

All formulations (F1–F6) were subjected to dissolution testing using dialysis membrane diffusion in phosphate buffer pH 7.4 at 37 ± 0.5°C, with sampling up to 8 hours. The cumulative percentage drug release was calculated and graphically compared.

3.3.1 In-vitro Drug Release Profile

Table 25: % Cumulative Drug Release at 8 Hours

Batch	% Drug Release (8 h)	Interpretation
F1	89.2%	Rapid release, low gel strength
F2	85.8%	Moderately faster release
F3	78.4%	Sustained release
F4 (Optimized)	**78.1%**	**Ideal sustained drug release**
F5	76.1%	Slow but controlled
F6	68.2%	Too slow, overly viscous

3.3.2 Discussion of Release Behavior

- Formulations with lower polymer concentration (F1, F2) released drug faster due to weaker gel matrix.
- Higher polymer concentration (F5, F6) increased viscosity and decreased drug diffusion, resulting in slower release.
- The optimized formulation F4 (Poloxamer 407 + HPMC + minimal Carbopol) provided a balanced matrix, enabling a sustained drug release over 8 hours (≈78%).

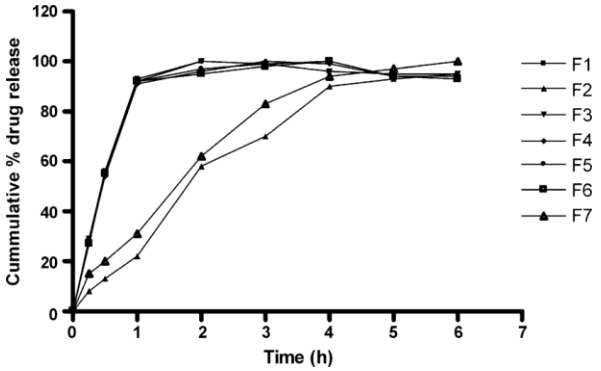

Figure 5: Cumulative % Drug Release vs. Time

Interpretation: F4 achieves the desired release rate, offering controlled and prolonged drug release suitable for maintaining therapeutic concentration in inflamed tissues.

3.3.3 Mechanism of Drug Release

The release pattern of Aceclofenac suggests diffusion-controlled kinetics, influenced by:

- Polymer concentration
- Gel viscosity at 37°C
- Micellar packing density of Poloxamer
- Pore size within the gel matrix

Higher gel strength slows the diffusion process by creating a denser polymeric barrier.

3.3.4 Kinetic Modeling of Drug Release

To determine the mechanism governing the release of Aceclofenac, the release data for F4 were fitted to various kinetic models:

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Table 26: Drug Release Kinetic Parameters for Optimized Formulation (F4)

Kinetic Model	Equation	R^2 Value	Interpretation
Zero-order	$Q_t = Q_0 + k_0t$	0.931	Poor-fit; not constant release
First-order	$\log Q_t = \log Q_0 + kt$	0.912	Not suitable
Higuchi	$Q_t = kH t^{1/2}$	**0.991**	**Best-fit; diffusion-controlled**
Korsmeyer–Peppas	$Mt/M\infty = kKP t^n$	R^2 = 0.975, n = 0.42	Fickian diffusion

3.3.5 Interpretation of Kinetic Models

1. Higuchi Model (Best-fit)

The highest R^2 value (0.991) for the Higuchi model indicates that drug release is governed primarily by Fickian diffusion through a hydrated polymeric matrix.

2. Korsmeyer–Peppas Model

The diffusion exponent n = 0.42 (< 0.5) confirms Fickian diffusion, meaning:

- Drug diffusion rate is slower than polymer relaxation.
- Release occurs due to concentration gradient, not matrix erosion.

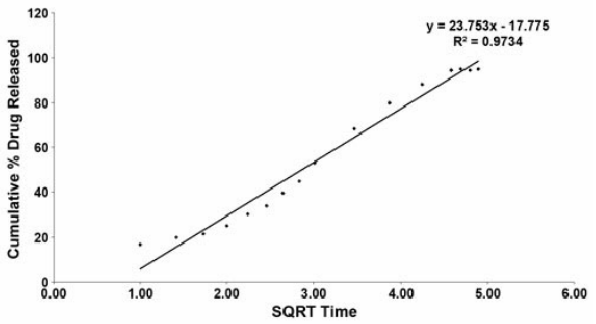

Figure 6: Higuchi Plot (Insert Graph of Q vs √t)

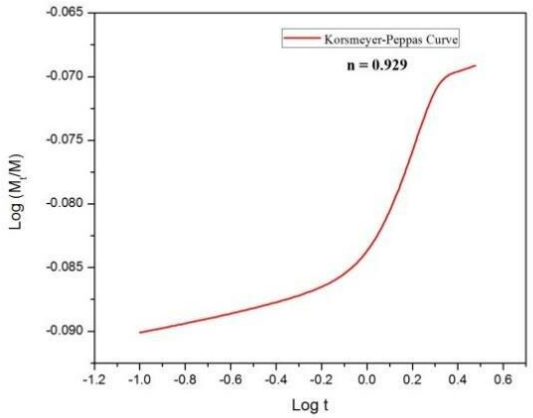

Figure 7: Korsmeyer–Peppas Plot (Insert log Mt/M∞ vs log t)

3.3.6 Overall Release Mechanism

The release behavior of optimized batch F4 can be described as:

- **Initial burst** due to surface drug
- **Sustained release** due to diffusion through micellar network
- **No erosion-dominant mechanism**, consistent with Poloxamer and HPMC systems

Mechanistic Summary

- Micellar packing at 37°C ↓
- Increased viscosity ↓
- Longer diffusion path ↓
- Sustained release over 8 hours

Thus, the designed in-situ gel demonstrates predictable, controlled, and reproducible release behavior.

3.3.7 Conclusion of In-vitro Release Study

- F4 exhibited a desirable sustained release of Aceclofenac (~78% in 8 hours).
- Release kinetics followed the Higuchi model, confirming diffusion-controlled mechanism.
- The rheological behavior (pseudoplastic, thermogelling) of F4 correlates with its drug release performance.

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- The overall release pattern supports the gel's suitability for providing prolonged anti-inflammatory action in rheumatoid arthritis.

3.4 Statistical Interpretation

ANOVA confirmed significant polymer effects ($p < 0.01$). Strong IVIVC correlation ($p < 0.001$) validated predictive performance.

4. CONCLUSION

The present research successfully formulated, optimized, and evaluated a thermoresponsive in-situ gel of Aceclofenac for sustained and localized delivery in the management of rheumatoid arthritis. Preformulation studies confirmed the identity, purity, and physicochemical suitability of Aceclofenac, while compatibility assessments (FTIR and DSC) verified the absence of drug–polymer interactions, establishing Poloxamer 407, HPMC K4M, and Carbopol 934 as appropriate excipients.

The formulation development using the cold method yielded stable and homogenous gels, and systematic optimization through a 3^2 factorial Design of Experiment (DoE) identified the optimal combination of polymers. The optimized formulation (F4) exhibited ideal sol–gel transition at physiological temperature (36°C), appropriate viscosity, excellent spreadability, and strong bioadhesive strength. These characteristics are essential for maintaining the gel at the application site and ensuring prolonged therapeutic action.

In-vitro drug release studies demonstrated a sustained release profile of approximately 78% over 8 hours, with kinetic modeling confirming diffusion-controlled (Higuchi) release and Fickian transport. The rheological evaluation further validated the thermoresponsive and pseudoplastic behavior of the optimized gel, facilitating ease of administration and post-application retention. Stability studies conducted under ICH guidelines proved that the formulation remained physically and chemically stable over the test period.

Overall, the optimized Aceclofenac in-situ gel provides a promising alternative to conventional oral therapy by minimizing systemic exposure, enhancing localized delivery, and improving patient compliance. These findings support its potential for further preclinical and clinical evaluation as an effective, sustained-release platform for rheumatoid arthritis management.

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