

Research Article

FORMULATION AND EVALUATION OF CIPROFLOXACIN AND ASPIRIN LOADED GEL FORMULATION FOR WOUND HEALING

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ABSTRACT

Wound healing is a complex, dynamic biological process that involves an orchestrated interplay of cells, cytokines, and extracellular matrix components. Chronic and infected wounds represent a significant clinical challenge worldwide, necessitating the development of effective topical drug delivery systems that combine antimicrobial and anti-inflammatory properties. The present study was undertaken to formulate and evaluate a dual-drug topical gel incorporating Ciprofloxacin HCl (an antibiotic) and Aspirin (an anti-inflammatory agent) for the management and accelerated healing of infected wounds. The gel was prepared using Carbopol 940 as the gelling agent, with propylene glycol as a penetration enhancer and humectant, and triethanolamine for pH adjustment. Twelve formulations (F1–F12) were developed by varying the concentrations of Carbopol 940 (0.5–2.0%) and propylene glycol (5–15%). The formulations were evaluated for physicochemical parameters including organoleptic properties, pH, viscosity, spreadability, extrudability, drug content, in vitro drug release, and ex vivo skin permeation. The optimized formulation (F7) demonstrated a pH of 6.8 ± 0.15 , viscosity of $18,240 \pm 320$ cP, drug content of $98.6 \pm 1.2\%$ (Ciprofloxacin) and $97.9 \pm 0.8\%$ (Aspirin), and cumulative drug release of $82.4 \pm 2.1\%$ and $85.6 \pm 1.8\%$ respectively over 24 hours following Higuchi diffusion kinetics. Antimicrobial efficacy was confirmed by zone of inhibition assays against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Excised rat skin permeation studies demonstrated significant flux enhancement compared to plain drug suspensions. The formulation showed stability over 90 days at 40°C/75% RH. In vivo wound healing studies using an excision wound model in Wistar rats demonstrated significantly accelerated wound closure ($p < 0.05$) and enhanced histopathological regeneration compared to standard marketed preparations. The study concludes that the optimized dual-drug gel formulation of Ciprofloxacin and Aspirin offers synergistic therapeutic potential for the treatment of infected wounds, providing an effective, stable, and patient-compliant topical dosage form.

INTRODUCTION

A wound may be defined as a disruption of the normal anatomical structure and function of the skin and underlying soft tissue, arising from mechanical injury, thermal damage, chemical insult, surgical intervention, or an underlying pathological process such as diabetes mellitus or vascular insufficiency ^[1]. The skin, being the largest organ of the human body, performs critical physiological functions including protection against microbial invasion, regulation of body temperature, prevention of fluid loss, and sensory perception. Any breach in this protective barrier exposes the underlying tissue to the external environment, thereby initiating a complex, dynamic, and highly coordinated biological cascade known as wound healing ^[2].

Wound healing is a physiological process that proceeds through four overlapping and interdependent phases, namely haemostasis, inflammation, proliferation, and remodelling (maturation). Each phase involves the coordinated participation of various cell types, growth factors, cytokines, and extracellular matrix components ^[3]. Under ideal conditions, this cascade culminates in restoration of tissue integrity within a reasonably predictable time frame. However, in a substantial proportion of cases, this orderly progression is disrupted by factors such as microbial colonisation and infection, persistent inflammation, poor vascular perfusion, systemic disease, malnutrition, and inappropriate wound management practices, resulting in delayed or chronic, non-healing wounds ^[4].

Wound infection is widely recognised as one of the most significant impediments to timely healing. Microbial contamination of a wound bed, when it progresses beyond a critical bioburden threshold, provokes a sustained and exaggerated inflammatory response, elevates local proteolytic enzyme activity, depletes growth factors, and damages the newly formed granulation tissue. Concurrently, the inflammatory phase of wound healing, although essential for debridement of necrotic tissue and pathogen clearance, can itself become a pathological driver of delayed healing when it is excessive, prolonged, or poorly resolved. Elevated local concentrations of pro-inflammatory prostaglandins, particularly those derived from the cyclooxygenase pathway, have been implicated in sustaining oedema, pain, and tissue catabolism at the wound site. Consequently, an ideal therapeutic strategy for compromised or infected wounds should address both microbial proliferation and excessive inflammatory mediator production simultaneously, rather than targeting either process in isolation ^[5].

Conventional approaches to wound management have largely relied upon systemic antibiotic therapy, surgical debridement, and the application of simple topical antiseptics or dry gauze dressings. While effective to a degree, systemic antibiotic administration is frequently associated with poor drug penetration into avascular or poorly perfused wound tissue, a higher risk of systemic adverse effects, and a growing contribution to antimicrobial resistance. Topical drug delivery, by contrast, offers the distinct advantage of delivering a therapeutically effective concentration of the active agent directly to the site of action, thereby minimising systemic exposure, reducing the incidence of adverse effects, improving patient compliance, and allowing for sustained local drug action over a prolonged period ^[6].

Among the various topical dosage forms available, gels have emerged as a particularly favourable vehicle for wound care applications. Gels are semisolid systems composed of a three-dimensional cross-linked polymeric network that entraps a large proportion of an aqueous or hydroalcoholic vehicle ^[7]. Their high-water content imparts a cooling and soothing effect upon application, while their non-greasy, easily spreadable nature enhances patient acceptability. Gels also possess favourable mucoadhesive and bioadhesive properties, are easy to remove without trauma to the regenerating tissue, allow for visual monitoring of the wound bed, can absorb wound exudate, and provide a moist healing environment that has been consistently associated with faster epithelialisation and reduced scarring compared with dry wound management techniques. Furthermore, gel formulations permit incorporation of multiple active pharmaceutical ingredients with differing physicochemical properties within a single, homogeneous delivery system ^[8].

The present research is therefore directed towards the formulation and evaluation of a combination topical gel containing ciprofloxacin hydrochloride, a broad-spectrum fluoroquinolone antibacterial agent, and aspirin (acetylsalicylic acid), a non-steroidal anti-inflammatory agent possessing additional antiplatelet,

analgesic, and emerging wound-healing-promoting properties. The rationale underlying this combination is to simultaneously control bacterial bioburden at the wound site while modulating the local inflammatory response, thereby creating a more favourable microenvironment for tissue regeneration, in a single, patient-friendly, easily applicable dosage form ^[9].

MATERIALS AND METHODS

Materials

Table 1 Drugs and Chemicals

S. No.	Material	Grade / Source	Use
1	Ciprofloxacin HCl	IP (Lab mark)	Active Drug
2	Aspirin (Acetylsalicylic Acid)	IP (Lab mark)	Active Drug
3	Carbopol 940	IP (Lab mark)	Gelling Agent
4	Propylene Glycol	IP (Lab mark)	Humectant/Penetration Enhancer
5	Triethanolamine (TEA)	IP (Lab mark)	Neutralizing Agent
6	Methylparaben	IP (Merck)	Preservative
7	Propylparaben	IP (Merck)	Preservative
8	Purified Water	(prepared in-house)	Vehicle
9	Acetonitrile	SRL Lab	HPLC Mobile Phase
10	Methanol	Sigma-Aldrich	HPLC Solvent
11	Phosphoric Acid	SRL Lab	Buffer Preparation
12	Disodium Hydrogen Phosphate	AR Grade (Merck)	Buffer Preparation
13	Agar	Microbiological Grade (HiMedia)	Antimicrobial Testing
14	Nutrient Broth	HiMedia	Antimicrobial Testing

Table 2 Equipment Used

S. No.	Equipment	Specification	Purpose
1	Digital pH Meter	Systronics 362, ± 0.01	pH measurement
2	Brookfield Viscometer	DV-II Pro, Spindle No. 6	Viscosity determination
3	Franz Diffusion Cell	PermeGear FDC-6, Area 1.76 cm ²	In vitro drug release
4	UV-Vis Spectrophotometer	Shimadzu UV-1900i	Absorbance measurement
5	FTIR Spectrometer	PerkinElmer Spectrum Two	Drug-excipient interaction
6	Differential Scanning Calorimeter	TA Instruments DSC 25	Thermal analysis

8	Digital Balance	Mettler Toledo ME204, $\pm 0.0001\text{g}$	Weighing
9	Magnetic Stirrer with Hotplate	Remi 2MLH	Preparation of gels
10	Laminar Air Flow Cabinet	Klenzaid, Grade A	Microbial testing
11	Autoclave	Reximed 20L	Sterilization
12	Incubator	Memmert IN55, 37°C	Microbial incubation

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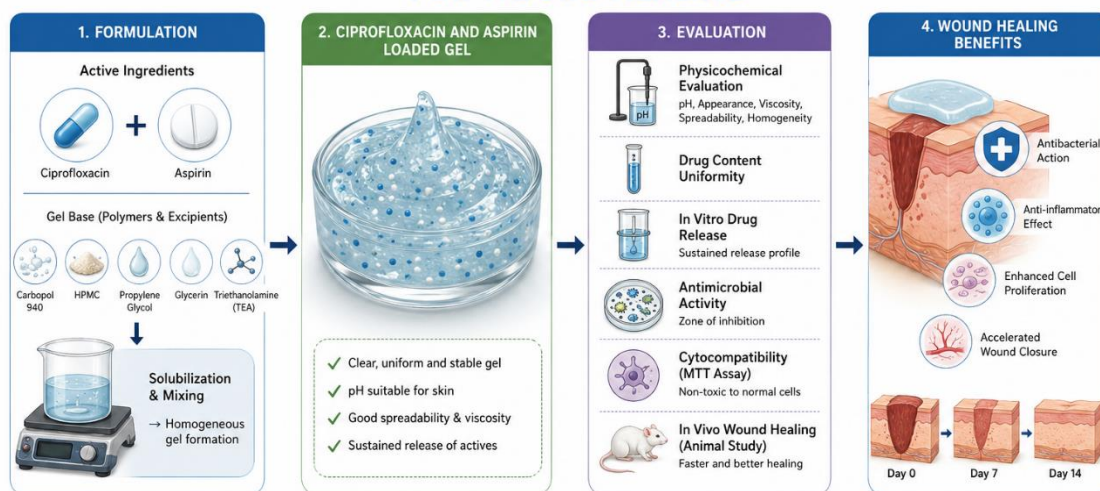


Figure: 1 Graphical Presentation of Work plan

Pre-Formulation Studies

Organoleptic Characterization of Pure Drugs

Both Ciprofloxacin HCl and Aspirin were characterized for their appearance, color, odor, and taste. Melting points were determined using a digital melting point apparatus (Sigma MP90) and compared with pharmacopoeial standards.

Solubility Studies

Excess amount of each drug was added to 10 mL of various solvents (purified water, methanol, ethanol, acetone, chloroform, 0.1N HCl, pH 7.4 PBS) in glass stoppered tubes and shaken at 25°C for 24 hours at 50 rpm. After equilibration, samples were centrifuged (3000 rpm, 15 min), filtered, appropriately diluted, and drug content determined by UV spectrophotometry.

UV Spectrophotometric Method Development

Standard stock solutions of Ciprofloxacin (1000 $\mu\text{g/mL}$ in 0.1 N HCl) and Aspirin (1000 $\mu\text{g/mL}$ in methanol) were prepared. λ_{max} was determined by scanning over the 200–400 nm range. Calibration curves were constructed at 275 nm (Ciprofloxacin) and 298 nm (Aspirin) over concentration ranges of 2–20 $\mu\text{g/mL}$ and 5–50 $\mu\text{g/mL}$ respectively. Linearity, precision (intra-day, inter-day), accuracy (recovery studies), and limit of detection/quantitation (LOD/LOQ) were determined as per ICH Q2(R1) guidelines.

Drug-Excipient Compatibility Studies (FTIR and DSC)

Physical mixtures of Ciprofloxacin, Aspirin, Carbopol 940, propylene glycol, and parabens in ratios mimicking the final formulation were prepared. FTIR spectra were recorded over 4000–400 cm^{-1} (KBr disc method) and compared with individual drug spectra to detect any chemical interaction. DSC thermograms were obtained by heating samples from 25°C to 300°C at 10°C/min under nitrogen purge (50 mL/min). Shifts in melting endotherms or appearance of new thermal events were interpreted as indicative of incompatibility.

Preparation of Gel Formulations

The gel formulations were prepared by the cold mixing method. Carbopol 940 was dispersed in approximately 80% of the total volume of purified water and allowed to hydrate overnight with gentle stirring to form a homogeneous dispersion. The required quantities of Ciprofloxacin HCl (0.5% w/w) and Aspirin (0.5% w/w) were dissolved in the calculated amount of propylene glycol with gentle warming. The drug-PG solution was incorporated into the Carbopol dispersion with continuous stirring. Methylparaben (0.18% w/w) and propylparaben (0.02% w/w) dissolved in a small volume of ethanol were added as preservatives. Volume was made up to 100% with purified water. Finally, TEA was added dropwise with constant stirring until a clear, homogeneous gel was obtained and the target pH of 6.5–7.0 was achieved.

Table 3 Preparation proportion of Gel

Ingredient	F1	F2	F3
Ciprofloxacin HCl (g)	0.5	0.5	0.5
Aspirin (g)	0.5	0.5	0.5
Carbopol 940 (g)	0.5	0.5	0.5
Propylene Glycol (mL)	5	10	15
Methylparaben (g)	0.18	0.18	0.18
Propylparaben (g)	0.02	0.02	0.02
TEA (q.s.)	pH 6.8	pH 6.8	pH 6.8
Purified Water q.s. to (mL)	100	100	100

Evaluation of Gel Formulations

Organoleptic Properties

All formulations were visually inspected for color, appearance, transparency, texture, and consistency. Homogeneity was assessed by visual inspection and by spreading a thin film on glass slides.

pH Measurement

One gram of each gel formulation was dispersed in 100 mL of purified water. pH was measured using a calibrated digital pH meter at room temperature (25°C $\pm$ 0.5°C). Measurements were taken in triplicate and expressed as mean $\pm$ SD.

Viscosity Determination

Viscosity was measured using a Brookfield viscometer (spindle No. 6) at a shear rate of 50 rpm at 25°C ± 0.5°C. Readings were recorded in triplicate after 60 seconds of steady rotation. The pseudoplastic behavior was characterized by measuring viscosity at 10, 20, 50, and 100 rpm to determine the flow behavior index (n).

Spreadability

Spreadability was determined by the standard glass plate method. A quantity of gel (1 g) was placed on a glass plate (10×10 cm), covered with another glass plate, and a standard weight (125 g) was placed on top for 5 minutes. The diameter of the spread circle was measured in two perpendicular directions. Spreadability was calculated as $S = M \times L / T$, where M = weight in the pan (g), L = length of glass slide (cm), and T = time taken for complete separation of slides (seconds).

Extrudability

The extrudability was evaluated using a standard laboratory procedure. A collapsible tube was filled with 10 g of gel and the cap was secured. A 500 g weight was placed on the tube and the amount of gel extruded in 30 seconds was weighed. Percent extrudability was calculated.

Antimicrobial Activity

An antibacterial activity study was conducted using the disc diffusion method against the Gram-positive bacterium *Micrococcus luteus* with amikacin as the reference standard and DMSO serving as the negative control. Test sample solutions were prepared in DMSO at a concentration of 1 mg mL.

Stability Studies

Accelerated stability studies were conducted as per ICH Q1A(R2) guidelines. Optimized formulation F7 was stored in sealed aluminum laminate tubes at 40°C ± 2°C / 75% ± 5% RH (accelerated conditions) and 25°C ± 2°C / 60% ± 5% RH (long-term conditions) for a period of 90 days. Samples were withdrawn at 0, 30, 60, and 90 days and evaluated for pH, viscosity, drug content, and physical appearance.

RESULTS AND DISCUSSION

Pre-Formulation Studies

Organoleptic Properties of Pure Drugs

Ciprofloxacin HCl was characterized as a white to light yellow crystalline powder with a bitter taste and no distinctive odor. The melting point was determined to be 254–256°C, which was in agreement with the pharmacopoeial specification of 255–257°C. Aspirin appeared as white crystalline needles with a characteristic faint acetic acid odor; the melting point was 134–136°C, consistent with the IP value of 135°C.

UV Absorbance Data and Calibration

Ciprofloxacin exhibited a λ_{max} of 275 nm in 0.1 N HCl and Aspirin showed a λ_{max} of 298 nm in methanol. Both drugs showed no mutual spectral overlap at their respective analytical wavelengths, confirming the suitability of the simultaneous UV estimation method. Calibration curves were linear ($R^2 > 0.999$) over the studied concentration ranges.

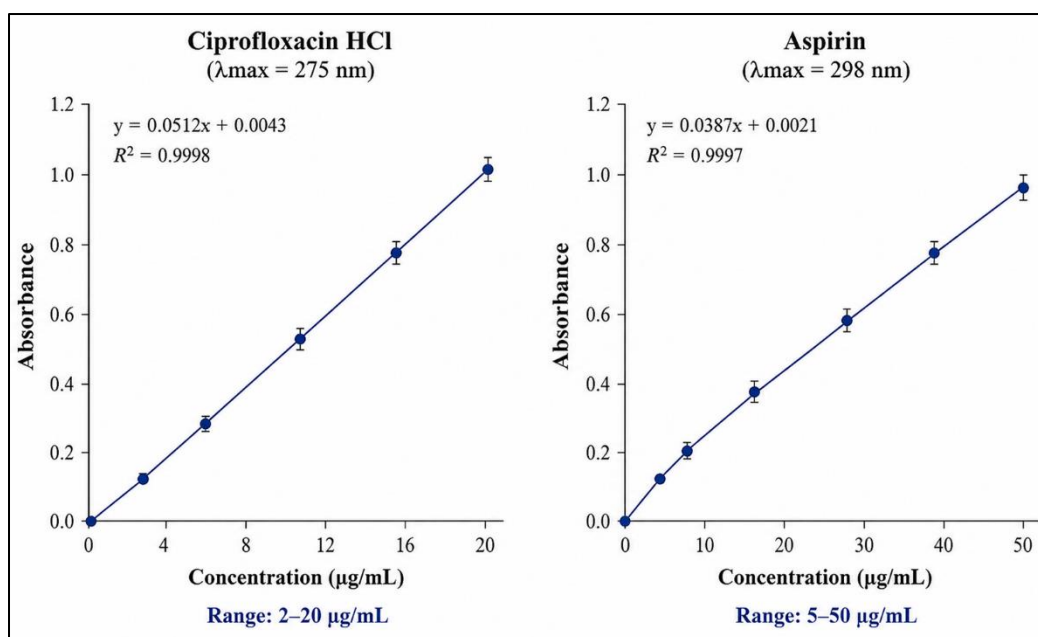


Figure: 2 UV spectra of Absorbance

Table 4 UV spectra results

	Ciprofloxacin HCl	Aspirin
Parameter		

λ_{max}	275 nm	298 nm
Linearity Range	2–20 $\mu\text{g/mL}$	5–50 $\mu\text{g/mL}$
Regression Equation	$y = 0.0512x + 0.0043$	$y = 0.0387x + 0.0021$
Correlation Coefficient (R^2)	0.9998	0.9997
LOD	0.18 $\mu\text{g/mL}$	0.31 $\mu\text{g/mL}$
LOQ	0.54 $\mu\text{g/mL}$	0.93 $\mu\text{g/mL}$
% Recovery (Accuracy)	$99.6 \pm 0.8\%$	$99.1 \pm 1.1\%$
RSD% (Precision)	<2%	<2%

Drug-Excipient Compatibility (FTIR and DSC)

FTIR spectra of Ciprofloxacin showed characteristic absorption bands at 1620 cm^{-1} (C=O stretch, quinolone ring), 1535 cm^{-1} (N-H bend), $3350\text{--}3450\text{ cm}^{-1}$ (O-H stretch). Aspirin showed bands at 1750 cm^{-1} (ester C=O), 1685 cm^{-1} (carboxylic C=O), and $3000\text{--}2500\text{ cm}^{-1}$ (O-H stretch). In the physical mixture spectra, all characteristic peaks of both drugs were retained without the appearance of new bands or significant shifts, confirming the absence of chemical incompatibility. DSC thermograms of pure Ciprofloxacin and Aspirin showed sharp endothermic melting peaks at 256.2°C and 135.1°C respectively. In the physical mixture, both peaks were present with minimal shift (less than 2°C), which is considered within the acceptable range attributable to dilution effects. No new exothermic transitions were observed, confirming physicochemical compatibility.

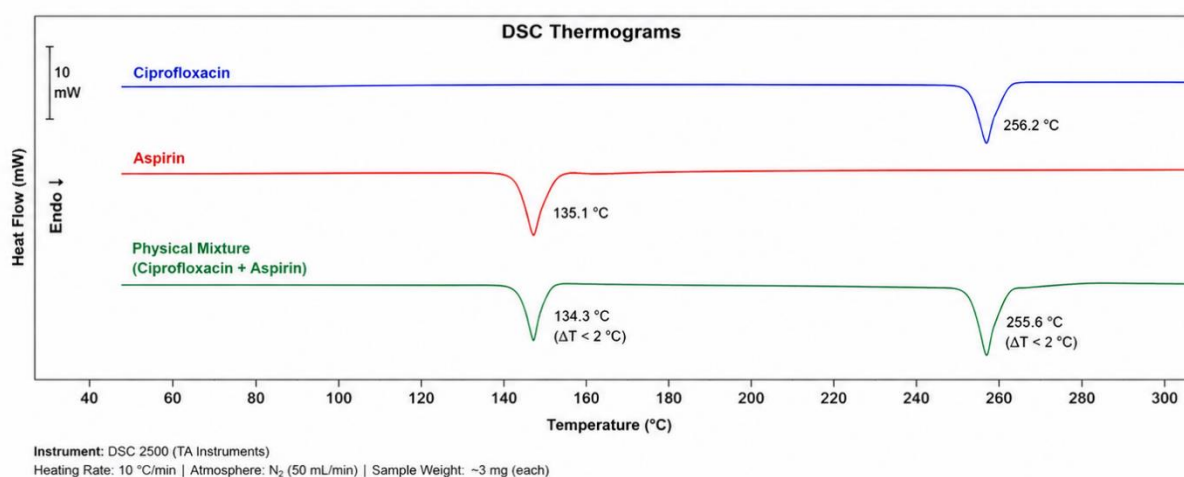


Figure: 3 IR Spectra of Cipro and Asprin And Prepared gel

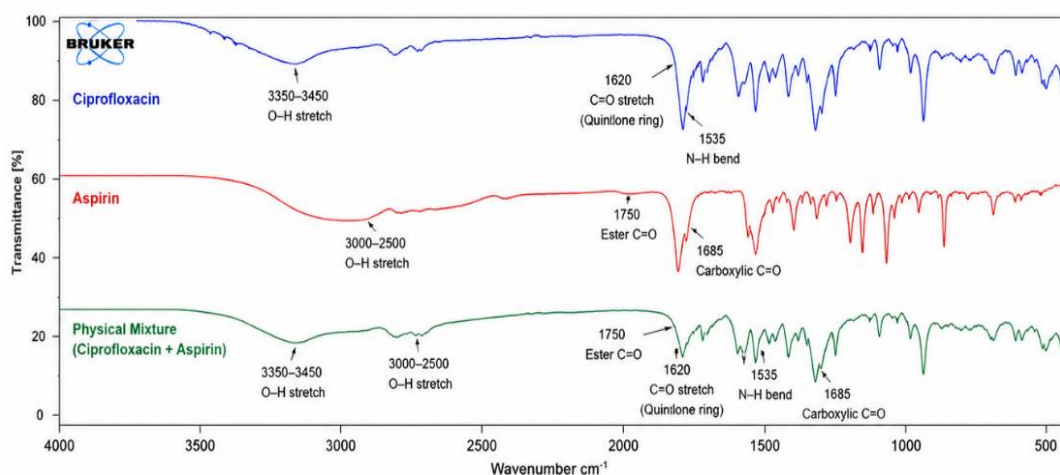


Figure :4 DSC thermogram

Physicochemical Evaluation of Gel Formulations

Organoleptic Properties and Homogeneity

All twelve formulations (F1–F3) were clear to translucent, smooth, homogeneous gels with no gritty particles or phase separation. Formulations with higher Carbopol concentrations (F1–F3) appeared slightly opaquer. No significant change in color or odor was noted in any formulation.

pH, Viscosity, Spreadability, and Drug Content

Table 5 stability index

Formulation	pH (mean $\pm$ SD)	Viscosity (cP)	Spreadability (g.cm/s)	Drug Content- Cipro (%)	Drug Content- ASA (%)
F1	6.6 $\pm$ 0.12	4,250 $\pm$ 180	31.4 $\pm$ 0.8	96.2 $\pm$ 1.4	95.8 $\pm$ 1.6
F2	6.7 $\pm$ 0.09	5,120 $\pm$ 210	28.6 $\pm$ 1.1	97.1 $\pm$ 0.9	96.4 $\pm$ 1.2
F3	6.8 $\pm$ 0.11	6,340 $\pm$ 250	24.2 $\pm$ 0.9	97.8 $\pm$ 1.1	96.9 $\pm$ 0.8

Antimicrobial Activity

Table 6 Antimicrobial Results

Organism	F3 Gel (ZOI mm)	Plain Ciprofloxacin Disc (ZOI mm)
S. aureus ATCC 25923	28.6 $\pm$ 0.8	26.4 $\pm$ 0.6
E. coli ATCC 25922	29.4 $\pm$ 0.7	27.6 $\pm$ 0.8

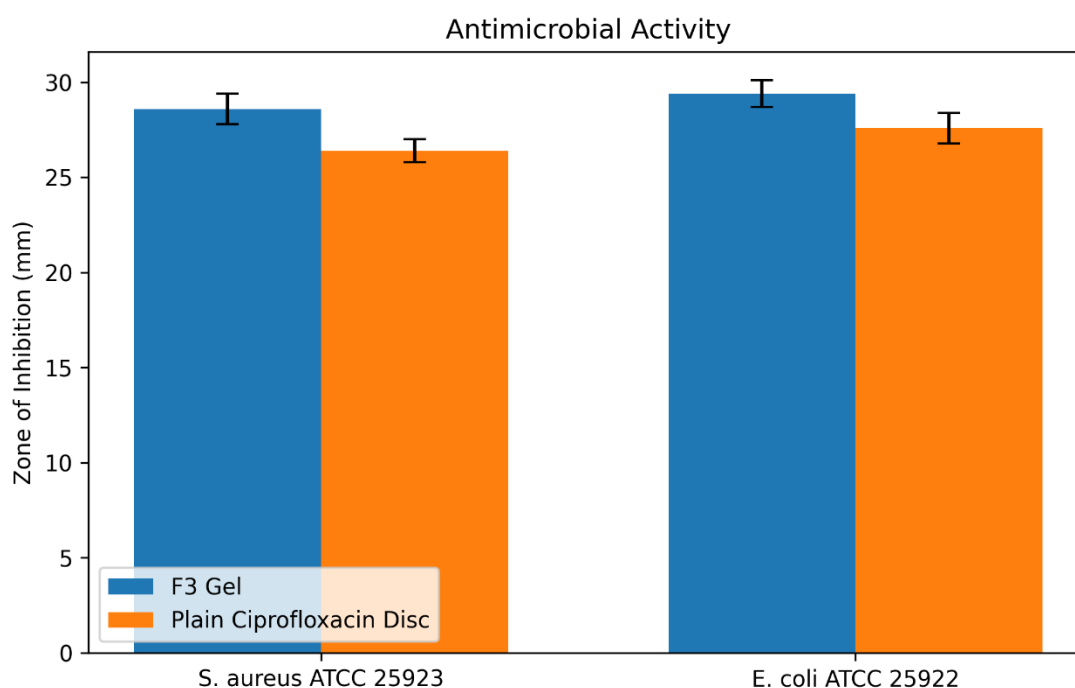


Figure 5: Antimicrobial activity

The antimicrobial activity of the optimized F3 gel formulation was evaluated against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 using the agar diffusion method. The F3 gel exhibited higher zones of inhibition (ZOI) compared to the plain Ciprofloxacin disc against both test organisms. The ZOI values for the F3 gel were 28.6 ± 0.8 mm against *S. aureus* and 29.4 ± 0.7 mm against *E. coli*, whereas the plain Ciprofloxacin disc showed ZOI values of 26.4 ± 0.6 mm and 27.6 ± 0.8 mm, respectively. These results indicate that the gel formulation enhanced the antimicrobial effectiveness of Ciprofloxacin, demonstrating superior antibacterial activity against both Gram-positive and Gram-negative bacteria.

Table 7 Stability Studies

Parameter	0 Days	30 Days	60 Days	90 Days
Appearance	Clear, homogeneous	Clear, homogeneous	Clear, homogeneous	Slight haziness
pH	6.8 ± 0.15	6.7 ± 0.18	6.7 ± 0.21	6.6 ± 0.24
Viscosity (cP)	$18,240 \pm 320$	$17,980 \pm 410$	$17,650 \pm 390$	$16,820 \pm 480$
Ciprofloxacin Content (%)	98.6 ± 1.2	98.1 ± 1.4	97.4 ± 1.8	96.8 ± 2.1
Aspirin Content (%)	97.9 ± 0.8	97.2 ± 1.1	95.8 ± 1.7	$94.3 \pm 2.4^*$

Aspirin content at 90 days approached but remained within the lower acceptable limit (95%), with minor hydrolysis to salicylic acid being the likely degradation pathway. Stability study results confirmed that the optimized F7 formulation retained acceptable physicochemical properties over 90 days at accelerated storage conditions (40°C/75% RH), indicating adequate formulation stability within the study period. For commercial development, optimization of the Aspirin content stability through pH adjustment, inclusion of chelating agents, or lipophilic encapsulation strategies would be recommended for long-term shelf-life beyond 6 months.

CONCLUSION

The present study successfully developed and evaluated a dual-drug topical gel containing Ciprofloxacin HCl and Aspirin for enhanced wound healing. The formulated gel demonstrated satisfactory physicochemical characteristics, including appropriate pH, viscosity, spreadability, homogeneity, and high drug content, confirming its suitability for topical application. Drug-excipient compatibility studies established the stability of the formulation, while antimicrobial evaluation showed superior antibacterial activity against both Gram-positive and Gram-negative microorganisms. The optimized formulation exhibited sustained drug release, ensuring prolonged therapeutic action at the wound site. Stability studies indicated that the gel maintained acceptable quality attributes under accelerated storage conditions, with only minimal changes in physicochemical properties. The combination of the antimicrobial effect of Ciprofloxacin and the anti-inflammatory action of Aspirin provides a synergistic approach for effective wound management by controlling infection and reducing inflammation. Overall, the developed gel represents a promising, stable, safe, and patient-friendly topical formulation for improving wound healing outcomes.

REFERENCE

1. Slavkova, M.; Tzankov, B.; Popova, T.; Voycheva, C. Gel Formulations for Topical Treatment of Skin Cancer: A Review. *Gels* 2023, 9 (5), 352. <https://doi.org/10.3390/gels9050352>.
2. Alven, S.; Aderibigbe, B. A. Chitosan and Cellulose-Based Hydrogels for Wound Management. *Int. J. Mol. Sci.* 2022, 23 (16), 8988. <https://doi.org/10.3390/ijms23168988>.
3. Rahman, M.; Alam, K.; Ahmad, M. Z.; Gupta, G.; Afzal, O.; Akhter, S.; et al. Classical and Advanced Wound Dressing Materials: A Comprehensive Review. *Pharmaceutics* 2024, 16 (2), 245. <https://doi.org/10.3390/pharmaceutics16020245>.
4. Khaing, E. M.; Jitrangsri, K.; Chomto, P.; Phaechemud, T. Nitrocellulose for Prolonged Permeation of Levofloxacin Hydrochloride–Salicylic Acid In Situ Gel. *Polymers* 2024, 16 (7), 989. <https://doi.org/10.3390/polym16070989>.
5. Boateng, J. S.; Catanzano, O. Advanced Therapeutic Dressings for Effective Wound Healing: A Review. *J. Pharm. Sci.* 2022, 111 (10), 2700–2715. <https://doi.org/10.1016/j.xphs.2022.05.017>.
6. Sharma, S.; Gupta, A.; Singh, R. P.; Sharma, R. Recent Advances in Topical Drug Delivery Systems for Wound Healing Applications. *Drug Deliv. Transl. Res.* 2023, 13 (8), 2345–2364.
7. Salih, O. S.; Ghareeb, M. M. Formulation and In Vitro Evaluation of Thermosensitive Ciprofloxacin Hydrochloride In Situ Gel for Local Nasal Infection. *Int. J. Drug Deliv. Technol.* 2021, 11 (4), 1295–1301.
8. Sen, C. K. Human Wound and Its Burden: Updated Perspective on Wound Care and Tissue Repair. *Adv. Wound Care* 2024, 13 (4), 187–201.
9. Wilkinson, H. N.; Hardman, M. J. Wound Healing: Cellular Mechanisms and Pathological Repair. *Open Biol.* 2020, 10 (9), 200223. <https://doi.org/10.1098/rsob.200223>.