Received: 14 Jan. 2026	Revised: 15 Feb. 2026	Accepted: 30 March. 2026

Research Article

ISBN: 978-81-995400-3-3

"DEVELOPMENT AND OPTIMIZATION OF AJOENE LOADED HYDROGEL FOR FUNGAL INFECTION."

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FUNDING INFORMATION:
Not Applicable

ABSTRACT:
The present study aimed to develop and evaluate a hydrogel-based topical formulation of Ajoene, a biologically active compound known for its potent antifungal properties. Given the poor aqueous solubility and instability of Ajoene, a hydrogel delivery system was formulated using Carbopol 940 and Propylene glycol to enhance its topical application potential. A series of nine formulations (AF1–AF9) were prepared and characterized for organoleptic properties, pH, viscosity, Spreadability, and drug content. The optimized formulation (AF1) exhibited a pH of 6.2, viscosity of 3248 cPs, and drug content of 98.56%. In vitro drug release studies demonstrated 79.67% cumulative release over 8 hours. FTIR and DSC analyses confirmed the compatibility and stability of Ajoene within the hydrogel matrix. A 32 factorial design revealed significant influence of polymer and solvent concentration on formulation performance. The stability of AF1 was maintained under accelerated storage conditions over three months. The results support the potential of Ajoene hydrogel as a stable and effective formulation for antifungal topical therapy.
Keywords
Ajoene, Hydrogel formulation, Antifungal therapy, Topical delivery, Carbopol 940, In vitro release

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Introduction

Hydrogel-based drug delivery systems have gained significant interest in pharmaceutical research due to their ability to provide localized, controlled release of drugs. These hydrophilic, three-dimensional polymer networks can absorb large amounts of water while retaining their structure, making them ideal for topical applications such as wound healing and antifungal treatments. Their biocompatibility, flexibility, and moisturizing effects also enhance skin permeation and patient comfort. [1]

Carbopol 940 and propylene glycol are widely used polymers in hydrogel formulations because of their excellent rheological and bioadhesive properties, which help control drug release through diffusion and swelling mechanisms. Incorporating additional functional agents like penetration enhancers or anti-inflammatory compounds can further improve therapeutic outcomes . [2]

Ajoene, a sulfur-containing compound derived from garlic, exhibits broad-spectrum antifungal activity by disrupting fungal membranes, inhibiting ergosterol synthesis, and causing oxidative stress. It is effective against fungi such as Candida albicans, Aspergillus species, and dermatophytes. However, Ajoene's poor water solubility and instability limit its conventional topical use. [3,4]

Importance of Localized Drug Delivery in Fungal Therapy

Localized drug delivery represents a crucial advancement in the treatment of superficial and mucocutaneous fungal infections. Unlike systemic administration, which involves the circulation of drugs throughout the body and often exposes healthy tissues to unnecessary pharmacological effects, localized therapy concentrates the therapeutic agent directly at the site of infection. [5] The increased efficiency, reduced systemic toxicity, and better patient tolerance associated with localized antifungal delivery make it an essential strategy in current dermatological and infectious disease pharmacotherapy. [6]

Superficial fungal infections are characterized by colonization and invasion of the outer keratinized layers of the epidermis, hair shafts, and nails. These regions are poorly vascularized, rendering systemic antifungal drugs less effective in achieving adequate therapeutic concentrations. Localized delivery overcomes the limitations of systemic therapy by offering higher drug concentrations at the infection site, improving bioavailability, and ensuring direct contact with fungal cells. This improves the fungicidal or fungistatic efficacy of the therapy while minimizing damage to surrounding healthy tissues . [7,8]

Advantages of Topical Over Systemic Antifungal Therapy

1. Site-specific delivery achieves high local concentrations for superficial infections like tinea and candidiasis, minimizing systemic exposure. [9]
2. Minimized systemic toxicity avoids hepatotoxicity, nephrotoxicity, and suits elderly/compromised patients. [10]
3. Lower risk of drug-drug interactions due to negligible systemic absorption. [11]
4. Improved patient compliance through ease of use, rapid relief, and minimal monitoring. [12]
5. Cost-effective and accessible as affordable OTC options (e.g., clotrimazole, terbinafine).
6. Safe for prolonged/prophylactic use in recurrent cases. [13]
7. Enables novel formulations (hydrogels, liposomes) for better penetration and controlled release against resistant strains. [14]

Challenges in Topical Delivery

Topical antifungal delivery encounters several hurdles that undermine efficacy. The stratum corneum acts as a formidable lipid-rich barrier, selectively permitting small, lipophilic molecules while blocking larger or hydrophilic ones, thus restricting drugs like Ajoene from reaching dermal infection sites. [15] Compounding this, formulations often suffer poor skin

retention due to daily activities like washing, sweating, or friction, which wash away the product and shorten therapeutic contact time—necessitating bioadhesive or sustained-release matrices. Skin permeability further varies by anatomical site (e.g., thicker palms/soles vs. thinner forearms), hydration, age, temperature, and pathologies like eczema, requiring site-specific optimizations. Patient compliance remains a major issue, with improper application, frequent dosing, or side effects prompting discontinuation and recurrence. To tackle these, our study engineered a Carbopol 940/propylene glycol hydrogel for Ajoene using factorial design, producing AF1 with skin-compatible pH (6.2), low viscosity for spreadability, 79.67% controlled release, potent antifungal zones against *C. albicans*/*A. niger*, and robust 3-month stability—collectively boosting penetration, residence time, uniformity, and user-friendliness for superior clinical outcomes. [16,17]

Materials

Ajoene, the active pharmaceutical ingredient, was obtained from Sigma-Aldrich. Carbopol 940 from HI Media Labs (Mumbai, India) served as the gelling agent. Propylene glycol and Tween 80 were used to improve solubility and skin penetration. Methyl paraben was added as a preservative, and triethanolamine was used to adjust pH. Ethanol was used in solubilization and extraction processes. Candida albicans and Aspergillus niger from the Microbial Type Culture Collection (MTCC) Chandigarh, India were used as test organisms for microbiological studies.

Methods

Preformulation Study

Ajoene was visually and olfactorily examined to check its colour, smell, and texture, aiding in quality assessment and early spoilage detection. For quantification, a stock solution in ethanol (100 µg/mL) was diluted to prepare a range of concentrations (2–12 µg/mL), and absorbance was recorded using UV-Vis spectroscopy to create a calibration curve. The same process was repeated using phosphate buffer (pH 7.4) to support drug content and release evaluations. To determine solubility, excess Ajoene was mixed with water, ethanol, buffer, and DMSO, then shaken at 37 ± 0.5°C for 48 hours. The mixtures were filtered, diluted, and analysed using spectrophotometry. Melting point was recorded using a digital apparatus and compared with known values for confirmation.

Differential Scanning Calorimetry (DSC) was carried out by heating a small amount of Ajoene (3–7 mg) from 50 to 300°C under nitrogen to observe thermal changes.

FTIR analysis, using KBr pellets, was performed in the range of 4000–400 cm^{-1} to identify functional groups and detect any shifts in peaks, indicating possible interactions with formulation components.

Experimental Design

The hydrogel formulation was optimized using a 32 full factorial design to study the effects of independent variables on key responses.

Software: Design Expert software (version 13.0, Stat-Ease Inc.) was used to generate and evaluate the experimental design.

Design: A 32 full factorial design was employed with two independent variables at three levels each:

A) Carbopol 940 (A): 0.5 g (-1), 1.0 g (0), and 1.5 g (+1).

B) Propylene glycol (B): 1% w/w (-1), 1.5% w/w (0), and 2% w/w (+1).

Responses: The dependent variables analyzed were Viscosity (R1) and In-vitro drug release at 8 hours (R2).

Tests: The data from the nine formulations were analyzed using Analysis of Variance (ANOVA) to determine the statistical significance of the independent variables and their interactions on the dependent variables.

Significance Level: The level of significance chosen was $p<0.05$. This threshold was used to identify statistically significant effects.

Table 1: Preparation of hydrogel batches using 32 factorial designs

(Ingredients and batch composition table as in your document.)

Preparation of Ajoene Hydrogel Formulations

General Procedures: Hydrogel formulations were prepared by first dissolving Carbopol 940 (at concentrations of 0.5 g, 1 g, or 1.5 g) in distilled water under gentle stirring until a uniform gel base was formed. Simultaneously, a solution of Ajoene (1 g) was prepared by dissolving it in varying concentrations of propylene glycol (1 g, 1.5 g, or 2 g). Tween 80 (2%) was added to this mixture to enhance the solubility of Ajoene. This solution was continuously stirred until it became clear and homogeneous.

The Ajoene–Tween 80–propylene glycol solution was then gradually incorporated into the hydrated Carbopol gel with continuous stirring to ensure uniform dispersion. Methyl paraben was added as a preservative. [18]

Key Parameters: Four drops of triethanolamine were then added to the mixture with gentle stirring to neutralize the pH and facilitate gel formation. The final volume of the formulation was adjusted to 100 g with distilled water, and the mixture was stirred until a homogeneous hydrogel was achieved. .

Characterization and Evaluation Methods

The hydrogel formulations were characterized for their physical, chemical, and antifungal properties to ensure their suitability for topical application. The following methods were used:

Physical and Chemical Characterization

1. **Visual Appearance and Homogeneity**: The formulations were visually inspected for their color, consistency, and transparency. A small sample was pressed between two glass slides to check for any undissolved particles or aggregates, confirming the homogeneity of the final product.
2. **pH Determination**: The pH of each formulation was measured using a calibrated digital pH meter. A 1 g sample was dispersed in 10 mL of distilled water and allowed to equilibrate before the measurement was taken, with all readings performed in triplicate.
3. **Spreadability**: The spreadability was determined using the parallel plate method. A 1 g sample was placed between two glass plates, and a weight of 500 g was applied. The increase in the diameter of the spread hydrogel was measured, and the spreadability was calculated using the formula $S = (M \times L)/T$.
4. **Viscosity**: Viscosity was measured using a Brookfield Digital Viscometer. A 50 g sample was placed in a beaker, and the spindle was rotated at 20 rpm. Readings were recorded in centipoise (cPs) after 2 minutes to ensure a stable measurement.
5. **Drug Content Uniformity**: To ensure a consistent drug concentration, the amount of Ajoene in each formulation was determined. A 1 g sample was dissolved in pH 7.4 phosphate buffer, filtered, and the absorbance was measured at 240 nm using a UV-visible spectrophotometer against a blank. The drug content was then calculated from a pre-established calibration curve.
6. **Stability Studies:** Accelerated stability studies were conducted according to ICH Q1A(R2) guidelines to predict the shelf-life of the formulations. Samples were stored in tightly closed containers at 40±2°C and 75±5% relative humidity for 3 months. The samples were evaluated at predetermined time intervals for any changes in their physical appearance, pH, drug content, and viscosity.
7. **In Vitro Drug Release Studies:** The release of Ajoene from hydrogel formulations was studied using Franz

diffusion cells with a 12,000 Da cutoff membrane pre-soaked in pH 7.4 phosphate buffer. The receptor chamber was kept at 37 ± 0.5°C with stirring at 100 rpm. One gram of hydrogel was placed in the donor compartment, and 1 mL samples were collected at intervals up to 8 hours, filtered, and analyzed at 240 nm using UV spectrophotometry.

8. **In Vitro Antifungal Activity:** The antifungal efficacy of hydrogels was assessed by agar well diffusion against Candida albicans and Aspergillus niger. Wells were filled with hydrogel on Sabouraud Dextrose Agar (SDA) plates inoculated with fungal suspensions and incubated for 72 hours. Inhibition zones were measured, using Clotrimazole as the positive control.

RESULTS

Organoleptic properties – API Characterization (Ajoene)

The observed properties of Ajoene matched its specifications. The Ajoene is a yellowish-white powder with a garlic-like odor. The observations for color, odor, and texture are all consistent with the expected specifications.

Solubility Studies

Solubility of Ajoene was checked in different solvents like water, ethanol, phosphate buffer pH 7.4, and DMSO. The Ajoene is slightly soluble in water, freely soluble in ethanol, sparingly soluble in buffer, and sparingly soluble in DMSO (dimethyl sulfoxide).

Melting Point

The melting point of Ajoene was observed to be 175.2ºC, which falls within the standard range of 171.1–176.5ºC.

Differential Scanning Calorimetry

The DSC thermogram of pure Ajoene exhibited a sharp endothermic peak at 175.2°C, corresponding to its melting point, confirming its crystalline nature and thermal stability.

Evaluation of UV Spectra Scan

A. Calibration Curve of Ajoene in Ethanol: Ajoene in ethanol was scanned from 200–400 nm and λmax was observed at 234 nm.

B. Calibration Curve of Ajoene in pH 7.4 buffer: Ajoene in pH 7.4 buffer was scanned from 200–400 nm and λmax was observed at 240 nm.

Evaluation of Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of pure Ajoene and its physical mixture with excipients were analyzed to assess potential chemical interactions.

FTIR Interpretation Showing Functional Groups in Pure Ajoene and Its Physical Mixture with Excipients

FTIR spectroscopy provided insight into the functional group integrity and compatibility of Ajoene with formulation excipients. The spectra of pure Ajoene exhibited characteristic peaks at 3598.00 cm^{-1} (O–H/N–H stretching), 2959.73 cm^{-1} (C–H stretching), and 2428.69 cm^{-1} (disulfide S=O stretch), confirming the presence of key functional groups. The physical mixture displayed similar peaks at 3500.89 cm^{-1}, 2961.73 cm^{-1}, and 2403.05 cm^{-1}, respectively, without any major shifts or disappearance. The fingerprint region also remained intact. These observations indicate no significant chemical interaction between Ajoene and excipients, affirming compatibility for hydrogel formulation. The preserved spectral signatures of Ajoene after formulation further affirm the drug's chemical stability within the hydrogel base.

Evaluation parameters of optimized batches hydrogel

S. No.	Parameter	Method	Key Results (e.g., AF1)
1	Physical Appearance & Homogeneity	Visual evaluation of color, clarity, consistency, phase separation, and particulate matter	All transparent/homogeneous; off-white, no separation.

S. No.	Parameter	Method	Key Results (e.g., AF1)
		for AF1–AF9.	
2	pH Determination	Calibrated digital pH meter (Table 2).	6.2–6.6
3	Viscosity Measurement	Brookfield Digital Viscometer (Table 2).	3043–3248
4	Spreadability	Parallel plate method (Table 2).	25.45–26.54 g·cm/s
5	Drug Content Uniformity	1 g in 100 mL pH 7.4 buffer; 240 nm (n=3; Table 2).	97.77–99.00%
6	In Vitro Drug Release	Franz cells (12,000 Da membrane), pH 7.4, 37°C, up to 8 h; 240 nm (Table 3).	79.67% release (highest).
7	In Vitro Antifungal Activity	Agar well diffusion vs. *C. albicans*/*A. niger* (72 h SDA); clotrimazole control (Figure 4).	Significant zones comparable to control.

Evaluation of Factorial Design Batches of hydrogel

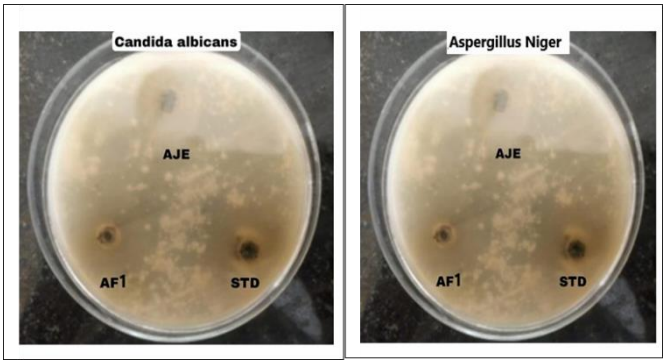
Physical Characteristics of Ajoene-loaded Hydrogel Formulations

Formulations, were off-white in color with a transparent appearance and demonstrated homogeneity with no phase separation. The consistency varied across the formulations, ranging from soft and flowable .

Antifungal activity

The antifungal activity against Candida albicans and Aspergillus niger showed that pure Ajoene exhibited inhibition zones of 28.4 mm and 26.8 mm, respectively, while the optimized batch AF1 demonstrated zones of 24.6 mm and 23.2 mm, and the standard Clotrimazole cream (1%) showed inhibition zones of 22.3 mm and 21.7 mm, respectively.

Figure 5: Zone of Inhibition of In-vitro antifungal activity of Ajoene (AJE), AF1 (Optimized batch) and Standard (Clotrimazole cream 1%).



Accelerated Stability

Over 3 months at 40 ± 2°C/75 ± 5% RH, hydrogel retained excellent physicochemical stability. It showed no phase separation, only slight yellowing after 2 months (off-white to mild yellowish tint, likely from oxidation). pH dropped mildly from 6.2 to 5.9, viscosity from 3248 cPs to 3043 cPs, and drug content from 98.56% to 96.89%—all within acceptable limits. Spreadability decreased marginally (26.54 to 25.45 g·cm/s), preserving usability.

Optimization Highlights

AF1 stood out as the ideal formulation with optimal physical traits, >98% drug content, and peak release (79.67%). Design-Expert analysis confirmed Carbopol 940 (0.5 g)

and propylene glycol (1 g) as key influencers on viscosity, spreadability, and performance.

Discussion

This study successfully developed and optimized a hydrogel-based topical delivery for Ajoene, a BCS Class II antifungal from garlic, overcoming its poor water solubility while ensuring stability and sustained release. met key criteria including pH 6.2–6.6, >97% drug content, and excipient compatibility (DSC/FTIR). Low-viscosity AF1 excelled with 79.67% release—due to inverse viscosity-diffusion dynamics—and was statistically validated ($R^2 > 0.99$), maintaining stability over 3 months under accelerated conditions.

Conclusion

the optimized hydrogel provides a stable, effective, and patient-friendly topical delivery system for Ajoene, markedly improving its solubility, sustained release (79.67%), and 3-month stability while minimizing viscosity-related barriers. This innovation holds strong promise for superior antifungal efficacy against dermal infections, enhancing patient compliance over conventional garlic extracts and paving the way for clinical advancement in topical therapeutics.

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