IJBPAS, May, 2026, 15(5): 2178-2194

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DESIGN, FORMULATION, AND OPTIMIZATION OF A COST-EFFECTIVE ANTIFUNGAL SHAMPOO FOR IMPROVED THERAPEUTIC EFFICACY

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Received 25th Feb. 2025; Revised 24th April 2025; Accepted 10th July 2025; Available online 1st May 2026

https://doi.org/10.31032/IJBPAS/2026/15.5.10139

ABSTRACT

Dandruff, a common scalp disorder affecting approximately 60% of the population, is primarily attributed to *Malassezia* spp. Conventional antifungal shampoos containing potent synthetic agents such as ketoconazole and miconazole are often associated with resistance development, adverse effects, and frequent relapse. This study aims to develop and optimize a cost-effective antifungal shampoo incorporating natural therapeutic agents and essential oils, formulated with sulfate-free surfactants to enhance scalp compatibility and minimize irritation. A systematic Design of Experiments (DoE) approach was employed to optimize key formulation parameters, including pH and viscosity. The antifungal activity against *Malassezia* spp. was evaluated using the agar cup plate method, demonstrating significant zones of inhibition due to the synergistic effect of the active components. Comprehensive physicochemical characterizations—such as pH, viscosity, foaming capacity and stability, wetting time, surface tension, particle size, zeta potential, and skin irritation—confirmed the formulation's stability, efficacy, and safety. The results suggest that this novel shampoo offers a promising, natural-based alternative to conventional formulations, with enhanced therapeutic efficacy, reduced risk of relapse, and improved user acceptability.

Keywords: Dandruff, *Malassezia*, Antifungal shampoo, Sulfate-free, Design of Experiments (DOE)

INTRODUCTION

Shampoo formulations have traditionally been designed as aqueous-based systems utilizing synthetic surfactants to facilitate scalp hygiene and hair aesthetics **[1]**. However, in response to increasing ecological concerns, sustainability imperatives, and the demand for minimalistic yet functional dermatocosmetic products, the pharmaceutical and cosmeceutical industries are shifting toward advanced formulations with therapeutic and environmental relevance **[2]**. Among these, solid-state and bio-enhanced antifungal shampoos are garnering considerable interest due to their enhanced shelf-life, reduced water content, and potential for improved drug loading and stability **[2, 3]**. Beyond their conventional role as cleansing agents, modern shampoos represent multifunctional delivery systems embedded with bioactive molecules that target specific scalp pathologies such as seborrheic dermatitis, tinea capitis, and dandruff—conditions frequently associated with *Malassezia* spp. overgrowth **[3]**. These shampoos serve as topical drug delivery platforms capable of facilitating localized antifungal activity while simultaneously preserving the physiological integrity of the hair shaft and scalp **[3]**.

Central to the therapeutic efficacy of such formulations are amphiphilic surfactants that function by reducing surface tension, promoting emulsification, and enabling the removal of sebum, microbial biofilms, and keratinized debris **[1, 4]**. However, surfactant choice, concentration, and compatibility with active pharmaceutical ingredients (APIs) significantly affect formulation stability, irritancy potential, and drug release dynamics **[4]**. Therefore, surfactant selection and rheological optimization must be aligned with pharmacokinetic and dermatopharmacologic considerations **[2, 4]**. This study explores the rational design, formulation, and statistical optimization of an antifungal shampoo incorporating organic acid-based active agents with proven fungistatic and keratolytic properties **[3]**. These agents not only disrupt fungal membrane integrity and inhibit ergosterol synthesis but also acidify the scalp microenvironment, thereby enhancing antifungal selectivity and bioavailability **[2, 3]**. Special attention is given to cost-effectiveness, ensuring accessibility without compromising formulation integrity or therapeutic performance. The novelty of this research lies in the integration of pharmaceutically acceptable excipients, pH-modulating agents, and clinically validated antifungal moieties into a stable, patient-compliant shampoo matrix. Employing

Quality-by-Design (QbD) principles and factorial design models, this investigation seeks to optimize critical formulation parameters such as viscosity, spreadability, foamability, drug content uniformity, and in vitro antifungal activity **[4]**. The overarching objective is to bridge the existing gap between dermatological therapy and cosmetic appeal by delivering a scientifically robust, economically viable antifungal shampoo that aligns with both Good Manufacturing Practices (GMP) and user-centric formulation standards **[2]**.

MATERIALS and METHODS

Formulation and Characterization of an Anti-Fungal Shampoo:

The formulation of the anti-fungal shampoo was optimized using a systematic approach based on Design of Experiment (DOE), employing varying concentrations of benzoic acid and salicylic acid across 13 different experimental formulations **[5]**. The process began by heating 120 mL of distilled water to 70°C, followed by the addition of the organic acids, which were thoroughly dissolved to ensure homogeneity. Therapeutic oils, such as 0.5 mL of tea tree oil and 1.5 mL of oregano oil, were incorporated for their antifungal properties, and the mixture was stirred to achieve uniform distribution **[6]**. Sodium cocoyl isethionate (30 g) was then added as a surfactant, followed by 14 mL of cocamidopropyl betaine to enhance foam stability **[5, 7]**. For stabilization, 1 g each of EDTA and magnesium stearate were dissolved separately and incorporated, while 6 g of hydroxypropyl methylcellulose (HPMC) was added for viscosity control **[7]**. Glycerin (14 mL) was included to enhance moisturizing, and 2 mL of lavender oil was added for fragrance. After cooling, the formulation was subjected to homogenization using a colloidal mill to ensure uniform texture and stability **[8]**. This process was repeated for all 13 formulations, each evaluated for key quality attributes like pH and viscosity, which were subsequently analyzed using DOE to optimize the formulation for an effective, cost-efficient antifungal shampoo with enhanced consumer acceptability **[5]**.

Table 1: Formulation of Shampoo

S. No.	INGREDIENTS	QUANTITY TAKEN (for 200ml)
1	Benzoic acid	As per formulation
2	Salicylic acid	As per formulation
3	Tea tree oil	0.5ml
4	Oregano oil	1.5ml
5	Sodium cocoyl isethionate	30gm
6	Cocamidopropyl betaine	14ml
7	Glycerin	14ml
8	Hydroxy propyl methyl	6gm

	cellulose	
9	**EDTA**	**1gm**
10	**Magnesium stearate**	**1gm**
11	**Lavender oil**	**2ml**
12	**Gel colorant**	**q.s**
13	**Distilled water**	**125ml**

Design of Experiment (DOE): A Scientific Framework for Optimization:

A 3^2 factorial Design of Experiment (DOE) was implemented as a systematic, data-driven optimization framework to investigate the influence of two organic acids—Benzoic Acid (BA) and Salicylic Acid (SA)—on the pH and viscosity of a shampoo formulation across 13 randomized experimental runs **[5]**. Quadratic polynomial regression models were developed for both responses, revealing that SA exerted a pronounced positive effect on pH, while BA showed a marginal negative influence, with a mild interactive synergy (BA×SA) contributing to response modulation **[5]**. The pH model ($R^2 = 0.7883$) exhibited significant curvature, indicating a nonlinear behavior predominantly driven by SA, and was statistically validated (Adequate Precision = 7.1874). Viscosity values ranged between 4352–8312 cP, and the corresponding model ($R^2 = 0.8897$) indicated strong positive contributions from both acids, with SA demonstrating a steeper linear and nonlinear (SA^2) impact **[6]**. The negative quadratic term for SA highlighted a parabolic viscosity profile, suggesting a critical concentration threshold **[7]**. Interaction effects in both models permitted fine-tuning of formulation attributes. Statistical adequacy (Adequate Precision > 7.1 for pH and > 8.2 for viscosity) confirmed model reliability for predictive applications. This DOE-based strategy offers a robust platform for optimization using Response Surface Methodology (RSM), aligning with Quality by Design (QbD) principles for the development of pH- and viscosity-controlled topical formulations **[5, 8]**.

Table 2: Formulation table using DoE

Ingredients For (100ml)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
OA-1	**0.085**	**1.5**	**2.91**	**2.5**	**1.5**	**1.5**	**1.5**	**0.5**	**2.5**	**1.5**	**1.5**	**1.5**	**0.5**
OA-2	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**1**	**2**	**3.41**	**0.58**	**1**

Table 3: DOE generated 3^2factorial designs

Std	Run	Organic acid-1	Organic acid-2	pH	Viscosity
5	**1**	**0.085**	**2**	**5.9**	**5874**
11	**2**	**1.5**	**2**	**6.1**	**6547**
6	**3**	**2.91**	**2**	**7.2**	**8211**
4	**4**	**2.5**	**3**	**7.9**	**8312**

9	5	1.5	2	6.1	6547
13	6	1.5	2	6.1	6547
12	7	1.5	2	6	6325
3	8	0.5	3	5.3	5124
2	9	2.5	1	6.5	6952
10	10	1.5	2	6.1	6547
8	11	1.5	3.41	5.8	5647
7	12	1.5	0.58	5.9	5874
1	13	0.5	1	4.5	4352

Response-1 (pH):3D Surface Plot for pH

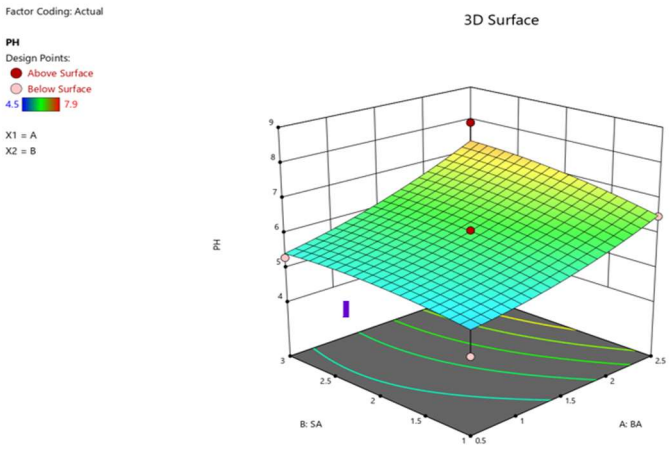

Figure 1: 3D Surface Plot for pH

Contour Plots for Desirability, pH, and Viscosity

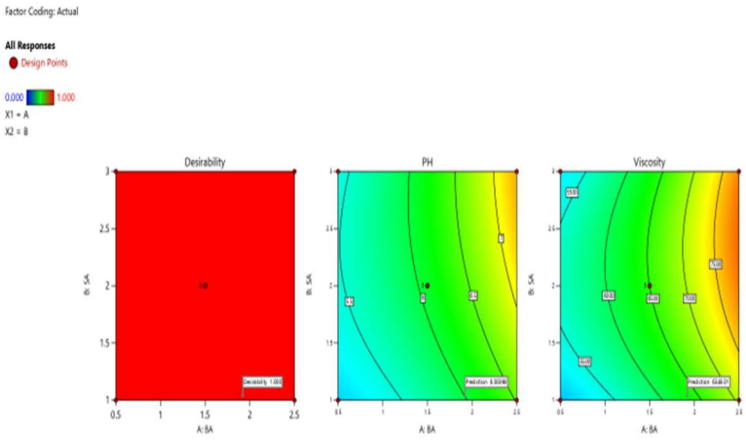

Figure 2: Contour Plots for Desirability, pH, and Viscosity

Contour Plots for viscosity:

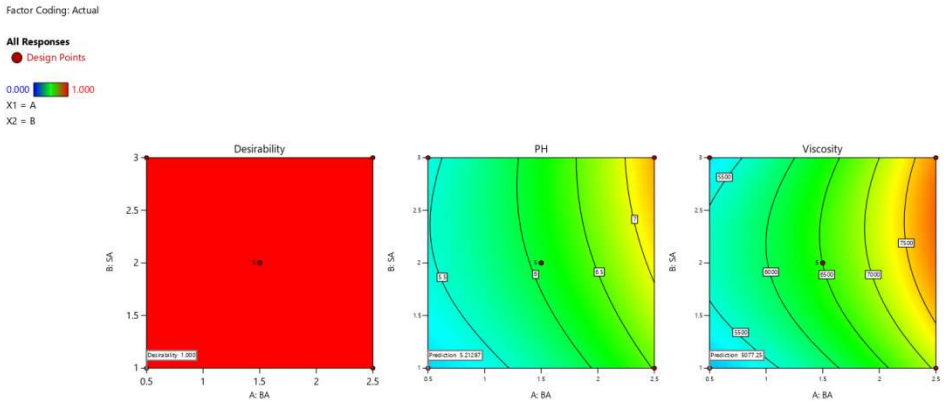

Figure 3: Contour Plots for viscosity

Evaluation of Antifungal Shampoo

Physico-Chemical Characteristic Features:

The formulated antifungal shampoo underwent a detailed physicochemical evaluation to assess key attributes related to its stability, functionality, and aesthetic appeal. Visual inspection was carried out to assess color, clarity, and the presence of any particulate matter or sedimentation. Organoleptic parameters such as odor, texture, and appearance were evaluated manually to determine product uniformity and user acceptability. The formulation's flow behavior, grittiness, and oily nature were examined through physical handling and observation, while the dispersion of active and excipient components was assessed to confirm homogeneity within the aqueous base. These assessments formed a foundation for confirming product consistency and suitability for further performance and stability testing.

Wetting Time of Shampoo Formulations:

Wetting time is a critical parameter indicating the surfactant efficiency and spreadability of shampoo formulations, reflecting their ability to displace air and penetrate hair or fabric. A shorter wetting time signifies superior cleansing efficacy. The wetting time was assessed using the cotton dipping method, where a standardized cotton swab was placed on the shampoo solution, and the time taken for saturation and sinking was recorded. The process was repeated in triplicate, and the average wetting time was calculated to ensure reproducibility and accuracy of results.

Surface tension is a critical physicochemical property influencing the wetting, penetration, and emulsification capabilities of shampoo formulations, directly affecting their cleansing efficiency. A surface tension range of 30–40 dynes/cm is optimal for effective

cleansing while maintaining mildness to the skin and scalp. The surface tension was evaluated using the drop count method, where the number of drops formed from a fixed volume was correlated with the liquid's surface tension. The surface tension of the shampoo was calculated using a formula considering the drop counts and densities of both the shampoo and reference (pure water). This provides a quantitative measure of the shampoo's spreading and cleansing efficiency. The skin irritation potential of the shampoo formulations was assessed through an animal model to ensure dermatological safety. Three optimal formulations, F9, F12, and F13, were selected based on their pH and viscosity, while high-concentration formulations were excluded due to sedimentation issues. Five rat groups were assigned different test conditions: formulations F13, F12, F9, a commercial shampoo, and a control group. A fixed amount of each formulation was applied to shaved dorsal skin, with irritation evaluated based on erythema, edema, and scar formation at 24, 48, and 72 hours. The irritation severity was scored using a standardized system from 0 (no reaction) to 4 (severe reaction).

The percentage of solid content in a shampoo formulation is a key determinant of its viscosity, application ease, and overall efficacy. Solid content encompasses non-volatile components, such as surfactants, thickeners, conditioning agents, and active ingredients. An optimal solid content ensures balanced viscosity, effective cleansing, and improved user experience, typically ranging between 20% and 30%. Excessive solid content may lead to overly thick formulations, complicating even application, while insufficient content could cause poor retention and reduced cleansing performance. Maintaining the ideal solid content ensures functional performance and desirable sensory characteristics. The cleansing efficacy of a shampoo is determined by its ability to remove dirt, oil, and sebum, closely linked to foam generation and lather stability. This study evaluated the shampoo’s cleansing performance through its foaming behavior in both soft and hard water, using foam formation as an indirect measure of surfactant activity. Surfactant performance was assessed in relation to water’s mineral content, which impacts the formulation’s cleansing potential. This approach highlights the shampoo's efficiency in emulsifying and removing impurities.

The pH stability of shampoo formulations was assessed over 24 weeks at 30°C using a digital pH meter. Various formulations (F1, F2, F3, F4, F8, F9, F11, F12, F13) containing different concentrations of Organic Acid-1

and Organic Acid-2 were compared to a commercial shampoo. pH measurements were recorded at 24 hours, 48 hours, and subsequent intervals (1, 4, 8, 12, and 24 weeks) to evaluate long-term stability. This study provided valuable insights into the pH stability of the formulated shampoos under controlled conditions. Viscosity of the formulated shampoos was evaluated using a Brookfield Viscometer at 30 ± 2°C and 50 RPM to determine flow resistance. The goal was to identify the optimal formulation with the desired semi-solid consistency. Viscosity readings were recorded in centipoise (cP) under controlled conditions to ensure consistency across formulations. The results were analyzed to select the formulation exhibiting ideal viscosity, ensuring optimal texture, spreadability, and ease of application.

The foam formation and stability of the shampoo formulations were assessed and compared to a commercial shampoo to evaluate cleansing efficiency and user experience. Foam formation was measured by mixing the shampoo with water and recording the peak foam volume and time to reach it. Foam stability was evaluated by measuring foam retention at 5 and 10 minutes post-formation, providing insights into foam persistence and durability over time. The particle size and zeta potential of the shampoo formulations were evaluated to assess their stability and dispersion characteristics, with comparisons made to a commercial shampoo. Shampoo samples were diluted with deionized water and sonicated for uniform dispersion. Particle size was measured using Dynamic Light Scattering (DLS) at 25°C, with results expressed in micrometers (μm). Zeta potential was determined using a Zetasizer to evaluate the surface charge of particles under electrophoretic conditions, with results recorded in millivolts (mV). The antifungal efficacy of the shampoo formulations was quantitatively assessed against *Malassezia furfur*, the etiological agent of dandruff, using a well-diffusion assay. Standardized fungal inocula were evenly spread on Sabouraud Dextrose Agar (SDA) plates, and wells were prepared for the test samples, including formulation F13, a commercial shampoo, and individual organic acids (OA-1 and OA-2) at 5% and 10% concentrations. The plates were incubated at 37°C for 48 hours, and antifungal activity was determined by measuring the zone of inhibition using a zone reader. The results were analyzed to compare the inhibitory effects of the formulations and components.

RESULTS

Physico-Chemical Characteristic Features:

The developed antifungal shampoo appeared as a slightly blue-colored, pleasant-smelling, free-flowing liquid with no grittiness or oily residue, indicating excellent homogeneity and aesthetic quality **[9]**. The absence of sedimentation and particulate matter confirmed uniform dispersion of actives and excipients, ensuring formulation stability and consistent dosing **[10]**. Optimized organoleptic and rheological properties contributed to superior user acceptability, cosmetic appeal, and enhanced therapeutic reliability **[9]**. The study employs a 3^2 factorial design methodology to optimize the development of an antifungal shampoo formulation using two natural organic acids (OA-1 and OA-2) and all-natural excipients **[11]**. Thirteen experimental runs were executed, varying the concentrations of OA-1 from 0.5% to 2.91% w/w and OA-2 from 0.58% to 3.41% w/w, with key response parameters being pH and viscosity.

pH was measured with a digital pH meter, and viscosity was determined using a Brookfield viscometer at 50 RPM and 30 ± 2°C **[10]**. The design framework enabled systematic evaluation of the effects of OA-1 and OA-2 on formulation stability and performance. Results from the 3D surface and contour plots reveal that OA-2 predominantly governs pH, with increasing concentrations leading to higher pH values. In contrast, viscosity is influenced more significantly by OA-1, with a notable increase in viscosity as OA-1 concentration rises **[11]**. The overlay plots confirm that the optimized formulation lies within the desired operational limits, offering flexibility in OA-1 and OA-2 concentration adjustments for the targeted formulation attributes. This comprehensive design space ensures robust, reproducible performance with minimal deviation, establishing the formulation's industrial applicability and scalability **[9, 10]**.

Table 3: DOE-generated 3^2 Factorial Designs

Std Run	Benzoic Acid (%)	Salicylic Acid-2 (%)	pH	Viscosity (cP)
1	0.085	2	5.9	5874
2	1.5	2	6.1	6547
3	2.91	2	7.2	8211
4	2.5	3	7.9	8312
5	1.5	2	6.1	6547
6	1.5	2	6.1	6547
7	1.5	2	6.0	6325
8	0.5	3	5.3	5124
9	2.5	1	6.5	6952
10	1.5	2	6.1	6547
11	1.5	3.41	5.8	5647
12	1.5	0.58	5.9	5874

The wetting time of the formulated shampoo demonstrated a wetting time of 44.7 seconds, while the commercial shampoo exhibited a slightly longer wetting time of 46.0 seconds. These results indicate comparable wettability characteristics between the two formulations, with a marginal difference observed in their wetting times **[12]**. Surface tension of the formulated shampoo exhibited a surface tension of 33.9 dynes/cm, falling within the ideal range of 30–40 dynes/cm for optimal cleansing and skin compatibility. In comparison, the commercial shampoo demonstrated a higher surface tension of 42.48 dynes/cm, indicating a slightly less mild formulation. This differential highlights the milder cleansing potential of the formulated shampoo, which is advantageous for sensitive skin and scalp **[13]**.

The skin irritation potential of the formulated shampoos (F9, F12, and F13) was assessed using an animal model to evaluate their dermatological safety, focusing on pH and viscosity as key parameters. High-concentration formulations were excluded due to sedimentation issues. The irritation response was compared against a commercial shampoo and a control group, with observations of erythema, edema, and scar formation over 72 hours. Results showed no significant irritation for the formulated shampoos, whereas the commercial shampoo exhibited mild erythema at 72 hours, underscoring the superior dermatological compatibility of the tested formulations **[14]**.

The percentage of solid content in shampoo formulations is a crucial determinant of their rheological properties, impacting spreadability, viscosity, and overall efficacy. Solid content encompasses non-volatile components such as surfactants, thickeners, conditioning agents, and active ingredients. An optimal solid content ensures a balanced formulation, facilitating effective cleansing and ease of application. The formulated shampoo demonstrated a solid content of 28%, significantly higher than the commercial shampoo's 15%, indicating superior viscosity and potentially enhanced performance **[15]**.

The cleansing property of the shampoo was assessed by evaluating its foaming performance in soft and hard water. The formulation exhibited superior foam formation in soft water, where the absence of divalent metal ions (Ca^{2+}, Mg^{2+}) minimizes interference with surfactant activity. In contrast, hard water, with its high ion content, reduced foam formation, indicating the detrimental effect of these ions on surfactant efficacy. Despite this, the shampoo demonstrated satisfactory foaming in both conditions, suggesting the need for further

optimization to maintain consistent performance in hard water regions **[16]**.

The pH stability of the formulated shampoos was rigorously assessed over a 24-week period at a constant temperature of 30°C using a calibrated digital pH meter. Various formulations, incorporating different concentrations of Organic Acid-1 and Organic Acid-2, were compared with a commercially available shampoo. The pH values of the formulations were monitored at 24-hour intervals, showing slight variations between different formulations. The results indicate that most formulations, particularly F9 and F13, maintained pH values within a consistent range, demonstrating good stability when compared to the commercially available shampoo **[17]**.

The viscosity of the formulated shampoos was meticulously evaluated to assess their resistance to flow using a Brookfield Viscometer, operating at a controlled temperature of 30 ± 2°C with varying rotational speeds (RPM). The objective was to identify the formulation exhibiting optimal viscosity, reflecting the desired semi-solid consistency for ease of application. The viscosity measurements at 50 RPM revealed significant variation among the formulations, with F4 and F3 showing the highest values, suggesting a thicker consistency, while F13 exhibited the lowest viscosity, indicating a relatively more fluid formulation **[18]**.

The foam formation and stability of the formulated shampoo were rigorously assessed and compared with a commercially available shampoo to evaluate their cleansing efficacy and user experience. The formulated shampoo exhibited superior foam formation, reaching a foam height of 50 mL within 30 seconds of initiation, compared to the commercial shampoo's foam height of 30 mL, which peaked after 50 seconds. This difference highlights the enhanced foaming performance of the formulated shampoo, contributing to a more effective and efficient cleansing process **[19]**. The particle size and zeta potential of the formulated shampoo were thoroughly evaluated and contrasted with a commercial counterpart to ascertain the dispersion stability and electrokinetic characteristics of the formulations. The formulated shampoo exhibited a particle size of 4.966 µm and a zeta potential of -1.2 mV, demonstrating superior stability compared to the commercial shampoo, which had a particle size of 6.462 µm and a significantly lower zeta potential of -50.1 mV. These results highlight the enhanced stability and dispersion properties of the formulated shampoo **[20]**.

The antifungal efficacy of the formulated shampoo was rigorously evaluated and

compared to that of a commercial shampoo and individual organic acid components (Organic Acid-1 and Organic Acid-2) by assessing their inhibitory activity against *Malassezia furfur*, a key pathogen in dandruff formation, using the zone of inhibition method and a zone reader. At a 5% concentration, the formulated shampoo (F13) demonstrated a remarkable inhibition zone of 20.2 mm, outperforming Organic Acid-1 (2.0 mm) and Organic Acid-2 (8.6 mm), as well as the commercial shampoo (6.8 mm). At 10%, the formulated shampoo maintained superior antifungal activity, achieving a 39.9 mm inhibition zone, significantly higher than Organic Acid-1 (15.7 mm), Organic Acid-2 (24.0 mm), and the commercial shampoo (13.1 mm). These findings underscore the potent antifungal potential of the formulated shampoo **[20]**.

DISCUSSION

The formulated antifungal shampoo exhibited superior physical characteristics, including aesthetic appeal and uniformity, which are essential for user acceptability and product stability. These attributes affirm the successful incorporation and dispersion of active ingredients and excipients **[21]**. The 3^2 factorial design adopted in this investigation provided a robust statistical framework to systematically decode the individual and interactive effects of two natural organic acids—OA-1 and OA-2—on the critical physicochemical properties of the antifungal shampoo formulation. Utilizing Response Surface Methodology (RSM), the study enabled precise modeling and optimization of formulation parameters, significantly enhancing the predictability and control of product attributes **[22]**. The response surface and contour plots indicated that OA-2 exerted a strong positive influence on pH, functioning potentially as a buffering agent or weakly basic component within the formulation matrix, thereby contributing to pH modulation and overall formulation stability **[23]**. This behavior underscores the importance of OA-2 in maintaining an optimal pH environment, which is crucial for scalp compatibility and preservative efficacy.

On the other hand, OA-1 showed a dominant and statistically significant effect on viscosity, suggesting a rheological synergy with the formulation's natural excipients, possibly mediated through hydrogen bonding, electrostatic interactions, or molecular entanglement phenomena with polymeric thickeners and surfactants **[21]**. These interactions likely enhance the formulation's semi-solid structure, contributing to better handling, spreadability, and user experience. The overlay plots generated from the design

space analysis confirmed that an optimized and reproducible formulation could be achieved within a broad yet controlled window of OA-1 and OA-2 concentrations. This highlights the robustness of the formulation, affirming its suitability for industrial-scale production with minimal variability, thereby ensuring product consistency and regulatory compliance **[22]**. In evaluating functional performance, the formulated shampoo displayed a wetting time of 44.7 seconds, marginally outperforming the commercial benchmark (46.0 seconds). This faster wetting behavior signifies a more effective reduction in surface tension, facilitating rapid spreadability over the hair and scalp surfaces. This was corroborated by surface tension measurements, where the formulated shampoo recorded 33.9 dynes/cm, which falls within the ideal dermatological range of 30–40 dynes/cm, indicative of mild and effective cleansing. In contrast, the commercial formulation exhibited a surface tension of 42.48 dynes/cm, suggesting relatively lower mildness and potentially higher irritancy **[24]**. These findings reflect the careful balance achieved between cleansing efficacy and skin compatibility in the newly developed formulation.

The dermatological safety of selected formulations (F9, F12, and F13) was further validated through in vivo skin irritation studies on animal models. Over a 72-hour observation period, the formulated shampoos showed no visible signs of erythema, edema, or scarring, in contrast to the mild erythema observed with the commercial product. This underscores the hypoallergenic potential and excellent tolerability of the new formulations, making them highly suitable for individuals with sensitive or compromised scalp conditions **[25]**. From a formulation science perspective, the solid content of the shampoo plays a crucial role in determining its rheology, stability, and overall efficacy. The developed formulation, with a solid content of 28%, demonstrated superior viscosity and performance, compared to the 15% solid content of the commercial shampoo, indicating a higher functional load of active ingredients and excipients. This enriched formulation base likely contributes to improved deposition, conditioning, and durability of cleansing action **[21]**.

The foam analysis revealed robust foaming in soft water conditions, achieving rapid and voluminous foam formation due to the uninhibited action of surfactants in the absence of divalent metal ions. Although foam volume was slightly reduced in hard water due to ionic interference (Ca^{2+} and Mg^{2+}), the overall performance remained acceptable

[24]. This suggests that while the formulation exhibits baseline robustness, future iterations may benefit from chelating agents or modified surfactant systems to further enhance performance in diverse water qualities. The long-term stability studies conducted over a 24-week period at 30°C established the pH stability of optimized formulations (F9 and F13). These formulations maintained pH within the acceptable dermatological range with minimal fluctuation, attesting to their chemical stability, shelf-life adequacy, and preservation of functional efficacy throughout storage **[25]**.

The viscosity evaluation using a Brookfield Viscometer confirmed distinct formulation-dependent flow behaviors. Formulations F3 and F4 exhibited higher viscosity, ideal for achieving a rich, semi-solid consistency preferred by consumers, while F13 displayed lower viscosity, indicating improved spreadability and rinseability, which may enhance sensory perception during use. This gradient in rheological behavior supports the customization of formulations based on consumer preferences and application context **[22]**. In terms of cleansing performance and user satisfaction, the formulated shampoo showed enhanced foam formation and faster foam generation (50 mL within 30 seconds) compared to the commercial product (30 mL at 50 seconds). These parameters directly correlate with surfactant efficiency, lather richness, and cleansing effectiveness, positioning the developed shampoo as a high-performance, consumer-friendly alternative **[23]**.

Physicochemical assessments extended to particle size and zeta potential analysis, key indicators of formulation homogeneity and colloidal stability. The formulated shampoo demonstrated a smaller average particle size (4.966 µm) and a moderate zeta potential (-1.2 mV), suggesting better dispersion and minimal aggregation, thereby enhancing the product's shelf-life and consistency. In comparison, the commercial shampoo exhibited a larger particle size (6.462 µm) and a high negative zeta potential (-50.1 mV), indicative of potential instability due to excessive repulsive forces **[24]**. Finally, the antifungal efficacy assessment via the zone of inhibition method established the formulated shampoo (F13) as significantly more potent against *Malassezia furfur*—a primary etiological agent of dandruff—compared to individual organic acids and a commercial reference product. At 5% concentration, F13 achieved a zone of 20.2 mm, while at 10%, the zone expanded to 39.9 mm, markedly surpassing the activity of OA-1 (15.7 mm), OA-2 (24.0 mm), and the commercial

shampoo (13.1 mm). This demonstrates a synergistic antifungal effect arising from the optimized blend of OA-1 and OA-2 within the formulation, and highlights its therapeutic relevance and superiority in managing scalp-related fungal infections **[25-27]**.

CONCLUSION

The application of a 3^2 factorial design enabled a statistically rigorous optimization of an antifungal shampoo formulation, revealing critical roles for OA-1 in viscosity modulation and OA-2 in pH stabilization. The formulation demonstrated superior physicochemical and functional performance, including enhanced wetting time, lower surface tension, and excellent dermatological safety. Rheological assessments and solid content analysis affirmed robust formulation stability and user-friendly texture. Colloidal characterization confirmed improved dispersion and shelf-life potential. Notably, the optimized formulation (F13) exhibited potent antifungal activity against *Malassezia furfur*, establishing its therapeutic viability and superiority over commercial counterparts.

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