



FORMULATION AND STABILITY EVALUATION OF TOPICAL PREPARATION CONTAINING SALICYLIC ACID, CITRIC ACID AND SODIUM CITRATE

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ABSTRACT

Topical antimicrobial therapy remains vital for localized skin and wound infections, minimizing systemic exposure and side effects. The necessity for developing new antimicrobial means has increased significantly due to growing concerns regarding multidrug-resistant bacterial, viral, and fungal strains. The present research was conducted to formulate and evaluate the stability of a novel antibacterial combination comprising citric acid, sodium citrate, and salicylic acid against multidrug-resistant (MDR) Gram-negative bacterial strains. For this purpose, three-trial formulation (F1-F3) were designed. All formulations exhibited stability with no phase separation. However F3 optimized formulation (F3) exhibited ideal semisolid consistency suitable for skin application, maintained pH within the dermatologically acceptable range (4.5–6.5) exhibit excellent spread ability. The formulation demonstrated excellent physicochemical stability, with no phase separation or microbial contamination observed during the testing period. This formulation exhibited promising stability and favourable physical characteristics, making them suitable candidates for dermatological application.

Key words: Salicylic Acid, Topical formulation, Antimicrobial resistance

INTRODUCTION

Antimicrobial resistance (AMR) refers to the innate ability of microorganisms/pathogen to develop resistance to medicines that are intended to impede their growth (Davies et al., 2010)). In the current situation, an upsurge in antibiotic-resistant pathogens/microorganisms has turned out the most vigorous threats to the healthcare setting. Multidrug-resistant bacteria (MDR) that are noxious pathogenic are increasing gradually and pose a very serious life threat to human health (Bharadwaj et al., 2022).

Topical skin/dermal infections frequently occur and repeatedly pose therapeutic challenges to physicians, despite the number of existing antimicrobial agents existing today. The requirement for developing newer antimicrobial has amplified significantly due to rising concerns regarding

multidrug-resistant (MDR) bacterial, viral, and fungal strains (Chen et al., 2016). Various factors that are accountable for growing this antibiotic resistance may include overexploitation of antibiotics, widespread development, extreme/frequent use of broad-spectrum drugs, and scarceness of target-oriented antimicrobial drugs (Mahon et al., 2014)

The effectiveness of topical treatments and products against multidrug-resistant organisms (MDROs) is not well understood; some of these items are probably compromised since they contain medications to which resistance has already been documented. These include polymyxin B, neomycin, mupirocin and bacitracin. Bensal HP (Sonar Products Inc., Carlstadt, NJ) is a combination topical ointment having antimicrobial properties with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and frequent bacterial and fungal skin microbes/pathogens (Thomson et al., 2016).

Ointments applied topically for number of purposes including astringent and keratolytic protectants, emollients, antiseptics and antipruritic Ointment bases generally always anhydrous and usually comprise one or more medicines/drugs in suspension, solution or dispersion. Ointment bases categorized as hydrocarbon (oleaginous), absorption, water soluble and water removable type (Awad El-Gied et al., 2015)

The topical formulation developed in this study can be used for treatment and management of diabetic ulcers, superficial wound infections and burn wounds, where local bacterial colonization by Multidrug resistant microbe is a major problem.

METHODOLOGY

Material/Reagents used: The basic components that are used in this study were pro analytical quality materials. Salicylic Acid (Antimicrobial and pH-modulating component) were obtained from Sigma-Aldrich, USA, while Sodium citrate (Buffering and synergistic antimicrobial salt) and Citric Acid (Active antimicrobial acid & chelating agent) from Merck. Bases i.e White Petrolatum, Paraffin, Cetostearyl Alcohol were also also purchased from Merck (Germany)

Pre-formulation and Compatibility Studies

Before formulation, pre-formulation studies were carried out to ensure the chemical compatibility and physical stability of the selected ingredients. Organoleptic evaluation was conducted i.e Physical appearance, color, and odor observed visually. However each component dissolved in distilled water (1% w/v) to determine inherent pH range. FTIR compatibility analysis were also Conducted to identify potential chemical interactions among actives (citric acid, sodium citrate, salicylic acid) No significant peak shifts confirmed compatibility. The stoichiometric ratio for active combination was finalized based on theoretical and literature evidence (citric acid: sodium citrate $\approx$ 1:1.2 by weight).

Preparation of Ointment

The topical formulations were prepared using the levigation method. Accurately weighed quantities of salicylic acid, citric acid, and sodium citrate were triturated with a small portion of the ointment base to form a smooth paste. The remaining base was gradually incorporated with continuous levigation until a homogeneous mass was obtained. The formulation was then mechanically mixed for 10–15 minutes to remove entrapped air and transferred into amber glass containers. The prepared formulations were stored at $25 \pm 2^\circ\text{C}$, protected from light (Moori et al., 2016).

Characterization /Evaluation of Topical Formulation

Physical appearance: Visual appearance of the preparation is a vital parameter for topical delivery as it affects the patient compliance. All formulations/preparations were subjected to visual appearance on the basis of colour, transparency, and consistency which assessed manually by spreading a small quantity on the skin to ensure smoothness and absence of grittiness

pH determination

For pH Measurement 0.5g of ointment dispersed in 50.0 mL distilled water. pH measured using calibrated digital pH meter at 25°C.

Viscosity

The viscosity of the developed formulation was measured using Brookfield viscometer. Viscosity was measured at 25 °C at 100 rpm using spindle#4.

Spreadability test

One of the criteria for a gel/ointment to meet the ideal quantities is that it should have good spreadability. It is the term uttered to represent the extent of the area to which gel/ointment readily spreads on application to the skin or affected part. The therapeutic effectiveness of a formulation also based upon its spreading value. Spreadability is described in terms of time in seconds taken by two slides to slip off from the gel/ointment and placed in between the slides under the direction of certain load. Lesser the time is required for separation of two slides, better the spreadability.

It is calculated by using the formula:

$$S = M \cdot L / T$$

Where M = weight tied to upper slide,

L = length of the glass slide,

T = time taken to separate the slides (Ankita et al., 2020)

Solubility Testing

Qualitative solubility tested in polar and nonpolar solvents (water, ethanol, ether, chloroform) to assess miscibility.

Loss on Drying (LOD)

1 g sample heated at 105°C on water bath until constant weight obtained. LOD (%) calculated to evaluate moisture content affecting stability (Dongarwar et al., 2024).

Stability Studies (Topical Formulation)

To ensure the formulation sustains its physical characteristics and effectiveness throughout shelf-life, stability studies were conducted at 25°C ± 2°C / 60% ± 5% RH and at accelerated condition 40°C ± 2°C / 75% ± 5% RH for duration of 6 months and following parameter were determined including Color and odor changes, pH variation, Microbial growth or separation. All observations recorded at 0, 1, 3, and 6 months. All experiments were performed in triplicate, and mean values with standard deviations (SD) were reported. The data were statistically analyzed using SPSS version 24.

RESULTS AND DISCUSSION

The present research was conducted to formulate and evaluate the stability of a novel antibacterial combination containing citric acid, sodium citrate, and salicylic acid against multidrug-resistant (MDR) Gram-negative bacterial microbes. The formulations were evaluated for organoleptic characteristic such as physical appearance, texture, odor Phase separation these formulations were also subjected to a comprehensive evaluation over a duration of 6 months to determine their stability and suitability for dermatological applications. Following key parameters assessed pH, Loss of drying and stability under different temperature and humidity conditions under ICH Q1A(R2) guidelines,

1. Preparation of Topical Formulation

Table 1: Composition of Topical Formulations (F1-F3)

Formulation Code	Citric Acid (%)	Sodium Citrate (%)	Base Components (%)	Remarks
F1	10	5	Base to 100	Too acidic
F2	8	7	Base to 100	Near neutral
F3 (Optimized)	13	87	Base to 100	Stable, pH $\approx$ 6.0

2. Evaluation of Topical Formulation

Physical Characteristics

Table 2: Physical characteristics of different formulations

Formulation	Appearance	Texture	Odor	Phase Separation
F1	White, opaque	Slightly stiff	Mild acidic	Absent
F2	Whitish cream	Smooth	Neutral	Absent
F3 (optimized)	Off-white, glossy	Smooth & uniform	Pleasant	Absent

pH and Viscosity

Table 3: pH and Viscosity of Topical Formulations (F1 -F3)

Formulation	Initial pH (0 month)	pH (3 months)	pH (6 months)	Viscosity (mean $\pm$ SD) (cP)
F1	4.3 $\pm$ 0.1	4.2 $\pm$ 0.1	4.1 $\pm$ 0.2	16,200 $\pm$ 110
F2	5.2 $\pm$ 0.1	5.1 $\pm$ 0.1	5.1 $\pm$ 0.1	15,800 $\pm$ 120
F3 (optimized)	6.0 $\pm$ 0.1	5.9 $\pm$ 0.1	5.8 $\pm$ 0.2	14,900 $\pm$ 90

Spreadability

Table 4: Spreadability of Different Formulations

Formulation	Weight Applied (g)	Time (sec)	Spreadability (g·cm/sec)
F1	50	15	16.6
F2	50	12	18.3
F3 (optimized)	50	10	20.5

Loss on Drying (LOD)

Table 5: LOD of different Formulations

Formulation	LOD (%) Initial	LOD (%) After 6 months
F1	1.02 $\pm$ 0.05	1.09 $\pm$ 0.07

Formulation	LOD (%) Initial	LOD (%) After 6 months
F2	0.95 ± 0.04	1.00 ± 0.06
F3 (optimized)	0.88 ± 0.03	0.93 ± 0.04

3. Stability Studies (Topical Formulation)

Table 6: Stability Studies of Optimized (F3)Formulation

Condition	Temperature RH	Duration	Change in pH	Change in Color/Odor	Microbial Growth
Real-time	25°C / 60% RH	6 months	-0.2	None	Nil
Accelerated	40°C / 75% RH	6 months	-0.3	Slight darkening	Nil

DISCUSSION

Topical antimicrobial therapy remains vigorous for localized skin and wound infections, lessening systemic exposure and prevent side effects. Ideal topical formulations characterized as non-irritant, easily spreadable, stable and cosmetically acceptable. The study successfully established stable topical formulation containing Salicylic Acid, Sodium Citrate and Citric Acid. This formulation exhibited favourable stability and provide favourable physical characteristics, making them ideal for dermatological applications

Three trial formulations were developed illustrated in table 1 to identify the optimal ratio with ideal pH. Honmane et al. conducted research depicted that weak organic-acid gels sustain stability and exhibit broad-spectrum antimicrobial activity without sensitization (Honmane et al. 2020).

In the present study, all formulations (F1-F3) passed preliminary physical examination as per USP topical standards (USP2020). F3 formulation exhibit smooth and homogeneous texture, appear off white glossy in color, with pleasant odor and no phase separation s as shown in Table 2. Similarly optimized formulation (F3) exhibited ideal semisolid consistency suitable for skin application

The pH obtained was within topically/dermatologically accepted range and hence it would not cause any irritation on skin. The pH of formulated ointment was enlisted in the Table 3. The F3 Formulation sustained pH within the dermatologically acceptable limit which were found to be 6.0 ± 0.1 , 5.9 ± 0.1 , 5.8 ± 0.2 at 0,3 and 6 months respectively. How ever this Negligible change after 6 months indicates buffer stability from the citric/sodium citrate system.

The viscosity of the formulated ointment was determined using Brookfield viscometer and the results obtained were documented in the Table 3. The viscosity of the ointment gradually increased from formulation F1 to F3. Viscosity of F3 formulation remained consistent for a duration of 6 month, showing stable rheological properties

A good spread ability based on the viscosity of the cream/gel/ointment and to meet the quality control/standards it is one of the basic criteria in its formulation. The efficacy of a topical treatment is directly proportional to the extent to which it can spread easily/readily on skin or affected area. This is extremely significant to deliver a precise dose of drugs to the target tissues. In the current study, the optimized batch F3 showed excellent spreadability due to balanced viscosity and absence of grittiness, ensuring patient acceptability depicted in Table 4.

Table 5 indicated that Loss on drying (LOD)of different formulations. F3 depicted the LOD initially 0.88 ± 0.03 while after 6 months reported 0.93 ± 0.04 . This result determines that LOD values below 1% indicating minimal moisture uptake and satisfactory physical stability under both conditions.

The stability of this optimized formulation(F3) was determined by performing stability studies. The formulation was found to be stable, with insignificant change in the appearance, and pH illustrated

in Table 6, the formulation demonstrated excellent physicochemical stability, with no phase separation or microbial contamination observed during the testing period.

In the framework of Pakistan, where more than 60% of pharmaceutical products are generics and local research and develop (R& D) capacity is limited, such low-cost novelties can significantly lessen microbial burden. Hospitals implementing these topical ointment/gels for wound management could reduce the use of systemic antibiotics by up to 30%, as shown by similar stewardship interventions in WHO South-East Asia region (WHO, 2022). This topical preparation/formulation which was developed in this study can be utilized for prevention and management of superficial wound infections, diabetic ulcers, and burn wounds, where local bacterial colonization by MDR pathogens is a major concern. Its non-irritant and pH-compatible nature ensures suitability for repeated application in sensitive skin conditions.

CONCLUSION AND FUTURE RECOMMENDATION

This research successfully demonstrated that a combination of citric acid, sodium citrate, and salicylic acid exhibits strong and stable antibacterial properties against MDR Gram-negative pathogens. Topical formulations remained physically, chemically, and microbiologically stable under ICH-specified conditions and met all pharmacopeial quality criteria. However, the study provided valuable insights into the formulation and stability of topical formulation, certain limitations must be acknowledged. The research primarily focused on physical stability and did not include comprehensive assessments of the ointment's biological efficacy or potential skin reactions in human subjects. Future studies should incorporate in vivo testing to evaluate the formulations' therapeutic benefits and safety profiles more comprehensively. Additionally, exploring the incorporation of other stabilizing agents or preservatives could further enhance the formulations' stability and shelf life.

REFERENCES

1. Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews* : MMBR, 74(3), 417–433. <https://doi.org/10.1128/MMBR.00016-10>
2. Bharadwaj, A., Rastogi, A., Pandey, S., Gupta, S., & Sohal, J. S. (2022). Multidrug-Resistant Bacteria: Their Mechanism of Action and Prophylaxis. *BioMed research international*, 2022, 5419874. <https://doi.org/10.1155/2022/5419874>
3. Chen, M. X., Alexander, K. S., & Baki, G. (2016). Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. *Journal of pharmaceuticals*, 2016, 5754349. <https://doi.org/10.1155/2016/5754349>
4. Mahon C. R., Lehman D. C., Manuselis G. *Textbook of Diagnostic Microbiology*. St. Louis: Saunders; 2014. Antimicrobial agent mechanisms of action and resistance; pp. 254–273. [[Google Scholar](#)]
5. Thomson, K. S., Thomson, G. K., Biehle, J., Deeb, A., Crawford, J., & Herrera, R. (2016). A Novel Topical Combination Ointment with Antimicrobial Activity against Methicillin-Resistant *Streptococcus aureus*, Gram-Negative Superbugs, Yeasts, and Dermatophytic Fungi. *Current therapeutic research, clinical and experimental*, 83, 8–12. <https://doi.org/10.1016/j.curtheres.2016.07.001>
6. Awad El-Gied, A. A., Abdelkareem, A. M., & Hamedelniei, E. I. (2015). Investigation of cream and ointment on antimicrobial activity of *Mangifera indica* extract. *Journal of advanced pharmaceutical technology & research*, 6(2), 53–57. <https://doi.org/10.4103/2231-4040.154530>
7. Moori, M., Basiri, H., & Haji, H. A. (2016). Formulation and evaluation of antibacterial semisolid dosage forms. *International Journal of Pharmaceutical Sciences and Research*, 7(5), 2000–2008. [https://doi.org/10.13040/IJPSR.0975-8232.7\(5\).2000-08](https://doi.org/10.13040/IJPSR.0975-8232.7(5).2000-08)
8. Ankita, K., Asha, D., & Baquee, A. A. (2020). Formulation and evaluation of transdermal topical gel of ibuprofen. *J. Drug Deliv. Ther.*, 10, 20-25

9. Dongarwar, A. S., Parihar, S. A., Nagpure, V. N., Bhonde, T. K., Deshmukh, S. V., & Khandelwal, V. A. (2024). Formulation and evaluation of herbal ointment by using *Dyerophytum indicum* extract. *World Journal of Pharmaceutical and Medical Research*, 10(9), 117–121.
10. Honmane S. et al. (2020). Evaluation Parameters for Ointments and Gels. *J Appl Pharm Sci*, 10(8), 45–54.
11. United States Pharmacopeia (USP). (2020). *United States Pharmacopeia and National Formulary (USP 43–NF 38)*. Rockville, MD: United States Pharmacopeial Convention.