

# Antimicrobial activity of Sulphalime water in the management of Mastitis: Formulation and Evaluation of Topical Cream

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## Abstract

**Background:** Mastitis is a common infectious disease of dairy animals that causes inflammation of the mammary gland and results in reduced milk production and economic losses. Sulphur possesses well-documented antibacterial properties, while calcium hydroxide has been used for its antimicrobial and alkaline characteristics. Combining these materials in a topical cream may provide a simple approach for the local management of mastitis.

**Objective:** The study was carried out to formulate a topical cream containing Sulphalime water and to evaluate its physicochemical properties and antibacterial activity against selected mastitis-causing microorganisms. Methods: Sulphalime water was prepared by dispersing sulphur in freshly prepared lime water and incorporated into an oil-in-water cream base. The formulations were designed using a factorial design and evaluated for appearance, pH, viscosity, spreadability, washability, and antimicrobial activity. Antibacterial activity was determined against *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* using different concentrations of Sulphalime water.

**Results:** Among the prepared formulations, F3 showed the most acceptable characteristics, with a smooth and uniform texture, pH of 6.54, viscosity of 31,280 cps, and spreadability of 14.29 g·cm/s. The formulation was easily washable and suitable for topical application. Sulphalime water inhibited the growth of all three test organisms, and the zone of inhibition increased with concentration. The highest antibacterial activity was observed at 75% concentration, producing inhibition zones of 22 mm against *Streptococcus agalactiae*, 20 mm against *Staphylococcus aureus*, and 19 mm against *Escherichia coli*.

**Conclusion:** The developed Sulphalime cream showed acceptable pharmaceutical properties and concentration-dependent antibacterial activity against the tested mastitis pathogens. The findings indicate that the formulation may be useful as a topical preparation for mastitis management. Further in vivo and clinical studies are required to confirm its therapeutic value.

**Keywords:** Mastitis, mammary gland, antibacterial property, Sulphur, calcium hydroxide, sulphalime.

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## 1. INTRODUCTION

Mastitis is an inflammation of the mammary gland that commonly affects dairy animals during lactation. It is considered one of the most important diseases in dairy farming because it reduces milk yield, alters milk composition, and affects animal health. The condition is mainly caused by bacterial infection, although physical injury or poor management practices may also contribute to its development. The organisms most frequently associated with mastitis include *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*. These pathogens enter the udder through the teat canal and multiply within the mammary tissue, leading to inflammation and tissue damage. Mastitis occurs in both clinical and subclinical forms. Clinical mastitis is characterized by visible changes in the udder, such as swelling, pain, redness, and abnormal

milk, whereas subclinical mastitis shows no obvious external signs but results in decreased milk production and elevated somatic cell counts. Early detection is important to prevent the spread of infection and reduce production losses.

In the chemical industry, calcium hydroxide (Ca(OH)<sub>2</sub>) is a soft, white powder. It is produced when calcium oxide and water combine to form a compound that has two hydroxide ions (OH<sup>-</sup>) for every calcium ion (Ca<sup>2+</sup>). Usually, calcium oxide (CaO) and water (H<sub>2</sub>O) react to produce calcium hydroxide. A substantial amount of heat is released during this reaction. It is also produced when dry Portland cement is mixed with water to make concrete. Calcium hydroxide is an essential ingredient in mortar, plaster, and cement used in construction. Additionally, it reacts with acids as a neutralising agent. Although calcium hydroxide is only marginally soluble

in water, it is soluble in glycerol and acids. It has a density of 2.24 grams per cubic centimetre, a melting point of 580°C (1,076°F), and a molar mass of 74.09 grams per mole [1].

Because sulfur is used so extensively in industrial processes, its consumption is frequently seen as a trustworthy indicator of both the level of the national economy and industrial activity. The production of fertilizers (phosphates and ammonium sulfate) uses sulfuric acid, which is produced from about six-sevenths of all sulfur. The manufacture of pigments, detergents, fibers, petroleum products, sheet metal, explosives, and storage batteries are just a few of the numerous significant uses. Paper, fungicides, insecticides, dyestuffs, and many other products are made from sulfur that is not transformed into sulfuric acid [2]. For centuries, dermatologists have utilized sulfur, a biologically active element. In addition to its anti-tumor properties, it possesses antibacterial, antifungal, antiviral, and keratolytic properties in the field of biomedicine [3], [4]. Acne, dandruff, scabies, and other dermatological conditions are treated with sulfur [5].

Cream was selected as the most suitable dosage form for sulphalime because it provides a non-greasy, easily spreadable and washable vehicle appropriate for topical application on inflamed mammary tissue in mastitis. The aqueous continuous phase also ensures uniform dispersion of sulphalime water and allows convenient removal during routine udder washing, supporting better hygiene and user acceptability.

## 2. MATERIALS AND METHODS

### a. Collection of chemicals

The primary active ingredient i.e. Sulphur is procured from LOBA Chemicals, PVT. LTD. Calcium hydroxide was bought from LOBA Chemicals, PVT.

LTD. Other ingredients used for cream preparation are of analytical grade.

### b. Preparation of Lime water

The preparation of Sulphalime cream starts with the preparation of lime water. To prepare the lime water, 1 gm of calcium hydroxide was dissolved in 100 ml distilled water. Stir this solution for 20-30 min. using magnetic stirrer. Then rest it for 3 hrs so that calcium hydroxide get proper dispersed in water. Now filter supernatant liquid. This prepared liquid is lime water, and further used for preparation of Sulphalime water.

### c. Preparation of Sulphalime water

The preparation of Sulphalime water starts with dissolving 2 gm of fine Sulphur powder in the freshly prepared 100 ml lime water. Stir the solution using magnetic stirrer for 45 min with constant heating at 60°C to uniform dispersion. Filter the whole solution using muslin cloth. Store this slight yellow colored suspension in amber bottle.

### d. Preparation of cream using DoE

The O/W cream base was prepared utilizing the Design Expert software. The oil phase prepared by adding emulsifying wax, cetyl alcohol (X1) and liquid paraffin as per Table no.01. The aqueous phase made by stirring freshly prepared sulphalime water (X3) with methyl paraben and propyl paraben. Both phases are heated at 70°C for a while so that the compounds gets melted in each other. Now Stop the heating and at room temperature, initiate the fusion of process by mixing the oil phase in aqueous phase with continuous stirring using magnetic stirrer, resulting in the formation of fresh sulphalime cream. The pH was adjusted with triethanolamine. After achieving room temperature store the cream in air tight wide mouth container.

Table no. 01: Experimental code of DoE

| Independent variables    | Coded levels |   |    |
|--------------------------|--------------|---|----|
| X1<br>(Cetyl alcohol)    | -1           | 0 | +1 |
| X2<br>(Liquid paraffin)  | -1           | 0 | +1 |
| X3<br>(Sulphalime water) | -1           | 0 | +1 |

**Table no. 02: Experimental runs by 3<sup>3</sup> factorial design**

| <b>Formulation</b> | <b>X1<br/>Cetyl alcohol (gm)</b> | <b>X2<br/>Liquid paraffin (ml)</b> | <b>X3<br/>Sulphalime water (ml)</b> |
|--------------------|----------------------------------|------------------------------------|-------------------------------------|
| F1                 | 2                                | 2                                  | 10                                  |
| F2                 | 10                               | 2                                  | 10                                  |
| F3                 | 2                                | 15                                 | 10                                  |
| F4                 | 10                               | 15                                 | 10                                  |
| F5                 | 2                                | 2                                  | 15                                  |
| F6                 | 10                               | 2                                  | 15                                  |
| F7                 | 2                                | 15                                 | 15                                  |
| F8                 | 10                               | 15                                 | 15                                  |
| F9                 | 2                                | 8.5                                | 12.5                                |
| F10                | 10                               | 8.5                                | 12.5                                |
| F11                | 6                                | 2                                  | 12.5                                |
| F12                | 6                                | 15                                 | 12.5                                |
| F13                | 6                                | 8.5                                | 10                                  |
| F14                | 6                                | 8.5                                | 15                                  |
| F15                | 6                                | 8.5                                | 12.5                                |
| F16                | 6                                | 8.5                                | 12.5                                |
| F17                | 6                                | 8.5                                | 12.5                                |

**e. Cream evaluation****i. Organoleptic properties**

The organoleptic evaluation parameters like color, odor, consistency, homogeneity, etc. were evaluated for cream formulations.

**ii. pH**

The digital pH meter was utilized to determine the pH. First, 4.0 and 9.2 pH capsules were used to calibrate the digital pH meter. One gram of formulated cream was dissolved in one hundred milliliters of distilled water to create the cream sample, and the pH was noted. To reduce error, the test was performed for three times [6].

**iii. Viscosity**

The Brookfield viscometer was applied to record the viscosity of the formulated cream at room temperature with spindle No. 63 rotated at 2.5 RPM [7].

**iv. Spreadability**

By recording the timing how long it takes for two slides to separate from the cream that was placed between them under a known load, the spreadability was calculated in seconds. A faster separation of the two slides means good spreadability. For the test, two sets of glass slides of standard size were used [8]. After that, the prepared cream formulation (1 gm) was placed on a slide. Another slide was then placed over the formulation. By applying a particular weight to the upper slide, the cream between the two slides was then uniformly compressed to form a thin layer. The weight was then removed after a few minutes, and any excess formulation that had adhered to the slides was wiped off. The slip-off time of upper slide is noted.

$$\text{Spreadability} = m \times l/t$$

Where,

m = Standard weight which is tied to or placed over the upper slide (20g) l = length of a glass slide (5 cm)

t = time taken in seconds.

**v. Washability**

Apply a consistent, pinch amount of the cream (around 1 gm) to a designed test area, typically the dorsal surface of the hand. Spread the cream gently with your fingers to create a thin, uniform layer which mimics how a product is generally used in daily life. Put treated hand under the stream of tap water. Check how quickly and completely the cream separates from the skin by gently rubbing the skin while you rinse [8].

**vi. Antimicrobial Analysis**

Antimicrobial activity was evaluated using the agar well diffusion method. Sterile molten agar,

previously inoculated with a standardized microbial suspension, was poured into Petri dishes and allowed to solidify. Wells of 6 mm diameter were then bored in the agar, and 100 µL of test samples at concentrations of 25%, 50% and 100% sulphalime water were added to each well. A standard streptomycin solution (1 mg/mL) served as the positive control, while DMSO was used as the negative control. The plates of *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus agalactiae* were incubated under appropriate conditions to allow diffusion of the samples through the agar, and the antimicrobial effect was determined by measuring the diameter of the clear inhibition zones around the wells in millimeters.

**vii. Stability Study**

The formulated F3<sup>rd</sup> cream was subjected for the stability study for 35 days. The cream was kept at 40°C ± 2°C / 75% RH ± 5% RH. The parameters like color, pH, spreadability, viscosity, phase separation and texture was evaluated.

**viii. Statistical analysis**

Design Expert®, version 12.0.3.0 was used to calculate the statistical significance ANOVA with extreme probability level of significance (p<0.05)

**3. RESULT****i. Organoleptic properties**

The formulated sulphalime cream was slightly yellow colored, characteristic odor with uniform texture.

**ii. pH**

The pH of formulated sulphalime cream was found to be 6.54. The pH was recorded at intraday and interday for 3 days in triplicates.

**iii. Viscosity**

The viscosity of all formulated cream batches (F1-F17) was determined using a suitable viscometer. The obtained results showed that the viscosity of the prepared formulations ranged from 21,760 cps to 31,280 cps. The following data shows that the F3 have good viscosity among all the creams.

**Table no. 03 Viscosity data of formulated creams**

| Sr.no. | Formulation | Viscosity (cps) |
|--------|-------------|-----------------|
| 1.     | F1          | 24860           |
| 2.     | F2          | 27540           |
| 3.     | F3          | 31280           |
| 4.     | F4          | 22940           |
| 5.     | F5          | 26730           |
| 6.     | F6          | 29180           |
| 7.     | F7          | 25460           |
| 8.     | F8          | 23890           |

|     |     |       |
|-----|-----|-------|
| 9.  | F9  | 28420 |
| 10. | F10 | 21760 |
| 11. | F11 | 27960 |
| 12. | F12 | 24380 |
| 13. | F13 | 26240 |
| 14. | F14 | 30140 |
| 15. | F15 | 23510 |
| 16. | F16 | 28760 |
| 17. | F17 | 25980 |

**iv. Spreadability**

As per the evaluation term faster the slide separation time means good spreadability. Among

the all batches F3 formulation gives good spreadability with seven seconds of slip time.

**Table no.04 Spreadability time of cream formulation**

| Sr.no. | Formulation | Time (sec) | Spreadability (g.cm/sec) |
|--------|-------------|------------|--------------------------|
| 1.     | F1          | 12         | 8.34                     |
| 2.     | F2          | 18         | 5.56                     |
| 3.     | F3          | 7          | 14.29                    |
| 4.     | F4          | 20         | 5                        |
| 5.     | F5          | 11         | 9.09                     |
| 6.     | F6          | 16         | 6.25                     |
| 7.     | F7          | 10         | 10                       |
| 8.     | F8          | 16         | 6.25                     |
| 9.     | F9          | 14         | 7.14                     |
| 10.    | F10         | 15         | 6.67                     |
| 11.    | F11         | 17         | 5.88                     |
| 12.    | F12         | 15         | 6.67                     |
| 13.    | F13         | 14         | 7.14                     |
| 14.    | F14         | 17         | 5.88                     |
| 15.    | F15         | 15         | 6.67                     |
| 16.    | F16         | 15         | 6.67                     |
| 17.    | F17         | 14         | 7.14                     |

**v. Washability**

The hand applied with formulated cream gets easily

Antimicrobial activity of Sulphalime water in the management of Mastitis: Formulation and Evaluation of Topical Cream cleaned under running tap water. This test was done for three time.

**vi. Antimicrobial Analysis**

The antimicrobial study demonstrated that Sulphalime water exhibited measurable antibacterial activity against all selected mastitis-

causing pathogens, namely *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*. The zone of inhibition increased progressively from 25% to 75% concentration, indicating that the antibacterial activity of Sulphalime water is concentration dependent.

**Table no. 05 Antimicrobial activity**

| Test Organism  | Zone of Inhibition (mm) |                      |                      |
|----------------|-------------------------|----------------------|----------------------|
|                | Sulphalime water 25%    | Sulphalime water 50% | Sulphalime water 75% |
| S. Aureus      | 14                      | 17                   | 20                   |
| St. agalactiae | 15                      | 19                   | 22                   |
| E. coli        | 13                      | 16                   | 19                   |

**vii. Stability study**

The optimized formulation was subjected to stability studies according to ICH guidelines. Samples were packed in suitable containers and stored at 40°C ± 2°C / 75% RH ± 5% RH for a period of 35 days. At

predetermined intervals (0, 14, 21, 28 and 35 days), the formulations were evaluated for physical appearance, pH, viscosity, spreadability. The results were compared to assess the stability of the formulation.

**Table no.06 Stability study of F3 formulation**

| Parameters          | Time (in days) |       |       |       |       |
|---------------------|----------------|-------|-------|-------|-------|
|                     | Initial        | 14    | 21    | 28    | 35    |
| pH                  | 6.54           | 6.53  | 6.52  | 6.51  | 6.51  |
| Spreadability (Sec) | 7              | 7.3   | 7.48  | 8.10  | 8.30  |
| Viscosity           | 31280          | 31270 | 31245 | 31233 | 31210 |

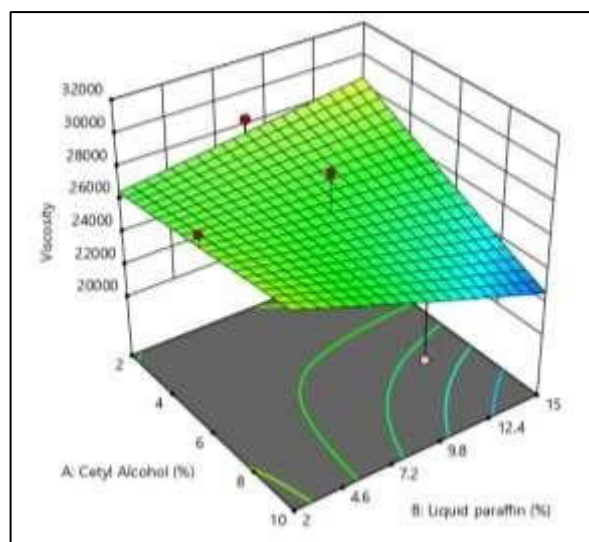
**viii. Statistical analysis**

The response surface plots show how cetyl alcohol (A) and liquid paraffin (B) influence viscosity and spreadability. Viscosity decreases with increasing liquid paraffin and increases moderately with higher cetyl alcohol, with nearly parallel contour lines

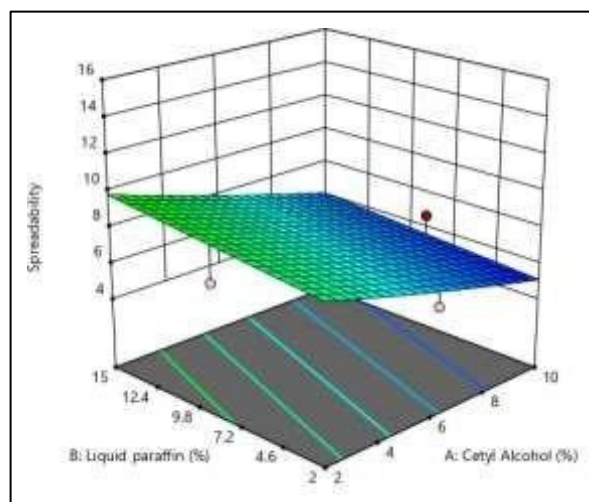
indicating weak interaction and largely linear effects. The experimental points lie close to the predicted surface, indicating good agreement between observed and model values. For spreadability, an inverse trend to viscosity is observed: higher cetyl alcohol reduces spreadability due to increased viscosity, while liquid paraffin

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 slightly improves spreadability by decreasing the variables.  
 consistency, again with minimal interaction between the variables.

**Fig. no. 01 Response Surface of Viscosity**



**Fig. no.02 Response Surface of Spreadability**



#### 4. DISCUSSION

The formulation of cream was starts with preparation of lime water. Leave the solution for some hours to properly disperse the calcium hydroxide in distilled water. Carefully disperse the sulphur in lime water so that the sulphur powder get evenly dispersed. The F3<sup>rd</sup> formulation have uniform texture among all other creams. It shows pH of 6.54 with slightly yellow color. Among all the batches, F3 exhibited the highest viscosity (31,280 cps) and showed good consistency, uniformity and acceptable rheological behavior. The viscosity values of the remaining formulations were found to be within an acceptable range for topical cream application. The higher viscosity of F3 may be attributed to the optimum concentration of emulsifying agents and other excipients, resulting in better internal structure and consistency of the cream. Thus, based on viscosity study, F3 was found to be the most suitable batch among all the prepared formulations. Among the tested organisms, *Streptococcus agalactiae* showed the

highest sensitivity to Sulphalime water, producing zones of inhibition of 15 mm, 19 mm, and 22 mm at 25%, 50%, and 75% concentrations, respectively. *Staphylococcus aureus* also exhibited significant inhibition with zones of 14 mm, 17 mm, and 20 mm, while *Escherichia coli* showed comparatively lower but still appreciable inhibition with zones of 13 mm, 16 mm, and 19 mm. The highest antimicrobial activity was observed at 75% concentration for all organisms, whereas the lowest activity was observed at 25% concentration. This confirms that increasing the concentration of Sulphalime water enhances its antibacterial action against mastitis pathogens.

#### 5. CONCLUSION

The sulphalime cream was prepared using sulphur and calcium hydroxide to achieve the antimicrobial activity against *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*. The prepared third cream formulation gives good viscosity as well as spreadability. The pH of third formulation was 6.54

Antimicrobial activity of Sulphalime water in the management of Mastitis: Formulation and Evaluation of Topical Cream which will be compatible with the skin surface, making it suitable to absorb as soon as possible.

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