PREPARATION AND CHARACTERIZATION OF TOPICAL HYDROGEL CONTAINING COMBINATION DRUGS (COMPARATIVE STUDY) FOR TOPICAL DELIVERY

Vijay kumar[1]*, Satkar Prasad[2]*, Prashant Singh[3]*

[1]Student- RKDF School of Pharmaceutical Science, Bhabha University, Bhopal, Madhya Pradesh

[2]Principal - RKDF School of Pharmaceutical Science, Bhabha University, Bhopal, Madhya Pradesh

[3]Assistant Professor - RKDF School of Pharmaceutical Science, Bhabha University, Bhopal, Madhya Pradesh

ABSTRACT

This study focused on developing a topical gel containing a combination of salicylic acid and aloin to improve treatment for skin problems. Both drugs were tested for their physical and chemical properties and were found to be compatible. Salicylic acid appeared as a white, odorless crystalline powder, while aloin was yellow with a characteristic odor. Both showed good solubility in polar solvents, making them suitable for gel formulation. UV–visible analysis confirmed their compatibility with absorption peaks at 235.5 nm, 297.0 nm, and 290.0 nm. The prepared gels were evaluated for pH, viscosity, spreadability, drug content, stability, skin irritation, and antimicrobial activity. The combination gel (F3) gave the best results, with a skin-friendly pH of 6.7, high viscosity for sustained application, excellent spreadability (26.17 gm.cm/sec), and a drug content of 98.65%. It also showed the strongest antimicrobial activity, with a maximum inhibition zone of 18 mm. No irritation was observed in animal tests, and stability studies over 90 days confirmed the formulation remained stable. These results suggest that a gel combining salicylic acid and aloin is safe, stable, and more effective than single-drug gels. It offers strong potential for use in treating skin infections and inflammatory conditions, with further in vivo and clinical studies recommended to confirm its therapeutic value.

Keywords: Salicylic acid, Aloin, topical hydrogel, combination therapy, antimicrobial activity.

1. INTROPDUCTION

Topical drug delivery systems offer several advantages, including localized action, avoidance of first-pass metabolism, improved patient compliance, and reduced systemic side effects. Hydrogels have gained prominence as carriers due to their high water content, biocompatibility, non-greasy texture, and ability to incorporate both hydrophilic and hydrophobic agents **(Zhao *et al.*, 2024).**

Combination drug therapy is increasingly being explored to achieve synergistic effects, reduce resistance, and enhance therapeutic outcomes. When delivered via hydrogels, such combinations may further improve stability, spreadability, and clinical effectiveness. However, limited studies have systematically compared single-drug and combination-drug hydrogel formulations in terms of their physicochemical properties and biological performance **(Poustchi *et al.*, 2021).**

Salicylic acid, a well-known beta-hydroxy acid, has been extensively used in dermatology for its keratolytic, comedolytic, and antimicrobial properties **(Arif, 2015).** It is particularly effective in treating acne, psoriasis, warts, and hyperkeratotic disorders. However, its frequent use can cause irritation, dryness, and erythema, which may limit patient adherence. To overcome these limitations, combining salicylic acid with a natural therapeutic agent may enhance efficacy while reducing side effects **(Urban *et al.,* 2022).**

Aloin, a bioactive anthraquinone glycoside derived from *Aloe vera*, exhibits strong anti-inflammatory, antimicrobial, antioxidant, and wound-healing properties. It has long been recognized in traditional medicine for skin protection and regeneration. Its soothing and moisturizing effects make it a promising adjunct to salicylic acid, as it may counteract irritation while contributing additional therapeutic benefits **(Egbuna *et al.,* 2020).**

The present work aims to formulate topical hydrogels containing single drugs and a drug combination, followed by comparative evaluation of their physical, chemical, and biological characteristics.

2. MATERIALS AND METHODS

2.1 Chemicals

Disodium hydrogen phosphate, Propylene glycol, Methyl paraben, Potassium dihydrogen phosphate and Sodium chloride was procured from merk. Salicylic acid was received from Molychem. Conc. Triethanolamine was acquired from Loba. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Sulab**,** Acetar Biotech, and Rankem.

2.2 Pre-formulation studies

2.2.1 Organoleptic properties

The organoleptic properties of salicylic acid and aloin were evaluated through visual and sensory observation **(Rasouli *et al.,* 2019).**

2.2.2 Solubility study

The qualitative solubility of salicylic acid and Aloin in various solvents was assessed in accordance with USP-NF (2007) guidelines **(Akay *et al.,* 2023).**

2.2.3 pH determination

pH was determined by Electrochemical method. Digital pH meter is used to determine the pH of Salicylic acid and aloin **(Miranda Mugica *et al.,* 2022).**

2.2.4 Melting point

Melting point of Salicylic acid and Aloin was analyzed by open Capillary method using Thiele's tube. **(Muir, 2019).**

2.2.5 Determination of Lambda max and calibration curve

Preparation of standard stock solution:

- **Preparation of standard stock solution:**

Approximately 5 mg each of salicylic acid and aloin were accurately weighed and separately transferred into 5 ml volumetric flasks. Each flask was then filled to volume with the appropriate solvent to prepare stock solutions with a concentration of 1000 µg/ml. From each of these stock solutions, 1 ml was withdrawn and further diluted to 10 ml using methanol, resulting in standard stock solutions with a final concentration of 100 µg/ml for both salicylic acid and aloin **(Jain and Patil 2024).**

- **Lambda max**

From the previously prepared stock solutions of salicylic acid and aloin, 3.5 ml of each sample was separately transferred into 5 ml volumetric flasks. The volume in each flask was then adjusted to the mark using the respective solvent to obtain a concentration of 70 µg/ml. These samples were scanned using a UV-Vis spectrophotometer over the wavelength range of 200–400 nm, with methanol serving as the blank. The wavelength at which maximum absorbance (λmax) occurred was recorded for both compounds **(Fang *et al.*, 2018).**

- **Linearity**

Aliquots of Salicylic acid working standard solution (100 µg/ml) were diluted to prepare concentrations of 5, 10, 15, 20, 25, and 30 µg/ml, while Aloin working standard solution (100 µg/ml) was diluted to obtain concentrations of 30,40,50,60,70 and 80 µg/ml. These solutions were accurately transferred into 5 ml volumetric flasks and diluted to the mark with the respective solvent. The absorbance of each solution was measured at 305 nm for Salicylic acid and 344 nm for Aloin using a solvent blank as reference. Calibration curves were constructed by plotting absorbance against concentration for each drug. A seven-point calibration curve was obtained over the concentration range of 30–100 µg/ml. The response was linear within the tested range, with the linear regression equation for Salicylic acid being $y = 0.009x + 0.059$ ($R^2 = 0.990$), and for Aloin, $y = 0.009x + 0.207$ ($R^2 = 0.998$) **(Sayed *et al.*, 2014).**

2.2.6 Drug loaded Gel Formulation Process

Carbopol-934 was initially dispersed in 50 ml of warm water (mixture A) and allowed to hydrate for 2 hours. The dispersion was then homogenized using a magnetic stirrer at 600 rpm to ensure uniform consistency. Meanwhile, in a separate container, carboxymethyl cellulose and methylparaben were dissolved in 50 mL of warm water (mixture B) and stirred continuously until a stiff gel was formed. Mixtures A and B were gradually combined with continuous stirring to achieve a uniform blend. Triethanolamine was added dropwise to neutralize the pH and facilitate gel formation. Subsequently, the active ingredients salicylic acid and aloin were incorporated into the neutralized gel base. Finally, propylene glycol, serving as a permeation enhancer, was added, and the entire mixture was stirred thoroughly until a smooth, homogenous gel free of lumps was obtained **(Shmakov *et al.*, 2023).**

Table 1: Composition of Drug loaded gel formulation

Name of Ingredient	Formulation I	Formulation II	Formulation III
Carbopol 940	1.5 gm	1.5 gm	1.5 gm
Carboxymethyl cellulose	1.5 gm	1.5 gm	1.5 gm
Propylene glycol	0.6ml	0.6 ml	0.6ml
Methyl paraben	0.1 ml	0.1ml	0.1ml
Salicylic acid	1 gm	----	1 gm
Aloin	----	1 gm	1 gm
Triethanolamine	q. s	q. s	q. s
Water	100 ml	100 ml	100 ml

2.3 Characterization of drug loaded Gel formulation

2.3.1 Physical appearance

The prepared gel formulations were evaluated for appearance, Colour, Odour, and homogeneity by visual observation **(Bhinge *et al.,* 2017).**

2.3.2 pH

pH of the formulation was determined by using Digital pH meter (EI) **(Vázquez-Blanco *et al.,* 2018).**

2.3.4 Spread ability

An ideal topical gel should exhibit adequate spreading ability when applied to the skin. This property was assessed by placing approximately 1 gram of the gel formulation on a clean glass slide. A second glass slide of the same size was carefully placed on top, creating a sandwich with the gel in between. A weight of 50 mg was then placed on the upper slide to allow the gel to spread evenly between the two slides. The distance the gel spread under the applied weight was observed, and the time taken for the gel to travel this distance was recorded. Spread ability was determined by the following formula

$$\text{Spreadability}(S) = \frac{M \times L}{T}$$

Where, S-Spread ability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T-Time for spreading gel in sec **(Sivaraman *et al.,* 2017).**

2.3.5 Skin irritation test

The hair on the dorsal topic area was carefully removed 1–2 days prior to the experiment to allow the skin to recover. The gel formulation was then applied to the shaved area. The treatment was carried out once daily for 2–3 consecutive days. Following each application, the skin was monitored for 24 hours to observe any adverse effects such as changes in color, alterations in skin morphology, erythema, or edema on the treated site **(Yampolsky *et al.,* 2024).**

2.3.6 Drug content

100 mg of formulation was accurately weighed and distributed in phosphate buffer at a pH of 7.4. Samples were obtained from the top, middle, and bottom of the formulation. The samples were then transferred to a volumetric flask and agitated well using a mechanical shaker for approximately 1 hour. The contents were filtered, and the filtrate was spectrophotometrically examined at 250-300 nm for drug content compared to a blank. The concentrations of samples were determined using the calibration curve (**Tiwari et al., 2021**).

2.4 Antimicrobial activity

2.4.1 Preparation of Nutrient Agar Media

28 g of Nutrient Media was mixed in 1 liter of distilled water. The pH of the media was tested prior to sterilization. The media was sterilized in an autoclave at 121ºC and 15 pounds pressure for 15 minutes. Nutrient media was poured into plates and exposed to laminar air flow until the agar solidified **(Terrones-Fernandez *et al.,* 2023).**

- **Well Diffusion Assay**

The bacterial suspension of *E. coli* was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate. The

agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the F1 (1mg/ml), F2 (1mg/ml) and F3 (1 and 2mg/ml) formulation solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well **(Diko, 2021).**

2.4.2 Stability studies

The drug-loaded gel formulation was packaged and subjected to stability testing in a controlled stability chamber under accelerated conditions at 25°C ± 2°C with 60% ± 5% relative humidity (RH) and 40°C ± 2°C with 70% ± 5% RH for a period of 3 months. The formulation was periodically evaluated for key parameters such as viscosity and pH at intervals of 30, 45, 60, and 90 days. These studies were conducted following the International Conference on Harmonization (ICH) guidelines to assess the formulation's stability under accelerated storage conditions. Any changes in viscosity and pH over the testing period were recorded and analysed **(González-González *et al.,* 2022).**

3. RESULT

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties

Table 2: Organoleptic properties of Salicylic acid and Aloin

Drug	Organoleptic properties	Observation (Salicylic acid)	Observation (Aloin)
Salicylic acid and Aloin	Color	White	Brownish-yellow
	Odor	Odorless	Similar to body odor
	Appearance	Crystalline powder.	Yellow crystal
	State	Solid	Crystaline Solid

3.1.2 Solubility study

Table 3: Solubility study of Salicylic acid and Aloin

Drug	Solvents	Observation/Inference (Salicylic acid)	Observation/Inference (Aloin)
Salicylic acid and Aloin	Water	Soluble	Soluble
	Ethanol	Freely soluble	Freely soluble
	Methanol	Freely soluble	Freely soluble
	Chloroform	Soluble	Very slightly soluble
	DMSO	Freely soluble	Soluble

3.1.3 Determination of pH, Melting point and Lambda max of Salicylic acid and Aloin

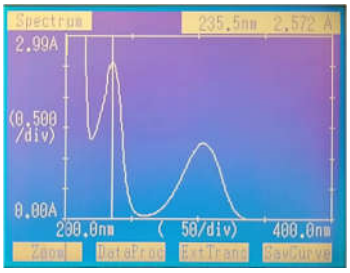
Table 4: pH, Melting point and Lambda max of Salicylic acid and Aloin

Drugs	Observed (pH)	References (pH)	UV absorption maxima (Lambda max)
Salicylic acid	3.0	155°C-160°C	235.5 nm
Aloin	4.3	148°C - 149°C	297.0 nm

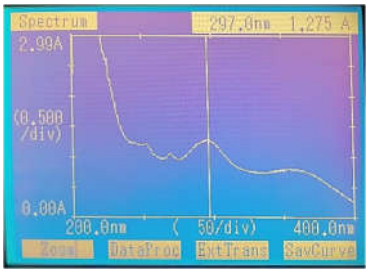
Table 5: Lambda max (overlay)

Drug	UV absorption maxima (Lambda max)

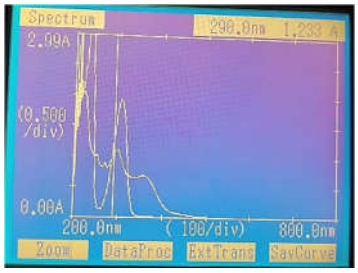
Salicylic acid and Aloin	290.0nm



Graph 1: Lambda max of Salicylic acid

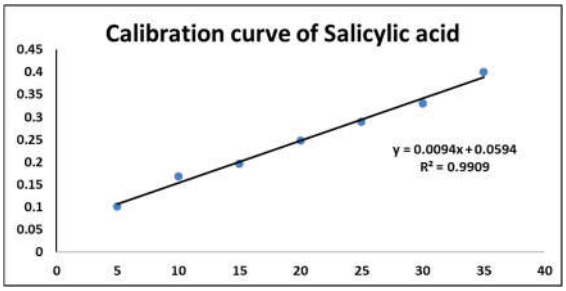


Graph 2: Lambda max of Aloin

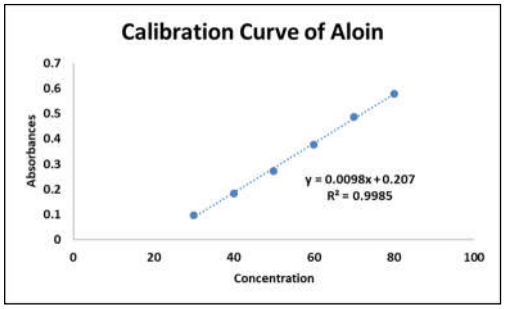


Graph 3: Salicylic acid and Aloin lambda max

3.1.4 Calibration curve of Salicylic acid



Graph 4: Calibration curve of Salicylic acid



Graph 5: Calibration curve of Aloin

3.2 Evaluation parameter of gel formulation

3.2.1 Organoleptic properties

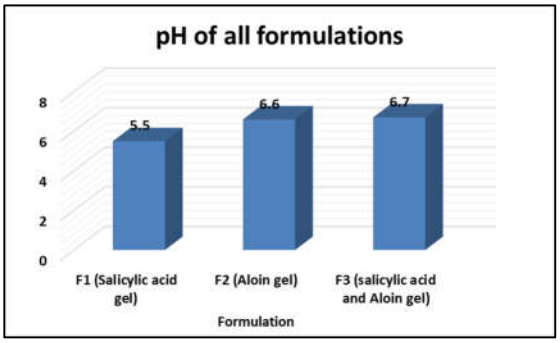
Table 6: Organoleptic properties gel formulation

Parameters	Results
Physical appearance	Semisolid gel
Colour	Slightly yellowish gel
Homogeneity	Absence of aggregates

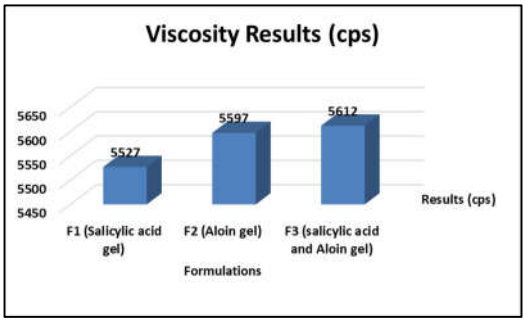
3.2.2 Determination of Viscosity, Ph, Skin irritation test and spreadability test

Table 7: Viscosity, pH, Skin irritation study, Drug content and Spreadability test

Formulation	Results (pH)	Skin irritation study	Viscosity Results (cps)	Spreadability test (gm.cm/sec)	Drug content
F1 (Salicylic acid gel)	5.5	Not irritant observed	5527±0.26	21.46	96.56%
F2 (Aloin gel)	6.6	Not irritant observed	5597±0.79	25.32	97.13%
F3 (salicylic acid and Aloin gel)	6.7	Not irritant observed	5612±0.89	26.17	98.65%

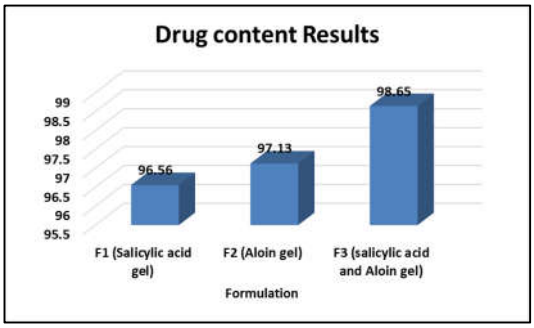


Graph 6: Graphical Presentation of pH



Graph 7: Graphical Presentation viscosity of

Determination **Determination**



Graph 8: Graphical Presentation of Drug Content

3.3 Results of antimicrobial activity of all formulations

3.3.1 Antimicrobial activity of Gel formulation

Table 8: Antimicrobial activity of all formulation (F1, F2 and F3 formulation)

Sample name	Zone of Inhibition (mm)
F1 (Salicylic acid gel)	9 mm
F2 (Aloin gel)	10 mm
F3 (salicylic acid and Aloin gel) (1mg/ml)	15 mm
F4 (salicylic acid and Aloin gel) (2mg/ml)	18 mm

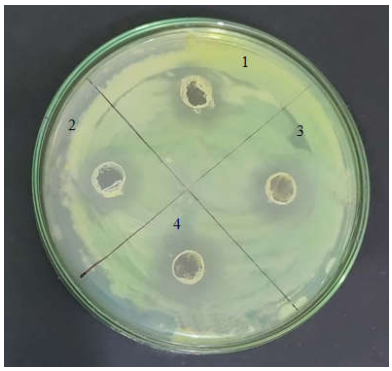
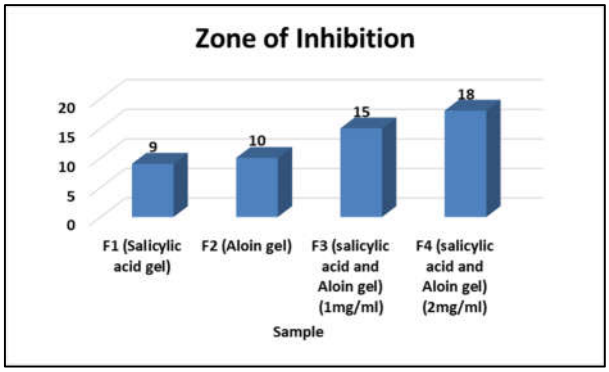


Figure 1: Antimicrobial activity of all Formulation (F1, F2 And F3 Formulation)

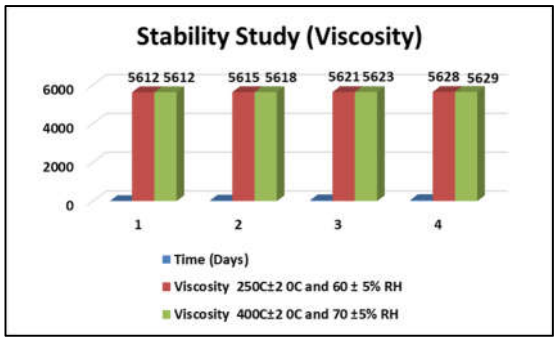


Graph 9: graphical representation of zone of inhibition

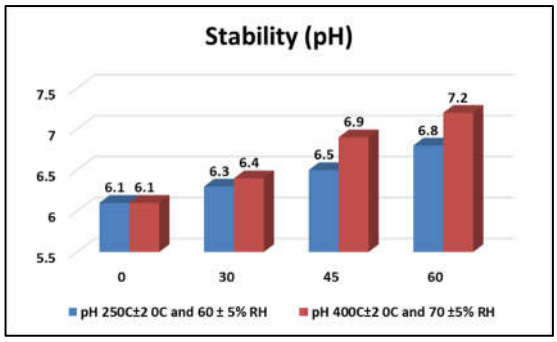
3.4 Stability studies

Table 9: Stability Study of formulation (Gel)

Time (Days)	25^{0}C±2 ^{0}C and 60 ± 5% RH		40^{0}C±2 ^{0}C and 70 ±5% RH	
	Viscosity	pH	Viscosity	pH
0	5612	6.1	5612	6.1
30	5615	6.3	5618	6.4
45	5621	6.5	5623	6.9
60	5628	6.8	5629	7.2
90	5632	7.1	5635	7.5



Graph 10: stability study of viscosity



Graph 11: Stability of ph

4. CONCLUSION

The findings of this thesis clearly demonstrate that a topical gel combining salicylic acid and aloin can be successfully formulated to produce a stable, safe, and highly effective topical delivery system. Both active agents exhibited favourable compatibility and physicochemical properties, supporting their incorporation into a single gel base. The formulated gel showed excellent rheological behavior, desirable spreadability, high drug content, and most importantly, a significant synergistic antimicrobial effect, surpassing the activity of individual formulations. The absence of skin irritation and the confirmed stability under accelerated conditions further validate the formulation's potential for clinical application.

In conclusion, the salicylic acid and aloin combination gel offers a promising alternative for the topical treatment of microbial skin infections and inflammatory conditions. Its enhanced antimicrobial action, ease of application, patient-friendly characteristics, and stability make it a strong candidate for future development into a commercial dermatological product. Further in vivo efficacy studies and clinical trials are recommended to fully establish its therapeutic value and market readiness.

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