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Formulation And Evaluation Of Salicylic Acid Loaded Gel For The Treatment Of Acne.

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ABSTRACT

Acne vulgaris is a long-term skin disorder that causes hair follicles to get clogged with dead skin cells and oil. These infections cause blackheads or whiteheads, pimples, splotchy skin, and scarring. Salicylic acid embedded anti-acne gel was formulated utilizing carbomer polymer as gelling agent. Biocompatible carbomer (carbopol 934) is very efficient gelling agent and preferably selected for skin care treatment preparations in very less concentration. The prepared formulations were characterized for drug excipient compatibility and stability studies. Distinct peaks of drug and excipients and satisfactory zeta potential demonstrated a compatible and stable formulation. Further, any change in physicochemical parameters (pH, viscosity, drug content, spreadibility and extrudability) was evaluated for 3 months that didn't display any characteristic change in gel. *In vitro* release studies were conducted at two different pH using acetate buffer (pH 5.5) and phosphate buffer (pH 9.0) to simulate normal and infectious state respectively. The high release concentration of salicylic acid in pH 9.0 suggested nicely drug pathogen interaction or anti-acne action. The agar diffusion technique was used to conduct *in vitro* antibacterial research against two devastating bacteria i.e. *Staphylococcus aureus* and *Escherichia coli*. Enhanced zone of inhibition (10.0 ± 0.1mm) of formulated gel was indicated against *Staphylococcus aureus* compared to *Escherichia coli* (3.7 ± 0.7 mm). The study concluded that formulated carbomer containing salicylic acid gel has potential to alleviate *Staphylococcus aureus* causing acne in very low concentration.

Keywords: Acne vulgaris, Skin disorder, Salicylic acid, Carbomer, *Staphylococcus aureus*

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INTRODUCTION

Acne vulgaris is a cutaneous inflammatory condition that affects a large number of people. Acne affects about 80% of all teens, and the problem can last far into adulthood [1]. Acne vulgaris has been linked to a high incidence of diet, medicine, occupational circumstances, pollution, climatic variables, as well as psychological and lifestyle factors [2]. Hyper seborrhea, abnormal keratinization of the pilosebaceous duct, reduced skin microbial variety, and inflammation are all caused by numerous conditions that alter the natural skin barrier and microorganisms [3]. However, because the number of sebaceous lobules per gland is thought to be larger in seborrhea acne-prone skin than in normal skin, these external exposure variables are seldom adequate to produce acne [4]. Acne is caused by increased sebum production, increased proliferation, and poor keratinocyte desquamation in the pilosebaceous unit[5].Acne is not a life-threatening disorder, but it can harm a patient's quality of life since it commonly occurs during puberty and adolescence, a sensitive period in life. Acne lesions that persist for a long time will ultimately scar [5, 6]. Acne is a common dermatologic condition that can cause severe inflammation and scarring, which appears as a result of the inflammation and is sometimes difficult to treat [7]. Acne is caused by four main processes: pilosebaceous follicle hyperkeratinization increased sebum production, increased Propionibacterium acnes colonization, and inflammation. There has been a recent spike in interest in acne treatments that entail modifying normal activities such as face washing, and studies have indicated that using specialized facial cleansers can help [8].

MATERIALS AND METHODS

Salicylic acid A.R. (HiMedia laboratory Dindory Nasik Mumbai), which is an ethanol-soluble drug, was selected as a model drug. Corbopol-934 L.R. (SD Fine-Chem Limited Mumbai) was used as Gelling agent, Propyl paraben L.R. (HiMedia laboratory Dindory Nasik Mumbai), Methyl paraben L. R. (HiMedia laboratory Dindory Nasik Mumbai), were used as preservatives, Propylene glycol. L. R. (LOBA CHEMIEMumbai) was used as humectants, Sodium lauryl sulfate A. R. (HiMedia laboratory Dindory Nasik Mumbai) was used as a surfactant, Ethanol, and Menthol oil was used in this study.

Preparation of salicylic acid loaded gel [9]

Appropriate amount of Salicylic acid was dissolved in a co-solvent (mixture of water and propylene glycol). The prepared solution was transferred in a beaker containing weighed quantity of Carbopol-934 with intent to obtain dispersion. The dispersion was rested for nearly 30 min. for efficient swelling followed by stirring at slow speed to obtain a homogenous gel formulation. The prepared gel was preserved by adding a mixture of methyl paraben and propyl paraben; Mint oil was added at final stage as perfuming agent. Table 1 depicts the details of optimized formulation.

Table 1: List of ingredients, functions and their quantities

Sr. No.	Name of ingredients	Functions	Quantities
1.	Salicylic acid	Active drug	2gm
2.	Carbapol-934	Gelling agent	1.5gm
3.	Methyl paraben	Preservative	0.06gm
4.	Propyl paraben	Preservative	0.03gm
5.	Propylene glycol	Humectant	2.5ml
6.	Sodium lauryl sulfate	Foaming agent	2.5gm
7.	Mint oil	Perfuming agent	0.5ml
8.	Distilled water	qs to 100ml	qs to 100ml

Evaluation of salicylic acid loaded gel

The prepared formulation was evaluation different parameters.

Organoleptic properties: The prepared gel formulation was assessed for various physical parameters like color, odor, homogeneity, and grittiness [10].

Determination of pH: The pH of gel was determined by using digital pH meter. 1 gm of gel was dissolved in 100 ml of distilled water. The pH of formulation was determined in triplicate and the average value for recorded [11].

Viscosity: The viscosity of gel was determined by using Brookfield viscometer (Brookfield DV-II + Pro) using spindle number RV 6 at 10rpm at 33±1°C [12].

Extrudability: The test was performed in order to determine the amount of gel that extrudes out of the collapsible tube on application of weight. Weighed quantity (20g) of gel was filled in collapsible aluminum tubes. The extrudability of the formulation was determined in terms of weights in grams required to extrude a 0.5cm ribbon of gel in10 seconds [13].

Spreadability: The extent to which a gel readily spreads on application to the skin referred to as spreadability. The gel's bioavailability is also influenced by the spreadability parameter. Under a given load, spreadability is defined as the time it takes the top slide to slip off the gel placed between the two slides in seconds. The higher the spreadability, the less time it takes to separate two slides. A total of 1 g of the formulation was sandwiched between two slides, each measuring 6 cm x 2 cm. On the upper slide, a 500 g weight was placed to evenly press the formulation for constant time. The change in diameter of gel was recorded as spreadability [14].

Homogeneity: The prepared gel formulation was physically analyzed for its homogenous nature and appearance [15].

Foamability: The required quantity of gel was placed in a water-filled beaker. The initial volume was recorded. The beaker was shaken for 10 times and the final volume was determined. Difference in volume represented the foaming ability of gel [16, 17].

Grittiness: The presence of any gritty particles was checked by applying the product to the skin [18].

Characterization

Fourier Transform Infrared (FTIR) Analysis

Identification and chemical drug-polymer interactions were studied by FTIR spectroscopy. Salicylic acid, polymer, and Physical mixtures of drug with polymer were individually mixed properly into a uniform mixture. A small quantity of the powder was compressed into a thin semi-transparent pellet by applying pressure. The IR spectrum of the samples from 500 - 3500cm^{-1} was recorded on FTIR 8400 Shimadzu instrument [19].

Zeta Potential Analysis

According to fundamental theory, the zeta potential is the electrical potential in the interfacial double layer (DL) at the sliding plane's location. The zeta potential is the potential difference between the dispersion medium and the stationary layer of the fluid coupled to the particle layer. It is an indicative of electrochemical equilibrium at the particle-liquid interface. Although, it measures the degree of electrostatic repulsion/attraction between particles, so it is one of the key properties used to define colloidal particle stability. It's worth mentioning that the term "stability" in the context of colloidal dispersions refers to the dispersion's resistance to change over time [20]. The zeta potential of prepared formulations was determined by using Malvern zetasizer. The study was performed in triplicates after appropriate dilution of sample with deionised water.

Drug content studies

Drug content was estimated in both acetate buffer (pH 5.5) and phosphate buffer (pH 9.0). Initially, accurately weighed 1g salicylic acid loaded gel was diluted separately in acetate and phosphate buffers and allowed to stand for 24 hours. There after their volumes were adjusted with 100 ml with appropriate buffers and filtered through Whatman filter paper. Drug content was determined by a UV spectrophotometer (Shimadzu, 1700) at 297.5 nm after applying below mentioned formula.

Percentage drug content = Actual drug content X100/ Total drug amount taken

***In-Vitro* Drug diffusion**

The in vitro diffusion studies were performed using Franz diffusion cell. Accurately weighed gel equivalent to 1 gm salicylic acid was placed on cellophane membrane fixed between donor and receptor compartments. The temperature was maintained at 37±1 ◦ C. The volume of the receptor cell was 50 ml and the effective surface area available for permeation was 6.9062cm^2. The receptor compartment is filled with acetate buffer (pH5.5) and phosphate buffer (pH9.0). The hydrodynamics of receptor fluid was maintained by stirring the fluid at 600 rpm and allowing for diffusion for 2-10 min. At predetermined time intervals (2, 4, 6, 8, and10 min), 5ml aliquots of sample were withdrawn and replaced with 5ml fresh acetate buffer (pH5.5) and phosphate buffer (pH9.0) respectively in separate franz diffusion cell. The samples were analyzed by the UV-Visible spectrophotometer at 297.5 nm and the drug release profile was estimated [21].

Stability Studies

The formulation was kept at 40±2°C and 75%RH for 0, 1, 2, and 3 months. In Freeze-thaw stability test, the formulation was kept in a refrigerator at 4±2°C for 24 hours and subsequently moved to 40±2°C for 24 hours and repeated all steps for 6 cycles.

***In-vitro*Anti-bacterial activity by agar well diffusion method**

Boiled 3g of Nutrient agar and PDA in 100ml distilled water to dissolve the contents completely. Sterilized the above contents in an autoclave maintained at 15 lbs pressure and 121 °C temperature for 15 minutes. Mixed well and checked the pH for 5.6 ± 0.2. Poured the sterilized media in sterilized petriplates and allowed it to solidify. With the help of a cork, the borer prepared 3 holes in two Petri plates. A separate petriplate for the standard drug, salicylic acid loaded gel, and sample gel with the help of spatula the solution was added to the wells and sub cultured the plates with a given standard of bacterial strain (*Staphylococcus aureus*) and (*E. coli*). All petriplates were incubated for temperature 37±0.2°C. After three days area of the zone of inhibition was observed [22].

RESULTS AND DISCUSSION

Evaluation of salicylic acid loaded gel

Various physicochemical parameters were evaluated and compiled in table 2. The formulated gel was white in color, uniform without having grittiness. pH of Salicylic acid loaded gel was found 6.21 which suggested compatibility with skin pH. Outcomes obtained from extrudability and spreadabiliy revealed desirable gel characters with ease of application at skin site. Further, the formulated gel was easily washable owing to adequate foam formation.

Table 2: Evaluation parameters of Salicylic acid loaded gel

S. No.	Parameters	Observations
1.	Colour	White
2.	Odor	Characteristics
3.	pH	6.21
4.	Viscosity (cps)	10618
5.	Consistency (60 sec)	7.46 mm
6.	Extrudability	1.01g/cm^2
7.	Spreadability	7.54gm.cm/sec
8.	Homogeneity	Excellent
9.	Washability	Easily washable
10.	Foamability	Foam volume 90ml in 3 minutes
11.	Grittiness	No gritty particles

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Fourier Transform Infrared Radiation (FTIR) Analysis

A drug-polymer compatibility study was done by FTIR spectroscopy. The FTIR spectrum of pure drug, physical mixture, corbapol-934, and the gel was obtained and compared to identify specific peaks by respective functional groups present in their chemical structure. Distinctive peaks of asymmetric C-H stretching at 3084.75cm^{-1}, symmetric C-H stretching at 2890.66cm^{-1}, C-N-C stretching vibration at 1501.40cm^{-1}, O-H stretching peak at 1282.42cm^{-1}, and C-O-H stretching vibration at 1056.32cm^{-1}, were seen. It shows that there was no chemical interaction between the drug and the polymer. This illustrated that Salicylic acid was compatible with the polymer and it was stable in gel formulations.

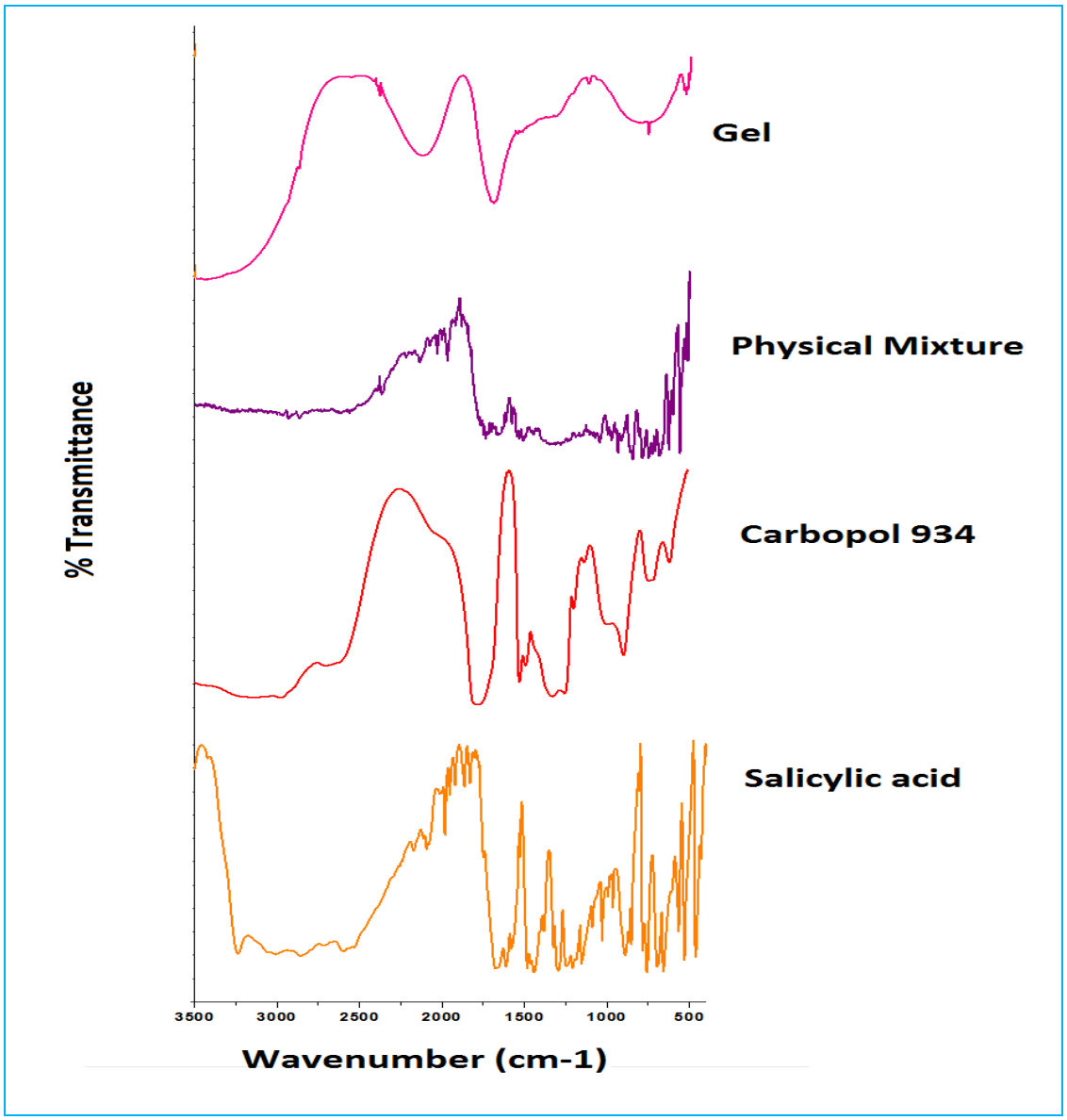

Figure 1: Compiled FTIR spectra of drug, polymer, physical mixture, and gel

Zeta Potential Analysis

The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles. For molecules and particles that are small enough, a high zeta potential (negative or positive) will confer stability, i.e. the particles will resist aggregation. The positive symbol in front of the zeta potential means that the net charge of the scattering object (including up to the slipping plane) is positive.

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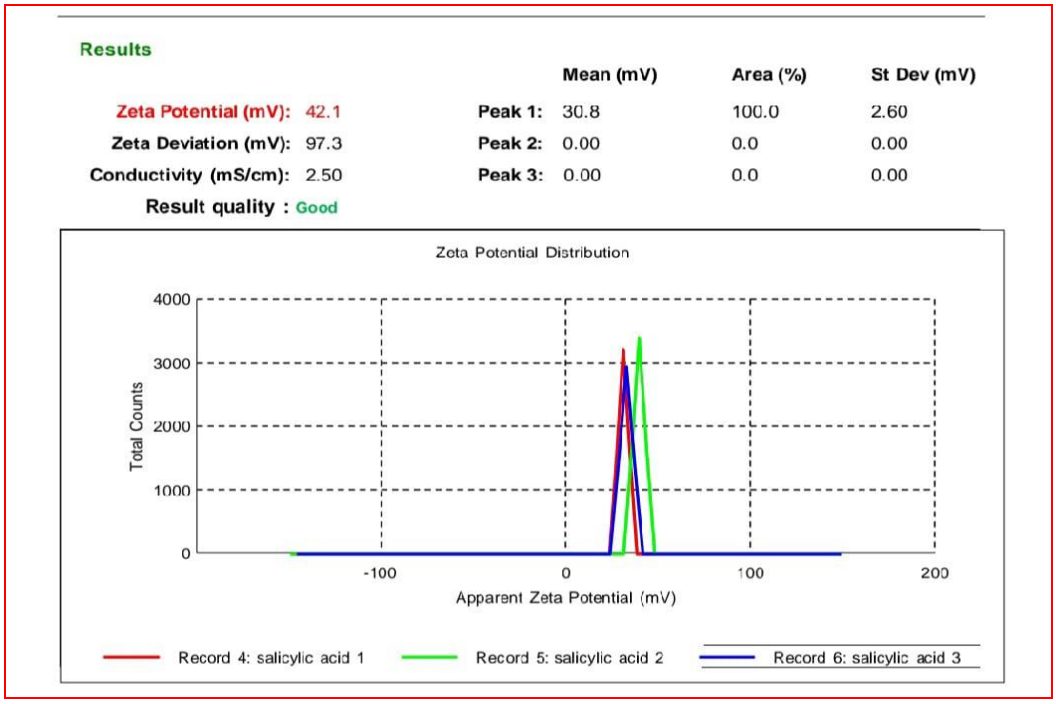

Figure 2: Zeta sizer of Salicylic acid loaded gel

***In-Vitro* Drug release**

Drug content was determined in phosphate buffer pH 9.0 through UV spectrophotometer at λ max 297.5nm. Formulated gels equivalent to 5 mg salicylic acid drug were selected for drug diffusion study. The drug release study from Salicylic acid-loaded gel indicated that the release of the drug was influenced by the type and chemical nature of the polymer. Very highly viscous solution and formed H-bond with drug molecule which reduced diffusion capacity. It was observed that maximum drug release from the gel was achieved within 2-10 min. The diffused study showed that the permeation of drug-containing gel showed good permeability of gel. Salicylic acid-loaded carbopol gel shows higher drug release. The result showed in shown in [Figure 2] [23].

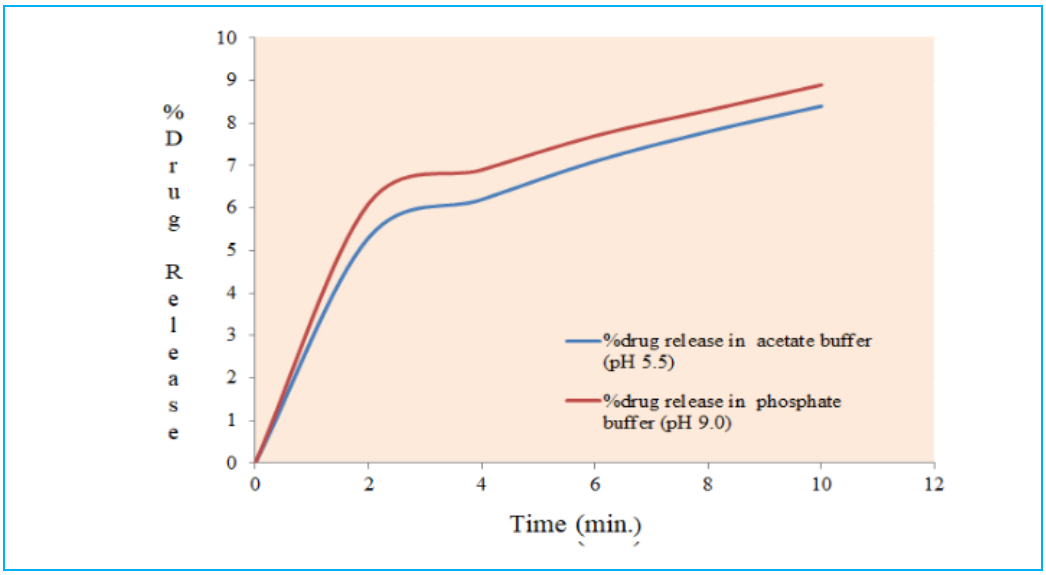

Figure 3. Drug release profile of Salicylic acid loaded gel formulation

The results obtained from the drug diffusion study suggested the release of salicylic acid at the site that is required for the treatment of acne caused by *Staphylococcus aureus* [24].

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Stability Studies

The figure shows the appearance of blank anti-acne gel (right) and Drug loaded anti-acne Gel (left). The appearance of the blank anti-acne gel was white while the intensity of its color. The intensity of its color increased with the addition of the drug which was a dark white color [Figure 4.] [25].

Figure 4: The appearance of Drug loaded gel (left) and Blank gel (right)

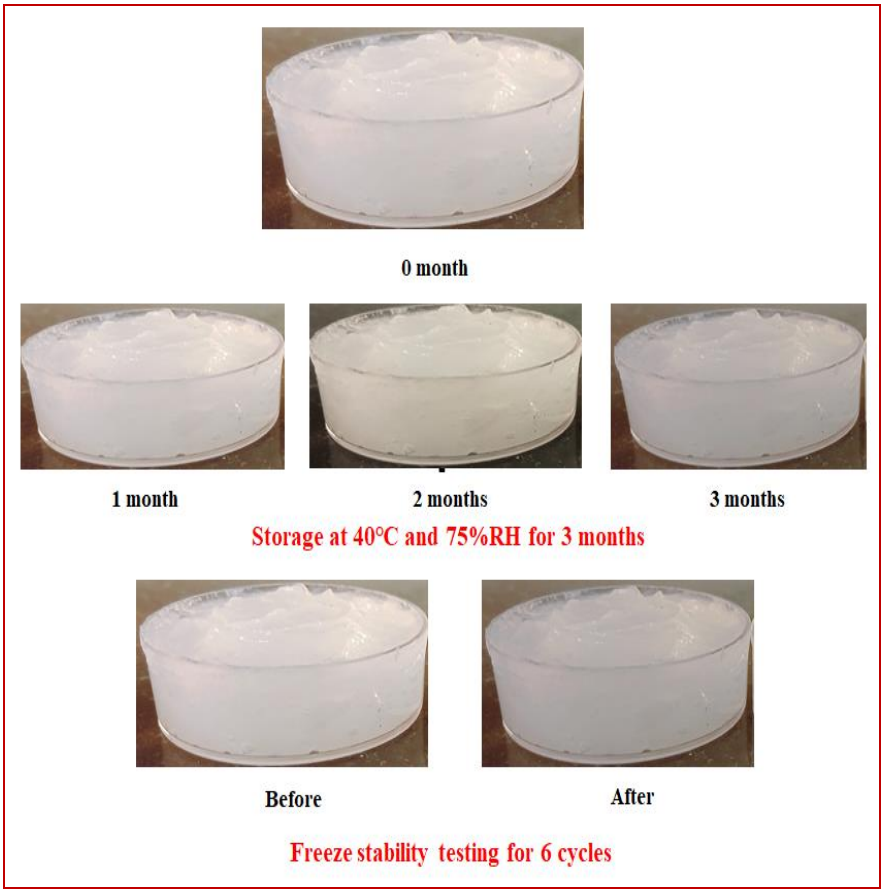

Figure 5: The appearance of Salicylic acid loaded gel formulations after stability testing

Different parameters including pH, viscosity, spreadability, extrudability, consistency, and Drug content of gel were shown in Table3. Addition of salicylic acid did not altered surface texture of gel. Thus, the drug is not affected by these values. The pH, Viscosity, Spreadability, Extrudability, Consistency, and Drug content value was found that varied after storage at 40±2°C for 3 months after the freeze-thaw test. However, slight decreased values of different parameters might be due to decreased movement of a

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polymer chain at high temperature. The appearance of anti-acne gel formulation after stability testing is shown in Figure5. Overall results showed that the anti-acne gel stood stable at stability testing condition, both kept at 40±2°C with 75%RH in stability chamber for 3 months and freeze-thaw test for 6 cycles, with a little change.

Table 3: pH, viscosity, Spreadability, Extrudability, Consistency, and Drug content gel formulation

Evaluation	0 month	1 month	2 month	3 month	Freeze-thaw stability
pH	6.21±0.02	6.12±0.03	6.07±0.01	6.03±0.02	6.01±0.01
Viscosity(cps)	10618	10330	10210	10105	10050
Spreadibility(gm)cm/sec	7.54	7.33	7.14	7.07	7.01
Extrudability g/cm^2	1.01	1	.98	.97	.95
Consistancy(60sec)	7.46	7.45	7.43	7.41	7.40
Drug content					
Acetate buffer(pH 5.5)	5.21mg	5.16mg	5.11mg	5.08mg	5.07mg
Phosphate buffer(pH 9.0)	5.71 mg	5.67 mg	5.64 mg	5.61 mg	5.60 mg

***In-vitro*Anti-bacterial activity**

In-vitro antibacterial activity of formulated salicylic acid-loaded gel against control is shown in the figure 6.

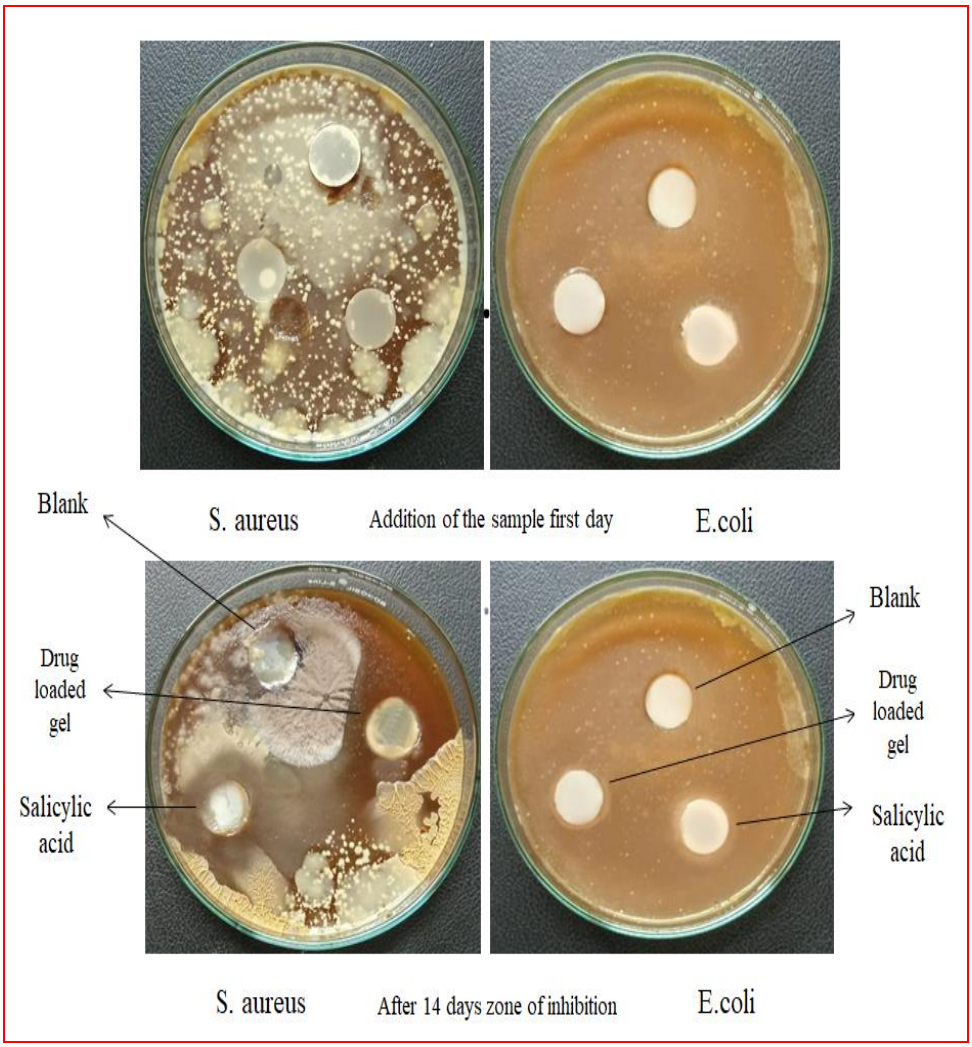

Figure 6: In-vitro antibacterial activities against *S.aureus* and *E.coli* (through agar well diffusion method)

The outcomes clearly portrayed antibacterial action of prepared salicylic acid gel against both selected acne pathogens **(***S.aureus* and *E.coli*). Although figure 6 displayed superior antiacne action for *S. aureus* (zonal inhibiton, 10.1 ± 0.1mm) compared to *E. coli* (3.7±0.7mm) owing to high susceptibility of salicylic acid for the former one. Hence, the formulated gel exhibited potential to alleviate acne causing pathogens and would be employ for the management of pimples and blackheads of skin.

CONCLUSION

A gel containing a Salicylic acid drug was formulated successfully by using carbopol-934 as a gelling agent.Salicylic acid-loaded gel formulation showed better results for the formation of the gel. Evaluation tests were carried out forSalicylic acid-loaded gel as color, consistency, pH, spreadability, washability, and foamabilitydrug content, drug release. The in-vitro drug release study of formulated salicylic acid-loaded gel. The in-vitro antibacterial study of gel showed effective results against *Staphylococcus aureus*. So from the studies, it was concluded that the prepared formulation can be effectively used for Acne.

Conflict of interest

The Author(s) declare (S) there is no conflict of interest

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