Research Article

Formulation and Characterization of Chlorhexidine Gluconate Non-Adhesive Dressing

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ABSTRACT

This invention relates to the pharmaceutical industry, more especially to the creation and description of a non-adhesive dressing containing chlorhexidine gluconate. Selecting the appropriate dressing can have a significant impact on wound care. Non-adhesive dressings are made specifically to avoid sticking to the wound, which speeds up the healing process and lessens discomfort when changing the dressing. For a variety of wounds, such as small cuts, burns, ulcers, surgical sites, and regions with delicate skin, non-adherent dressings are perfect. They aid in achieving the ideal moisture balance, which is essential for the best possible healing. Chlorhexidine gluconate's (CHG) broad-spectrum antibacterial action mainly aids in wound healing by preventing infection. It lowers the microbial burden in the wound region by efficiently eliminating or preventing the growth of bacteria, fungus, and some viruses. As a result, the wound environment becomes cleaner, which is necessary for appropriate healing. CHG promotes quicker re-epithelialization, lowers inflammation, and supports natural tissue repair processes by lowering the risk of infection. It is a useful agent in wound care, particularly for superficial and partial-thickness burns, because of its residual effect, which permits extended antibacterial action. In this experiment gauge bandages are used for the non-adhesive dressing. Other excipients were used in the preparation of the chlorhexidine gluconate ointment. Then, other batches of dressing bandages were created. Evaluation tests for the manufactured patches were completed, and a stable, potent chlorhexidine gluconate ointment was successfully made.

Keywords: Chlorhexidine gluconate, Non-adhesive dressing, Wound Healing.

INTRODUCTION

The molecular formula $C_{22}H_{30}C_{12}N_{10}$, chlorhexidine gluconate (CHG), which has antiseptic and antibacterial qualities, is used to sanitize surgical equipment and disinfect skin before surgery. Gram-positive and gram-negative bacteria are rendered inactive by the biguanide chlorhexidine, which penetrates both the inner and outer cell membranes. It has a propensity to adhere to mucous membranes and skin. It does not affect spores, mycobacteria are resistant to it, and its antiviral efficacy varies. Chlorhexidine gluconate (CHG) is frequently used in conjunction with gluconic acid[1-4].

As the concentration of CHG rises, so does its bactericidal action. A controlled in-vitro study6 showed that CHG might affect a variety of bacteria at doses as high as 1%. The most vulnerable bacteria to CHG were Salmonella and Escherichia coli, which showed 100% bacterial suppression at doses less than 0.01%. Additionally, it is used to cure oral yeast infections, reduce tooth plaque, clean wounds, and avoid urinary catheter blockages.

A broad-spectrum antimicrobial agent, chlorhexidine gluconate (CHG) solution acts on microorganisms and aids in wound healing. CHG interacts with the lipids in bacterial cell membranes, causing structural and functional disruptions that ultimately lead to cell lysis and death; it also inhibits vital enzymes in microorganisms, causing metabolic disruptions and preventing reproduction; and it binds to DNA, preventing transcription and microbial replication[5-7].

The largest organ in our body, the skin serves as a barrier against bacteria, fungi, viruses, and other microorganisms. Any injury that ruptures the skin is considered a wound and increases the possibility of bacteria infiltrating the body and resulting in an infection. One cannot undervalue the significance of providing effective wound care. Care for wounds should always address the patient's needs, encourage natural healing, and avoid complications. An essential component of the healing process is tending to wounds following surgery, an accident, or an illness. In addition to preventing infection and other problems, good wound care promotes quicker healing and reduced scarring. Chronic wounds can occur as a result of wounds, particularly in complex burn patients and those that are not adequately managed[7].

The community is consequently left with a heavy financial and social burden. Therefore, it is essential to pay close care to the bandages and dressing on the wound in order to prevent infection and to keep an eye out for any potential issues.

The following characteristics are often required for wound dressings:

(a) The dressing material must be able to absorb the liquid that is released from the injured area

(b) It should allow water to evaporate at a specific rate and prevent the spread of microorganisms

(c) The substance shouldn't adhere to the surrounding tissue because removing it after it has healed could harm the newly formed skin.

By lowering the amount of microorganisms present in the wound, CHG promotes healing. It increases tissue oxygenation, granulation tissue development, and collagen synthesis—all of which are critical for wound healing. All things considered, chlorhexidine gluconate solution is a useful antibacterial that is essential for encouraging wound healing and avoiding infection[7-10].

MATERIALS AND METHODS

Materials

Table 1: List of Chemicals used

SL. NO.	Ingredients	Source
1	Chlorhexidine gluconate	IPCA
2	White petrolatum	Loba Chemical PVT. LTD
3	Mineral oil	Sisco Research Laboratory
4	White paraffin wax	Sisco Research Laboratory
5	Yellow Soft paraffin wax	Sisco Research Laboratory
6	Cetyl alcohol	Loba Chemical PVT. LTD
7	Glycerol	Sisco Research Laboratory
8	Sodium lauryl sulphate	NICE Chemicals PVT. LTD
9	Stearyl alcohol	Loba Chemical PVT. LTD
10	Sodium benzoate	Sisco Research Laboratory
11	Purified water	Laboratory source
12	Propylene Glycol	Sigma Aldrich Chemical
13	Tween 80	Mana Scientific product
14	Beeswax	NICE Chemicals PVT. LTD
15	Menthol	Loba Chemical PVT. LTD
16	Sodium CMC	Sisco Research Laboratory
17	Propyl paraben	Avra synthesis Pvt. Ltd

Method of preparation

In this process F_1 & F_2 batch was prepared with a variation of different excipients. Dipped 4 gauge in "Chlorhexidine Gluconate" solution for 1.5hrs and then dried for 30mins. Begin by separately melting white paraffin wax and soft yellow paraffin wax using a water bath. Once melted, incorporate the white paraffin wax into the yellow soft paraffin solution. Next, blend in liquid paraffin (mineral oil) with the melted white petrolatum solution, mixing vigorously to ensure a smooth consistency. Add glycerol and SLS into the mixture of yellow soft paraffin wax, stirring well. Finally, combine the paraffin mixture with the white petrolatum mixture and set aside to cool.

In this process F_3, F_4 & F_5 batch was prepared with a variation of different excipients. Measure the white soft paraffin and melt it. Then add glycerol and polyethylene glycol to the melted paraffin mixture. Incorporate sodium carboxymethyl cellulose (CMC) and propylparaben into the mixture. Add menthol and stir continuously. Measure the beeswax in another beaker and melt it using a water bath and added to the paraffin mixture and remove it from the water bath. Introduce chlorhexidine gluconate solution into the mix and stir vigorously until it forms a thick ointment.

FORMULATION TABLE

Table 2: List of the ingredients which are used

Ingredient	Batch					
	F1	F2	F3	F4	F5	F6
Chlorhexidine gluconate	5 ml	5ml	5ml	5ml	5ml	5ml
White petrolatum (g/ml)	-	-	70gm	50gm	35gm	35gm
Mineral oil (g/ml)	10ml	10ml	20ml	10ml	-	-
Glycerol (g/ml)	10 ml	10ml	-	-	5ml	7 ml
White paraffin wax (g/ml)	50gm	20gm	10gm	5gm	-	-
Propylene Glycol (g/ml)	-	-	-	-	5gm	5gm
Yellow Soft paraffin wax (g/ml)	60 gm	60 gm	60 gm	30gm	-	-
Cetyl alcohol (g/ml)	-	-	5gm	5gm	-	-
Sodium lauryl sulphate	1 gm	1 gm	0.2 gm	-	--	-
Beeswax (g/ml)	-	0.7 ml	0.7 ml	0.6 ml	2.5gm	1.5gm
Menthol (g/ml)	-	-	-	-	1gm	1gm
Sodium CMC (g/ml)	-	-	-	-	1gm	0.5gm
Propyl Paraben (g/ml)	-	-	-	-	0.05gm	0.05gm

Characterization of CHG non-adhesive dressings

The dressing undergoes many physicochemical and performance tests to guarantee user acceptance, safety, and effectiveness-

1. Physical Characteristics and Density - The dressing needs to be flexible, smooth, and devoid of air bubbles or fissures. A digital micrometer measures the thickness, which needs to remain constant for reliable drug delivery[11].

2. pH- To prevent irritation, the dressing's surface pH should fall between 4.5 to 6.5, which is the skin-compatible range.

3. Uniformity and Drug Content- HPLC or UV-Vis spectroscopy are used to analyse the CHG content.

Consistent therapeutic impact is ensured by homogenous drug content.

4. Water Vapour Transmission Rate (WVTR) and Moisture Content- A. WVTR assesses the dressing's capacity to sustain a moist wound environment while avoiding exudate buildup; B. Moisture content influences dressing flexibility and shelf life.

5. Swelling Index and Fluid Absorption- The ability of the dressing to absorb wound exudate and swell is assessed by these tests, which is essential for moist wound healing[12-15].

6. In Vitro Drug Release and Permeation- Diffusion cells, such as the Franz diffusion cell, and simulated wound fluid are used to study the release of CHG. To lessen the frequency of dressing changes, the release should be man Moisturaged and maintained over time.

7. Antimicrobial activity- Agar diffusion methods (zone of inhibition) or time-kill assays against common pathogens like Staphylococcus aureus, Pseudomonas aeruginosa, and E. coli are used to test the dressing's antimicrobial activity[16-19].

8. Adhesion & peel test- The dressing should remain in place despite not being sticky. Peel testing makes sure the surrounding skin is not harmed during removal.

9. Biocompatibility and Skin Irritation- To evaluate any negative reactions and make sure the dressing is safe for topical administration, in vivo or ex vivo testing is carried out (such as rabbit skin irritation tests)[20].

RESULT AND DISCUSSION

Calibration of Chlorhexidine gluconate solution:

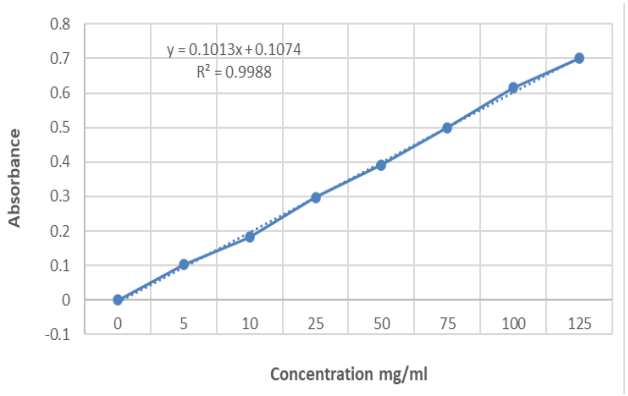
A pure drug sample of Chlorhexidine gluconate was taken in the volumetric flask of 5ml capacity and the volume was made up with distilled water at pH 1**.**

Apparatus: UV-VIS Spectrophotometer (UV-1900i, SHIMADZU, A125361)

Preparation: To prepare the UV stock solution of the CHG drug, a known quantity of 5 ml (equivalent to 5,000 µl) of the drug was accurately weighed and dissolved in a small volume of a suitable solvent such as methanol or distilled water. The solution was transferred into a 50 mL volumetric flask and the volume was made up to the mark with the same solvent to obtain a primary stock solution of 50 µg/mL. From this stock, working standard solutions of different concentrations—5, 10, 25, 50, 75, 100, 125 µg/mL—were prepared by pipetting appropriate aliquots (e.g., 1 mL for 5 µg/mL, 2 mL for 10 µg/mL, etc.) into separate 10 mL volumetric flasks and diluting each to volume with the same solvent. These solutions were then used for UV spectrophotometric analysis to construct a calibration curve and to study the linearity of CHG drug concentration with absorbance.

Table 3: Concentration & Absorbance of Chlorhexidine gluconate solution

Concentration (µg/ml)	Absorbance
0	0
5	0.102
10	0.182
25	0.298
50	0.392
75	0.5
100	0.615
125	0.7



Graph 1: Concentration & Absorbance of Chlorhexidine gluconate solution

Determination of % yield: The Chlorhexidine gluconate non adhesive dressing were calculated by considering the initial drug (Chlorhexidine gluconate) loaded ointment and weight of final theoretical drug amount (drug and others excipients). Using these two values, the yields were determined by the formula-

$$\text{Yield (\%)} = \frac{\text{Actual CHG loaded}}{\text{Theoretical CHG amount}} \times 100$$

Table 4: Percentage (%) yield results of batches F_1 to F_6

Formulation	Actual CHG loaded (gm)	Theoretical CHG amount (gm)	Yield (%)
F1	46.6	51.87	89.84
F2	48.76	51.87	94
F3	46.20	51.87	89.06
F4	48.28	51.87	93.07
F5	47.72	51.87	92
F6	49.80	51.87	96

From the calculation it was found that F3 formulation is low percentage yield and F6 formulation highest percentage yield.

Determination of drug loading

The amount of active pharmaceutical ingredient (API) added per unit weight or volume of the formulation is known as drug loading. One gram of the prepared CHG ointment is precisely weighed, dissolved in distilled water, and stirred to guarantee full extraction of CHG from the base. An aliquot of the resultant solution is diluted after it has been filtered. A UV-Visible spectrophotometer is then used to spectrophotometrically measure the concentration of CHG at its maximum absorbance wavelength. Standard solutions with established concentrations of CHG are used to create a calibration curve.

$$\text{Drug loading study (\%)} = \frac{\text{Particle drug content}}{\text{Weight of gauge}} \times 100$$

Table 5: Drug loading study results of batches F_1 to F_6

Formulation	Particle drug content	Gauge weight (mg)	Drug loading (%)
F1	36.67	3100	1.182
F2	35.68	2900	1.23
F3	36.68	3000	1.222
F4	36.67	3200	1.145
F5	36.71	3100	1.184
F6	36.71	3200	1.147

***In vitro* dissolution release study**

Apparatus: USP Type-I (Paddle)

Volume of medium: 900 mL

Temperature: 37± 0.5°C

Speed: 50 rpm

Dissolution medium used: Phosphate Buffer (pH: 7.4)

Aliquot taken at each time interval of 5 ml: 5 ml

Time: 30mins, 60mins, 90 mins upto 180 min

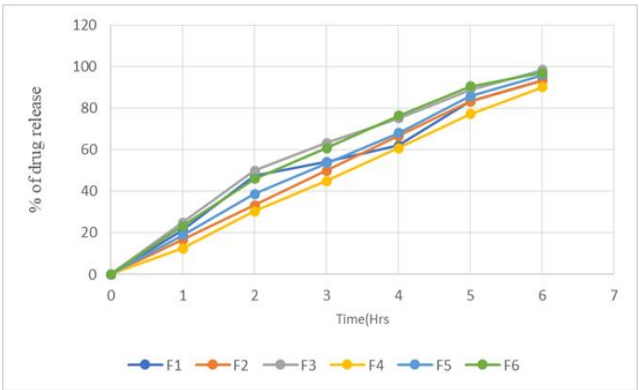
Filter: Whatman filter paper

Procedure: In vitro release studies of the drug were performed on the USP Type I & type-II Dissolution test apparatus (Electro lab Model TDT-08L) at a paddle speed of 50 rpm. The dissolution was performed in 900 mL of phosphate buffer at pH 7.4 at 37± 0.5°C**.**

First, weigh the sample as 2 milligrams of CHG cream. Put it in a pouch made of dialysis membranes or cellophane. To create a bag, seal the two ends. This simulates diffusion by functioning as a semi-permeable barrier. The bag was submerged in the dissolution medium. At every time point, take 5 mL of the dissolving medium and filter it through Whatman filter paper. To keep sink conditions stable, replace the withdrawn volume with new, pre-warmed buffer. At the end use UV spectrophotometry to analyze at λmax.

Table 6: Dissolution study of Chlorhexidine gluconate non-adhesive of batches F_1 to F_6

Time (min)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	21.34	16.67	25	12.5	18.98	23.34
2	47.65	33.33	50	30.45	38.76	46
3	54.2	50	63.34	45	53.43	60.76
4	62.05	66.67	75.12	60.78	68.12	76.34
5	83.41	83.33	88.87	77.12	85.67	90.45
6	93.45	93.45	98.34	90	95.89	97



Graph 2: Dissolution plot CHG ointment

Anti-microbial study:

For this investigation, nutrient agar was chosen as the culture medium. It was made by dissolving the proper quantity of nutritious agar powder in distilled water, then sterilizing it for 15 minutes at 121°C in an autoclave. Following sterilization, the molten agar was allowed to cool to between 45 and 50°C before being aseptically transferred to sterile Petri plates until it reached a consistent thickness. Laminar airflow was used to allow the plates to harden.

E. coli was subcultured into nutrient broth once a pure culture was obtained. For 18 to 24 hours, the broth culture was incubated at 37°C in order to achieve the exponential growth phase. To ensure a standardized bacterial load, the turbidity of the bacterial suspension was adjusted to meet the 0.5 McFarland standard.

Using a sterile cotton swab, the standardized E. coli and Staphylococcus aureus culture was applied to the surface of the solidified nutrient agar plates. A consistent bacterial lawn was created by rotating the swab to guarantee even distribution throughout the whole agar surface.

In the middle of the inoculated agar plates, sterile CHG non-adhesive dressing ointment samples were carefully positioned. For comparison of the antibacterial impact, a simple, non-medicated (without CHG) was utilized as a control.

To avoid condensation on the agar surface, all infected and sample-loaded plates were incubated for 24 hours at 37°C while inverted.

Following incubation, zones of inhibition surrounding the dressing were inspected on the plates. The transparent region around the dressing where bacterial growth is inhibited is known as the zone of inhibition. A caliper or ruler was used to measure the zone's diameter in millimeters. Greater antibacterial action is indicated by a bigger zone. The outcomes of the CHG dressing and the control were contrasted.

The antibacterial activity of CHG non-adhesive dressings against E. coli and Staphylococcus aureus was shown in this investigation. The effectiveness of CHG in preventing bacterial growth was validated by the existence of a distinct and quantifiable zone of inhibition. The conclusion that the CHG content was specifically responsible for the antibacterial activity was supported by the control dressing's minimal or nonexistent zone. This technique provides a straightforward yet efficient way to screen antimicrobial wound dressings in advance.

Zone sizes are often greater for CHG, which makes it more effective against S. aureus than E. coli.

Because of its superior efficacy against S. aureus, even at lower concentrations, F6 is the most promising batch shows that formulation quality is just as important as concentration.

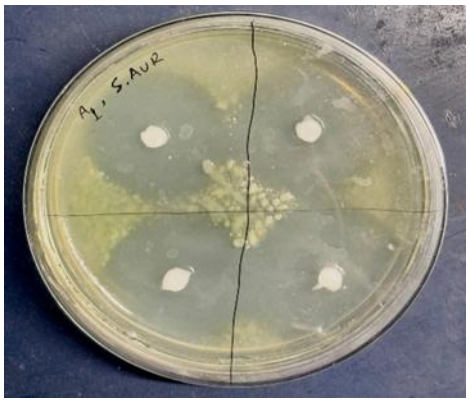


Figure 1: Zone of inhibition of F_6 batch against *S. aureus*

Spreadability test

A critical evaluation criterion for topical formulations such as ointments, particularly those designed for non-adhesive dressings, is the spreadability test. Patient comfort, treatment effectiveness, and uniformity of application are all directly impacted by how easily the ointment spreads across the skin or dressing surface. Optimal spreadability guarantees even coverage of the wound area without using excessive pressure that can irritate delicate or injured skin for a CHG-based ointment, which is utilized for its antibacterial qualities in wound care.

The parallel plate method is usually used to perform the test. A set amount of the ointment sandwiched between two glass slides in this process. To give the ointment time to spread, a normal weight is placed on the upper slide for a set amount of time. Next, the spread sample's diameter or covered area is measured.

The equation which is used to calculate the Spreadability (S) is:

$S = (M \times L) / T$

Where:

M = weight applied,

L = length the slide moved, T = time taken

This graph shows Batch F6 has the highest spreadability at 0.375gm.cm/sec, which define it spreads more easily than the other batches. That is desirable for better patient compliance and application ease.

Table 7: Spreadability study data of Chlorhexidine gluconate base ointment

Batch	Weight applied (gm)	Time taken (sec)	Spreadability (gm.cm/sec)
F1	6.10	135	0.338
F2	6.10	140	0.326
F3	6.10	130	0.351
F4	6.10	130	0.351
F5	6.10	125	0.266
F6	6.10	120	0.375

CONCLUSION

The present research work was targeted at the development and characterisation of a non-adhesive wound dressing loaded with Chlorhexidine Gluconate (CHG), a commonly used antibacterial agent renowned for its broad-spectrum activity. The fundamental objective was to develop a biocompatible, effective, and easy-to-apply dressing system that assures continuous medication release, minimizes microbial contamination, and promotes patient compliance without inflicting skin trauma upon removal.

In order to establish a flexible, non-irritating base that does not stick strongly to the wound or skin, a non-adhesive dressing matrix was prepared in this study using appropriate polymeric components (such as polyethylene glycol, glycerol, white petrolatum, and several other suitable excipients). A regulated drug loading technique was used to include CHG into the dressing, guaranteeing uniform dispersion and potent antibacterial activity.

A number of factors, such as appearance, weight, % yield, and drug loading, were assessed for the formulation. Spectrophotometric analysis was used to determine the drug loading and entrapment efficiency. In vitro drug release tests were also performed on the refined formulation. Furthermore, the functional efficacy of the CHG-loaded dressing was validated by antibacterial efficacy testing against common wound pathogens as Escherichia coli and Staphylococcus aureus.

The formulation also exhibited excellent physical stability, good compatibility with skin pH. These results collectively indicate that the developed dressing system can be a

promising alternative to traditional adhesive bandages and topical antiseptics.

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