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DEVELOPMENT AND EVALUATION OF MEDICATED BIODEGRADABLE FILM FOR WOUNDS AND BURNS

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Keywords:

Chitosan, HPMC, Silver sulfadiazine, Biodegradable film, Wound healing

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ABSTRACT: As a part of our ongoing effect to develop a biodegradable medicated film for wound healing using chitosan and HPMC as polymers. The drug used is Silver sulfadiazine. This formulation produces better biodegradability and bioadhesion. The biodegradable film is non-toxic and non-irritant to the skin. An animal wound model was performed on the back of the rats and treated, respectively with medicated biodegradable film and with a marketed product. There are no infections found during the period of study. The wound healed within 6 days, in the case of medicated film formulation, but not with the marketed cream. Medicated films could be a good alternative to conventional wound dressings, which is painful and injurious. The film adheres to open wounds, and therefore it is good protection providing a moist healing environment. Better patient compliance is an added advantage as it does not require repeated application.

INTRODUCTION: Medicated films are used for wounds and burns with extended antibacterial activity. Biodegradability erodes the polymer film and releases the drug at the site of action. It will also provide wound protection against external matters and physical injury. It will provide a dry environment over the wound surface and suppress microbial growth and also enhance tissue granulation. Wound healing is an intricate process whereby the skin (or another organ-tissue) repairs itself after injury and the wounds may get infected [1]. To prevent infection, we can use different wound healing medicaments including antibiotics.

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In this research work both dressing and medicament incorporated together as a biodegradable film. The potential of using the intact skin as port of drug administration has been recognized for several decades. Transdermal therapeutic systems have been designed to provide controlled continuous delivery of drugs via the skin to the systemic circulation.

Topical Drug Delivery: Topical delivery can be defined as drug or medication applied to a specific area of the skin and affecting only the area to which it is applied. Topical treatment of dermatological disease as well as skincare, a wide variety of vehicles ranging from solids to semisolids and liquids preparations is available to clinicians and patients. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most accessible organs of human body for topical administration and main

route of topical drug delivery systems. It is necessary to understand the anatomy, physiology, physicochemical properties of the skin to utilize the phenomenon of percutaneous absorption successfully [2, 3, 4].

Wounds: A wound can be described as a defect or a breaking the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physiological condition. According to the Wound Healing Society, a wound is a result of 'disruption of normal anatomic structure and function'. Based on the nature of the repair process, wounds can be classified as acute or chronic wounds. Acute wounds are usually tissue injuries that heal completely, with minimal scarring, within the expected time frame, usually 8-12 weeks.

The primary causes of acute wounds include mechanical injuries due to external factors such as abrasions and tears which are caused by frictional contact between the skin and hard surfaces. Mechanical injuries also include penetrating wounds caused by knives and gunshots and surgical wounds caused by surgical incisions to for example remove tumors. Another category of acute wounds includes burns and chemical injuries, which arise from a variety of sources such as radiation, electricity, corrosive chemicals and thermal sources. The temperature of the source and the exposure time influence the degree of a thermal burn. Burns will normally require special care because of the associated trauma [5, 6].

Chronic wounds, on the other hand, arise from tissue injuries that heal slowly, that is have not healed beyond 12 weeks and often reoccur. Such wounds fail to heal due to repeated tissue insults or underlying physiological conditions such as diabetes and malignancies, persistent infections, poor primary treatment, and other patient-related factors. These result in a disruption of the orderly sequence of events during the wound healing process. Chronic wounds include decubitis ulcers (bedsores or pressure sores) and leg ulcers (venous, ischemic or of traumatic origin). Wounds are also classified based on the number of skin layers and area of skin affected. An injury that affects the epidermal skin surface alone is referred to as a superficial wound, while injury involving both the epidermis and the deeper dermal layers, including the blood vessels, sweat glands, and hair follicles is referred to as partial-thickness wound. Full-thickness wounds occur when the underlying subcutaneous fat or deeper tissues are damaged in addition to the epidermis and dermal layers.

Burn Wound Infection: A burn is defined as damage to the skin caused by excessive heat or caustic chemicals. The most common burn injuries result from exposure to heat and chemicals. Burn wound infection is defined as bacterial invasion into viable tissue beneath the burn eschar. Because all burns are colonized with bacteria, a positive swab culture does not mean infection is present. Infection is diagnosed by quantitative culturing of a small full-thickness biopsy of the burn wound, including some viable tissue Infection can also be diagnosed clinically, although this method is less reliable. Burn wounds are dynamic and can evolve into deeper injuries over time, depending on the initial injury and subsequent environmental insults. Burn wounds are composed of an outer layer of nonviable tissue, known as the zone of necrosis. This involves both layers of skin in a full-thickness burn. In a partial-thickness burn, the viable tissue beneath the layer of necrosis is still injured known as the zone of injury, and can become nonviable over time, depending on the degree of injury and subsequent insults, such as infection. This process is known as wound conversion [8, 9].

METHODS:

Formulation of Medicated Film: Medicated Biodegradable film was prepared using Chitosan and HPMC as Polymers. First Chitosan was dissolved in 1% acetic acid solution with constant stirring for 2 h using mechanical stirrer. To this HPMC solution in water was added. The Drug suspension was prepared in distilled water. Then the prepared drug suspension was poured into the polymer solution with continuous stirring. The final volume was then made up. Finally, the above solution was poured into the film mold. Air-dried the film [22].

Evaluation of Medicated Film:

Physical Characterization:

Physical Appearance: All the Films were visually inspected for colour, clarity, flexibility and smoothness [24, 27].

Thickness of the Films: The thicknesses of the drug-loaded polymeric films were measured at five different points using a screw gauge. The average and standard deviation of five reading were calculated for each film.

Folding Endurance: The folding endurance was performed manually for the prepared films. A strip of film (2 × 2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance.

Percentage Moisture Content: The prepared films were weighed individually and kept in desiccators containing calcium chloride at room temperature for 24 h. The films were weighed repeatedly until they showed a constant weight. Values for the percentage of moisture content were calculated using the formula

Percentage of moisture content = Initial weight – Final weight / Final weight × 100

Percentage Moisture Uptake: The weighed films were kept in desiccators at room temperature for 24 h and then exposed to 84% RH using a saturated solution of potassium chloride. The films were weighed repeatedly until they showed a constant weight. Values for the percentage of moisture uptake were calculated using the formula.

Percentage of moisture uptake = Final weight – Initial weight / Initial Weight × 100

Drug Content: Films were cut into 1 × 1 cm and taken in a 10 ml volumetric flask add 5 ml of 1% acetic acid, dissolve with continuous shaking in a shaker for 3 h. Then 2 ml ammonia solution and 1ml PDAB was added and make up the volume with distilled water. The solutions were filtered and the absorbance was measured at 452 nm.

Drug entrapment (%) = Concentration × Dilution factor

Biodegradability Test: The *in-vitro* degradation of Chitosan and HPMC Films (1 × 1 cm) was followed in 1 ml phosphate buffer solution (PBS, pH 7.4) at 37 ºC containing 1.5 µg/ml lysozyme. The concentration of lysozyme was chosen to correspond to the concentration in the human serum. Briefly; films of known dry weights were sterilized by autoclaving (120 1C, 20 min) and incubated in the lysozyme solution with gentle mechanical agitation for the period of study. The lysozyme solution was refreshed daily to ensure continuous enzyme activity. After 6, 12, 18 and 24 days, samples were removed from the medium, rinsed with distilled water, dried under vacuum and weighed. The extent of *in-vitro* degradation was expressed as percentage of weight loss of the dried films after lysozyme treatment. To separate between enzymatic degradation and dissolution, control samples were stored for 24 days under the same conditions as described above, but without the addition of lysozyme [40, 41].

Cumulative Drug Release: The cumulative drug release study was done simultaneously with the biodegradability test. The drug content in the replaced media of the biodegradability test was estimated by spectrophotometrically. The color was developed using PDAB solution and the same was measured at 452 nm.

***In-vivo* Wound Healing:** The rats were anesthetized by intravenous injection of 30 g/ml sodium Phenobarbital at the ear marginal vein, at a dose of 30 mg/kg body weight. Each rat was then shaved over an area of 3 × 3 cm in the thoracic region. Then a 1 × 1 cm^2 area of the skin was cut and removed by using sterile surgical blade. Then the medicated film of SSD was cut into same wound size and placed over the wound. The rat was observed regularly till the wound was completely healed [44, 45, 46, 47].

Comparison of Medicated Film with Marketed Cream: Six rats (3 × 2 groups) were anesthetized by intravenous injection of 30 g/ml sodium Phenobarbital in the ear marginal vein, at a dose of 30 mg/kg body weight. Each rat was then shaved over an area of 3 × 3 cm in the thoracic region. Then a 0.5 × 0.5 cm^2 area of the skin was cut and removed by using sterile surgical blade. The film was cut into same size and applied over the wound for the first group. Similarly medicated cream was applied to the three rats in the second group. The rats were examined until the wound was healed.

Stability Studies: In any rational drug design or evaluation of dosage forms for drugs, the stability of the active component and formulation must be a

major criterion in determining their acceptance or rejection. Stability of a drug can be defined as the time from the date of manufacture and the packing of the formulation until its chemical or biological activity was not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously [50].

Objective of Stability Study: The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, enabling recommended storage conditions, retest periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. ICH guidelines were followed for the study; only 45 days study was performed.

ICH Guidelines Accelerated Testing: 40 ºC ± 20 ºC at 75% RH ± 5% for 6 Months.

Procedure: The selected formulation F4 was exposed to 40 ºC and 75% RH for stability studies for 45 days. Folding endurance, Thickness, and Drug content were evaluated before and after the stability study.

RESULTS:

Preformulation:

Physical Appearance: Silver sulphadiazine was found to be white crystalline powder.

Solubility: Solubility of silver sulphadiazine was performed in various solvents like Ethanol, Ammonia solution, and water. The results are shown table.

TABLE 1: SOLUBILITY PROFILE OF THE DRUG

Drug	Water	Ethanol	Ammonia solution
Silver sulphadiazine	Insoluble	Partially soluble	Soluble

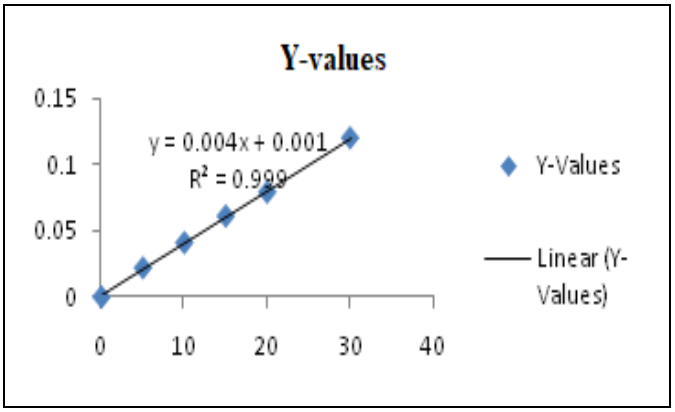
Analytical Methods:

Determination of λ_{max}: The λ_{max} of silver sulphadiazine was determined by Scanning 10 µg /ml solution of the drug between 400-800 nm regions using a UV spectrophotometer and was found to be 451 nm.

Development of Calibration Curve of Silver Sulphadiazine: A standard calibration curve for the drug was obtained by measuring absorbance at 451 nm and by plotting the graph of Concentration *vs.* Absorbance.

TABLE 2: DATA FOR CALIBRATION CURVE OF SILVER SULPHADIAZINE AT 451 NM

Concentration (µg/ml)	Absorbance (451 nm)
0	0
5	0.022
10	0.041
15	0.061
20	0.079
30	0.120



GRAPH 1: CALIBRATION CURVE OF SILVER SULPHADIAZINE

Formulation of Medicated Biodegradable Film:

FIG. 1: MEDICATED BIODEGRADABLE FILM

TABLE 3: FORMULATION CODE FOR MEDICATED FILM

Ingredients	Drug polymer ratio 1:40				Drug polymer ratio 1:40			
	F1 (4:0)	F2 (3:1)	F3 (2:1)	F4 (1:1)	F5 (4:0)	F6 (1:1)	F7 (2:1)	F8 (3:1)
Drug (mg)	10	10	10	10	10	10	10	10
Chitosan (mg)	400	300	266.67	200	300	150	200	225
HPMC (mg)	-	100	133.3	200	-	150	100	75

Evaluation of Medicated Film:

Physicochemical Characterisation:

TABLE 4: PHYSICAL APPEARANCE OF FILMS

Formulation code	Visual appearance	Clarity	Surface	Flexibility
F1	Transparent	Clear	Smooth	Flexible
F2	Transparent	Clear	Smooth	Flexible
F3	Transparent	Clear	Smooth	Flexible
F4	Transparent	Clear	Smooth	Flexible
F5	Transparent	Clear	Smooth	Flexible
F6	Transparent	Clear	Smooth	Flexible
F7	Transparent	Clear	Smooth	Flexible
F8	Transparent	Clear	Smooth	Flexible

All the drug-loaded films were found to be transparent, flexible with a smooth surface

TABLE 5: PHYSICO-CHEMICAL EVALUATIONS DATA OF FILM

Formulation Code	Thickness (mm)	Folding Endurance (number of times)	Moisture Content (%)	Moisture Uptake (%)
F1	0.25 ± 1.26	65 ± 0.96	6.6 ± 1.24	8.2 ± 0.92
F2	0.22 ± 1.18	125 ± 1.19	7.7 ± 0.84	8.9 ±1.12
F3	0.24 ± 0.98	115 ± 0.86	7.5 ± 0.96	9.3 ± 0.89
F4	0.25 ± 0.74	130 ± 0.67	11.5 ± 0.73	15.6 ± 0.65
F5	0.23 ± 0.92	76 ± 0.84	6.2 ± 1.07	7.7 ± 0.95
F6	0.22 ± 1.31	89 ± 1.24	10.1 ± 0.92	11.8 ± 1.05
F7	0.28 ± 1.02	118 ± 0.96	9.6 ± 1.18	10.4 ± 0.86
F8	0.28 ± 0.94	127 ± 0.88	10.5 ±0.89	12.6 ± 1.26

S.D., n=3

The thicknesses of all the films were measured by a screw gauge. Results showed that thickness of all formulations was varied from 0.22 ± 1.31 mm to 0.28 ± 1.02 mm. Low deviation value in film thickness measurements ensured uniformity of the medicated films.

In order to evaluate the flexibility, the films were subjected to folding endurance test. The values were between 65 ± 0.96 to 130 ± 0.67 foldings. This revealed that the prepared films were having capacity to withstand the mechanical pressure along with good flexibility.

Moisture evaluation indicates that the formulation F4 and F8 have shown high percent moisture Content and it shows the highest maximum moisture absorption than the other formulation. Moisture content and moisture uptake studies indicated that the increasing conc. of HPMC may be attributed to the hygroscopic nature of the polymeric films. In both cases there is linear relation.

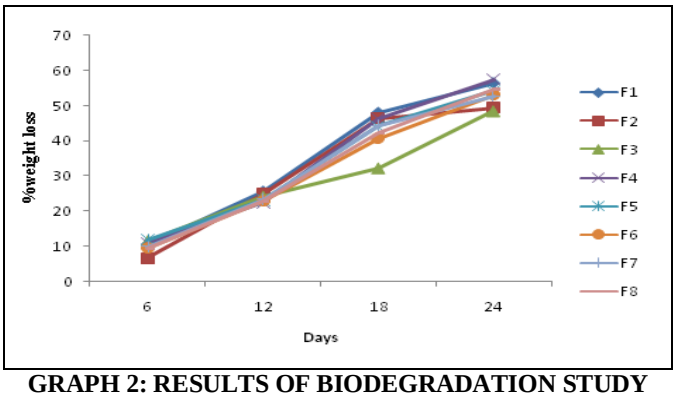
Drug Content: The % drug entrapments of all medicated films were found to be in between 95.24 ± 1.15 to 98.71 ± 0.45. All medicated biodegradable formulations showed the presence of high drug content. The values confirmed the uniformity of the Films.

TABLE 6: DRUG CONTENT ESTIMATION OF MEDICATED FILMS

Formulation code	Percentage drug entrapped
F1	95.24 ± 1.15
F2	97.43 ± 0.93
F3	96.96 ± 0.78
F4	98.71 ± 0.45
F5	95.76 ± 0.82
F6	96.12 ± 0.68
F7	97.76 ± 1.27
F8	96.98 ± 0.75

S.D., n = 3

Biodegradation Test: The *in-vitro* degradation study revealed that the Polymers can be degraded by lysozyme, which indicates their excellent and controllable biodegradability. Formulation F4 shows maximum biodegradability since its weight loss is 57.44 on 24th day.

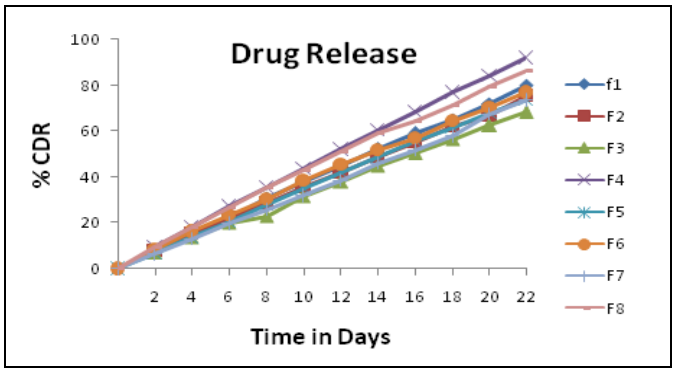


GRAPH 2: RESULTS OF BIODEGRADATION STUDY

TABLE 7: RESULTS OF BIODEGRADATION STUDY

Formulation code	Percentage(%) weight loss (different days)			
	6th day	12th day	18th day	24th day
F1	10.52	25.51	47.91	56.38
F2	6.76	25	46.47	49.47
F3	11.53	24.13	32.2	48.52
F4	11.11	22.58	46.31	57.44
F5	11.95	23.15	44.21	54.25
F6	9.57	22.91	40.86	53.12
F7	9.89	23.4	44.32	52.68
F8	9.47	22.82	42.39	54.73

Cumulative Drug Release:



GRAPH 3: CUMULATIVE DRUG RELEASE OF MEDICATED FILMS F1-F8

TABLE 8: PERCENTAGE CUMULATIVE DRUG RELEASE OF MEDICATED FILMS

Time in Days	F1	F2	F3	F4	F5	F6	F7	F8
2	7.45	7.74	7.06	9.64	6.80	8.32	6.45	9.24
4	14.45	15.22	13.61	18.47	13.90	16.32	13.04	18.28
6	22.50	22.30	19.94	27.30	20.54	23.58	19.68	26.88
8	30.80	28.62	22.75	35.48	28.23	30.42	25.75	35.53
10	37.80	35.50	31.69	43.53	35.18	38.59	31.81	43.27
12	44.96	41.77	38.02	52.07	41.99	45.24	38.42	51.32
14	52.10	48.85	44.70	60.62	48.63	51.89	45.98	58.93
16	58.97	55.12	50.27	68.67	55.77	57.12	51.65	64.34
18	65.06	62.21	56.46	76.89	61.71	64.65	57.89	71.34
20	71.93	66.88	62.40	84.11	67.81	70.42	67.23	79.18
22	80.05	75.61	68.21	91.88	73.61	76.92	73.45	86.23
24	88.46	83.57	74.15	98.93	79.80	82.67	79.16	93.47

Animal Study (IAEC No.: 014/M.Pharm 2014): To study the effect of formulation, in a rat with a deep wound (about 1 × 1 cm size). The photographs show that there is effective wound

healing happened, and complete healing was achieved on the 18th day. The biodegradable film was completely degraded within the wound. During the period of study, there is any infection does not occur.

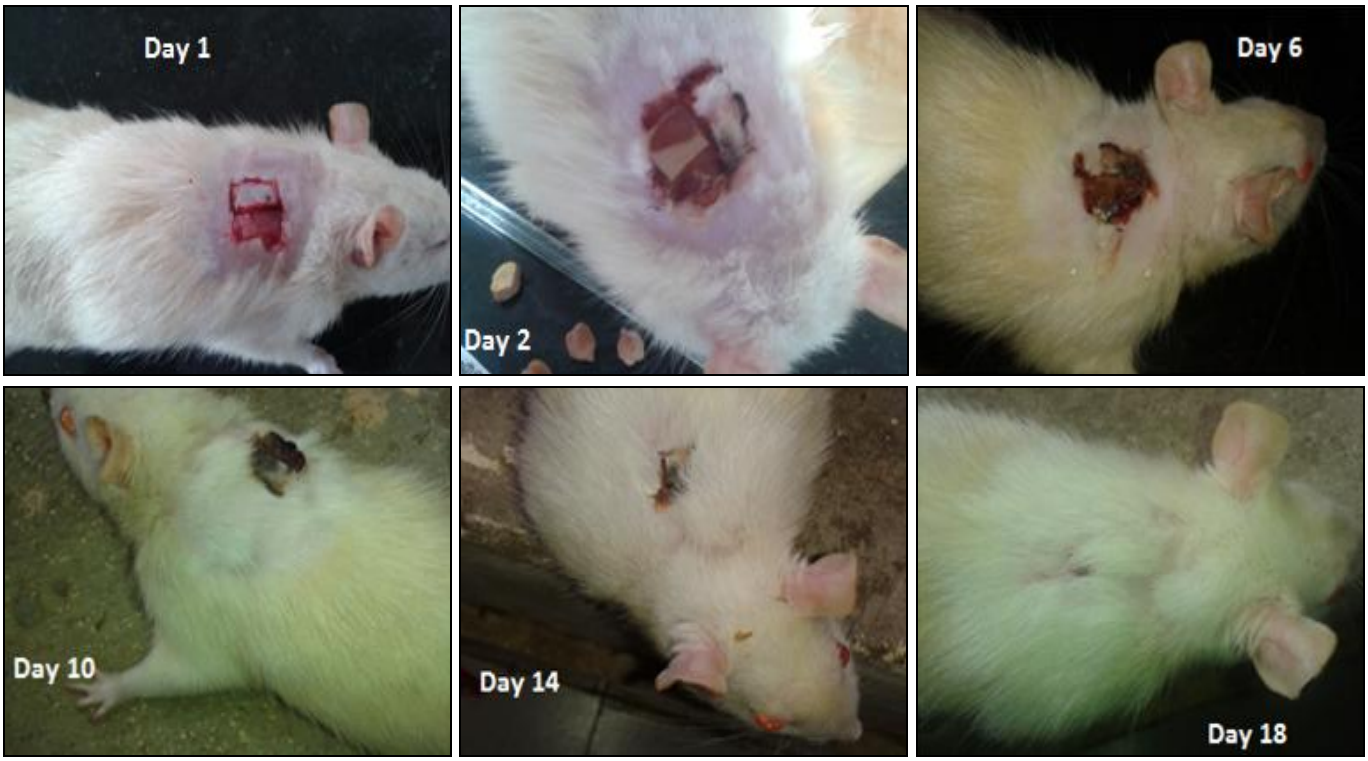


FIG. 2: STUDY OF WOUND HEALING PROPERTIES USING MEDICATED FILM, MORE THAN 2 WEEKS

Comparison with Marketed Product: a Comparison study between best formulation (F4) and marketed cream. The wound size of both in F4 and in marketed is about 5 × 5 mm.

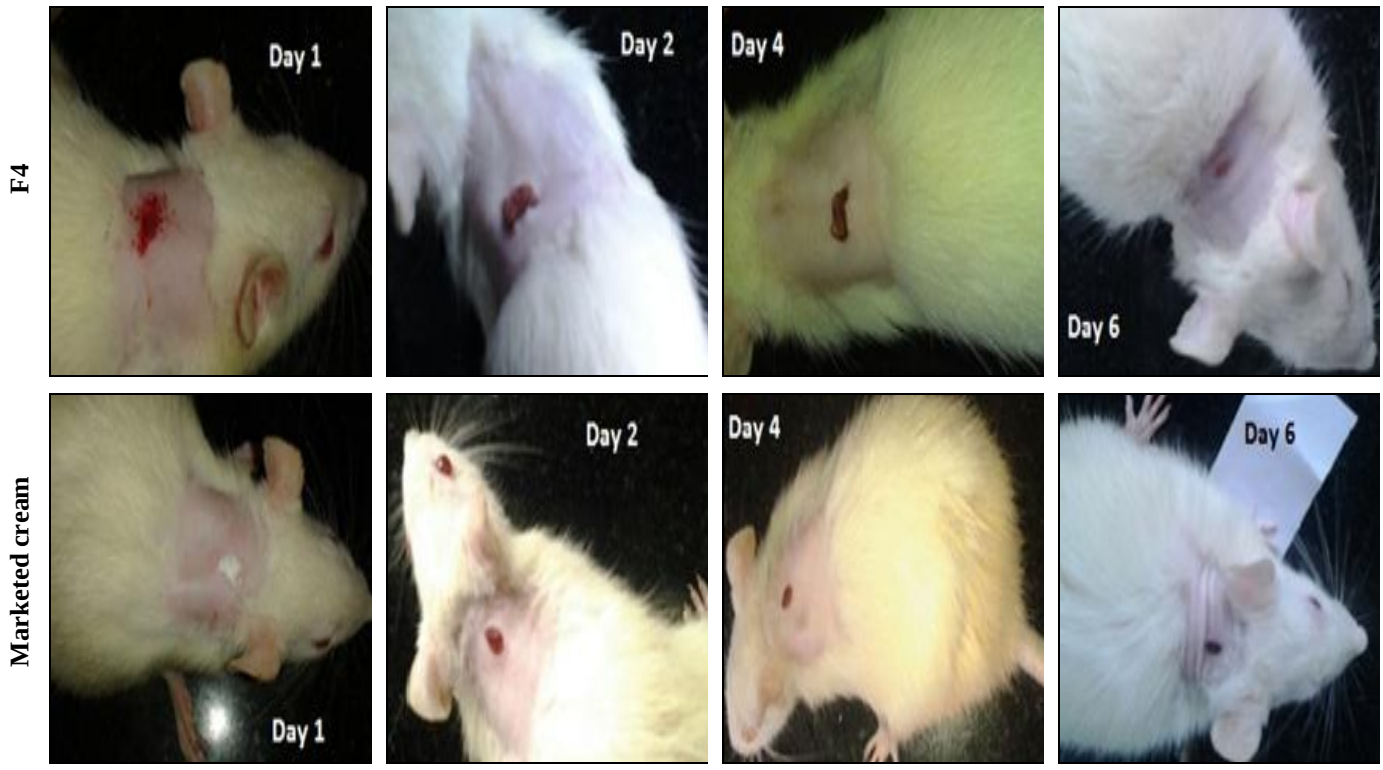


FIG. 3: COMPARISON OF WOUND HEALING BETWEEN F4 AND MARKETED PRODUCT

The wound healed within 6 days, in the case of F4 formulation, but not with the marketed cream. The group of rats treated with medicated cream took 10 to 12 days for complete wound healing. No indication of infection was noticed during the period of study.

CONCLUSION: Medicated Biodegradable film of Silver sulphadiazine was successfully developed and evaluated. The preformulation studies (compatibility study-FTIR) revealed that there was no interaction between drug and excipients. All the prepared Medicated Biodegradable films were flexible and smooth. The results of Physico-chemical characteristics of Medicated films were satisfactory with respect to thickness, weight variation, folding endurance, and moisture loss and moisture uptake. Drug content estimation of all the formulations revealed that the drug content was uniform in all the films. Films prepared using chitosan and HPMC Polymers showed satisfactory biodegradability.

Formulation F4 showed maximum drug release and better biodegradability. Kinetics study of selected formulation revealed that the drug release followed zero-order release with a super case II diffusion mechanism. *In-vivo* wound healing study was evaluated on animal model, and it showed complete healing without any signs of infections or scar Formulation F4 showed a better Wound healing (wound heals within eight days) when compared to the marketed Cream (More than 14 days) Formulation F4 was found to be stable and retained their original properties under accelerated storage conditions. Medicated films could be a good alternative to conventional wound dressings which is painful and injurious. The film adheres to open wounds and therefore it is good protection providing a moist healing environment. Better patient compliance is an added advantage as it does not require repeated application. The film could be suitably cut to fit into the wound of any size/shape and provides a sustained release of drug.

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CONFLICTS OF INTEREST: Nil

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