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FORMULATION, EVALUATION AND OPTIMIZATION OF NOVEL SILVER SULFADIAZINE LOADED FILM FORMING HYDROGEL FOR BURNS

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ABSTRACT

Plan: *Formulation, evaluation and optimization of Silver sulfadiazine loaded film forming hydrogel using different hydrophilic polymers for the treatment of burns.*
Preface: *Burn injuries are very complex. One of the golden treatments in topical burn is silver sulfadiazine (SSD).The film forming hydrogels (FFHs) are non- solid dosage forms that produce a flexible, occlusive film in-situ after application on the skin or any other body surface.*
Methodology: *The hydrogel matrix was prepared with combinations of PVA & PVP, HPMC E5LV, and HPMC E15LV. The prepared FFHs were evaluated for their various physicochemical properties. The optimized formulation was selected by antimicrobial activity by agar diffusion technique. The in-vitro drug release study of optimized formulation was carried out and analyzed by potentiometric titrations.*
Outcome: *All the formulations showed better antimicrobial activity when compared with marketed SSD cream. The formulation F3 was found to be the optimized formulation.*

1. INTRODUCTION

A burn is considered as an injury to the skin or any other organic tissue which is primarily caused by heat or due to a radiation, radio activity, friction, electricity or contact with any other chemicals. When the skin is burned its normal functions are altered and will become vulnerable to the invasion of various microorganism such as gram-negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Actinobacter* species, *Proteus* species, *Klebsiella* species, and gram-positive bacteria like *Staphylococcus aureus*, *Enterococcus* species etc [1]. This will ultimately lead to burn wound infection and non-healing of the wound which results in mortality.

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An appropriate antibacterial therapy should be initiated in time to avoid serious damage to the skin by delivering topical antibiotics as an adjunctive therapy to the systemic dosing. This will reduce an overall serum antibiotic concentration, at the same time increases the local concentration to bactericidal levels[2, 3]. The silver sulfadiazine (SSD) is one of the golden treatments in topical burn wounds. It combines the inhibitory action of silver salt and the antibacterial effect of sulfadiazine. SSD binds to various cell components including DNA and causes bacterial cell membrane damage[1, 4].

There are many silver sulfadiazine creams available in the market, but these have tendency to form pseudo eschar, which is difficult to differentiate from burn–eschar and these may also impede the penetration of SSD into the burn wound. But hydration can clearly improve permeation of SSD and possibly other drugs through burn eschar[2, 5, 6]. Recent studies revealed that moist wound environment promotes better epithelialization of superficial burn wounds compared to the dry bandaged wounds. Hydrogel dressings were found to be more effective for reducing pain of burns than the conventional dressings with Silver Sulfadiazine & dry gauze[7].

The conventional topical preparations like gels, ointments or creams can be easily wiped off from the treatment site which will result in ineffective antimicrobial activity. To overcome these problems this work is concerned with the formulation of a film forming hydrogel loaded with silver sulfadiazine[8, 9, 10].

The formulation of film–forming hydrogel (FFH) with ability to transform from hydrogel to a flexible, protective and occlusive film after application to burn site, has the advantage of retaining in the site for more duration with more improved release of antibacterial agents[8,9,11].

2. MATERIALS AND METHODS

2.1. Materials

Silver sulfadiazine was purchased from Yarrow Chem Pvt. Ltd (Mumbai, India); polyvinyl alcohol was purchased from LOBA Chemie Pvt. Ltd (Mumbai, India); polyvinylpyrrolidone from SRL Pvt Ltd (Mumbai, India); HPMC E5LV from Hi Media Laboratories (Mumbai, India); and HPMC E15LV from Yarrow Chem Pvt. Ltd (Mumbai, India). Propylene Glycol, Ethanol and Ammonia solution were purchased from Nice chemicals Pvt. Ltd. (Cochin, India). Nutrient broth and Mueller Hinton Agar Medium were purchased from Hi Media laboratories Pvt. Ltd (Mumbai, India).

2.2. Formulation of film forming hydrogel [8, 9, 10,11]

Weighed accurately required quantity of polymer (Table 1) and transferred to a bottle containing mixture of water and ethanol, which was then tightly closed. The polymer was allowed to swell overnight. Propylene glycol and drug was added to the above polymer dispersion and mixed thoroughly in a mechanical stirrer until a uniform gel was formed. The formulation was stored in a well closed air tight black color bottle and protected from light.

Table 1: Formulation of Silver sulfadiazine loaded film forming hydrogels

Ingredients (%w/w)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}
Silver Sulfadiazine	1	1	1	1	1	1	1	1	1	1
Polyvinyl alcohol	12.04	15.48	17.2	-	-	-	-	-	-	-
Polyvinylpyrrolidine K30	1.96	2.52	2.8	-	-	-	-	-	-	-
HPMC E15LV	-	-	-	5	7	9	10	-	-	-
HPMC E5LV	-	-	-	-	-	-	-	14	18	20
Propylene glycol	3	3	3	3	3	3	3	3	3	3
Ethanol	8	8	8	8	8	8	8	8	8	8
Water	74	70	68	83	81	79	78	74	70	68

2.3. Evaluation of film forming hydrogel

2.3.1. Morphological characteristics of film forming hydrogel[2, 5, 8,9,10]

The prepared hydrogels were evaluated for their appearance, viscosity, pH, spreadability and film forming time on acrylic plate (2cm x 2cm) at 25^0C.

2.3.2. Drug content uniformity of film forming hydrogel [5]

Drug content uniformity was determined by dissolving the formulation in a minimum volume of ammonia solution (25% w/w) and made up to 100 ml using pH 7.4 phosphate buffer. The absorbance was measured using UV spectrophotometer at 240nm.

2.3.3. Ex-vivo bioadhesive strength of film forming hydrogel[2]

The bioadhessive strength of hydrogels were evaluated by employing method described by Peh and Wong[12] with slight modification of balance. The measurement was conducted with chicken pouch as the model tissue. The proximal portion of a chicken pouch was used to represent the mucous-like texture. The freshly slaughtered chicken pouch, after the removal of all the fats and debris was washed with physiological saline at 4°C. The chicken pouch membrane was then affixed on the cylindrical support (diameter, 2 cm; length, 4 cm; surface area, 3.14 cm^2). The support was then attached to the left hand side of the balance by a string. Another membrane was affixed to a cylindrical support of similar dimension, which was clamped at the bottom. The two supports were aligned to ensure that the gel came into direct contact between the surfaces of the chicken pouches when the upper support was lowered. The balance was kept in this position for 5 min contact time. The weight was added to the right hand pan until the upper support detached from the lower support.

The mass in grams required to detach the support from the surface gave the measure of bio-adhesive strength.

2.3.4. Morphological characteristics of hydrogel film [8,9,10]

Prepared hydrogel films were evaluated for their appearance, outward stickiness, and thickness uniformity.

2.3.5. Mechanical properties of hydrogel film [13, 14, 15]

Prepared hydrogel films were evaluated for their folding endurance and tensile strength. Folding endurance was obtained by folding the film several times at the same place without breaking. Tensile strength of batches of the all formulations was determined by using the Shimadzu tensile testing machine.

2.3.6. Water-Vapour Transmission Rate (WVTR) of hydrogel film [15, 16, 17]

The polymeric films were tied on the top of open glass vials containing 5 g of anhydrous $CaCl_2$. The positive control (P) was air tight vial and negative control (N) was open vial. All the vials were then placed in desiccator, containing a saturated solution of NaCl at 37^OC (approximately 70% RH). The equilibrium moisture penetration was determined by weighing the vials after specific interval of time and the rate of water vapour permeability was calculated. The vapor penetration was determined by weighing the vials on day 0, 1, 2, 3, 4 and 5, respectively. Linear regression was used to estimate the slope of this line in g/day and WVTR (g/cm^2.day) was calculated by dividing the slope by the area (cm^2).

2.3.7. Antimicrobial activity of silver sulfadiazine [2,4,5,18]

The E. coli and *S. aureus* subcultures were prepared by inoculating the organisms in sterilized nutrient broth medium separately and incubated for 18-24 hrs in BOD incubator to allow the growth of bacteria. The sub cultured nutrient broth was then introduced into the Mueller Hinton Agar medium for *S. aureus* and *E. coli* separately. About 50 ml of Mueller Hinton Agar medium was then poured into sterilized petridishes, allowed to solidify and the wells were cut by using sterile 1ml syringes.

10 mg of each film forming hydrogels containing 100μg drug, their corresponding blank hydrogels (without drug) and a marketed silver sulfadiazine cream (MR) were filled in separate wells. The petridishes were then inverted and incubated under aerobic conditions at 37 ± 0.5^OC during 24 hr. Then the petridishes were visually inspected for observation (or not) of any growth inhibition halos.

2.3.8. In vitro drug release of silver ions from film forming hydrogel [18, 19, 20]

The hydrogels equivalent to 4 mg of silver sulfadiazine was accurately weighed and added to 40 ml of pH 7.4 phosphate buffer as dissolution medium, maintained at 37 ± 0.5^OC. The release of silver ions from the hydrogels was determined by measuring the potential difference using a combined silver electrode connected to a millivolt meter.

The electrode was immersed in the dissolution medium and the readings were noted directly from the potentiometer.

2.3.9. Surface imaging by scanning electron microscopy[18]

The surface analysis of the optimized hydrogel film formulation was studied by using scanning electron microscopy (SEM). The formulations were sputter coated with gold in an autofine coater and examined for 20 seconds. The images were taken at different magnification for silver sulfadiazine loaded hydrogel film.

3. RESULTS AND DISCUSSION

3.1. Formulation of film forming hydrogel

The silver sulfadiazine loaded film forming hydrogels were prepared by dispersing 1 % w/w of micronized drug in a polymeric gel matrix of various hydrophilic polymers such as PVA, PVP, HPMC E15LV and HPMC E5LV in a mixture of water and ethanol (Table1). The propylene glycol was used as plasticizer in 3% concentration. The ethanol was used to reduce film forming time which evaporates on application, and also enhance the solubility of polymers. Ethanol concentration was taken as 8% to reduce any irritation or damage to the burned skin.

3.2. Evaluation of film forming hydrogel

3.2.1. Morphological characteristics of film forming hydrogel

All the formulations were found to be white in colour, due to the dispersion of creamy white drug particles rather than the solubilized form. The formulations F1 to F6 was more desirable as it showed optimum viscosity values. All the formulations showed pH within the range of pH of the burn site; hence no irritation was expected. The spreadability increased with decrease in polymer concentration. All the formulations showed film forming time with in 7.06 min (Table 2). The hydrogel prepared by PVP, PVA combination showed rapid film forming property than with HPMC.

3.2.2. Drug content uniformity of film forming hydrogel

All the formulations showed more than 90% of drug content. This suggested the uniform distribution of drug in the polymer matrix.

3.2.3. Ex-vivo bioadhessive strength of film forming hydrogel

The bio adhesion is an important property required for the effective adhesion of formulation to the burn surface for prolonged period of time. The bioadhessive strength of hydrogels was much higher than that of marketed silver sulfadiazine cream. The bio adhesion property was found to increase with increase in polymer concentration. More over the formulation prepared by PVA, PVP combination showed more bio-adhesion than those with HPMC (Table 2).

3.2.4. Morphological characteristics of hydrogel film

All the films were semi-transparent, uniform, free from grittiness and air bubbles. The formulations F1, F2, and F3 produced flexible and tacky films as PVP act as a tackifier and secondary film forming agent. For a film to be retained on a burn wound surface all over the day it should posses' good integrity, flexibility and tackiness on the burn wound surface. The flaky and brittle films will cause partial missing of the formed film over the burn surface, resulting in the exposure of burn wound to further infections (Table 2).

The formulations F1 and F2 showed medium outward stickiness. All other formulations showed low outward stickiness. This will prevent unwanted adhering of the formed film on to the clothes and dressings of patients. The medium outward stickiness of F1, and F2 may be due to the presence of PVP, a tackifier and also due to more volume of water content presented in the formulation which will slow down the film forming time. All of the formulations showed uniform thickness (Table 2).

Table 2: Morphological characteristics and bioadhesive strength of formulations

Formulation	*Appearance*	*Outward stickiness*	*Film forming time(min)**	*Bioadhesive strength(g)*
F1	Flexible and tacky	Medium	6.10 ±0.09	108
F2	Flexible and tacky	Medium	6.07 ±0.07	122
F3	Flexible and tacky	Low	5.12 ±0.11	135
F4	Flexible and non-tacky	Low	7.06 ±0.07	65
F5	Flexible and non-tacky	Low	6.17 ±0.11	68
F6	Flexible and non-tacky	Low	6.16 ±0.05	95
F7	Flaky and brittle	Low	6.11 ±0.05	96
F8	Flaky and brittle	Low	6.18 ±0.03	97
F9	Flaky and brittle	Low	6.15 ±0.02	97
F10	Flaky and brittle	Low	6.13 ±0.03	95
MARKETED	-	-	-	45

*(n=3) Mean ± SD

3.2.5. Mechanical properties of hydrogel film

Folding endurance measures the ability of the film to withstand rupture, higher the folding endurance lower will be the chances of film rupture. Formulations showing folding endurance >200 indicated that the films have good physical properties. The films formed from a combination of PVA and PVP was much flexible than those prepared with HPMC.

Since the formulations F7 to F10 showed physical properties which were not desirable such as low viscosity, higher film forming time, flaky and brittle films and low folding endurance, they were omitted from the further evaluations.

The formulations prepared by PVP, PVA combinations exhibited higher percentage elongation and lower tensile strength compared to that of HPMC (Table 3).

3.2.6. Water-Vapour Transmission Rate (WVTR) of hydrogel film

The results indicated that the film formed by HPMC has highest WVTR. The relatively low water vapour permeability value of PVA films might be due to large number of crystalline regions of PVA that are impermeable to water vapour diffusion (Table 3).

Table 3: Mechanical properties and water vapour transmission rate of formulations

Formulation	*Water vapour transmission rate(g/cm^2.day)*	*Elongation at break (%)**	*Tensile strength (n/mm^2)**
F1	0.298	157.23±6.58	0.96±0.10
F2	0.298	176.01±7.33	1.10±0.08
F3	0.199	165.02±16.42	1.31±0.20
F4	0.298	5.47±0.69	2.08±0.70
F5	0.348	3.26±0.68	1.53±0.26
F6	0.348	3.34±0.10	2.23±0.04
POSITIVE CONTROL (P)	0	-	-
NEGATIVE CONTROL(N)	0.497	-	-

*(n=3) Mean ± SD

3.2.7. Antimicrobial activity of silver sulfadiazine

When compared with marketed SSD cream, all the formulations showed greater zone of inhibition for gram positive and gram negative microorganism. The formulation F3 prepared from PVA, PVP combination (20%) was found to be more active against both gram positive and gram negative microorganisms. It showed greater zone of inhibition in comparison with marketed formulation. This might be due to the improved solubility of silver sulfadiazine in presence of PVA and PVP. The polymers PVA and PVP were known to increase wettability of the hydrophobic drugs and there by showed enhanced antimicrobial activity in agar well diffusion method.

More over in silver sulfadiazine, the silver ions were highly reactive and affect multiple sites within bacterial cells, ultimately causing bacterial death. Since the formulation F3 showed maximum zone of inhibition, it was selected as optimized formulation and considered for *in vitro* dissolution studies (Table 4).

Table 4: Zone of inhibition obtained from antimicrobial study

Organism	Zone of inhibition (cm) of formulations		
	F3	Std	MARKETED (MR)
Gram +ve (*E.coli*)	1.2	1.3	0.9
Gram –ve (*S. aureus*)	1.5	1.7	0.9

3.2.8. In vitro drug release of silver ions from film forming hydrogel

Based on the antimicrobial analysis, formulation F3 was selected for further analysis. The antimicrobial property was predominantly due the release of silver ion. The silver ions were determined by potentiometric titrations. The formulation F3 was compared with marketed silver sulfadiazine cream [Vivek Pharmachem (India) Ltd, Mfg: 06/15, Exp: 06/17, Batch No. S511]. The *in vitro* release studies showed (Figure 1) that the formulation F3 showed a sustained release of silver ions for 24hr. At the end of 24hrs, the formulation showed a release of 92.38%. This might be due to the sustained release property of hydrogel polymer. The marketed formulation (MR) showed release of 96.02 % at the end of 8th hr. The release study revealed that the marketed formulation has to be applied several times a day. The formulation F3 can be applied as once daily on the burn wound site. Based on the antimicrobial activity and *in vitro* drug release, formulation F3 can be considered as optimized formulation.

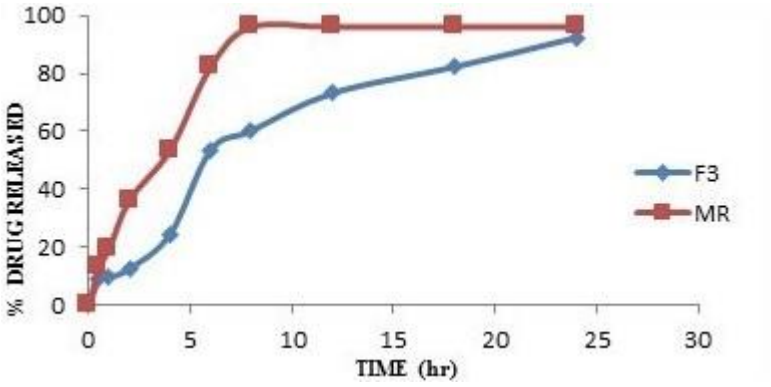

Figure 1: *In vitro* drug release of F3 and marketed formulation (MR)

3.2.9. Surface imaging by scanning electron microscopy

The surface analysis of the hydrogel film (F3) showed that the film possess uniform surface morphology with homogenous dispersion of silver sulfadiazine. The porous nature of the hydrogel film contributed for the enhanced water vapour permeability which facilitates the water vapour transmission and accelerates the wound healing process (Figure 2).

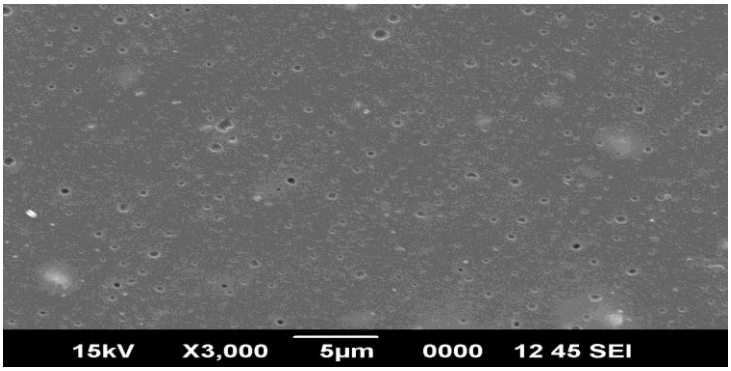

Figure 2: SEM image of formulation F3 at 3000 magnification

4. CONCLUSION

Burn injuries are very complex and associated with large amount of inflammation that can lead to worsening of the tissue damage caused by the initial thermal injury. Among the novel formulations to be applied on the damaged skin, hydrogels have shown the superiority as they can provide a moist environment for the wounds or burns and at the same time deliver the incorporated drug to the burn site. Silver sulfadiazine is the drug of choice for the treatment of burn wounds, as it possesses broad spectrum of activity against microorganisms. The antimicrobial property was mainly due to the release of silver ions. The application of film forming hydrogel incorporated with silver sulfadiazine (formulation F3) to the burn site has the ability to transform to a flexible, occlusive, and a protective film with the release of silver sulfadiazine for 24 hrs. This enhances the wound healing property along with improved patient compliance.

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