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Topical spray of silver sulfadiazine for wound healing

Bhadra Sulekha[1]* and Gajera Avin[2]

[1]*Parul Institute of Pharmacy, Vadodara, India*
[2]*Gowrie Healthcare Pvt. Limited, Vadodara, India*

ABSTRACT

Silver sulfadiazine creams or ointments are used as antimicrobial agents in the treatment of second and third degree burns. But application by touching the wounded surface is painful and may spread secondary infection. The objective of the present study was to formulate topical spray for wound healing to provide more patient compliance and reduce the chances of further contamination or spread of infection at the site of wound. An aqueous spray formulation of silver sulfadiazine was prepared using HPMC E5LV as film forming polymer and PEG 200 as plasticizer. The concentrations of polymer and plasticizer were optimized by 3^2 full factorial design, considering film forming time, volume per spray, area of film, % cumulative drug release as dependent factors (responses). The optimized batch with drying time 332±4 Sec and % drug release 71±1.4% in 270 min., showed comparable in-vitro antimicrobial activity and wound healing capacity in Wistar rats with marketed formulation. Thus, a topical spray of Silver sulfadiazine can be used for effective treatment of open wounds.

Keywords: Silver sulfadiazine, HPMC E5 LV, PEG 200, 3^2 factorial designs, wound healing.

INTRODUCTION

An open wound is a break in the skin's surface resulting in external bleeding. It may allow bacteria to enter to enter the body, causing an infection [1]. Standard burn wound care involves prevention of infection by using topical antimicrobials (bacitracin, neomycin, polymyxin B sulfate, and silver sulfadiazine) [2]. Silver sulfadiazine (SSD) is a topical antibiotic which is widely used in the form of cream. There are practical shortcomings of the medicated cream, such as: the necessity of wearing sterile gloves for its application, applying at least a 1.6mm layer of cream, maceration after long usage, soaking to clothing, bandage and pain while applying cream on injured site etc. [3]. FDA label also suggest application of SSD topical cream 'Silvadene' under sterile conditions [4]. These topical creams may cause few side effects like itching. Many sustained release formulations have been developed over past two decades to overcome these side effects by slowing down the release rate of formulations, like gels [5, 6], emulgels [7], lipid based gel [8], sprays [9, 10] etc. Application of spray formulation can also reduce the chances of secondary contamination of the wound site. Spray formulation for delivery of SSD developed by Foroutan et al. is aerosol formulations, where the drug is dispersed in an organic solvent acetone [9], whereas a non-aerosol spray gel has been patented by Lulla et al. [10]. Aerosol formulations need special containers and gels are difficult to spray. Therefore, present formulation was aimed to develop aqueous spray formulation, which can be easily sprayed on the open wound and also reduce the chances of secondary infection. Aqueous spray formulations are also cheaper and less likely to produce the stinging action of hydro-alcoholic sprays. Topical sprays prepared for many other drugs by different scientist also contain propellant or hydro alcoholic solvent systems [11-13].

EXPERIMENTAL SECTION

Materials

Silver Sulfadiazine was gift sample from SKant healthcare Pvt. Ltd., India. Low viscosity Hydroxypropyl methyl cellulose (HPMC E5LV) from Ozone International, India., polyethylene glycol (PEG 200) from Loba Chemie Pvt. Ltd, India., ammonia solution from Aatur Instruchem, India, sodium hydroxide and potassium dihydrogen phosphate from Rankem, India.

Methods

Preparation of spray

The spray solution was prepared by simple solution method. Briefly, the polymer was dissolved in water by constant stirring using magnetic stirrer. Drug was dissolved in ammonia solution and drug solution was added into a polymeric solution with constant stirring. Then the plasticizer and methyl paraben (0.1%) were added in solution and a final volume was made upto the mark with water.

Screening of components

Screening of polymer and plasticizer was based on preliminary batches decided using D-optimal design using main effect model. Eleven batches of SSD spray were prepared using different polymers (HPMC E5 and E50, PVP and Carbopol 934 and 940) and different plasticizers (PEG, Propylene glycol, Honey). The amount of silver sulfadiazine was kept constant (1%). The polymer and plasticizer were selected depending on their drying time, area of film, volume per spray.

Optimization

The formulation contains plasticizer and film forming polymer, which may have impact on properties like its drying time, area of film, and volume per spray & % drug release. Therefore, two independent variables were selected for optimization studies. Three levels (coded as -1, 0, +1) of these independent variables were decided to be studied. A factorial design 3^2 was applied, which resulted into 13 batches (Table 1). These batches were prepared and evaluated for dependent variables drying time (Y_1), area of film (Y_2), volume per spray (Y_3), % drug releases (Y_4).

Table 1: 3^2 Design with coded & actual values of independent variables

	X_1 = Conc. of Polymer		X_2 = Conc. of Plasticizer	
Run	**Coded values**	**Actual values (%)**	**Coded values**	**Actual values (%)***
1	-1	1.0	-1	30
2	0	1.5	-1	30
3	1	2.0	-1	30
4	-1	1.0	0	40
5	0	1.5	0	40
6	1	2.0	0	40
7	-1	1.0	1	50
8	0	1.5	1	50
9	1	2.0	1	50
10	0	1.5	0	40
11	0	1.5	0	40
12	0	1.5	0	40
13	0	1.5	0	40

** % of polymer concentration*

Characterization of topical spray

Drying Time

Film forming time was determined according to the method given Paradka et. al. [11]. Briefly, 5 sprays were actuated into a petridish and the time to form a film was recorded.

Volume per Spray

Volume of solution delivered upon each actuation was determined by method suggested by Lu et. al. [12] to calculate the number of sprays required to administer required amount of drug. Ten sprays were actuated in a measuring cylinder and an average was calculated as volume per spray.

Area of Film

The area of film was also measured by method suggested by Paradka *et. al* [11], with little modification. A water soluble dye, methylene blue was added to the spray solution, and 5 sprays (equivalent to one dose) were actuated on a paper and then the area of film was calculated by using equation πr^2.

Drug release study

The *in-vitro* drug release of silver sulfadiazine from prepared formulations was studied using Franz diffusion cell as suggested by Lu *et. al* [11]. A dialysis membrane was sandwiched between donor and receptor compartment of Franz diffusion cell. The temperature was kept constant at 32 ± 0.2^oC. One ml of spray solution was taken in the donor compartment and phosphate buffer pH 7.4 in receptor compartment. Diffusion medium was continuously stirred using magnetic stirrer to avoid diffusion layer effect. Samples were withdrawn at 30 min. intervals till approx. 90% of the drug is released from at least one of the formulation. The withdrawn samples were analyzed by UV spectrophotometer.

pH

The pH of formulation was determined using calibrated digital pH meter. About 25 ml of spray solution was taken in a small glass beaker and the electrode of pH meter was dipped into it for a minute and the pH was noted. The measurement of pH of each formulation was done in triplicate and mean values were calculated.

Drug content

One ml of spray solution was taken and its absorbance was determined using UV spectrophotometer after adequate dilution using water at 256 nm. Concentration was determined from the standard plot and the drug content was calculated as % of theoretical value.

$$\text{Drug Content} = \frac{\text{Actual Drug content}}{\text{Theoretical Drug content}} \text{ X } 100$$

Dose Uniformity

The dosing uniformity of the spray formulation was measured using method for dose uniformity prescribed by EMA [14] with little modification. Volume of 10 consecutive sprays was measured after every 40 sprays. Similarly, dose uniformity was performed to check chances of in-use clogging due to polymer deposition at the spray-nozzle. In this study total 50 sprays were sprayed after an interval of seven days.

Antimicrobial activity

In vitro antimicrobial activity was studied using cup-plate method. The cylinder plate method depends upon diffusion of antimicrobial drug from a vertical cylinder through a solidified agar layer in a petridish or plate to an extent such that growth of added micro-organism is prevented entirely in a zone around the cylinder containing antimicrobial agent [15]. Soybean-casein digest agar medium was prepared as it is the official nutrient medium for *Staphylococcus aureus* [16]. Overnight grown culture of *Staphylococcus aureus* was inoculated into the sterilized petridish containing soybean-casein digest agar media. After 20 minutes, wells were formed in agar plate and they were filled with the optimized formulation. Then it was incubated at 32.5 ± 2.5^oC for 24h. After 24h, the diameter of inhibition zone was noted.

In-vivo study

For *in-vivo* wound healing study, 12 healthy Wistar albino rats of 220 ± 20 g were used, after obtaining approval from CPCSEA 921/AC/05. Animals were maintained on standard animal feed and had free access to water. The animals were divided into two groups randomly, with six animals in each group. The abdominal hairs of the rats were shaved using an electric clipper carefully and incision wound was created with help of sterile cutter [17]. The first group was treated with the optimized formulation spraying 3 sprays, once daily and to the third group, equivalent dose of the marketed formulation i.e. silver sulfadiazine cream U.S.P. (~0.6 g) was applied daily. Then wound healing was checked for 14 days.

RESULTS AND DISCUSSION

Screening Studies

The results of screening batches indicated that out of different batches, the preliminary trial batch S10, containing HPMC E5 and PEG200, exhibited least drying time, highest volume per spray and area of film as compared to other batches (Table 2). The results indicate that use of HPMC as film forming polymer forms least viscous solution. This is evident from the fact that 1% HPMC solutions have greater area than 0.1% carbopol and takes less time to dry as film. So, HPMC E5 LV was selected as film forming polymer and PEG 200 as plasticizer.

Table 2: Screening for Film forming Polymer and Plasticizer

Ingredients	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Silver Sulfadiazine (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	**1.0**	1.0
Film forming polymers											
Carbopol 940 (%)	0.1	-	-	-	-	0.1	0.1	0.1	0.1	-	-
Carbopol 934 (%)	-	0.1	-	-	-	-	-	-	-	-	-
HPMC E5 LV (%)	-	-	1.0	-	-	-	-	-	-	**1.0**	1.0
PVP K 30 (%)	-	-	-	1.0	-	-	-	-	-	-	-
HPMC E 50 LV (%)	-	-	-		1.0	-	-	-	-	--	-
Plasticizer (% of polymer concentration)											
PEG 600	40	40	40	-	-	-	-	-	-	-	-
PEG 400	-	-	-	40	-	-	40	-	-	--	-
Propylene Glycol	-	-	-	-	40	40	-	-	-	-	40
PEG 200	-	-	-	-	-	-		40	-	**40**	-
Honey	-	-	-	-	-	-	-	-	40	--	-
Drying time (sec)	390± 2.02	330± 2.05	230 ± 2.07	310 ± 3.04	290 ± 3.02	340 ± 3.89	350 ± 2.98	330 ± 2.96	370 ± 2.87	**270 ± 2.98**	320 ± 2.76
Area of Film (cm^2)	78.5± 1.25	176.6± 1.98	162.7± 1.65	153.8± 1.47	226.8± 1.32	153.8± 1.62	200.9± 1.54	226.8± 1.54	176.6± 1.38	**283.3± 1.74**	226.8± 1.62
Volume per Spray (ml)	0.16± 0.02	0.18 ± 0.04	0.18 ± 0.03	0.20 ± 0.04	0.18 ± 0.05	0.18 ± 0.07	0.19 ± 0.05	0.20 ± 0.03	0.16 ± 0.04	**0.19 ± 0.04**	0.20 ± 0.07

Optimization

Optimization was done by using 3^2 Factorial design. Concentration of polymer (HPMC E5 LV) and concentration of plasticizer (PEG 200) was the independent variables used. Responses considered were drying time, area of film, volume per spray, % *in vitro* drug release at 270 min.

Drying time

Drying time of formulations varied from 270 sec to 330 sec (Table 3). With an increase in concentration of HPMC and PEG 200, increase in drying time was observed. The batch F1 showed the lowest drying time i.e. 270 sec and batch F9 showed highest drying time i.e. 330 Sec. The variation in a drying time may due to the HPMC E5 LV as it is a viscosity enhancing agent and also PEG 200 is also viscosity enhancing agent so due to this there is an increase in drying time with the increase in concentration of HPMC E5 LV and PEG 200.

Concentration of PEG showed greater impact on drying time. The data obtained were treated using Stat Ease Design Expert Software (DX7) and analysed statistically using analysis of variance (ANOVA). Significant effect of the conc. of HPMC E5 LV & PEG 200 were observed on drying time (p value <0.05). No interaction was found between the two independent variables (p value 0.518). Therefore, the equation was further reduced to:

$$Y_1 = +289.31 + 9.16A + 20.00B + 12.41B^2$$

Area of film

Area of film of formulations varied from 140.95 cm^2 to 186.17 cm^2. With an increase in concentration of HPMC and PEG 200, decrease in area of film was observed (Table 3). These may be due to the viscous nature of HPMC E5LV and PEG 200. As the concentration of HPMC E5 LV and PEG 200 increase, the viscosity of solution also increase and due to that there is a less volume of spray actuated so area of film is also decreasing with increase in the concentration of polymer and plasticizer.

A probability F 0.0006, which is less than 0.05, indicated that the model is significant. P value of independent factors A & B were also less than 0.05. Thus significant effect of the conc. of HPMCE5 LV & Conc. of PEG 200 was observed on the area of film. Similar to drying time, area of film is independent of interaction of independent variables. Therefore, the equation was further reduced to:

$$Y_2 = +163.82 - 10.49A - 11.32B$$

The negative coefficients of HPMC E5LV and PEG 200 showed that as the concentration of HPMC E5LV and PEG 200 increases, area of film decreases. Response surface plot (Fig. 1b) graphically shows that with an increase in concentration of HPMC E5LV and PEG 200, area of film decreases.

Table 3: Characteristics of Optimization batches F1 to F13

Batch	HPMC conc. (%)	PEG conc. (%)	Drying time (Sec)	Area of film (cm^2)	Volume of spray (ml)	%CDR at 270 min
F1	1.0	30	270 ± 3.02	186.17 + 1.65	0.19 ± 0.02	91.27 ± 1.54
F2	1.5	30	285 ± 2.78	171.94 + 1.62	0.18 ± 0.07	86.27 ± 1.23
F3	2.0	30	290 ± 2.96	162.77 + 1.74	0.16 ± 0.06	77.27 ± 1.51
F4	1.0	40	280 ± 2.89	176.00 ± 1.53	0.18 ± 0.06	89.51 ± 1.42
F5	1.5	40	290 ± 2.87	162.77 ± 1.78	0.17 ± 0.04	84.71 ± 1.84
F6	2.0	40	300 ± 3.54	158.28 ± 1.85	0.16 ± 0.03	76.13 ± 1.62
F7	1.0	50	315 ± 3.27	162.77 ± 1.06	0.17 ± 0.08	85.73 ± 1.51
F8	1.5	50	320 ±3.84	149.19 ± 1.42	0.16 ± 0.02	83.59 ± 1.86
F9	2.0	50	330 ± 3.42	140.95 ± 1.74	0.15 ± 0.04	70.91 ± 1.53
F10	1.5	40	285 ± 3.75	162.77 ± 1.27	0.18 ± 0.05	84.91 ± 1.96
F11	1.5	40	295 ± 3.14	171.94 ± 1.85	0.18 ± 0.08	85.94 ± 1.72
F12	1.5	40	290 ± 3.68	162.77 ± 1.98	0.16 ± 0.06	83.24 ± 1.24
F13	1.5	40	295 ± 3.41	158.28 ± 1.62	0.17 ± 0.09	85.96 ± 1.37

** % of Polymer Concentration*

Volume per spray

Volume per spray of formulations was found to be from 0.15 ml to 0.19 ml. With an increase in concentration of HPMC and PEG 200 it was found that there was decrease in volume per spray. The batch F1 showed highest volume per spray i.e. 0.19 ml and batch F9 showed lowest volume per spray i.e. 0.15 ml (Table 3). All the factors showed similar impact on vol. of spray as on area of film. Therefore, the equation was further reduced to

$$Y_3 = +0.17 - 0.011A - 8.33B$$

Increase in both independent factors cause a decrease in volume of spray, with conc. of plasticizer showing more negative impact on volume of spray.

***In vitro* drug release**

In vitro release of formulations was found to be varied from 70.91% to 91.27% in 270 mins. With an increase in concentration of HPMC and PEG 200 it was found that there was decrease in drug release. The batch F1 showed highest drug release i.e. 91.27% in 4 hours and batch F9 showed lowest % in vitro drug release i.e.70.91% in 270 mins. There was decrease in drug release with increase in concentration of HPMC E5 LV and PEG 200. These effect is been obtained due to the viscosity property of HPMC E5 LV and PEG 200. As the viscosity of solution increases the drug releases decreases, according to following equation:

$$Y_4 = +85.15 - 7.03A - 2.43B - 2.85A^2$$

Evaluation of Optimized Batch

The optimized batch was selected using Stat-Ease DX 7, keeping the goal of minimum drying time and release rate and maximum area of film and volume of delivery. Optimized formulation, containing 2% HPMC E5LV and 50% PEG, was clear and transparent homogeneous solution. The pH was found to be 7.6±0.3, drying time 332±4 sec, volume per spray 0.15±0.01 ml, Area of film 142.6±0.8 cm^2, drug content 95-100% and in vitro drug release 71±1.4% in 270 min.

Dose uniformity

The dose uniformity was performed to check chances of in-use clogging due to polymer deposition at the spray-nozzle by checking the volume of spray after an interval of seven days. The average volume per spray was found to be 0.16 ml initially, and after 7 days it was found to be 0.15 ml. So, it can be assumed that polymer is not deposited in the nozzle during in-use storage.

Antimicrobial activity

The zone of inhibition was found to be 24 mm in antimicrobial activity for optimized batch. The zone of inhibition was equivalent or more than plain drug suspension of similar concentration (Fig. 1). This proves that the formulation components do not cause adverse effect on the efficacy of silver sulfadiazine.

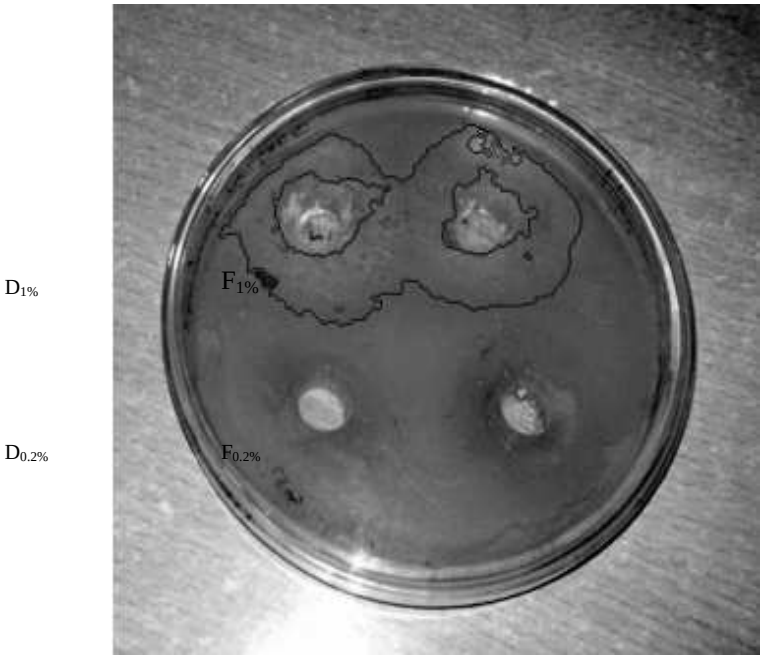

Figure 1: Comparison of antimicrobial activity of suspension (D) and spray formulation (F) of silver sulfadiazine

***In-vivo* study**

The wound healing activity conducted on albino rats revealed that the optimized formulation was equivalent to the marketed formulation in wound healing activity (Fig. 2).

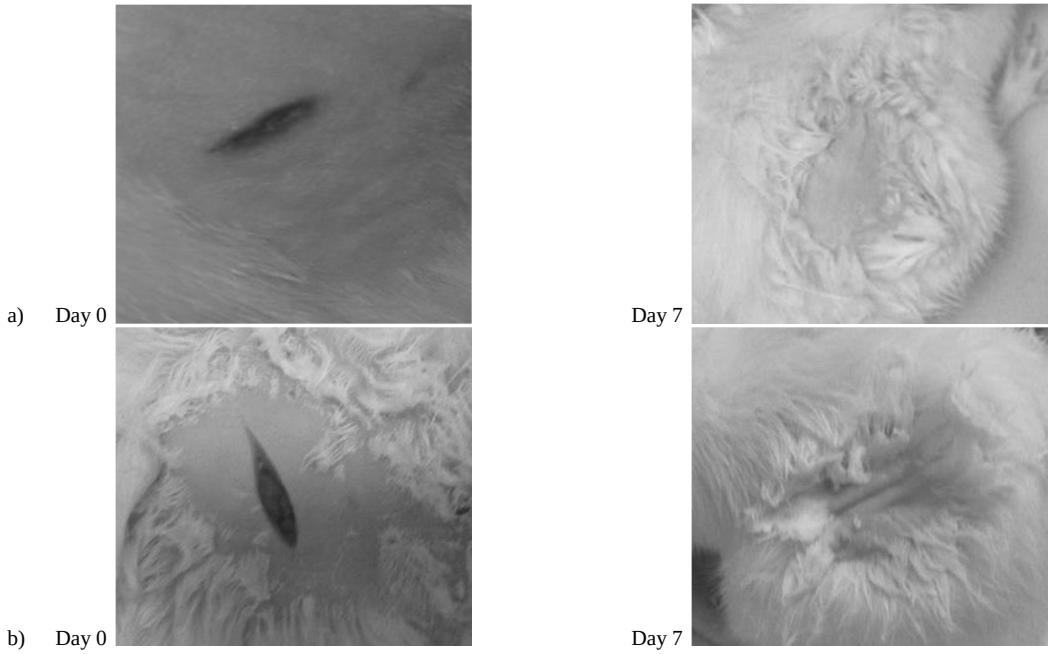

Figure 2: Effect of a) optimized spray formulation and b) marketed formulation of SSD

CONCLUSION

The present study showed that silver sulfadiazine spray could be used because of its advantages such as equivalent microbial activity compare to marketed formulation and more, eliminating potential contamination and greatly reducing pain associated with dressing changes, preserving a good physical and chemical stability, avoiding the need for rubbing the product on the skin, and guaranteeing content sterility through application, over the cream form.

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