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Development and evaluation of silver sulfadiazine loaded microsponge based gel for partial thickness (second degree) burn wounds

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ABSTRACT

Silver sulfadiazine has been frequently used as an antibacterial agent for topical treatment of partial thickness burn wounds. In this study, we present the preparation of silver sulfadiazine microsponges by w/o/w emulsion solvent evaporation method. Formulation variables were optimized by using 3^2 factorial design. The optimized microsponges were characterized by FTIR, DSC, PXRD, particle size analysis, SEM analysis and mercury intrusion porosimetry studies. Viscosity, texture analysis and *ex vivo* drug deposition study of optimized microsponge loaded gel were also evaluated. The safety of the optimized gel was assessed by MTT assay using epidermal keratinocyte (HaCaT) and mouse embryonic fibroblast (NIH-3T3) cell lines. *In vitro* antibacterial studies were carried out to compare the antibacterial inhibitory efficiency of the optimized gel against the commercial product. The efficacy of the optimized gel was evaluated by the partial thickness (second degree) burn wound model in mice. Optimized microsponge loaded gel enhanced the drug retaining capacity in the skin layers, by 3 fold higher to that of a commercial product. The antibacterial inhibitory efficiency of optimized gel was similar to the commercial product against the *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Optimized gel showed reduced frequency of application, no skin irritation, low cytotoxicity on dermal cell lines and enhanced wound contraction.

1. Introduction

Skin acts as a protective barrier and protects the body from intentional and accidental injuries. Burn injuries are significant causes of mortality and morbidity, and burn therapy usually requires prolonged hospitalization and rehabilitation which increases health care costs. Wound healing process is impaired and the healing time gets extended in burn injuries due to vascular obstruction (Church et al., 2006). Topical delivery of antibacterial agents becomes an essential component of the burn wound therapy due to vascular obstruction which hinders the transport of systematically administered antibiotics to the burned area (Fajardo et al., 2013). Topical therapy allows the delivery of active ingredient directly to the targeted area.

The ultimate goal of burn wound therapy is to prevent the infection effectively and promote the healing process. Burn wound demands topical treatment of antibacterial agents to prevent infection, reduce wound eschar formation, and promote wound healing process (Cribbs et al., 1998). Partial thickness (second degree) burns mainly affect the epidermis and hypodermis thereby increasing the susceptibility for an infection (Ito et al., 2015). *Pseudomonas aeruginosa and Staphylococcus aureus* are the most common opportunistic pathogens generally associated with burn wound infections. Moreover, untreated burn wound infection delays healing, encourages scarring and may result in bacteremia, sepsis or multiple-organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention (Church et al., 2006).

Silver sulfadiazine (SSD) is widely used as an antibacterial agent for topical treatment of burn wounds (Miller et al., 2012). The existing marketed products of SSD are usually available as a 1% *w*/w cream. The drawbacks associated with the existing commercial products of SSD are immediate burst release of the drug into the exposed targeted areas, increased frequency of application (usually two to four times daily), increased inflammation by the vehicles of the product, cytotoxicity towards keratinocytes and fibroblasts, pseudo eschar formation and heavy metal poisoning in case of long term usage (Sandri et al., 2014; Brandt et al., 2012; Muller et al., 2003 and Tsipouras et al., 1995). The adverse effects associated with immediate burst release of SSD have increased the sensitivity of the skin towards sunlight, blistering, peeling or loosening of the skin, skin lesions, intense itching of burn wounds, skin rashes, allergic reactions and cytotoxicity. All of these contribute to retard the healing process (Shao et al., 2015 and Fuller and Frederick, 2009). Therefore, an alternative strategy is required to control the release rate of SSD and promote the drug efficiency. In this context, a recent interest had developed to use microsponge based gels for

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achieving the sustained release of active pharmaceutical ingredients (APIs).

Microsponge drug delivery system is an advance delivery system that could act as a locally targeted delayed release drug depot (Kumar and Ghosh, 2015). Microsponges are porous polymeric microspheres usually 5–300 μm in diameter. Microsponges are spherical particles enriched with full of lacunae can entrap a wide variety of APIs (Hong et al., 2015 and Kaity et al., 2010).

Currently, there is no commercially available product with sustained release topical preparation of SSD. Recently a report described the sustained SSD delivery through bio-polymeric wafers for the treatment of infected chronic wounds (Boateng et al., 2015) which validated the necessity of sustained release topical dosage form of SSD. Hence, in the present investigation we aimed to fabricate a sustained release topical preparation of SSD by incorporating SSD into microsponges and dispersing the drug loaded microsponges into carbopol gel as a carrier. SSD is a highly ionizable drug. Formulation of ionizable drug into microsponges by w/o/w emulsion solvent evaporation technique is a challenging task to the formulator. A few reports are available where entrapment of ionizable drug into microsponges or microspheres (Ahmed et al., 2014; Jones and Pearce, 1995 and Rojas et al., 1999) has been illustrated. If the drug gets ionized during formulation, it drastically affects the entrapment of the drug as the most of the drug gets leaches into the external aqueous phase. Thus, a strategy is required to maintain the drug in unionized state during the formulation and prevent its leaching into the external aqueous phase. The formulation parameters were statistically optimized using 3^2 factorial design. The effects of independent variables Polymer amount (X_1) and pH of external aqueous phase (X_2) on the drug entrapment efficiency (Y_1), particle size (Y_2) and percentage cumulative drug permeated at 8 h (Y_3) were evaluated. Response surface plots and contour plots assisted in optimizing the formulation.

Finally, the optimized formulations were characterized for particle size analysis, mercury intrusion porosimetry study, scanning electron microscopy (SEM), Fourier transform infrared analysis (FTIR), differential scanning calorimetric analysis (DSC) and powder X-ray diffraction analysis (PXRD). Texture analysis was used to evaluate the mechanical properties of the optimized gel. Safety of the optimized formulation gel was assessed by MTT assay using epidermal keratinocyte (HaCaT) cell line and mouse embryonic fibroblast (NIH-3T3) cell line. Acute skin irritation study was performed in New Zealand white albino rabbits. *In vitro* antibacterial studies were evaluated to compare the antibacterial inhibitory efficiency of the optimized formulation gel against the commercial product. The efficacy of the optimized formulation gel was evaluated by partial thickness burn wound model in Swiss albino mice and the results were compared with the commercial product. The efficiency increased by 3 times than that of the commercial product.

2. Materials and methods

2.1. Materials

Silver sulfadiazine (SSD) was purchased from Yarrow Chemicals., Mumbai, India. Xanthan gum (XG) was obtained as gift sample from Hindustan Gum and Chemical Limited, Bhiwani, India. Ethyl cellulose (18–22 cps viscosity grade) (EC) was obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. All other reagents (Carbopol 934, triethanolamine were purchased from Loba Chemie Pvt. Ltd., Mumbai, India.) obtained commercially were of analytical grade and used as received. Milli-Q® grade water was used in all the experiments. NIH 3T3 (Mouse embryonic fibroblast) cell line and HaCaT (Human keratinocyte) cell line was received from National Centre for Cell Sciences (NCCS), Pune, India. 3 - (4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Dulbecco's modified eagle's medium (DMEM) and trypsin were procured from Sigma-Aldrich Co, St Louis, USA.

2.2. Methods

2.2.1. Preparation of microsponges and microsponge loaded gel and their statistical optimization

2.2.1.1. Preparation of microsponges. The microsponges (MSPs) were prepared by w/o/w emulsion solvent evaporation method as reported by Orlu et al., 2006. Accurately weighed 400 mg of SSD was dispersed in 1 mL of 0.1% *w/v* xanthan gum solution to form an aqueous phase. The polymer solution was prepared by dissolving ethyl cellulose (X_1 = 400, 800 and 1200 mg) in a mixture of dichloromethane and methanol (25 mL DCM + 5 mL methanol) to form an organic phase. The aqueous phase was added dropwise to organic phase under continuous stirring to form a primary (w/o) emulsion. The external aqueous phase was prepared by dissolving 1 g of PVA in 100 mL of water under continuous stirring at a temperature of 60 °C to form a clear solution. The pH of external aqueous phase (X_2 = 2.0, 2.5 and 3.0) was adjusted by the using of 1 *N* HCl. The primary (w/o) emulsion was poured into the external aqueous phase (1% *w/v* aqueous PVA solution) with continuous stirring at 500 rpm to form w/o/w emulsion. Following 2 h of stirring, the mixture was filtered to separate the microsponges (MSPs). The MSPs were dried in an air-heated oven at 40 °C for 12 h.

2.2.1.2. Factorial design. A 3^2 full factorial design (Two factors, three levels) was used to optimize SSD MSPs. Total of nine formulations of SSD MSPs were prepared by varying two independent variables.

Polymer amount (X_1) and pH of external aqueous phase (X_2) were considered as independent variables whereas percentage drug entrapment efficiency (Y_1), particle size (Y_2) and percentage cumulative drug permeated at 8 h (Y_3) were considered as dependent responses. The effects of various independent variables upon dependent responses were modeled using following mathematical model equation generated by 3^2 factorial design (Acharya et al., 2014).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

Design Expert software (Design Expert 8 Trial version 8.0.7.1., Stat Ease Inc., Minneapolis, Minnesota) was used to structure the design layout and analyze the data from all formulations.

The experimental design was validated by preparing the four batches of optimized formulations. The responses of optimized formulations were evaluated. The predicted values were compared with experimental values and the % of bias was estimated.

2.2.1.3. Drug entrapment efficiency. 20 mg of drug loaded ethyl cellulose MSPs were dissolved in 2 mL of dichloromethane and methanol mixture, 48 mL of 0.05% *v/v* aqueous ammonia solution was added and the mixture was heated at 50–55 °C for 30 min. The final volume was adjusted to 50 mL with the same aqueous ammonia solution. After cooling and filtration a clear phase was obtained. It was analyzed spectrophotometrically (UV-1800, Shimadzu, Japan) at 241 nm after suitable dilution with aqueous ammonia solution.

The drug content and the drug entrapment efficiency of MSPs were calculated. Each test was performed in triplicate and represented as average ± SD. The drug entrapment efficiency was calculated by the following relationship.

$$\%\text{Drug entrapment efficiency} = [\text{Actual Drug Content in Microsponges}/\text{Theoretical Drug Content}] \times 100 \quad (2)$$

2.2.1.4. Particle size determination. Particle size distribution of SSD microsponges were measured by laser diffraction technique (Malvern Mastersizer, Mastersizer 2000, Malvern Instruments, UK) where water