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Formulation and Evaluation of Innovative Anti-septic Solution For Wound healing

Garemella Lakshmi Gayathri, Narsa Reddy K, Amit B Patil*

Department of Pharmaceutics, Industrial Pharmacy, JSS College of Pharmacy, Mysuru JSS Academy of Higher Education and Research, Mysuru-570015, Karnataka, India

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ABSTRACT

Wound healing is a complex process in which the skin and their tissues are undergone self repairing after injury. The wound healing is not only complex but also fragile, and also susceptible to interruption or failure leading to the formulation of non healing chronic wounds, Tissue repair is a simple linear process in which the growth factor cause the proliferation which eventually leads to the skin healing process. Currently in market there is no antiseptic spray solution is available for wound healing or retention process. The objective of current work is to formulate and optimize and evaluate a innovative wound healing solution. Silver sulfadiazine (0.5%) was optimized and finalized the concentration of excipients to make a prototype formulation. The batches were prepared under room temperature and it contains ingredients like 3% of PVP k30 which is used as thickening agent and stabilizing agent and it enhances retentivity property, 5% preservatives, 0.05% rosemary oil is used as a flavour, 0.05% brilliant blue was used as a colouring agent. Evaluation studies such as Appearance, Retentivity, Sensorial analysis was done. Silver sulfadiazine content was determined on the given stability condition and content found to be within specification.

*Corresponding Author

Name: Amit B Patil
Phone: +917975787977
Email: amitbpatil@jssuni.edu.in

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INTRODUCTION

Wound healing is a essential physiological process consisting of collabration of many cell strains and their products(1).attempts to restore lesion induced by local aggression beginning of inflammatory stage. In the end, they result in repair, three collagen and regeneration which corresponds to the process of cell proliferation and posterior differencation through pre-existing cells in the tissues or stem cells (Eming *et al.*, 2007). Due to trauma or resulting from specific pathological condition one lesion created by all stimuli that break physical continuity of functional tissues. Lesions can be external or internal physiological chemical electrical or thermal. Moreover, the lesions can result in damage to specific organelles or to cells as a whole (Shaw and Martin, 2009).

Tissue repair is the simple linear process in which the growth factors cause cell proliferation leading to an integrated dynamic changes that involves soluble mediators blood cells production of extra cellular matrix and parenchymal cells. The skin healing process according to Mitchel et al., illustrates the principle repair of majority of tissues.

Stages of wound healing,

1. Inflammatory stage

2. Proliferatory stage
3. Re modelling stage

Classification of wounds

Wounds can be classified according to various criteria. Time is an important factor in injury management and wound repairing.wounds can be clinically classified into acute and chronic according to the time frame of healing (Farrington-Rock *et al.*, 2004; Martin, 1997; Medrado *et al.*, 2003).

Acute Wounds

Wounds that repair themselves and that proceed normally by following a timely and orderly healing pathway with the end result of both functional and anatomical restoration are classified as acute wounds.the time course of healing usually ranges from 5 to10 days or maximum of 30 days. Acute wounds can be acquired as a result of traumatic loss of tissues or surgical procedure (Martin, 1997).

For ex an operation to remove soft tissue toumr located in skin and underlying parenchyma can sometimes result in large albeit no contaminated wound that cannot be healed by primary intentions or defective tissues. Traumatic wounds are frequently encountered they may involve only soft tissues or might be associated with bone fractures the combined injuries have been classified by classification system of AO foundation which is one of the most comprehensive and widely used included within this classification system are closed and open fractures with assessment of skin muscle tendons and neurovascular injuries (Ribatti *et al.*, 2011).

Chronic Wounds

These are those that fail to progress through normal stages of healing and they cannot be repaired in a orderly and timely manner (Nayak *et al.*, 2009).

The healing process is incomplete and disturbed by various factors which prolong one or more stages in the phases of haemostatic inflammation proliferation or remodelling. These factors include infection tissue hypoxia necrosis extrudates and levels of excess inflammatory cytokines (Armulik *et al.*, 2011).

A continuous state of inflammation in a wound creates a cascade of tissue response that together perpetuate a nonhealing state. Because the healing process is in an uncoordinated manner functional and anatomical outcome are poor. These wounds are frequently relapse (Xian, 2006). They include naturopathic pressure arterial and venous insufficiency burns and vasculitis (Xian, 2006; Alon and Nourshargh, 2013).

Complicated Wounds

A complicated wound is a special entity and is defined as a combination of an infection and tissue defect (Medrado *et al.*, 2003). Infection poses a constant threat to the wound which causes a defect in contrast, evolves due to the traumatic or post infectious aetiology or a wide tissue resection e.g. tumour management. Every wound gets contaminated irrespective of cause size location and management. Typical characteristics of infection are redness, heat pain, edema loss or limited function of affected part.

According to degree of contamination wounds are classified into three groups

1. Aseptic wounds (bone and joint operations)
2. Contaminated wounds (abdominal and lung operations)
3. Septicwounds(abscesses bowel operations)

Factors Affecting Wound Healing

Many factors controlling the efficiency speed and manner of wound healing falls under two types local and systemic factors.

Local factors

Moisture keeping a wound moist rather than dry makes wound healing more rapid and with less pain and scaling

Mechanical factors

Edem, Ionizing radiation, Faculty technique of wound closure, Ischemia and necrosis, Low oxygen tension, Perfusion

Systemic factors

Nutrients -malnutrition or nutritional deficiencies, Metabolic disease, Immuno suppressants, Connected tissue disorders, Smoking, Age, Alcohol, Metabolic disease, ImmunosuppressantsConnected tissue disorders

Complications of Wound Healing

1. Deficient scar formation - results in wound dehiscence or rupture of the wound due to the inadequate information of granulation tissue.
2. Excessive scar formation
3. Exuberant granulation (proud flesh)
4. Deficient contraction or excessive skin contraction(skin grafts, in burns)

MATERIALS AND METHODS

Prototype Formulation Development

To develop a stable prototype formulation, optimization studies were planned to get a product of below mentioned attributes

- Desired Sensorials
- Stable formulation
- Ease of manufacturing
- Cost effectiveness

Optimization Studies

Various concentrations of Isopropyl alcohol, Benzyl benzoate, polymer, colour, rosemary oil are taken by which trial and error method is done till optimized formulation is achieved.

Method of wound healing antiseptic liquid

Silver sulfadiazine was weighed accurately and dissolved in a clean, dry beaker containing water and mix it properly for 5 min. PVP K30 was weighed accurately dissolved in another beaker of water until it gets clear and this solution was added to the above solution. Benzyl benzoate, IPA, and rosemary oil were weighed accurately and added in another beaker under constant mixing and add this solution to the above solution (PVP K30 in water). Finally, made up to the final weight with water and mixed it for further 15 min (Table 1).

Evaluation Studies

Physiochemical analysis

Appearances

The prepared formulations are evaluated in terms of colour, odour, consistency and fluidity

Ph. determination

The ph of the gels determined by using digital meter at room temperature.

Specific gravity

It's the ration of the density of the substance ton the ration of the reference substance for the same given volume

Specific gravity =

$$\frac{(W3 - W1)\ g}{(W2 - W1)g}$$

Retentivity

The ability of the substance to retain on skin area and its done manually and the retention is about 6 hours and its found to be 10 ppms.

Content Uniformity Studies

The content of the liquid dosage form can be determined by active ingredients extraction and then using the analytical procedures such as titrations, to determine the quantitative in the respective amounts. The choice of method of extraction from the dosage form will depend on the physicochemical properties of the active ingredients in the formulations (Rodero and Khosrotehrani, 2010).

Sample preparation and titration

Pipette 2.5ml of the sample in 250 ml volumetric flask and diluted it up to mark with distilled water.

Sample titration

Pippet out 25ml of the sample solution into 250ml volumetric flask and to make the solution alkali add 10% of sodium carbonated solution. Add 50ml of chloroform and SLS solution along with 5to 10 drops of the bromo phenol indicator and titrate with 0.001 M standard silver sulfadiazine solution by shaking, after addition of the each titrant till the end point of appearance of the permanent blue in chloroform layers with rapid separation of aqueous and chloroform layers

Make a control solution with all the above reagents and 25ml distilled water instead of the sample solutions and continuous with the titration as mentioned above.

Stability studies

Formulation is packed in high density polyethylene and studies were carried out in stability chambers at 30°C and 65% RH and 45°C and 75% RH as per ICH guide lines (Table 2).

RESULTS AND DISCUSSION

Optimization Studies

Optimization of polymers

Optimization of polymers was done to check for the sensorial performance pf PVP K30 based following,

The formulation ASPO1,ASPO2, ASPO3, ASPO4, ASPO5 checked for polymer appearance, stickiness and ease of uses where its determined in the range of 6-9 grade

Since we got very good product flow with 3% W/V PVP K30 and it was acting as emulsifier cum gelling agent. It was decided to use PVP K30 as gelling and emulsifying agents and further trial were conducted using PVP K30

- It is cost effective
- It consumes less time

Table 1: Different solution formulations were prepared with the final optimized formula base

Batch Number	Ingredients						
	Silver sulfadi-azine	PVP K30	IPA	Benzyl benzoate	Rosemary oil	Brilliant blue	Water
ASPO11	0.5	3	3	0.01	0.05	0.05	Qs
ASPO12	0.5	3	10	0.01	0.05	0.05	Qs
ASPO13	0.5	3	10	0.01	0.05	0.05	Qs
ASPO13	0.5	3	3	0.01	0.05	0.05	Qs
ASPO14	0.5	3	3	0.01	0.05	0.05	Qs
ASPO15	0.5	3	3	0.01	0.05	0.05	Qs

Table 2: Stability conditions

Sl No	Stability conditions	Intervals
1	45°C and 75% RH	1,2,3 and 6 months
2	30°C and 65% RH	3 and 6 months

Table 3: Retentivity Analysis

Sl.No	Attributes	Individual Batches	Time of Retentivity (Hr)			
			2	4	6	8
1	Retentivity	ASAPO01	100	25	0	0
2		ASAPO02	100	75	50	25
3		ASAPO03	100	50	25	10
4		ASAPO04	100	25	10	0
5		ASAPO05	100	75	25	10

Table 4: Retentivity Score

Sl.No	Attributes	Individual	Score			
			A	B	C	D
1	Retentivity(6HR)	ASAPO01	2	1	1	2
2		ASAPO02	4	3	3	2
3		ASAPO03	3	2	2	1
4		ASAPO04	2	1	2	1
5		ASAPO05	2	1	2	3

- Retentivity period is more

Optimization of IPA

The formulation ASPO1,ASPO2, ASPO3, ASPO4, ASPO5 checked for polymer appearance, stickiness and ease of uses where its determined in the range of 7-9 grade. The formulation ASPO02 is best formulation obtained.

Optimization of Perfume concentration

A base was prepared with batch no-ASPO02 with above finalized batches pf excipient and divided into 3 parts

ASPO02 – A contains 0.05% concentration of fragrance

ASPO05 – B contains 0.04% concentration of fragrance

Evaluation Studies

Physiochemical Analysis

Appearance

The prepared formulation were evaluated in terms of colour were found to be stable and has a pleasant

Table 5: Sensorial Analysis Results

Sl.No	Attributes	Score 1	Score 10	Rating
		Pre-Use Product Sensorials		
1	Appearance	Extremely Disliked	Extremely liked	10
2	Fragrance Profile Like ability	Extremely difficult	Extremely easy	10
3	Fragrance Profile	Extremely Disliked	Extremely like	8
4	Fragrance strength	Extremely strong	Extremely mild	4
5	Fragrance strength Likeability	Extremely Disliked	Extremely like	8
		In-Use Product Sensorials		
1	Retentivity	Extremely sticky	Not at all sticky	1
2	Fragrance profile	Extremely disliked	Extremely like	8
3	Fragrance strength	Extremely strong	Extremely mild	4
4	Fragrance strength Likeability	Extremely disliked	Extremely like	8
		Post-Use Product Sensorials		
1	Stickiness	Extremely sticky	Not at all sticky	1
2	Fragrance profile	Extremely strong	Extremely weak	2
3	Fragrance strength	Extremely strong	Extremely mild	2
4	Fragrance strength likeability	Extremely disliked	Extremely like	2
5	Fragrance profile likeability	Extremely disliked	Extremely like	2

Table 6: Sensorial Analysis Result

Sl.No	Attributes	Score 1	Score 10	Rating
		Pre- use Product sensorials		
1	Appearance	Extremely disliked	Extremely liked	10
2	Ease of use	Extremely Difficult	Extremely Easy	10
3	Fragrance profile	Extremely Disliked	Extremely like	9
4	Fragrance Strength	Extremely Strong	Extremely mild	4
5	Fragrance strength Likeability	Extremely Disliked	Extremely like	8
		In-Use product Sensorials		
1	Stickiness	Extremely Sticky	Not at all Sticky	7
2	Fragrance strength	Extremely Disliked	Extremely Like	9
3	Fragrance Profile	Extremely strong	Extremely Mild	5
4	Fragrance Strength Likeability	Extremely Disliked	Extremely like	8
		Post-use product Sensorials (after 6 Hours)		
1	Stickiness	Extremely Sticky	Not at all sticky	3
2	Fragrance strength	Extremely strong	Extremely mild	3
3	Fragrance strength Likeability	Extremely Disliked	Extremely like	4
4	Fragrance profile	Extremely Disliked	Extremely like	3

smell

pH Determination

The pH of the solution was found to be 6 – 7

Specific Gravity

Specific gravity is the ratio of the density of the substance to the density of the reference substance for the same given volume

Specific gravity =

$$\frac{(w3-w1)g}{(w2-w1)g}$$

$$= \frac{42.1-18}{43.2-18}$$

$$= 0.956$$

Table 7: Sensorial Analysis Result Score

Sl. No	Attributes	Score 1	Score 10	Rating
		Pre- use Product Sensorials		
1	Appearance	Extremely disliked	Extremely liked	10
2	Ease of use	Extremely Difficult	Extremely Easy	10
3	Fragrance profile	Extremely Disliked	Extremely like	8
4	Fragrance Strength	Extremely Strong	Extremely Mild	4
5	Fragrance strength Likeability	Extremely Disliked	Extremely like	7
		In-Use product Sensorials		
1	Fragrance Profile	Extremely Disliked	Extremely Like	8
2	Fragrance Strength	Extremely strong	Extremely Mild	4
3	Fragrance Strength Likeability	Extremely Disliked	Extremely like	7
		Post-use product Sensorials (after 6 Hours)		
1	Stickiness	Not at all Sticky	Extremely sticky	2
2	Fragrance Profile	Extremely Disliked	Extremely Like	2
3	Fragrance strength	Extremely Strong	Extremely Mild	1
4	Fragrance Strength likeability	Extremely Disliked	Extremely like	1

Where,

W1= weight of empty specific gravity bottle

W2 = weight of empty specific gravity bottle + D1 water

W3= weight of empty specific gravity bottle + Sample

Retentivity

The ability of a substance to retain or resist on skin and it is frequently shown manually, it was found that to be stable for 6hrs on the skin and the retentivity is found to be 10 ppm

Retentivity study procedure

For the study of retentivity of formulated solution a group of five panel list have been chosen and optimized formulation is selected based on the feedback obtained from the participants (Tables 3, 4, 5, 6 and 7).

Report

From the above studies performed based on the concentration retentivity of the drug, ASPO2 formulation was found to be long lasting and it was also found to be selective and compatible to more number of patients.

Content Uniformity

Content Uniformity of the Formulation was done by sampling the manufactured batch of the three samples (F-1 , F-2 ,F-3) manufacturing vessel and samples were started titrated. It was found that active was uniformity distributed throughout gel matrix.

Summary

The aim of the study was to formulate the antiseptic liquid for wound healing and its retention properties.

All proposed excipients for the formulation of the antiseptic solution were found to be good,Sincewe got a very good product rententivity with 3% PVP 30K and its was acting emulsifier cum gelling agent. Ease of manufacturing was also quite easy with PVP K30 Its cost effective. It consumes less time.

Among the batches taken for different concentration of IAP, it was observed that the emolliency level of the batch with 5% (ASPO02) of IAP is equivalent to batch with 10 % (ASPO03) of IAP. So it was decided to go with 5% (ASPO02) of IAP in the formulation to have desired moisturized feel and reproducible batch was prepared.

Fragrance with 0.05%(ASPO02) has got high score in evaluation of the product fragrance, batch 0.04% fragrance was not liked much due to low impact and 0.06% (ASPO06) fragrance was intense. So it was decided to keep the fragrance level at 0.06% (ASPO06) while the colour with 0.05%has got high score in evaluation. The final mixing time is optimized as 15 min. the physical status of the prepared antiseptic spray solution was blue clear with ph of 6-7.

The active ingredient in the optimized formulation was found to be uniformly distributed well within the product specification limit.

Formulation is packed in high density polyethylene

and stabilitystudies were carried out in stability chambers at 30°C with 65% RH and 45°c with 75% RH as per ICH guide lines for 3 months and was found to be stable.

CONCLUSIONS

Formulation development and optimization of the antiseptic solution with enhanced sensorial which may help in treating wound.A simple solubility method was employed for the development of antiseptic solution.PVP K30 AND IAP are successfully utilized for the development of retention properties of antiseptic solution containing silver sulfadiazine for treatment of wound healing. The optimized formulation was stable up to 3 months at 40°Cwith 75% RH and 30°C with 65%RH. Among all the formulation ASPO02 formulation was found to be long lasting and its more selective by the patients.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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