FORMULATION AND EVALUATION OF TOPICAL PREPARATION CONTAINING ANTIBACTERIAL DRUG LOADED METALLIC NANOPARTICLES FOR WOUND HEALING

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Abstract- Wound is damage to the integrity of biological tissue, including skin, mucous membranes, and organ tissues. Various types of traumas can cause these, and it is critical to ensure wounds are cleaned and appropriately dressed to limit the spread of infection and further injury. The skin has vital role of regulating fluid balance, infection control, and thermogenesis. Disruption of this regenerating protective layer can be devastating to the patient and society. More than 2 million burn cases and 7 million chronic skin ulcers caused by pressure, arterial or venous insufficiency, and diabetes mellitus each year in the United States alone is affected by abnormal wound healing. This translates to annual costs of $9 billion in attempt to reduce the major disability and consequent death of such severe skin injury. In everyday pathology, wounds remain a challenging clinical problem, with early and late complications presenting a frequent cause of morbidity and mortality. The immense social and economic impact of wounds worldwide is a consequence of their high rate of occurrence in general and their increasing frequency in the ageing population.

Keywords-Topical Preparation. Antibacterial drugs, Wound Healing

NANOPARTICLES:
Nanoparticles can be defined as objects ranging in size from 1 - 100 nm that due to their size may differ from the bulk material. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver Nanoparticles (NPs) research is an emerging branch of science. Tuning size and shape of NPs alter their properties and offer huge opportunities for surprising discoveries. Nanoparticles can be classified into different types according to the size, morphology, physical and chemical properties. They are as follows
- Carbon-based nanoparticles,
- Ceramic nanoparticles,
- Metal nanoparticles,
- Semiconductor nanoparticles,
- Polymeric nanoparticles
- Lipid-based nanoparticles

Ideal Properties of nanoparticles:
- Nanoparticles are stable in blood
Nanoparticles are having nontoxic nature.
Non thrombogenic
Non inflammatory in nature is one of the property of nanoparticles.
The nanoparticles are Biodegradable

Metallic Nanoparticles:
Silver Nanoparticles
Silver nanoparticles (AgNPs) are a class of materials with sizes in the range 1–100 nm and atomic mass 107.87. The interest in the study of AgNPs with respect to their various different behaviors has recently increased because of their unique and attractive physical, chemical, and biological properties (Lee and Jun, 2019; Sánchez-López et al., 2020). AgNPs are also known to have unique properties in terms of toxicity, surface plasmon resonance, and electrical resistance.

I. Methods of preparation of silver nanoparticles:
A) Physical method
- Ball milling
- Laser ablation
- Chemical itching
- Vapor deposition

B) Chemical method
- Chemical reduction
- Photochemical method
- Son chemical method
- Electrochemical method

C) Biological method
- Bacteria mediated
- Fungi mediated
- Algae mediated
- Plant mediated

D) Microwave approach
- Polyol method

II. Chemical Reduction Method:
The chemical approach is widely used for synthesizing AgNPs using water or organic solvents. It is an easy way to synthesize AgNPs in solution. However, a certain amount of toxic material may be produced as residue.

Some reducing agents such as borohydride, citrate, ascorbate, and glucose have been used to address this problem. This is by continuing through a single process to generate a colored silver solution, this is due to the surface of a metal having free of charge electrons in the conduction band and positively charged nuclei

(i) Metal precursors,
(ii) Reducing agents
(iii) Stabilizing/Capping agents.

The initial nucleation and the subsequent growth of nuclei can be controlled by adjusting the reaction parameters such as temperature, pH, precursor types, solvents, reduction agents, and stabilizing/capping agents.
MATERIALS AND INSTRUMENTS

MATERIALS
The following drugs, polymers, excipients and chemicals were used for the formulation and characterization of drug loaded Nanoparticles and topical preparation.

List of materials

<table>
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<tr>
<th>MATERIALS</th>
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<tr>
<td>Nitrofurazone Silver Nitrate.</td>
<td>TCI CHEMICALS PVT. LTD</td>
</tr>
<tr>
<td>Sodium borohydride.</td>
<td>LOBA CHEMIE PVT.LTD.</td>
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<tr>
<td>Polyvinyl Pyrrolidone (PVP).</td>
<td>HIMEDIA PVT.LTD.</td>
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<tr>
<td>Polyvinyl alcohol (PVA).</td>
<td>SRL PVT.LTD.</td>
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CHEMICALS

- Polyethylene Glycol (PEG), Dimethyl Formamide (DMF)
  LOBA CHEMIE PVT.LTD.
- Potassium Dihydrogen Phosphate, Disodium Hydrogen Phosphate
  LOBA CHEMIE PVT.LTD.
- Bee’s wax, Cetosteryl alcohol, White Soft Paraffin
  SDFCL FINE CHEM LIMITED SDFCL FINE CHEM LIMITED LOBA CHEMIE PVT.LTD.
- Liquid Paraffin (Light), Propylene Glycol, Cetomacrogol 1000
  LOBA CHEMIE PVT.LTD.
- CRODA COLORCON. PVT LTD

Preservatives
- Methyl paraben, Propyl Paraben
  HIMEDIA PVT.LTD.

Preformulation:

1. Identification and confirmation of Nitrofurazone (NFZ)
   Identification and confirmation of NFZ were carried out by determination of melting point, Ultraviolet (UV) spectroscopy studies, Fourier transform infrared (FT-IR) spectroscopy studies,
   a) Determination of melting point of NFZ
      The digital melting point test apparatus was used for the determination of melting point. In this method, the drug NFZ was filled into a capillary tube which one bottom side is sealed, then capillary tube was dipped into the liquid paraffin bath and was slowly heated.
      The temperature range for the beginning of melting and the completion of the melting process was noted(ALGAS ORGANICS InterLab, 2019; No, 2021a, 2021b).
   b) Determination of partition coefficient of Nitrofurazone
      The partition coefficient study was performed using Shake flask method in that n-octanol as oil phase and distilled water or phosphate buffer saline pH 7.4 as the aqueous phase. Take the two phases in conical flasks and to each, 10 mg weighed the amount of NFZ was added.
      The flasks were at 37 oC for 6 h to achieve a complete partitioning.
      The two phases were separated by separating funnel and analyzed by UV-visible spectroscopy at λ max of the NFZ to determine the concentration of drug (w/v) against blank. The partition coefficient of NFZ Ko/w was calculated using equation (Cross et al., 2003; Srinivasarao et al., 2017).

Ultraviolet (UV) visible Spectroscopy Study of Nitrofurazone

i) Determination of λ max of Nitrofurazone (NFZ) in the Polyethylene Glycol 400 (PEG 400)
The stock solution was prepared by dissolving 10 mg of NFZ in 10 mL Polyethylene glycol (PEG) to obtain the working standard of 1000 (μg/mL). Standard solution, 0.1mL further diluted up to 10 mL with distilled water, two different volumetric flasks to obtain a sample of 10 (μg /mL). Then the solution was analyzed spectroscopically to determine λ max(Savjani, Gajjar and Savjani, 2012).

ii) Standard calibration curve in Polyethylene Glycol 400 (PEG 400)
From the standard stock solution 1000 (μg/mL), 1 mL was transfer to 10 mL volumetric flask using a micropipette and the volume was diluted up to 10 mL with distilled water to obtain100 (μg/mL) solution. From this, different concentrations were made by taking 0.2 0.4 0.6 0.8 1.0, 1.2, 1.4 and 1.6 mL and making the volume up to 10 mL of each with distilled water to obtain 2, 4, 6, 08, 10, 12, 14,16 (μg/mL) solutions respectively. The absorbance of each solution was taken at λ max and concentration versus absorbance was plotted(Sastry et al., 1992; Tubino, Vila and Palumbo, 2009; Paramelle et al., 2014; Pfeifer, Kemmer and Heinze, 2021).

iii) Determination of λ max of Nitrofurazone (NFZ) in the Dimethyl Formamide (DMF).
The stock solution was prepared by dissolving 10 mg of NFZ in 10 mL DMF to obtain the working standard of 1000 (μg/mL). Standard solution, 0.1mL further diluted up to 10 mL with distilled water, two different volumetric flasks to obtain a sample of 10 (μg /mL). Then the solution was analyzed spectroscopically to determine λ max.

iv) Standard calibration Curve in Dimethyl Formamide (DMF)
From the standard stock solution 1000 (μg/mL), 1 mL was transfer to 10 mL volumetric flask using a micropipette and the volume was diluted up to 10 mL with distilled water to obtain100 (μg/mL) solution. From this, different concentrations were made by taking 0.2 0.4 0.6 0.8 1.0, 1.2, 1.4 and 1.6 mL and making the volume up to 10 mL of each with distilled water to obtain 2, 4, 6, 08, 10, 12, 14, and 16 (μg /mL) solutions respectively. The absorbance of each solution was taken at λ max and concentration versus absorbance was plotted.

Fourier Transform Infrared (FT-IR) spectroscopy of Nitrofurazone
The alpha Bruker FTIR instrument is used for determination. The sample of the Nitrofurazone was triturated and mixed well with IR grade potassium brom ide 1:100 ratios. The mixture was place in the sample holder of the FT-IR instrument and scanned in the range from 4000 to 400 cm⁻¹ to obtain the spectrum. The FT-IR spectrum obtained was compared with the standard FT-IR spectrum of the NFZ.

Drug-excipient compatibility studies
1. FT-IR Spectroscopy study of Nitrofurazone
The sample of the NFZ was triturated and mixed well with IR grade potassium bromide in 1:100 ratios. The mixture was place in the sample holder of the FT-IR instrument and scanned in the range from 4000 to 400 cm⁻¹ to obtain the spectrum. The alpha Bruker FTIR is used for determination.

2. FT-IR Spectroscopy study of silver nitrate (AgNO3)
The sample of the AgNO3 was triturated and mixed well with IR grade potassium bromide in 1:100 ratios. The mixture was place in the sample holder of the FT-IR instrument and scanned in the range from 4000 to 400 cm⁻¹ to obtain the spectrum. The alpha Bruker FTIR instrument is used for determination.

3. FT-IR Spectroscopy study of Nitrofurazone and Sodium borohydride (NaBH4)
The sample of the NFZ and Sodium borohydride was triturated and mixed well with IR grade potassium bromide in 1:100 ratios. The mixture was place in the sample holder of the FT-IR instrument and scanned in the range from 4000 to 400 cm⁻¹ to obtain the spectrum. The FT-IR spectrum obtained was compared with the standard FT-IR spectrum of the NFZ(Ghorab et al., 2016; Qais et al., 2019; Vega-Baudrit et al., 2019).
Formulation of Nitrofurazone loaded silver nanoparticles cream

1. Drug excipient compatibility study
The Fourier transform infrared spectroscopy study is used to identify the interaction and compatibility between the drug and excipient. NFZ were mixed with the emulsifying wax to find out the compatibility and different frequencies were observed by using alpha Bruker FTIR.

2. Method of preparation of cream
The oil phase was prepared by melting the waxes at 75°C and mixing the ingredients uniformly. The liquid solution of nitrofurazone loaded silver nanoparticles add into the oil phase. The aqueous phase was prepared by dissolving the water-soluble ingredients in deionized water. The water phase was warmed to 75–80°C until all ingredients were dissolved. When the water and oil phase were at the same temperature, the aqueous phase was slowly added to the oil phase with moderate agitation and was kept stirred until the temperature dropped to 40°C. The emulsion was cooled to room temperature to form a semisolid cream (Chen, Alexander and Baki, 2016; Rai, Poudyl and Das, 2019).

Evaluation of Nitrofurazone loaded silver nanoparticles cream
a) Physical appearance
The physical appearance of the cream observed by its colour & roughness.

b) Determination of pH:
The pH of the cream can be measured on a standard digital pH meter at room temperature by taking adequate amount of the formulation diluted with a 100 ml of pH 7.4 buffer solvent.

c) Spreadability
Where,
m = weight applied to upper slide. l = length moved on the glass slide. t = time taken.

d) Viscosity
Electronic pH meter
Adequate amount of sample is taken between two glass slides and a weight of 100gm is applied on the slides for 5 minutes. Spreadability can be expressed as,

\[ S = \frac{ML}{T} \]

A Brookfield instrument was used with concentric spindle #64 to determine the viscosity of optimized cream. These tests were carried out at room temperature. The spindle was allowed to rotate at 100 rpm value.

e) Homogeneity
The optimized cream was tested for the homogeneity by visual inspection and tested for their appearance and presence of any aggregates.

f) Drug content
To determine the amount of drug present in the formulation 10 gm of NFZ loaded AgNPs cream (was taken in 100 ml amber coloured volumetric flask to it 30ml distilled water was added and shaken for 30 min on mechanical shaker (REMI).

In-vitro diffusion study.
The diffusion studied by using static flow through cell Franz -Diffusion apparatus. The instrument was assembled for the study along with continuous circulation of water and in the accepter compartment the buffer pH 7.4 is added. The desirable cut of cellulose membrane is putted in such manner it should attach the upper layer of buffer 7.4 of donor compartment. One gram of cream is applied on the membrane and the sampling was done on predetermined intervals and analyzed by UV method.

Skin irritation study:-
On the basis of the results obtained from in vivo study, batch of nitrofurazone loaded silver nanoparticles cream was selected as an optimized formulation to investigate the dermatological safety and storage stability. In skin irritation
study, healthy male Wistar albino rats weighing between 250-300 gm were used. The backside of rats was carefully shaved and segregated into two groups containing six rats. The rats were treated with the cream (equivalent to the dose of 1 mg of nitrofurazone) and control (formalin) to the shaved area (0.4 cm²). The skin was observed carefully over 24 h for any signs of erythema and oedema which are indicators of irritation (Rupal et al., 2010; Pathan et al., 2019).

**Optimization of formulation by using 32 Design**

**a) Experimental design and analysis**

Full factorial design for two factors at three levels each was selected to optimize the response of variables. The two factors, the white soft paraffin (%) and Emulsifying wax (%) used varied and the factor levels were suitably coded. The viscosity, drug release and Spreadability were taken as the response variables. In the design, the two factors were evaluated each at three levels and experimental trials were executed for possible combinations. All other formulation variables and processing variables were kept constant throughout the studies.

The formulae were developed as 9 sets varying the variables following 32 full factorial design (3 levels) using Design expert. Dependent variables were Y1 = Viscosity, Y2 = Drug release and Y3 = Spreadability. The effect of two independent variables were recorded.

**Wound Healing activity**

**a) Screening of animal model**

Male and female Wister rats (150–200 g) were procured and performed the study Experimental protocol & Treatments

Untreated group Marketed Formulation NFZ-AgNPs cream formulation

Route of administration

- Topical

Number of animals: 6

**b) Excision wound model on rats**

Three groups of animals containing six in each groups taken. The animals anaesthetized under light ether anesthesia. One full thickness paravertebral incision of 1 cm² made including the cutaneous muscles of the depilated back of each rat. Wound area was measured by tracing the wound on a millimetre’s graph paper. The marketed formulation and NFZ-AgNPs, were applied to the wound injury models using an applicator. The all formulations were applied one time on the site of injury for 12 consecutive days at a specified time until the wound was completely healed. The number of days required for complete epithelialization was noted (Mukherjee, Verpoorte and Suresh, 2000; Excision made on the Wister rats of 1cm² for wound healing activity.)
c) Determination of wound recovery time

The optimized cream of NFZ loaded silver nanoparticles and marketed Nitrofurazone cream were applied with appropriate amount on the wounds and observed the days required to heal the wound completely.

The effect of optimized cream of Nitrofurazone loaded silver nanoparticles and marketed Nitrofurazone cream was recorded.

Stability studies

Accelerated stability study

The stability study was performed as per ICH guidelines. The optimized topical cream stability studies were studied for 30, 60 & 90 days at temperatures 4°C ± 1°C and 37°C ± 1°C.

REFERENCES:


Fig no.7.8. Excision made on the Wister rats of 1cm² for wound healing activity.