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Original Research Article

Formulation of Neomycin Sulfate Wound Dressing

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Abstract

Dermatological problems are always a worry in case of wounds¸ ulcers, warts etc., as they are infections caused by microorganisms. The infecting microorganism, or pathogen, interferes with normal function of the host cell and lead to chronic wound, gangrene, loss of an infected organ, and even death. In the present work formulation and development of non-adherent, absorbent ready-made medicated wound dressing has been designed with Neomycin sulfate which has antibacterial activity. The dressing contain drug impregnated cotton gauge supported by cotton pad with dressing cloth to tie on infected wound, entire readymade dressing will be sterile one. The formulated dressing were evaluated for absorption capacity of cotton used, weight variation, size, drug content uniformity, pH, FT-IR. In vitro drug release studies were carried out from the prepared gauge in phosphate buffer pH 7.5. Stability studies were conducted for a period of 6 months. Primary skin irritation test studies were carried out on guinea pigs/ rabbits and on healthy human volunteers with the permission of Ethical committee.

Keywords: Neomycin sulfate, cotton gauge, dressing.

INTRODUCTION

The skin often has been referred as the largest of the body organs with a surface area of about 2m². The total area of the skin ranges from about 2500 cm² at birth to 18000 cm² in the adults, when it weighs about 4.8 kg in men and 3.2 kg in women[1, 2]. A wound is defined as a physical injury where the skin or mucous membrane is torn, pierced, punctured, cut, or otherwise broken

Healing of skin wounds provides a classical example of combination of regeneration and repair[3] accomplished in one of the following two ways i.e. Healing by first intention (primary union); and Healing by second intention (secondary union)[4]. Traditional wound management involves disinfection, debridgement and provision of a moist environment to encourage the establishment of the best environment for natural healing process[5, 6]. Synthetic wound dressings consisted of two types; gauze – based dressings and paste bandages such as zinc past bandages. Wound dressings were introduced which delivered important characteristics of an ideal wound dressing: moisture keeping and absorbing (e.g. polyurethane foams, hydrocolloids) and moisture keeping and antibacterial (e.g. iodine – containing gels)[7].

New Generation of medicated dressing has to two part, drug containing gauze and porous membrane i.e. absorbent pad which absorbed exudates which exude from wound and maintain sufficient moist atmosphere around the wound which promotes healing. Drug containing porous membrane allowed the exudates to pass through it and absorb on absorbent pad while it release the drug which through percutaneous absorption reaches to the systematic circulation and promote the healing. In the present work formulation and development of non-adherent, absorbent ready-made medicated wound dressing has been designed with Neomycin sulfate which has antibacterial activity. The dressing contain drug impregnated cotton gauge supported by cotton pad with dressing cloth to tie on infected wound, entire readymade dressing will be sterile one.

MATERIALS AND METHODS

Neomycin sulfate was Gift sample from Anmol healthcare, Gujarat. White soft paraffin, hard paraffin, was purchased from S D fine chemical, Boisar. Liquid paraffin, Bees wax was purchased from Loba chemical, Mumbai.

Preparation of medicated gauge

To the mixture of base add require quantity of drug at same temperature, mix it thoroughly. Cotton gauze of appropriate size (4×4cm) is dipped in to the hot mixture for 5 second removes it from mixture and cool it to room temperature (Table-1). These wax impregnated medicated gauze is placed below the absorbent pad of same size having backing member with cotton gauge to fix the dressing on site of application. The prepared dressing was packed and sterilized by B-radiation in industry (Figure-1).

Sterilization of prepared dressing

Aluminum foil packed dressing were sent for β-radiation sterilization to Anmol healthcare, Gujarat. The prepared dressings were sterilized by a dose of 2.5×106 rods[8].

Evaluation of medicated gauge

- **Water absorption studies**

The study was carried out among the three cotton pads available in local market to find out and select best among them. Amount of water absorbs is determine by dipping specific size of cotton pad in beaker of water for specified period of time i.e. 10, 20, 30, 40, 50 and 60 second in different beaker. The difference in initial and final weight of pad gives water absorption and water holding capacity of absorption pad.

- **Weight variation and size**

The weight was determined by using digital weighing balance. The size of 4×4cm was chosen fro the preparation medicated gauge and complete dressing. Three samples were randomly chosen from each formulation were for a length determination and mean values were considered (Table-3).

- **pH determination**

pH of 10% w/v aqueous solution of the formulation were determine using digital pH meter.

- **Drug content uniformity:**

Single unite of medicated gauge was socked in 25ml of chloroform, slightly warm it, extract with 20ml of phosphate buffer of pH 7.5. Filter through Whatman filter paper in 50ml volumetric flask[10]. To these add 2ml of glycerin ninhydrine (0.1w/v) solution to volumetric flask make up the volume up to the mark. Heat on water bath for 15 min. the absorbance of solution is masseur at 570.5nm in Shimadzu UV-1700 spectrophotometer against appropriate solution as blank. The data was subjected to statistical analysis[11]. An average of three reading was considered.

- **In vitro drug release studies**

Single unit of Neomycin sulfate impregenetated gauze is directly placed in medium. At internal of every 15 minutes. 5 ml of samples were withdrawn from beaker and replaced by same quantity of fresh phosphate buffer pH 7.5 for period of 210 minutes[12]. To the sample add 2ml of glycerin ninhydrine (0.1% w/v) solution and heat for 15 min. on water bath. The concentrations of sample were estimated by measuring the absorbance at 570.5nm in Shimadzu UV – 1700, Spectrophotometer.

- **Primary skin irritation on Rabbits**

6 healthy rabbits (2 male and 1 female) were selected for the study. These rabbits were kept in three different cages and supplied with fresh food and water during the test period, 24 hours prior to test. Neck region portion was shaved to expose sufficiently large test area. The test site was cleaned with surgical spirit then medicated dressing is applied to test area. The test site was observed for erythema and edema for 24 hours, 48 hours & 72 hours after application. This test was conducted to evaluate the irritancy of the prepared medicated dressing on the intact skin of rabbit. None of the prepared medicated dressing showed any erythema or edema, indicating that the prepared formulations were non-irritant on the skin of rabbit. These studies were carried out in the animal house of M.R. Medical College, Gulbarga.

- **Primary skin irritation on Human volunteers**

6 healthy human were selected for the study. The test site (5×5 cm) of forehand was cleaned with surgical spirit then medicated dressing is applied to test area. The test site was observed for erythema and edema for 24 hours, 48 hours & 72 hours after application. This test was conducted to evaluate the irritancy of the prepared medicated dressing on the intact skin of forehands of volunteers. None of the prepared medicated dressing showed any erythema or edema, indicating that the prepared formulations were non-irritant on the skin of volunteers.

- **Sterility studies**

For conforming sterility of sterile dressing fluid thioglycolate medium modified by adding 0.5% of gelatin to increase the viscosity and enhance nutritional qualities. The raised viscosity improves the dispersion of sample and reduces

oxygenation during and after shaking. 50 ml of medium was taken in wide mouth bottle and closed with aluminum foil. The medium is warm at 52ºC and single unite of dressing is inoculated in it. The bottle is shaken in a reciprocating shaker for 10 minutes. After shaking the medium as transferred in to Petri dish, allow to cool and incubated at 32±2º C for 72º hours[13].

- **Antimicrobial studies:**

For study the antimicrobial activity Agar media is use[14]. For study the antimicrobial activity of dressing microorganisms was obtained from the push of a patient were grown on agar media. Microorganisms obtain from wound at lower leg part, on cotton and transferred to peptone media which act as transfer medium. The organisms obtained from the pus of patient was inoculated in the prepare medium for 24 hours at 32ºC in incubator. After 24 hours of incubation single unite of dressing is plunged in to medium by folding single unit of medicated gauze to1×1cm size and incubated at 32ºC for 72 hours. After 72 hours zone of inhibition is measured (Table-2).

- **FI-IR spectral analysis**

The spectra of Neomycin Sulfate and its formulation were obtain by potassium bromide plate method using Perkin Elmer FTR Series model 1615 spectrometer and compared.

Photograph- 1: Model of wound dressing

Figure-1: Water absorption and water handling studies

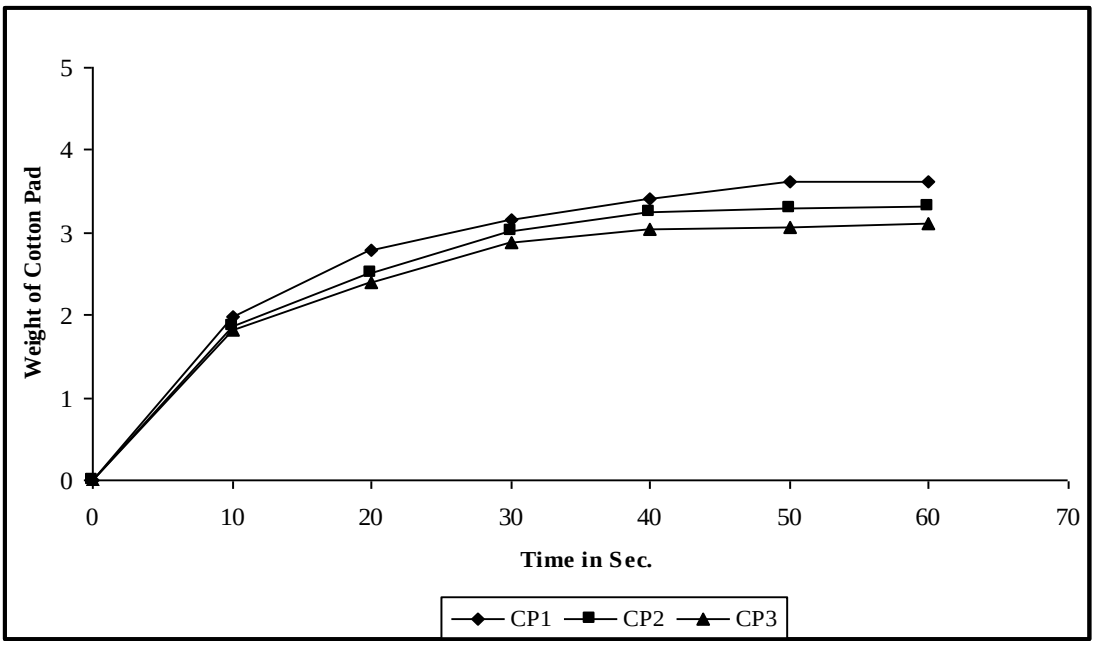


Table-1: Formulae of medicated gauge

Ingredient	NSG1	NSG2	NSG3	NSG4	NSG5	NSG6	NSG7	NSG8
Neomycin sulfate	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Hard paraffin	30%	-	-	15%	-	15%	10%	-
Liquid paraffin	-	30%	-	15%	15%	-	10%	-
Bees wax	-	-	30%	-	15%	15%	10%	-
White soft paraffin q.s.	100%	100%	100%	100%	100%	100%	100%	100%

Table-2: Zone inhibition study

Sl. No.	Sample	Zone of inhibition in cm			
		Plate -I	Plate-II	Plate –III	Average
1	Pure drug	2.8	2.9	2.8	2.85
2	Formulation NSG1	2.4	2.3	2.4	2.35
3	Formulation NSG2	1.9	1.9	2.1	2.05
4	Formulation NSG3	2.6	2.5	2.5	2.55

Dry weight of cotton pad is 550mg
Each reading is mean of three determinations

Table-3: Water absorption and water handling studies

SL. No.	Time (second)	Water Absorbance of cotton pad (4×4×0.4cm) in gm		
		CP-1	CP-2	CP-3
1	10	1.98	1.86	1.82
2	20	2.78	2.51	2.39
3	30	3.16	3.02	2.89
4	40	3.42	3.25	3.04
5	50	3.61	3.29	3.07
6	60	3.62	3.31	3.10

Each reading is mean of three determinations
CP-1: cotton pad 1, CP-2: cotton pad 2, CP-3: cotton pad 3

RESULTS AND DISCUSSION

Neomycin sulfate is drug use for treatment of infectious wound due to its antimicrobial activity on causative organism (i.e., gram positive and gram negative). In the present work different waxes combination were used for preparation of eight neomycin sulfate medicated dressing formulation and evaluated for the utility in the treatment. The cotton pad-I shows maximum amount of water absorption among the studied three different cotton pads available in the local market. Cotton pad-I shows highest amount of water absorption, handling capacity. Hence cotton pad-I is used throughout the formulation for better absorption of excaudate coming out from wound. The *in vitro* drug release was carried out in pH 7.5 phosphate buffer. The formulation NSG8 shows the maximum drug release (89.94%). All the formulations were found to follow first order release kinetics (Table-4, Figure-2).

In the proposed work the formulation NSG1, NSG2, NSG3 were subjected to primary skin irritation test on experimental animal (Rabbits) and health human volunteers. The results shown that the formulations are devoid of any primary skin irritation or sensation of erythema or edema, even after 72 hours of application of the prepared selected formulation.

Table-4: In vitro drug release of neomycin sulfate impregnated gauge (NSG 8)

SL. No.	Time (min)	Square route of time (min)	Log Time	% Cum drug released	Log % cum. drug released	% Cum drug remaining	Log % cum. drug remaining
1	00	0.00	0.00	0.00 ± 0.00	0.00	0.00	0.00
2	15	3.872	1.176	5.94 ± 0.121	0.773	94.06	1.973
3	30	5.477	1.477	12.79 ± 0.313	1.106	87.21	1.940
4	45	6.708	1.653	19.06 ± 0.230	1.280	80.94	1.908
5	60	7.745	1.778	25.69 ± 0.180	1.409	74.31	1.871
6	75	8.660	1.875	31.95 ± 0.250	1.504	68.05	1.832
7	90	9.486	0.954	38.86 ± 0.230	1.589	61.14	1.786
8	105	10.246	2.021	44.91 ± 0.420	1.652	55.09	1.741
9	120	10.954	2.079	51.98 ± 0.590	1.715	48.02	1.681
10	135	11.618	2.130	57.82 ± 0.720	1.762	42.18	1.625
11	150	12.247	2.176	66.31 ± 0.820	1.821	33.67	1.527
12	165	12.845	2.217	70.83 ± 0.700	1.850	29.17	1.464
13	180	13.416	2.255	77.82 ± 0.750	1.891	22.18	1.345
14	195	13.964	2.290	83.90 ± 0.480	1.923	16.90	1.227
15	210	14.491	2.322	89.94 ± 0.400	1.953	10.06	1.002

***** Each reading is mean of three determinations
***** Each medicated gauze (4 x 4cm) contains 1.8mg of the drug.

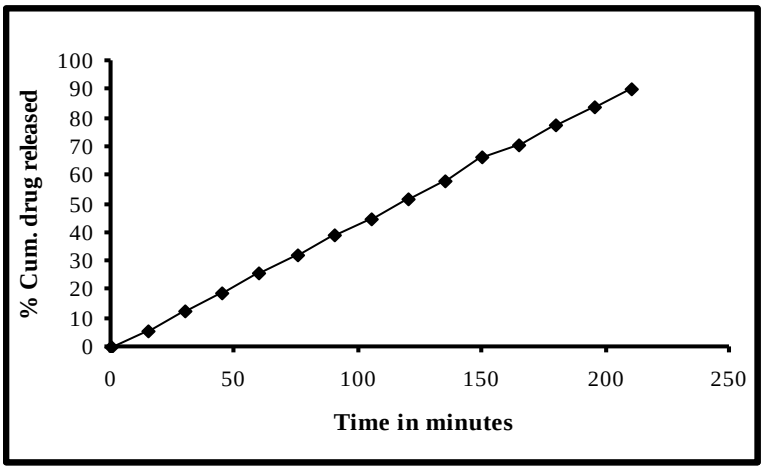


Figure-2: Zero order

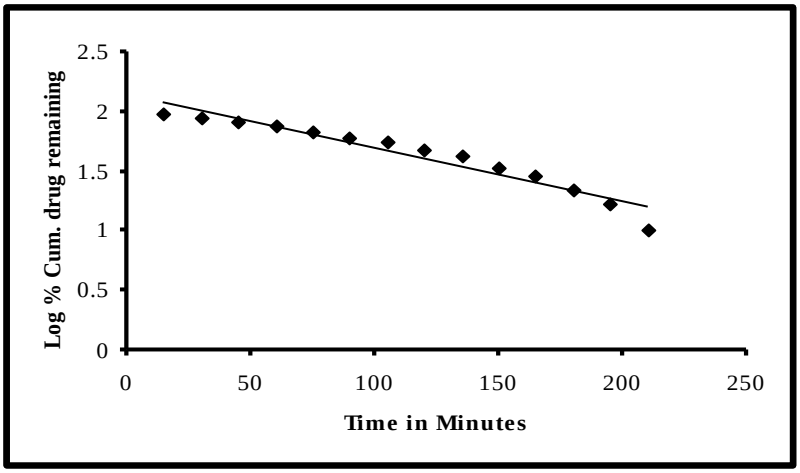


Figure-3: First Order

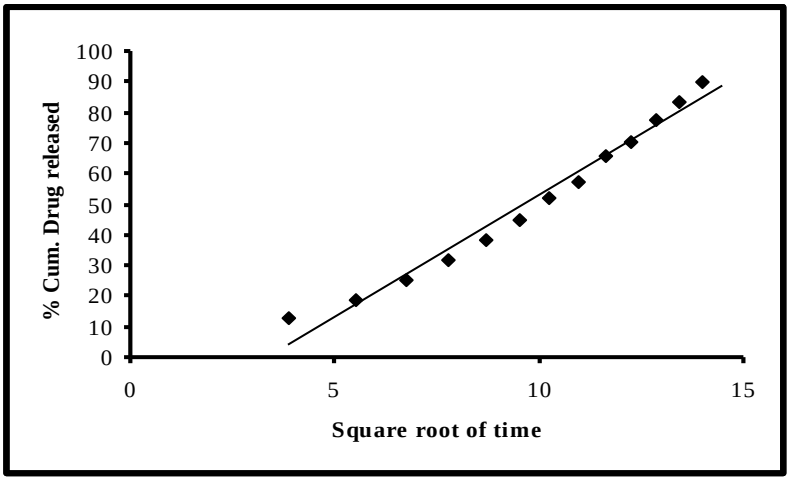
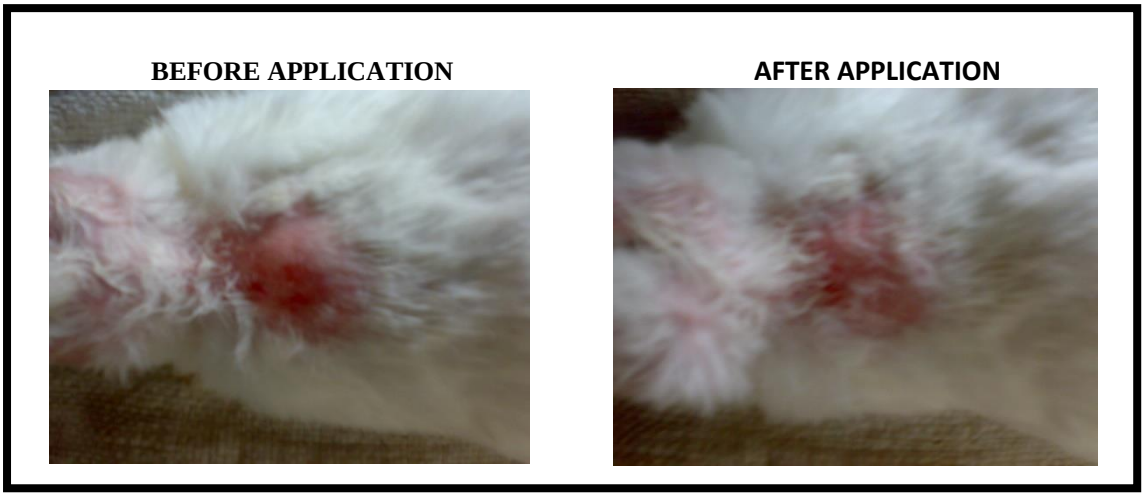
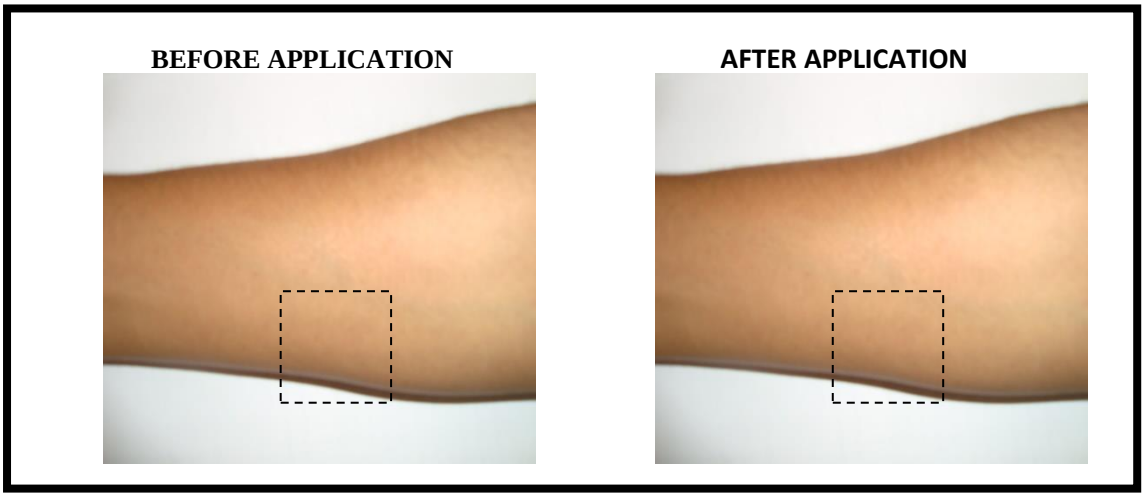


Figure-4: Higuchi's plot

Photograph-2: Primary Skin Irritation test of Neomycin sulfate gauge on animal



Photograph-3: Primary Skin Irritation test of Neomycin sulfate gauge on human



CONCLUSION

Neomycin Sulfate medicated gauge with supportive cotton pad can be prepared for the treatment of wounds. These will have additional advantages of water absorption capacity nonadherant, patient compliances, convenience and comfortness with efficient treatment. The physico chemical characterization revealed that all the formulations were found

to have uniform weight and size. The primary skin irritation test reveals that the prepared formulations were devoid of any skin irritation of sensitization or erythema or edema in experimental animals like rabbits. IR spectra analysis showed that there was no drug excipient interaction.

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