

FORMULATION AND EVALUATION OF NEOMYCIN SULFATE HYDROGELS FOR WOUND HEALING

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DOI: 10.47750/pnr.2022.13.S01.30

Abstract

Purpose: The present study was designed to study the Neomycin Sulfate (NS)-loaded hydrogels to heal wounds.

Material and Method: NS-loaded hydrogels were prepared by simple stirring method.

Results: The prepared formulation exhibited good consistency, homogeneity and gelling strength. The application of gel on healthy volunteers revealed gels to be safe for skin. It also exhibited pH between 7.4-8.0 and drug content between 100±4% evaluated through microbial assay. In-vitro drug release data showed a fast initial drug release during the first 1 h, followed by a slower and sustained release over 5 h.

Conclusion: The finding suggests that NS based hydrogel is suitable for drug delivery through topical application to enhance the dermal contact time and improved patient compliance.

Keywords: Hydrogels, Neomycin Sulphate, Wound Healing.

INTRODUCTION

The continuum of skin

The skin acts as a covering to the body and is firmly incorporated through retinacular tendons to the basic fascial endoskeleton [1], veins, nerves [2] and lymphatic system. The fascial endoskeleton or retinacular framework [3] is significant in deciding the restrictions of skin development. Explicit tying focuses from this retinacular framework characterize the appearance of skin; for instance, well-defined holding tendons have been concentrated around the head that characterize explicit skin compartments of the face, and retinacula of the skin are thick in glabrous regions like the foot sole and hand so the skin doesn't shear rapidly in these specific areas.

Skin of human is made out of three particular layers: dermis, epidermis and hypodermis, with fluctuating levels of specialization inside each layer.

The epidermis

The most superficial and active biological layer of these layers as the basal layer of the epithelium (layer basale) is continually renewing. [4]

Epithelial cells thickly pack the epidermis to a depth of in between the range of 75 and 150 µm (up to 600 µm thick on palms/soles). The epidermis goes through a cycle of cornification which is one of the adaptable cycles to furnishing the body with an obstruction to the elements common to numerous species aside from fish.

The high lipid content from cholesterol and unsaturated fats produced by the layer stratum granulosum, represents the immobility and fractional clarity of the epidermis. These sit on an unpredictable assembly of collagen IV, laminin, nidogen, perlecan, heparin sulfate proteoglycans and junction atoms called as the cellar layer. This is an important piece of the dermoepidermal intersection [5] that characterizes and furthermore adheres the epidermis and dermis together, giving a strong

mechanical obstruction against microbes.

The dermis

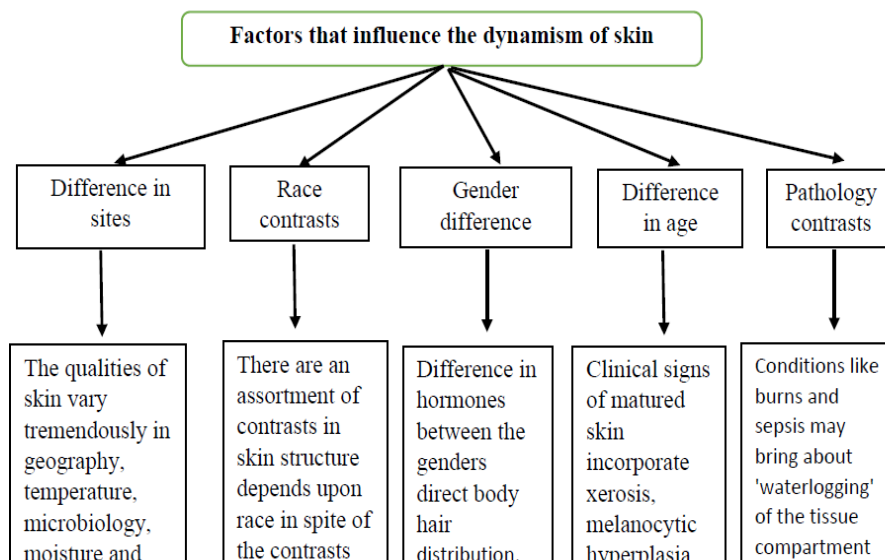
This layer is mostly <2 mm thick, yet perhaps up to 4 mm thick (like grown-up back) and provides greater part of the mechanical strength to skin. Shear powers and breaking power of this layer are 5–15 MPa on the face and up to 27 MPa from layer on the back [6]. Forces opposed in the hypodermis are just 1–5 MPa. The direction where skin is extended influences its elasticity, subsequently applying footing corresponding to Langer's lines have the most grounded extreme rigidity [5, 6] however least extendability [7] which starts to fail whenever skin is extended past 1.5 times its length.

The dermis of papillary interfaces closely with the rete edge projections from the superficial layer just as encompassing individual hair follicles. The designing of hair follicles and related adnexal constructions and eccrine glands either emerge from nearby signalling from with cell precommitment or long reach signalling and pattern inferred through diffusion response.

The hypodermis

The hypodermis mostly comprises of loose connective tissue which relying upon site structures gliding layers or enormous pockets of fat cells which protects the skin. The tissue is especially rich in proteoglycan and glycosaminoglycans, which draws in liquid into the tissue giving it mucous-like properties [8]. The variety of cells found in the hypodermis are fibroblasts, fat cells and macrophages which have a specific part in adipocyte homoeostasis in obesity [9], conceivably connected with tissue redesigning [10] and may start thermogenesis of fat during exposure to cold and exercise [11, 12]. Adipocytes are incorporated into lobules with the fibrous septa and rich blood and lymphatic supply in the middle.

The 'microvacuolar' tissue that makes up the hypodermis [8] goes about as active repository for interstitial fluid that can progressively adjust the stiffness of the tissue [13, 14].

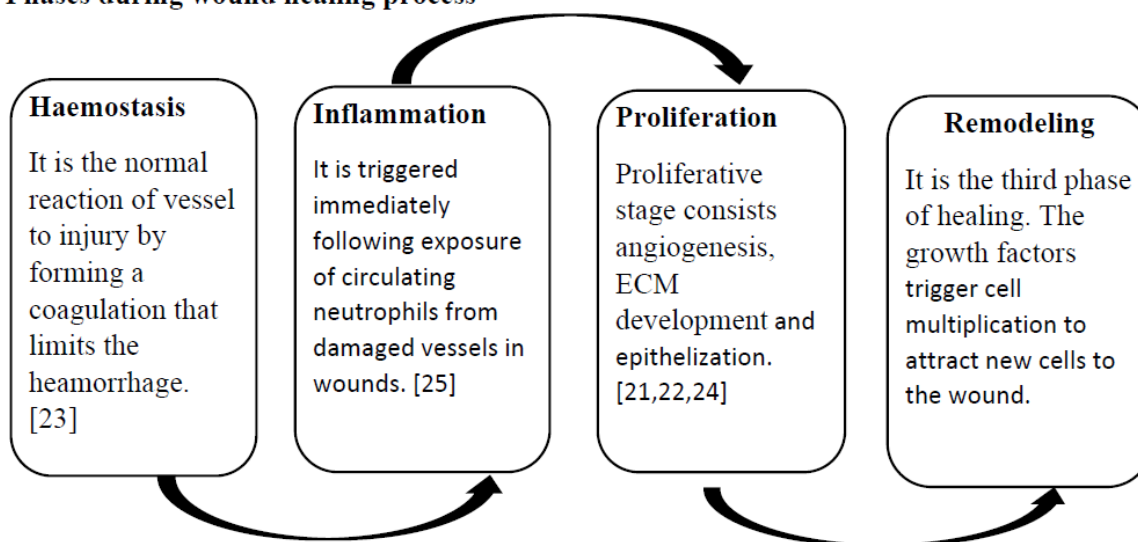


Flow chart 1 Factors influencing dynamism of skin

Wound

Wound [18] recuperating is quite possibly the most complex cycles in multicellular living beings, including multi stages during process which incorporate hemostasis/aggravation phase, multiplication/proliferation phase, and remodeling phase [19]. Unbalancing at least one of these phases could prompt two particular harming results: either development of chronic wound or a hypertrophic scar/keloid formation [20].

Phases during wound healing process



Flow chart 2 Phases of wound healing

Neomycin sulphate- Antimicrobial agent

Neomycin is an aminoglycoside antimicrobial that is created by *Streptomyces fradiae* and was first depicted in 1949. Neomycin involves three chemically related components particularly neomycin A (neamine), neomycin B (framycetin; likewise called neomycin sulfate) and neomycin C, with the amount of each varying with the manufacturing cycle.

Because of its relative toxicity when given systematically, neomycin is by and large utilized just topically, either alone or in mix with different antimicrobials, especially polymyxin B or potentially bacitracin. Topical formulations are accessible in variety of preparations like gels, solutions, eye drops, and eardrops. Neomycin is effective against staphylococci and most oxygen consuming Gram-negative microbes, in spite of the fact that streptococci and Gram-positive bacilli are resistant.

Like other aminoglycosides, neomycin acts by binding to the 30S subunit of the bacterial ribosome to hinder protein synthesis. Resistance is intervened through various systems, with the most huge being enzymatic inactivation of the medication by chromosomally or plasmid-encoded aminoglycoside-modifying catalysts [26]. Topical neomycin-containing preparations are most generally utilized for treatment of local skin infections because of staphylococci and Gram-negative bacilli. Notwithstanding, neomycin formulations perform less well than other topical antimicrobials, for example, fusidic acid and mupirocin, for the treatment of common skin diseases, like impetigo [27]. Likewise, one of the significant concerns in regards to the utilization of topical neomycin is the clearly high pervasiveness of hypersensitive contact dermatitis, which has been assessed at 1% to 6% however is believed to be higher in patients with impaired skin obstruction [28]. Additionally, systemic complexities, like ototoxicity, can happen following topical neomycin treatment, remembering occasions of extreme ototoxicity for patients with eardrum hole getting neomycin eardrops [29].

Topical Drug Delivery System

Topical delivery [30] formulations are localized medication delivery system any place inside the body through ophthalmic, rectal, vaginal and derma as topical routes.

Skin is perhaps the most promptly available organ on body of human for topical administration of drug and is primary route for topical delivery of drug in the system.

Topical formulations are applied to the skin for systemic or localized impacts. At times, the base might be utilized alone for its therapeutic actions like emollient, protection or soothing effects. Numerous topical formulations contain active pharmaceutical ingredients or excipients which are dissolved or dispersed in the base. The blend of therapeutic agents and base gives the chance to a wide scope of topical formulations, suitable for some sorts of medication delivery and treatment terms used to group the bases of topical formulations wherein therapeutic ingredients are added, might be based on their physical properties (suspension) or on their desired use (liniments) or on their ingredients (hydrophilic creams). [31]

Difficulties in developing topical drug delivery system

The challenge of formulating a fruitful topical product originates from the few necessities that a formulation should meet:

1. Selection of container and Product Stability

2. Skin Penetration
3. Acceptability of cosmetic

Dermatological bases

Topical formulations implied for local or systemic impact are classified as-

- Solids – Dusting powder
- Semi-solids – Creams, Gel, Ointments, Paste and other
- Fluids – Solution, Emulsion, Liniments, Suspension, Soaps, Shakes, Collidons, Lotion, Paints and other. [32]

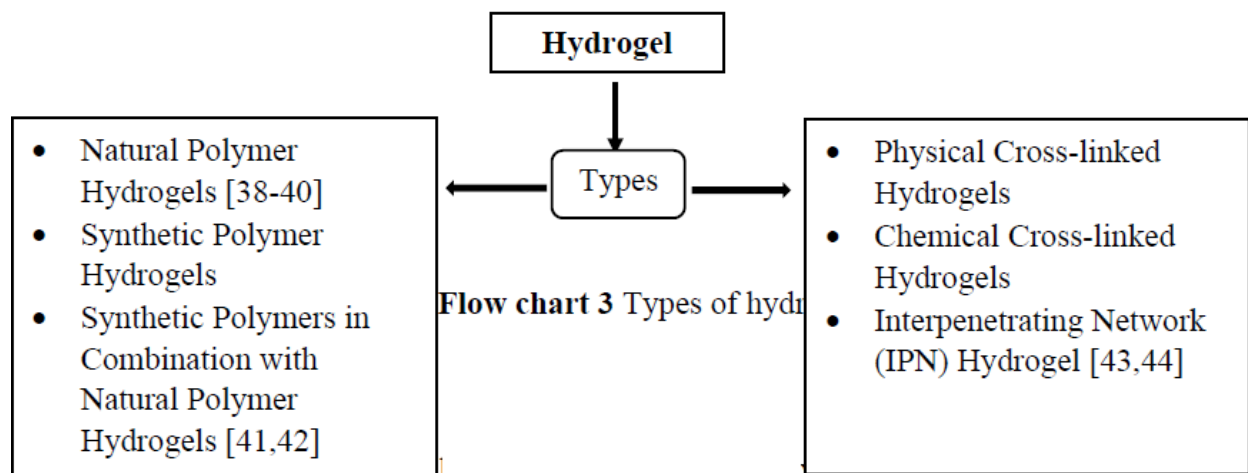
In these semi-solid preparations are more encouraging over solid and liquids considering its property to stick to surface of utilization for sensible span before they worn off.

Pharmaceutical semi-solid formulations incorporate pastes, cream emulsions, gel and ointments.

Introduction of Hydrogels

Hydrogels [33], with their design of three-dimensional crosslinked polymer meshwork, tend to retain impressive measures of water inside their interstices and continue holding it while keeping up their structure in the swollen state. The exhibit of such network in hydrogels is due to the presence of polar hydrophilic moieties like SO₃H, OH, NH₂, COOH, CONH₂, and so forth, along the polymer network as branched groups. The propensity of water retention in hydrogels is because of their swelling property, which is because of the hydrophilicity of connected moieties, swelling media, and crosslinked bonding strength. Crosslinking controls water absorption just as aiding in keeping up the structure in the swollen state [34-37]. The crosslinkers assume a large part for secondary interactions with natural tissues alongside the support of hydrophilic groups for water take-up [36].

Because of the distinct properties like biodegradability, biocompatibility, hydrophilicity, superabsorbancy, viscoelasticity, non-abrasiveness, and featheriness, hydrogels assume an excellent part in biomedical applications. Aside from that, hydrogels additionally react to different stimuli like temperature, electric and magnetic field, biological atoms, and ionic strength [37]. A number of hydrogels can increase the dwelling period of active ingredients because of the mucoadhesive and bioadhesive attributes which advance them as reasonable chosen people as medication transporters [34].



Flow chart 3 Types of hydrogel

Flow chart 3 Types of hydrogel

Properties of Hydrogels

- i) The utilization of hydrophilic polymers (natural or synthetic) not only encourage water absorption yet in addition aid the interaction with biological tissues (epithelial tissues and mucous films).
- ii) Hydrogels in the completely swollen state are visco-elastic, soft, rubbery, and low in interfacial point with biological fluids, which diminishes the odds of a negative immune reaction.
- iii) Every one of these components add to the biocompatibility of hydrogels.
- iv) The hydrogels are degradable to various degrees depending on the sort of cross-linker included.
- v) Besides, hydrogels have a swelling property, which is the main one in their existence. The swelling of hydrogels happens in three stages:

- v.i) penetration of water into the hydrogel network (primary bound water),
- v.ii) unwinding of polymer chains (secondary bound water),
- v.iii) extension of the hydrogel network (free water).

As per the Flory–Rehner hypothesis, swelling is a function of the flexible nature of the polymer chains and their compatibility with water atoms [45].

Hydrogels show various reactions to changes in environmental stimuli, which may by and large be categorized into-

- i) physical (temperature, light, and so on)
- ii) substance (pH and ionic strength), and
- iii) Biological stimuli.

DISADVANTAGES

- Hydrogels should be utilized for dry or low degree of exudate wounds. The abundance dampness can prompt maceration of the skin.
- Due to the higher content of water (70-90%) hydrogels don't have the capacity of retention of exudates.
- Accumulation of liquid in hydrogels gives a reasonable climate to bacterial development and can create tainted smell a short time later. Consequently, hydrogels should be changed frequently.
- The mechanical strength of hydrogels is weak.
- Problems like high crystallinity, undesired mechanical and thermal properties, less solubility, unreacted monomers, non-biodegradability and the utilization of toxic cross-linkers are the restrictions of the hydrogel innovation.

ADVANTAGES

- Under physiological conditions, they have ability to hold a lot of water or biological fluids.
- They are portrayed by a delicate rubbery consistency like living tissues, making them an ideal substance for variety of uses.
- Hydrogels with the characteristic properties, for example, desired usefulness, reversibility, sterilizability and biocompatibility meet both material and natural prerequisites to treat or replace tissues and organs, or the capacity of living tissues, just as to associate with the body's biological system.
- Hydrogels can be applied and taken out with negligible injury and torment from wound bed.
- Because of the cooling impact that hydrogels have on wound bed, they can give a soothing feeling to patients.

MATERIAL AND METHOD

Neomycin Sulfate and benzoic acid were purchased from Central Drug House (P) Ltd., carbopol 934 purchased from LOBA Chemie Pvt. Ltd. Triethanolamine (TEA) was purchased from Fisher Scientific, U.K. Analytical grade chemicals were used.

7 hydrogel formulations were tested, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6% and 0.7% w/w.

FORMULATION OF TOPICAL GEL

For 30 g gel

Table 1. Formulation of Topical Gel

Formulation code	Neomycin sulfate (g)	Ne (% w/w)	Carbopol 934 (g)	TEA (ml)	Propylene glycol (ml)	Benzoic acid (g)	Distilled Water (ml)
F1	0.03	0.1	0.25	2	14.17	0.05	13.5
F2	0.06	0.2	0.25	2	13.64	0.05	14
F3	0.09	0.3	0.25	2	13.11	0.05	14.5
F4	0.12	0.4	0.25	2	12.58	0.05	15
F5	0.15	0.5	0.25	2	13.05	0.05	14.5
F6	0.18	0.6	0.25	2	13.52	0.05	14
F7	0.21	0.7	0.25	2	14	0.05	13.5

All the quantity of excipients were taken in accordance with Inactive Ingredient Database.

PREPARATION

Preparation of hydrogel base

1. Carbopol gels were formulated by first preparing a solution of Carbopol in distilled water and propylene glycol.
2. Specifically, 0.25 gm of Carbopol was slowly added to a mixture of water and propylene glycol.
3. The mixture was stirred slowly with a magnetic stirrer at 25°C, until all the Carbopol was dissolved and kept undisturbed in a cool and dry place for 24 hours for proper swelling.

Preparation of neomycin sulfate hydrogels

4. After 24 hours, separately appropriate quantities of neomycin sulphate were dissolved in distilled water and triethanolamine to yield five different drug solutions (0.1%, 0.3%, 0.5% and 0.7% drug content).
5. Neomycin sulfate solution of desired quantity for different formulations were incorporated into base gels by using slow magnetic stirring (50 rpm) for 10-15 minutes.
6. Gel stored for another 24 hours at room temperature to stabilize.

PHYSICOCHEMICAL EVALUATION OF GEL:

1. Physical Appearance/clarity

The topical gel formulations were observed carefully visually for appearance/clarity, colour, homogeneity, consistency and presence of suspended particulate matter if any. It was further assessed by observing them against a dark and white background. [46]

2. Determination of pH

The pH of various gel formulations was determined by using previously calibrated digital pH meter. 1 gram of gel was dissolved in 10 ml distilled water. The pH of 10% (w/w) topical gel was determined using electronic digital pH meter, standardized using pH 4.0, 7.0 and 10.0 standard buffers before use at 27°C. The values were recorded immediately after preparation and after storage for 24hrs at room temperature. [47]

3. Determination of Viscosity

The measurement of viscosity of the prepared gel was measure by using Brookfield Viscometer (model LV DV - II+Pro) using spindle number S64. The gels were rotated at 100 rotations per minute and the viscosity values were noted [46]

4. Spreadability

1 gm topical gel was placed within a circle of 1 cm diameter on petridish over which a second petridish is placed inverted. A weight of 500 g was allowed to rest on the upper glass plate. The increase in diameter due to spreading of the gel was noted. [48]

5. Tube Extrudability

A known weight was placed over collapsible tube containing topical gel at crimped end. The cap was removed, topical gel extrudes out until pressure was dissipated. Weight in grams required to extrude topical gel in 10 seconds was determined. Then average pressure required to extrude 1 gm of gel was determined. [49]

6. Drug Content

It was done using microbial assay method mentioned in Indian Pharmacopoeia 2018 edition.

Methods opted were-

- i. Direct phosphate buffer dilutions
- ii. Mild heating of stock solution
- iii. Drug extraction with chloroform

Test organism- *Staphylococcus epidermidis*

Antibiotic assay medium 11 [50]

Formula used-

$$a = (U1 + U2) - (S1+S2) / (U1-U2) + (S1-S2)$$

$$\% \text{ Potency} = \text{Antilog} (2.0 + a \log 4)$$

7. Skin irritation test

It was performed on healthy human volunteers. 5 volunteers were selected for each gel and 1 g of formulated gel was applied on back hand skin area of 2 sq mm. The volunteers were continuously observed for lesions and skin irritation.

8. HPTLC

Standard drug solution (3.5mg/ml), mobile phase containing methanol, isopropyl alcohol, methylene chloride, ammonium hydroxide and water (4:2:2:2:1.5) were prepared and HPTLC was performed for standard drug, drug and hydrogel formulation (0.3% w/w) according to procedure mentioned in USP. [51]

9. In vitro drug dissolution studies

It was performed using dissolution apparatus using dialysis membrane including following conditions-

Temperature- 37± 0.50C

RPM- 50

Alliquotes- 4ml

Phosphate Buffer- pH 7.4

1g topical gel was transferred to a pre activated dialysis membrane. The bag is then put in the dissolution apparatus (paddle type) having dissolution media 250ml.

Dissolution studies were carried out using phosphate buffer with 0.3%, 0.5%, 0.7% w/w topical gel formulations at above mentioned conditions.

2ml sample withdrawn at interval of 15, 30, 1, 2, 3 and 4 hours. Samples were replaced by equivalent volume of fresh dissolution medium. The samples were analyzed using UV Spectrophotometer at 205nm. [48]

10. Drug Release kinetic study

The data obtained from the in vitro release study were analyzed using various kinetic models to describe the mechanism of drug release from the hydrogels. Three kinetic models including zero-order (1), first-order (2), Higuchi square root (3), Korsmeyer-Peppas (4) models were applied on the release data

$$Q = K_0 t \quad (1)$$

where Q is the amount of drug release at time t , K_0 is zero order rate constant expressed in units of concentration/time and t is the time. Consider

$$\log Q = \log Q_0 - \frac{K_1 t}{2.303} \quad (2)$$

where C_0 is the initial concentration of drug and K_1 is first order constant. Consider

$$Q = K_H t^{1/2} \quad (3)$$

Where K_H is the constant reflecting the design variables of the system.

To find out the mechanism of drug release, the release data was fitted in Korsmeyer-Peppas model as follows:

$$\frac{M_t}{M_\infty} = K t^n \quad (4)$$

Where M_t/M_∞ is fraction of drug released at time t , K is the release rate constant incorporating structural and geometric characteristics of the tablet, and n is the release exponent. The n value (diffusion exponent) is used to characterize different

release mechanisms. [52]

11. Stability studies

Hydrogels F3, F5 and F7 which showed a promising sustained drug release were kept in air tight plastic container and subjected to accelerated stability study. They were stored at room temperature (25°C) and in the refrigerator (4°C) over a period of three months. Physical evaluation of the samples were carried out by visual inspection for any change in color, odor or precipitation. Rheological properties (viscosity) was also examined. Furthermore evaluation of chemical stability was done by pH determination and spectrophotometric analysis of drug content. [52]

Table 2. Result and Discussion

Batch code	F1	F2	F3	F4	F5	F6	F7
Appearance	Clear	Clear	Clear	Ppt (24 hrs)	Clear	Ppt (24 hrs)	Clear
pH	8.0±0.02	8.1±0.05	7.3±0.01	-	7.3±0.04	-	7.4±0.03
Viscosity	1490±50	1380±20	1284±50	-	1184±40	-	1098±10
Extrudability	17.00±0.02	17.30±0.03	15.50±0.01	-	12.25±0.05	-	13±0.01
Spreadability	4.5±0.02	5.1±0.05	5.3±0.03	-	6.2±0.02	-	6.6±0.01
Drug Content	-	-	103%	-	104%	-	96%
In-vitro (CPR)	-	-	98.62%	-	98.71%	-	95.08%
Release kinetics	-	-	First order	-	First order	-	First order
Stability studies	Unstable	Unstable	Pass	-	Pass	-	Fail

Above values indicate mean±SD (n=3)

Table 2 Summary of all results

From all the evaluation parameters undergone we can conclude that among all the batches prepared of hydrogel formulation, F3 showed the most promising results.

Preformulation Studies

Table 3 Physical appearance of drug

Properties	Neomycin Sulfate
Form	Powder
Color	Yellowish white
Odor	Odorless

Melting point

The melting point was determined to be 195 °C.

UV Spectroscopy

Maximum wavelength for absorbance was found to be 205nm after triplication of method.

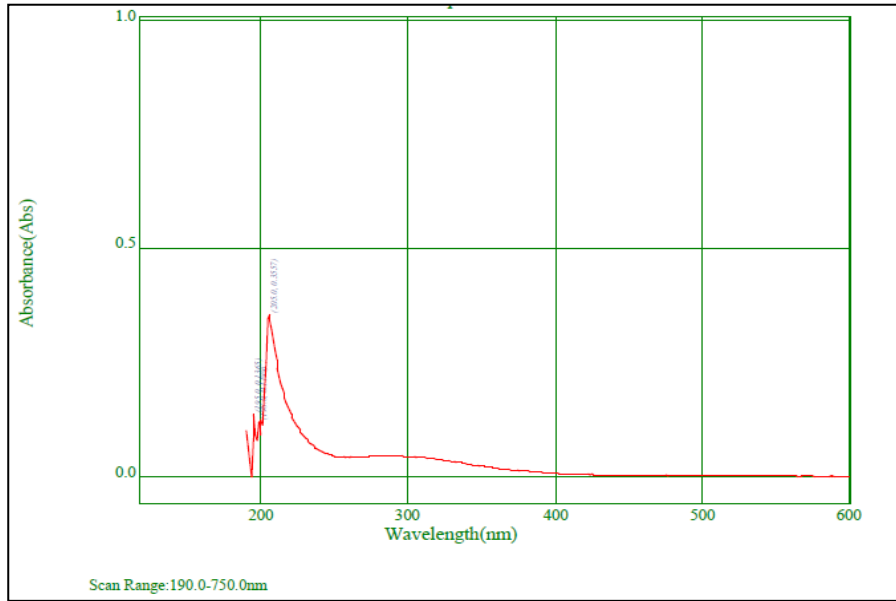


Figure 1 λ_{max} of Ne

Standard curve

Table 4. Standard curve of Ne in phosphate buffer pH- 7.4

S. NO.	Concentration ($\mu\text{g/ml}$)	Absorbance (at 205nm)
1.	0	0.000
2.	10.00	0.060
3.	20.00	0.078
4.	30.00	0.124
5.	40.00	0.144

Absorbance values of neomycin sulfate in phosphate buffer at 205nm

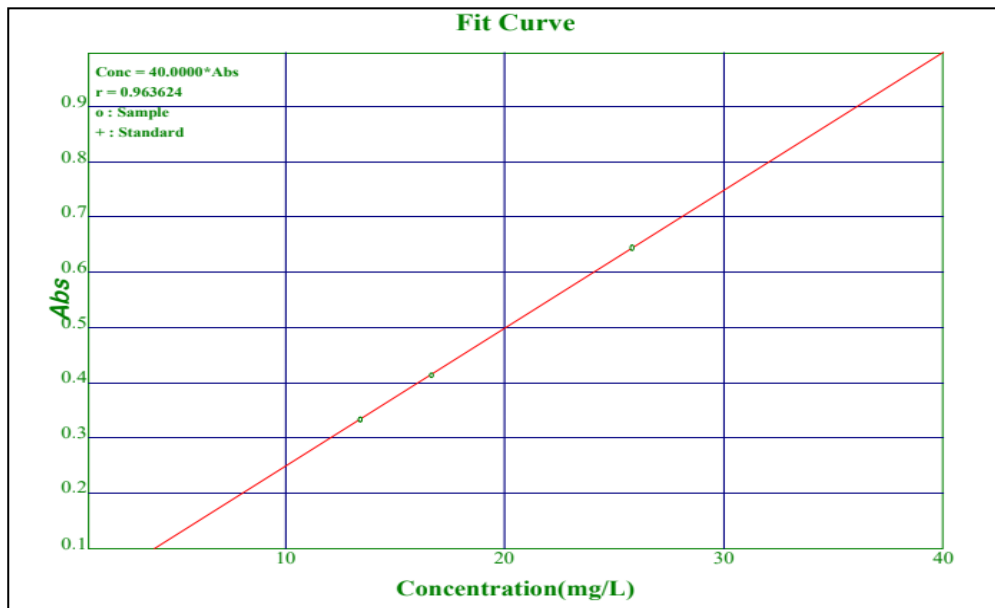


Figure 2 Standard curve in phosphate buffer pH- 7.4

IR Spectrum of Standard Neomycin Sulfate

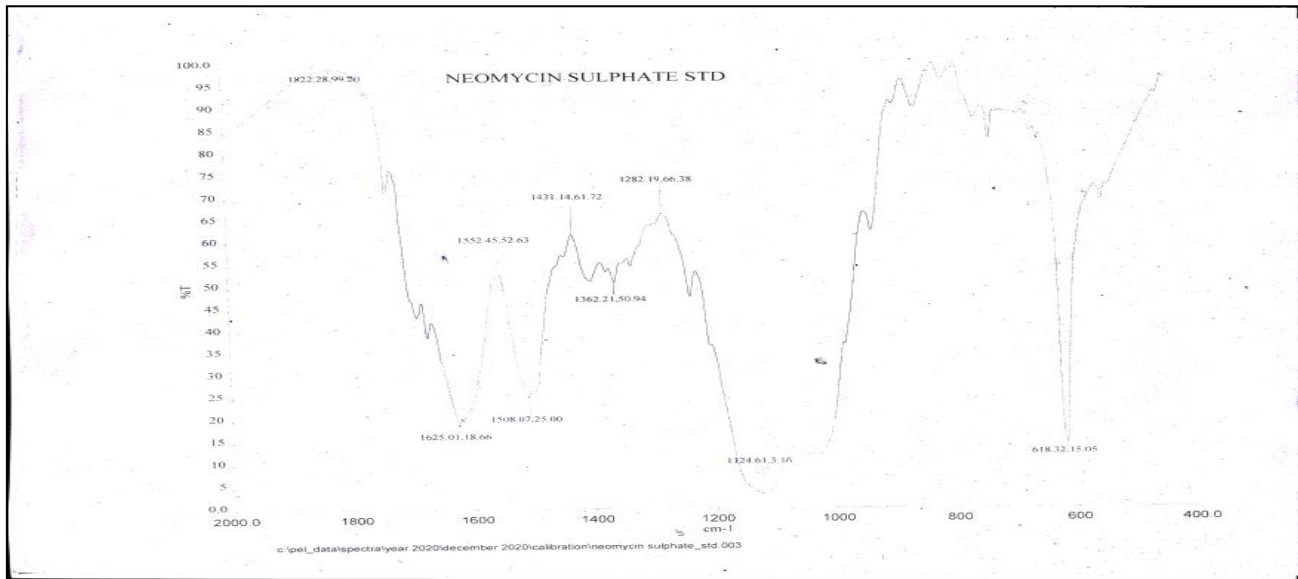


Figure 3 IR Spectrum of Neomycin Sulfate IPRS

IR Spectrum of Neomycin Sulfate (Test)

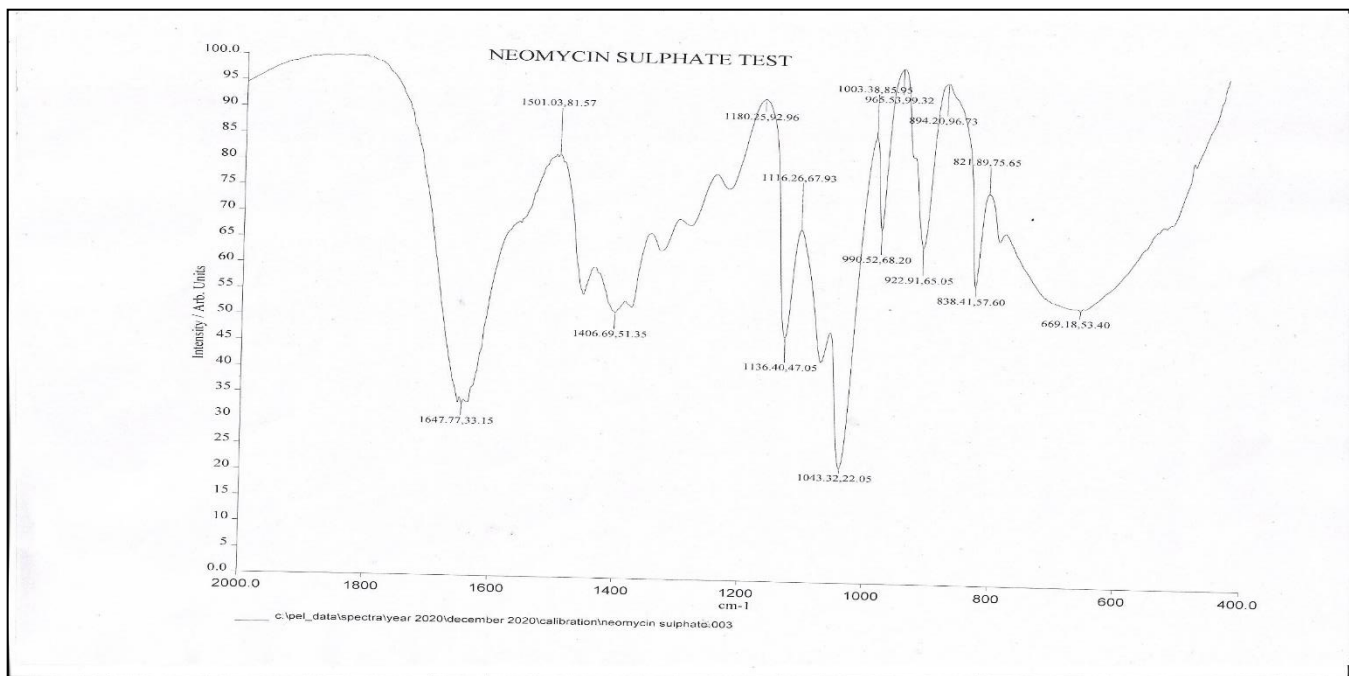


Figure 4 IR Spectrum of Neomycin Sulfate (Test)

Table 5. IR spectra determination of Ne Test and standard.

Functional group	Observed cm ⁻¹
O-H bending (aromatic)	1647
O-H bending (alcohol)	1406
CO stretching (secondary alcohol)	1043

It was observed that all the major functional peaks present in std were also present in test. Therefore there is no impurity or

adulteration in the purchased drug.

IR Spectrum of Carbopol

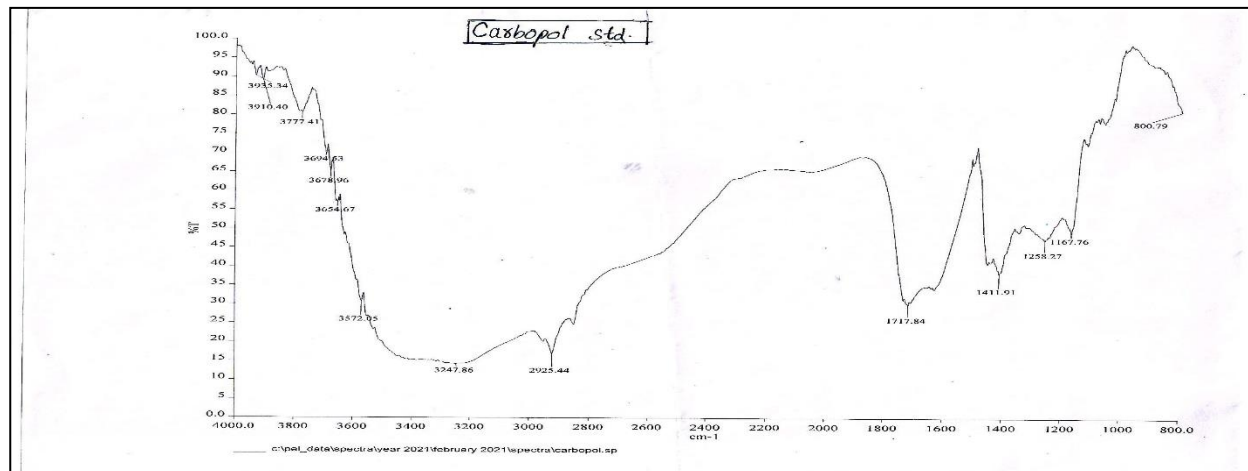


Figure 5 IR Spectrum of Carbopol 934

IR Spectrum of Carbopol+ Neomycin Sulfate

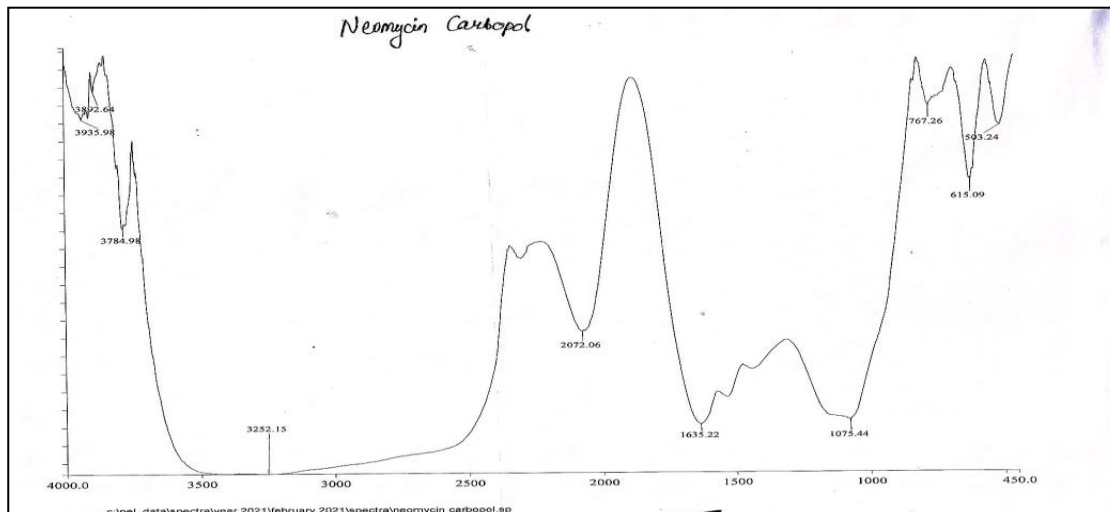


Figure 6 IR Spectrum of Carbopol 934+Ne

Table 6 IR Spectra determination of Carbopol and mixture of carbopol+Ne

Functional groups	Observed (cm ⁻¹)
O-H stretching alcohol	3252
C=O stretching	1635

It was observed that all the major peaks present in Carbopol 934 were also present in IR spectra of Ne-Carbopol. Therefore, there is no interaction between the following drug and excipient.

Water Dilution	+	+	+	+++	+++
Stickiness	+++	+++	+++	+	+
Homogeneity	+++	+++	+++	+++	+++
Odor	+	+	+	0	0
Transparency	O	O	O	T	T
Oily feel	0	0	0	0	0

0-Absent, +- moderate, ++ good, +++- best, Translucent- T, Opaque- O

Table 8. Organoleptic tests

The topical gels were prepared and subjected to physical evaluations such as appearance, pH, viscosity, spreadability, extrudability and skin irritation.

Formulation code	Smoothness/Roughness	pH	Viscosity (rpm)	Spreadability (gm.cm/sec.)	Extrudibility (gm./cm ²)	Skin Irritation
F1	Smooth	8.0±0.02	1490±50	4.5±0.02	17.00±0.02	Nil
F2	Smooth	8.1±0.05	1380±20	5.1±0.05	17.30±0.03	Nil
F3	Smooth	7.3±0.01	1284±50	5.3±0.03	15.50±0.01	Nil
F5	Smooth	7.3±0.04	1184±40	6.2±0.02	12.25±0.05	Nil
F7	Smooth	7.4±0.03	1098±10	6.6±0.01	13±0.01	Nil

Above values indicate mean±SD (n=3)

Table 8 Result of appearance, pH, viscosity, spreadability, extrudability and skin irritation

The gels were clear throughout the evaluation. The pH was constant (8.0 – 7.3) and did not produce any type of irritation when applied on the skin. Viscosity, Extrudability and spreadability were excellent. Furthermore, the stability studies results of selected formulation revealed that F3 and F5 formulation was stable under normal storage condition.

2. Drug content through microbial assay

Methods

1. Direct phosphate buffer dilutions-



Figure 9 0.5% w/w Neomycin Sulphate Hydrogel microbial assay

The zones were small and not very sharp.

We concluded that the gels were not able to release drug easily because of viscous nature. So we tried other methods as per IP to get more accurate results.

2. Mild heating of stock solution

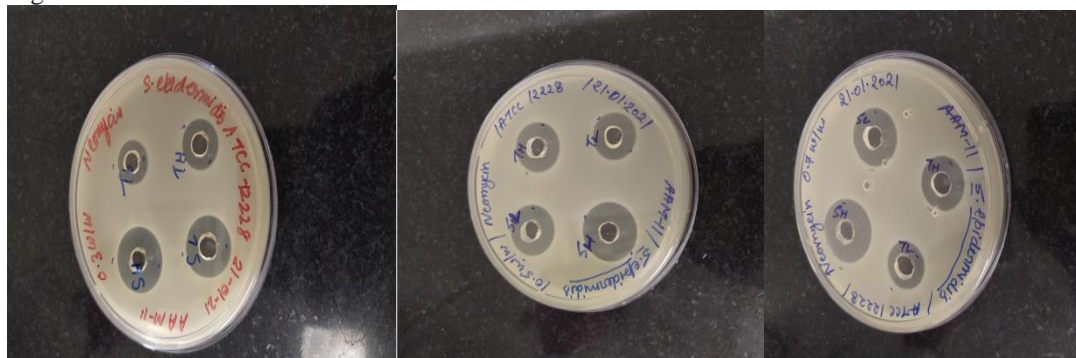


Figure 10 0.3%,0.5%, and 0.7 % w/w Neomycin Sulphate Hydrogels microbial assay

Heating of stock solutions was done to reduce the viscosity of the gels and to remove the haziness from the dilutions for a better release of drug.

The results were better than the previous assay but the % potency were still less than the acceptance criteria.

So we tried to perform the assay as per IP method of microbial assay of neomycin sulphate ointments.

3. Drug extraction with chloroform

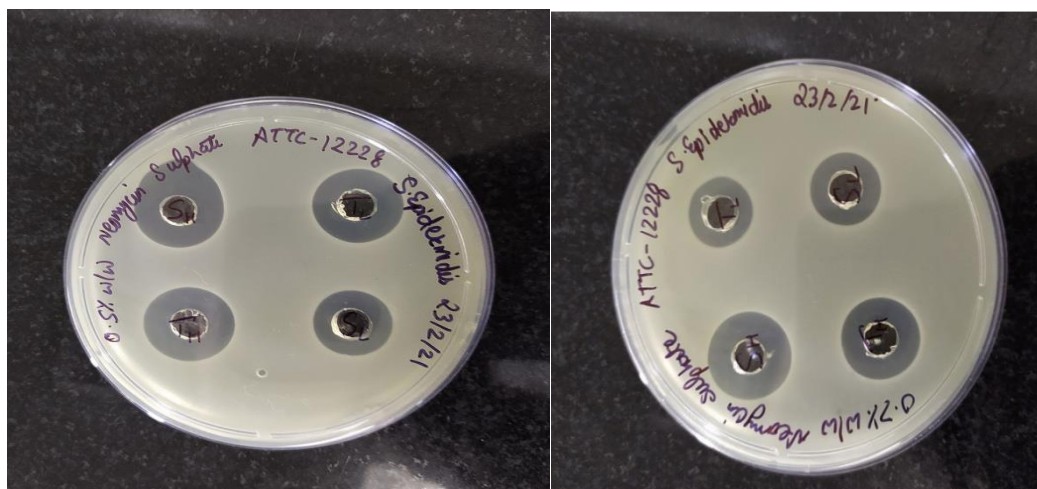


Figure 11 0.5% and 0.7% w/w Neomycin Sulphate Hydrogels microbial assay

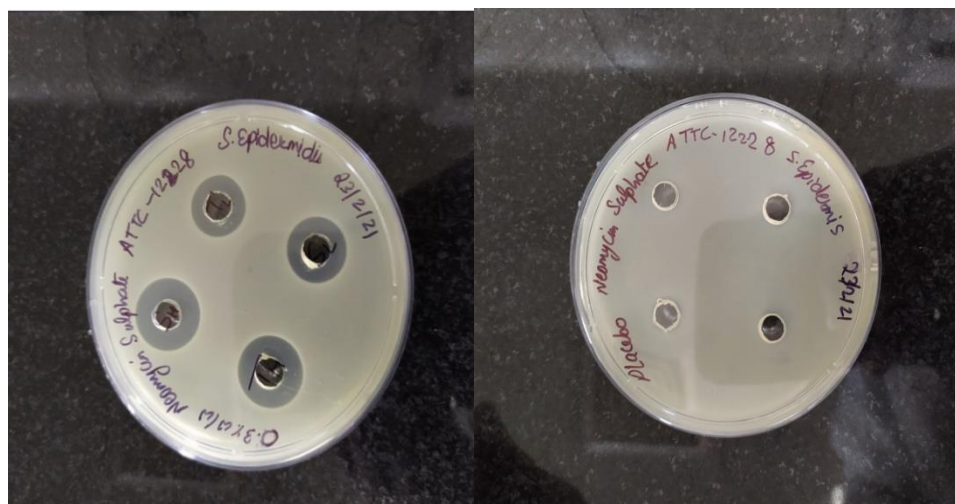


Figure 12 0.3% and placebo of Neomycin Sulphate Hydrogels microbial assay

Table 9 Result of zone of inhibition

Sample	S _H	T _H	S _L	T _L
0.3% w/w	17.3	17.4	15.4	15.4
0.5% w/w	19.2	19.4	14.8	14.9
0.7% w/w	19.2	18.9	15.8	15.8

% potency of samples were found to be-

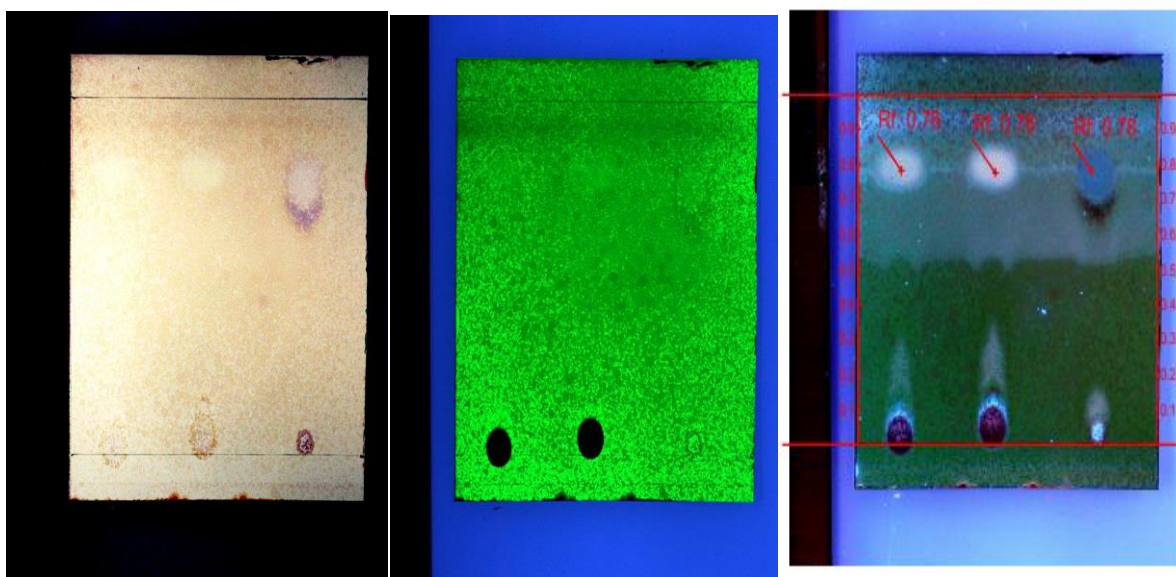
- 0.3% w/w neomycin sulphate hydrogel- 103.6%
- 0.5% w/w neomycin sulphate hydrogel- 104%
- 0.7% w/w neomycin sulphate hydrogel- 96%

The zones formed were very sharp and clear indicating effectiveness of drug.

Microbial assay of placebo was done to ensure that the efficacy of formulation is only because of drug and there is no interference of excipients.

Out of opted 3 batches F3 showed the drug content nearest to 100%.

3. High Performance Thin Layer Chromatography



(a)

(b)

(c)

	(a)	(b)	(c)
Illumination type	White transmission	254 nm remission	366 nm remission

Figure 13 HPTLC of Ne std, test and 0.3% w/w hydrogel formulation

No spots were seen at 254 nm transition but sharp spots of Rf value 0.78 were seen at transmission of 366 nm.

The Rf value of std. test and formulation (0.3%w/w) were observed to be equal indicating presence of desired qty of drug in formulation.

4. DSC (Differential Scanning Calorimetry)

The peak value of the mixture (drug+ excipients) and formulation (0.3%w/w) was 284.27°C and for the test sample it was found to be 285.30°C.

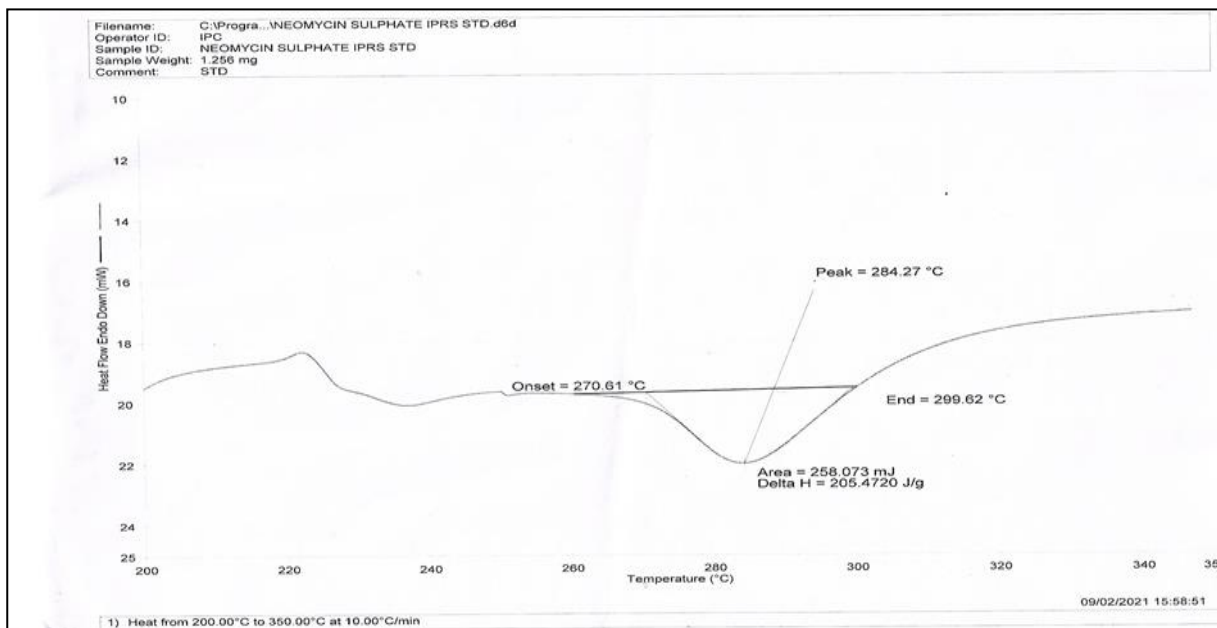


Figure 14 DSC report of mixture of drug and excipients

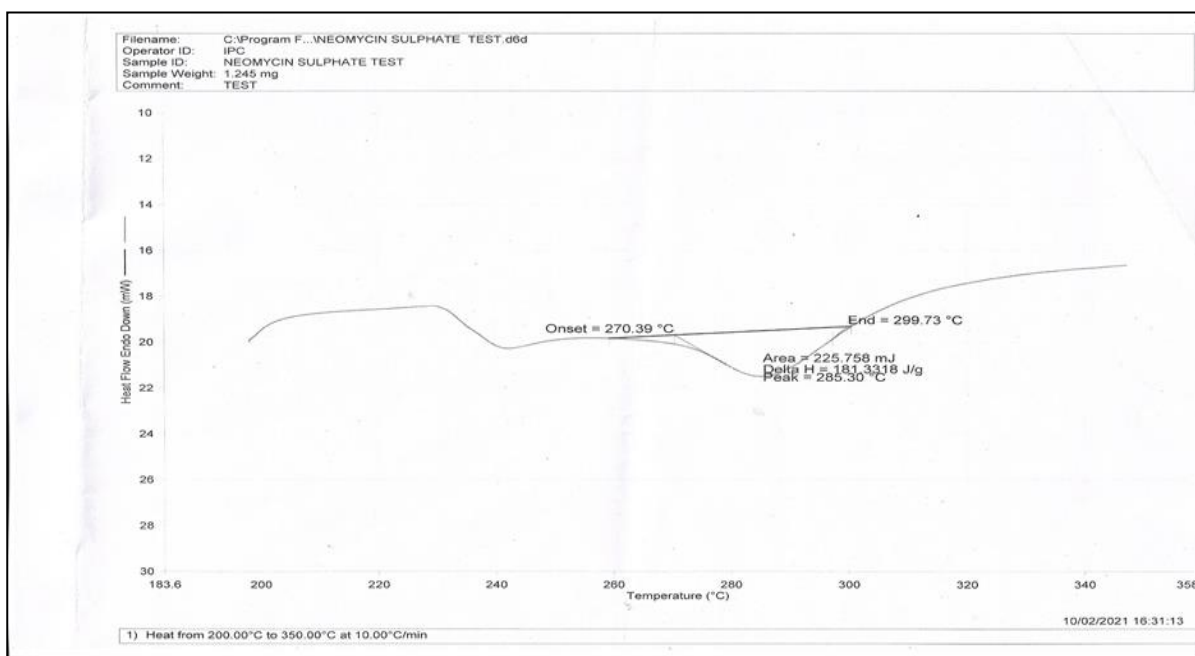


Figure 15 DSC report of 0.3% w/w hydrogel formulation

DSC results of the drug and the formulation showed that there is not much significant difference between the results.

5. In-Vitro drug release studies

The major driving forces for the release of drug from the hydrogel networks are the partition coefficient of the drug between the aqueous phase and the polymeric phase and diffusion of the drug across the dialysis membrane. The pre activated dialysis membrane retained the hydrogel formulations and allow the diffusion of the Ne into the release media. In vitro release profiles of the Ne from formulation are provided in figure 22.

The Ne topical hydrogels showed an initial fast drug release during the first one hour followed by a slower and sustained release for five hours. The initial fast release of drug was attributed to surface associated drug and the slow release was attributed to the drug entrapped in the hydrogel network. Moreover, the drug release efficiency was found to reach the maximum level after 5 h for all the samples analyzed.

Table 10 Result of In-vitro drug diffusion study

Time (Hr)	CPR		
	F3 (%)	F5 (%)	F7 (%)
0	0	0	0
0.25	16.57	16.57	16.57
0.50	27.63	28.19	27.48
0.75	39.99	39.55	39.44
1	53.70	53.04	54.14
2	64.85	65.75	66.06
3	78.75	79.50	80.32
4	90.90	90.76	92.68
5	98.62	98.71	95.08

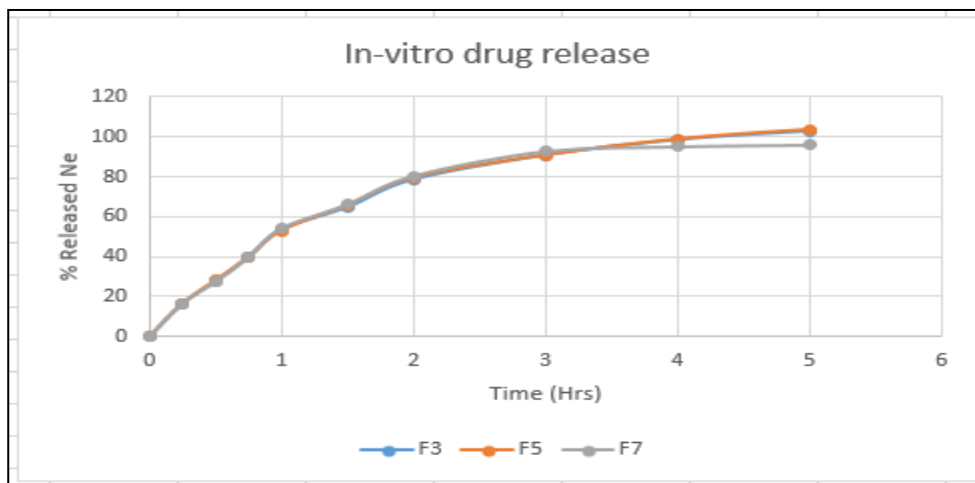


Figure 16-time v/s % released of Ne graph

6. Release Kinetics

Table 11 Result of release kinetics study

Batch no.	F3	F5	F7
Model	r ²	r ²	r ²
Zero Order	0.9648	0.9695	0.9693
First Order	0.9948	0.9972	0.9957
Higuchi	0.9672	0.9673	0.9634

Hixson-Crowell	0.9931	0.9933	0.9907
Korsmeyer- Peppas	0.4583	0.4608	0.4654

Observing the data of release kinetics study all the formulations are following first order kinetics comparing the r² value of all models which shows that rate of drug elimination is directly proportional to drug concentration in the body.

7. Accelerated Stability Studies

Table 12 Result of accelerated stability studies

Sr. no.	Batches	Months	Appearance	pH	Viscosity (RPM)	Drug Content (%)
1	F3	0	Clear	7.3±0.01	1284±50	103.00
		1	Clear	7.2±0.03	1287±20	101.75
		2	Clear	7.2±0.01	1279±60	100.50
		3	Clear	7.2±0.05	1281±30	99.00
2	F5	0	Clear	7.3±0.04	1184±40	104.00
		1	Clear	7.4±0.01	1180±20	103.25
		2	Clear	7.2±0.06	1139±50	101.00
		3	Clear	7.2±0.02	1092±20	100.00
3	F7	0	Clear	7.4±0.03	1098±10	96.00
		1	Clear	7.4±0.02	1095±30	96.35
		2	Clear	7.2±0.04	1101±30	95.00
		3	Clear	7.2±0.05	1097±20	94.00

Above values indicate mean±SD (n=3)

F1 and F2 didn't pass the accelerated stability test as the precipitation occurred in first month of observation.

F5 viscosity decreased to a certain level with time, whereas F3 and F7 showed minimal variation in viscosity.

F7 failed the accelerated stability test because the drug content reached 94% in 3 months which do not lie in the acceptable range of drug content for formulations i.e. 95-105%.

CONCLUSION

Various approaches are available in healthcare for topical drug delivery system which fulfills therapeutic aspects as well as patient compliance. However, for treatment of topical wound, compare to cream and ointment topical gel shows better results. Neomycin Sulfate together with its excipients used are a promising alternative to conventional drug therapy used for wound healing.

In present study carbopol used as a gelling agent also possesses wound healing activity. Physicochemical compatibility between drug and excipients checked and confirmed by FTIR shows promising results. The gel were prepared and evaluated for various physicochemical and performance parameters.

Experimental data for the assessment of wound healing activity by microbial assay shows good results. Hence we summarized and concluded that additive and synergistic effects of drug and excipients were responsible for potent wound healing activity. The investigations of present study supports scientific evidence to the folkloric use of Neomycin Sulfate in wound healing.

ACKNOWLEDGEMENT

This work was supported by the Amity Institute of Pharmacy by Amity University.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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