"DESIGN, DEVELOPMENT AND EVALUATION OF ACCELERATED DIABETIC WOUND HEALING TOPICAL FORMULATION OF NEOMYCIN-SULPHATE AND CURCUMIN"

A Thesis
Submitted to

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY

IN THE PARTIAL FULFILLMENT OF REQUIREMENT FOR THE AWARD OF DEGREE IN MASTERS OF PHARMACY (M.PHARM) IN THE SUBJECT

IN THE FOURTH SEMESTER
MASTERS OF PHARMACY

UNDER THE SUPERVISION OF

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DECLARATION

I hereby declare that this thesis entitled **"Design, development and evaluation of accelerated diabetic wound healing topical formulation of neomycin-sulphate and curcumin"** is a bonifide and genuine research work carried out by me under the supervision of **Dr. Bhupen Kalita, Asst. Professor, Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science, AZARA, Guwahati-17**. The work embolished in this thesis is original and has been submitted in part or fulfillment for the award of any degree, diploma, associate-ship or fellowship of any other university or institution.

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I wish her all success in life.

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I wish her all success in life

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Abstract

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cell below the dermis begins to increase collagen production. Later, the epithelial tissue is regenerated. There are three stages to the process of wound healing: inflammation, proliferation and remodeling. The diabetic accelerated wound healing properties of Neomycin sulphate as well as Curcumin has been reported for treatment of incision as well as excision wounds. The main aim of this study is to design, development and evaluation of accelerated diabetic wound healing topical formulation of neomycin-sulphate and curcumin. Gel formulations were prepared with concentration of 0.5% Neomycin sulphate and 0.3% Curcumin. Materials like carbopol-934 and HPMC K4M as main gelling agent was collected from the Merck Specialities Private Ltd., methyl paraben, propyl paraben, triethanolamine, and PEG 400, was collected from the Merck Specialities Private Ltd. Ethanol was collected from China, (Changshu Yangyuan Chemicals). Gel was prepared by continuously homonising by magnetic stirrer. Evaluation of the herbal gel formulation was done to determine the viscosity, ph, spreadibility, homogeneity, drug content study and physical parameters or organoleptic properties, which would conclude the formulation to have a good transdermal dosage form of drug to the human skin.

Keywords: Neomycin sulphate, curcumin, excipients, wound healing, gel formulation and evaluation.

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ABBREVIATION	
ºC	Degree centigrade
E.g.	Example
Fig	Figure
gm	Gram
Hrs.	Hours
mg	Milli gram
µg	Micro gram
µm	Micrometer
rpm	Rotation per minute
i.e.	That is
M	Meter
% w/v	%weight by volume
cps	centipoises
IR	Infrared Spectroscopy
DSC	Differential Scanning Calorimeter

CHAPTER- 1
INTRODUCTION

CHAPTER- 2
LITERATURE REVIEW

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1. Introduction:

1.1 Skin

The human skin is the outer covering of the body. It is the largest organ of the body. The skin has up to seven layers of tissue and guards the underlying muscles, bones, ligaments and internal organs. Human skin is comparable to that of most other mammals. Though virtually all human skin is covered with hair follicles, it can even be hairless to few people. There are generally two types of skin, hairy skin and glabrous skin. Because it interfaces in the midst of the environment, skin plays an significant immunity role in protecting the body pathogenic and excessive water loss, its additional functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B. Severely damaged skin tries to heal by forming scar tissue. It often gets discolored and pigmented. In humans, skin pigmentation varies in the midst of populations, and the skin type ranges from dry to oily skin type. Such skin variation provides a affluent and varied habitat for bacteria that are present on the human skin.

1.1.1. Structure

Skin has mesoderm cells, pigmentation, such as melanin provided by melanocytes, which take in some of the potentially unsafe ultraviolet radiation (UV) from sunlight. It moreover contains DNA repair enzymes that facilitate reverse UV damage, such that people deficient of the genes for these enzymes undergo high rates of skin cancer. One form predominantly produced by UV light, malignant melanoma, is particularly persistent, causing it to spread quickly, and can habitually be deadly. Human skin pigmentation varies in the midst of populations in a striking manner.

In terms of surface area, the skin is the second largest organ in the human body (inside the small intestine it is 15 to 20 times larger). For the average adult human, the skin has a surface area of between 1.5-2.0 square metres (16.1-21.5 sq ft.). The thickness of the skin varies considerably over all parts of the body, and between men and women and the young and the old. An example is the skin on the forearm which is on average 1.3 mm in the male and 1.26 mm in the female. The average square inch (6.5 cm^2) of

skin holds 650 sweat glands, 20 blood vessels, 60,000 melanocytes, and more than 1,000 nerve endings. The average human skin cell is about 30 micrometres in diameter, but there are variants. A skin cell usually ranges from 25-40 micrometres (squared), depending on a variety of factors. Skin is composed of three primary layers: the epidermis, the dermis and the hypodermis.

1.1.2. Layers of skin

1.1.2.1. Epidermis

Epidermis stands for, "epi" comes from the Greek word "over" or "upon", is the outermost or uppermost layer of the skin. It forms the waterproof, protective layer or covering over the body's surface which also serves as a barrier to infection and is made up of stratified squamous epithelium with an underlying layer basal lamina.

The epidermis contains no blood vessels, and cells in the deepest layers are nourished almost exclusively by diffused oxygen from the surrounding air and to a far lesser degree by blood capillaries extending to the outer layers of the dermis. The main type of cells which make up the epidermis are Merkel cells, keratinocytes, with melanocytes and Langerhans cells also present. The epidermis can be further subdivided into the following *strata* (beginning with the outermost layer): corneum, lucidum (only in palms of hands and bottoms of feet), granulosum, spinosum, basale. Cells are formed through mitosis at the basale layer. The daughter cells (see cell division) move up the strata changing shape and composition as they die due to isolation from their blood source. The cytoplasm is released and the protein keratin is inserted. They eventually reach the corneum and slough off (desquamation). This process is called "keratinization". This keratinized layer of skin is responsible for keeping water in the body and keeping other harmful chemicals and pathogens out, making skin a natural barrier to infection.

Sublayers

Epidermis is divided into the following 5 sublayers or strata:

- Stratum corneum

- Stratum lucidum
- Stratum granulosum
- Stratum spinosum
- Stratum germinativum

1.1.2.2. Dermis

The dermis is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis is tightly connected to the epidermis by a basement membrane. It also harbors many nerve endings that provide the sense of touch and heat. It contains the hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels. The blood vessels in the dermis provide nourishment and waste removal from its own cells as well as from the Stratum basale of the epidermis. The dermis is structurally divided into two areas: a superficial area adjacent to the epidermis, called the *papillary region*, and a deep thicker area known as the *reticular region*.

Papillary region

The papillary region is composed of loose areolar connective tissue. It is named for its fingerlike projections called *papillae*, that extend toward the epidermis. The papillae provide the dermis with a "bumpy" surface that interdigitates with the epidermis, strengthening the connection between the two layers of skin. In the palms, fingers, soles, and toes, the influence of the papillae projecting into the epidermis forms contours in the skin's surface. These epidermal ridges occur in patterns (*see:* fingerprint) that are genetically and epigenetically determined and are therefore unique to the individual, making it possible to use fingerprints or footprints as a means of identification.

Reticular region

The reticular region lies deep in the papillary region and is usually much thicker. It is composed of dense irregular connective tissue, and receives its name from the dense concentration of collagenous, elastic, and reticular fibers that weave throughout it.

These protein fibers give the dermis its properties of strength, extensibility, and elasticity.

1.1.2.3. Hypodermis

The hypodermis is not part of the skin, and lies below the dermis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue, adipose tissue and elastin. The main cell types are fibroblasts, macrophages and adipocytes (the hypodermis contains 50% of body fat)

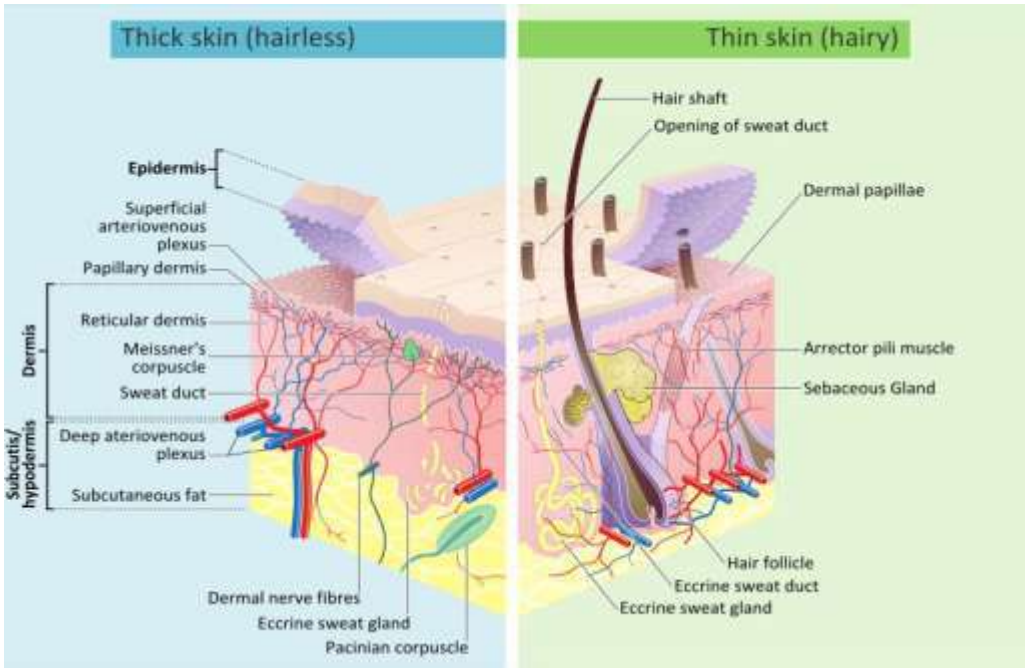


Fig 1.1.2: Structure of layers of skin

1.1.3 Functions of the Skin

1. Protection of the human body

2. Sensation i.e. transmitting to the brain information about surroundings

3. Temperature regulation

4. Immunity i.e. the role of the skin within the immune system

5. Enables movement and growth without injury

6. Excretion from the body of certain types of waste materials

7. Endocrine function e.g. re. Vitamin D

1.2. Wound healing

1.2.1. Definition

Wound healing refers to a living organism's replacement of destroyed or damaged tissue by newly produced tissue. Wound healing is depicted in a discrete timeline of physical attributes (phases) constituting the post-trauma repairing process. In undamaged skin, the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment. When the barrier is broken, a regulated sequence of biochemical events is set into motion to repair the damage. This process is divided into predictable phases: blood clotting (homeostasis), inflammation, tissue growth (cell proliferation), and tissue remodeling (maturation and cell differentiation).

The wound healing process is not only complex but also fragile, and it is susceptible to interruption or failure leading to the formation of non-healing chronic wounds. Factors that contribute to non-healing chronic wounds are diabetes, venous or arterial disease, infection, and metabolic deficiencies of old age.

Wound care encourages and speeds wound healing via cleaning and protection from re-injury or infection. Depending on each patient's needs, it can range from the simplest first aid to entire nursing specialties such as wound, ostomy, and continence nursing and burn center care.

1.2.2. Stages of wound healing

- **Homeostasis (blood clotting):** Within the first few minutes of injury, platelets in the blood begin to stick to the injured site. They change into an amorphous shape, more suitable for clotting, and they release chemical signals to promote clotting. This results in the activation of fibrin, which forms a mesh and acts as "glue" to

bind platelets to each other. This makes a clot that serves to plug the break in the blood vessel, slowing/preventing further bleeding.

- **Inflammation:** During this phase, damaged and dead cells are cleared out, along with bacteria and other pathogens or debris. This happens through the process of phagocytosis, where white blood cells engulf debris and destroy it. Platelet-derived growth factors are released into the wound that cause the migration and division of cells during the proliferative phase.
- **Proliferation (growth of new tissue):** In this phase, angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur. In angiogenesis, vascular endothelial cells form new blood vessels. In fibroplasias and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin. Concurrently, re-epithelialization of the epidermis occurs, in which epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue. In wound contraction, myofibroblasts decrease the size of the wound by gripping the wound edges and contracting using a mechanism that resembles that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis.
- **Maturation (remodeling):** During maturation and remodeling, collagen is realigned along tension lines, and cells that are no longer needed are removed by programmed cell death, or apoptosis.

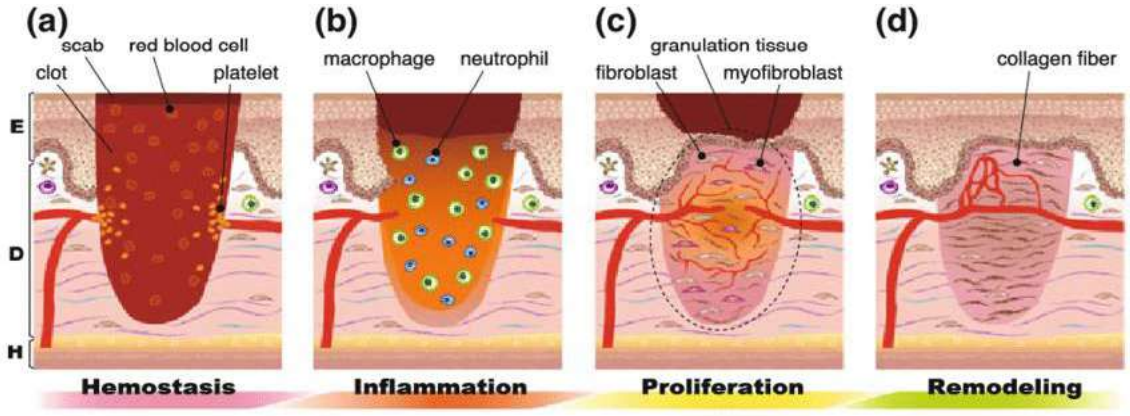


Fig 1.2.2: Stages of wound healing

1.2.3. Factors affecting wound healing

Many factors controlling the efficacy, speed, and manner of wound healing fall under two types: local and systemic factors.

1. **Local factors**

- Moisture: keeping a wound moist rather than dry makes wound healing more rapid and with less pain and less scarring.
- Mechanical factors
- Edema
- Ionizing radiation
- Faulty technique of wound closure
- Ischemia and necrosis
- Foreign bodies. Sharp, small foreign bodies can penetrate the skin leaving little surface wound but causing internal injury and internal bleeding. For a glass foreign body, "frequently, an innocent skin wound disguises the extensive nature of the injuries beneath". First-degree nerve injury requires a few hours to a few weeks to recover. If a foreign body passes by a nerve and causes first-degree nerve injury during entry, then the sensation of the foreign body or pain due to internal wounding may be delayed by a few hours to a few weeks after entry. A sudden increase in pain during the first few weeks of wound healing could be a sign of a recovered nerve reporting internal injuries rather than a newly developed infection.
- Low oxygen tension
- Perfusion

2. **Systemic factors**

- Inflammation
- Diabetes – Individuals with diabetes demonstrate reduced capability in the healing of acute wounds. Additionally, diabetic individuals are susceptible to developing chronic diabetic foot ulcers, a serious complication of diabetes which affects 15% of people with diabetes and accounts for 84% of all diabetes-related lower leg amputations. The impaired healing abilities of diabetics with diabetic foot ulcers

and/or acute wounds involves multiple pathophysiological mechanisms. This impaired healing involves hypoxia, fibroblast and epidermal cell dysfunction, impaired angiogenesis and neovascularization, high levels of metalloproteases, damage from reactive oxygen species and AGEs (advanced glycation end-products), decreased host immune resistance, and neuropathy.

- Nutrients – Malnutrition or nutritional deficiencies have a recognizable impact on wound healing post trauma or surgical intervention. Nutrients including proteins, carbohydrates, arginine, glutamine, polyunsaturated fatty acids, vitamin A, vitamin C, vitamin E, magnesium, copper, zinc and iron all play significant roles in wound healing. Fats and carbohydrates provide the majority of energy required for wound healing. Glucose is the most prominent source of fuel and it is used to create cellular ATP, providing energy for angiogenesis and the deposition of new tissues. As the nutritional needs of each patient and their associated wound are complex, it is suggested that tailored nutritional support would benefit both acute and chronic wound healing.
- Metabolic diseases
- Immunosuppression
- Connective tissue disorders
- Smoking – Smoking causes a delay in the speed of wound repair notably in the proliferative and inflammatory phases. It also increases the likelihood of certain complications such as wound rupture, wound and flap necrosis, decrease in wound tensile strength and infection. Passive smoking also impairs a proper wound healing process.
- Age – Increased age (over 60 years) is a risk factor for impaired wound healing. It is recognized that, in older adults of otherwise overall good health, the effects of aging causes a temporal delay in healing, but no major impairment with regard to the quality of healing. Delayed wound healing in patients of increasing age is associated with altered inflammatory response; for example delayed T-cell infiltration of the wound with alterations in the production of chemokines, and reduced macrophage phagocytic capacity.
- Alcohol – Alcohol consumption impairs wound healing and also increases the chances of infection. Alcohol affects the proliferative phase of healing. A

single unit of alcohol causes a negative effect on re-epithelialization, wound closure, collagen production and angiogenesis.

1.2.4. Types of wound healing intensions

Successful wound healing is dependent on various cell types, molecular mediators and structural elements.

1. **Primary intention**

Primary intention is the healing of a clean wound without tissue loss. In this process, wound edges are brought together, so that they are adjacent to each other (re-approximated). Wound closure is performed with sutures (stitches), staples, or adhesive tape or glue.

Primary intention can only be implemented when the wound is precise and there is minimal disruption to the local tissue and the epithelial basement membrane, e.g. surgical incisions.

This process is faster than healing by secondary intention. There is also less scarring associated with primary intention, as there are no large tissue losses to be filled with granulation tissue, though some granulation tissue will form.

- Examples of primary intention include: well-repaired lacerations, well reduced bone fractures, healing after flap surgery.
- Early removal of dressings from clean or clean-contaminated wounds does affect primary healing of wounds.

2. **Secondary intention**

- Secondary intention is implemented when primary intention is not possible because of significant tissue damage or loss, usually due to the wound having been created by major trauma.
- The wound is allowed to granulate.
- Surgeon may pack the wound with a gauze or use a drainage system.
- Granulation results in a broader scar.
- Healing process can be slow due to presence of drainage from infection.

- Wound care must be performed daily to encourage wound debris removal to allow for granulation tissue formation.
- Using antibiotics or antiseptics for the surgical wound healing by secondary intention is controversial.
- Examples: gingivectomy, gingivoplasty, tooth extraction sockets, poorly reduced fractures, burns, severe lacerations, pressure ulcers.
- There is insufficient evidence that the choice of dressings or topical agents affects the secondary healing of wounds.
- There is lack of evidence for the effectiveness of negative pressure wound therapy in wound healing by secondary intention.

3. **Tertiary intention:** (Delayed primary closure or secondary suture):

- The wound is initially cleaned, debrided and observed, typically 4 or 5 days before closure.
- The wound is purposely left open.
- Examples: healing of wounds by use of tissue grafts.

If the wound edges are not re-approximated immediately, delayed primary wound healing transpires. By the fourth day, phagocytosis of contaminated tissues is well underway, and the processes of epithelization, collagen deposition, and maturation are occurring. Foreign materials are walled off by macrophages that may metamorphose into epithelioid cells, which are encircled by mononuclear leukocytes, forming granuloma. Usually the wound is closed surgically at this juncture, and if the "cleansing" of the wound is incomplete, chronic inflammation can ensue, resulting in prominent scarring.

1.3. Gel

1.3.1. Definition

A gel is a solid jelly-like soft material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state. By weight, gels are mostly liquid, yet they behave like solids due to a three-dimensional cross-linked network within the liquid. It is the cross linking within the fluid that gives a gel its structure (hardness) and contributes to the adhesive stick (tack). In this way gels are a dispersion of molecules of a liquid within a solid in which liquid particles are dispersed in the solid medium. Gels are thicker than liquids. Gels are often a semisolid emulsion sometimes using alcohol as a solvent for the active ingredient. Some gels liquefy at body temperature. Gel tends to be cellulose cut with alcohol or acetone. Gels tend to be self-drying. Gels tend to have greatly variable ingredients between brands. Gels carry a significant risk of inducing hypersensitivity due to fragrances and preservatives. Gel is useful for hairy areas and body folds. In applying gel one should avoid fissures in the skin, due to the stinging effect of the alcohol base. Gel enjoys a high rate of acceptance due to its cosmetic elegance.

1.3.2. Interest and advantage

Topical gel formulations are of increasing interest in the dermatology industry. Gel formulations are typically transparent or translucent, water-based semisolids with good spreading properties and pleasing aesthetic characteristics. Gels derive their consistency and rheological properties from polymers that can swell in water and then interact in such a way as to thicken the water and increase viscosity. Polymers may interact physically, by chain entanglement, or by ionic or hydrophobic/hydrophilic interactions. In each case, the polymers form a matrix that increases the viscosity of the water and allows for

(1) physical stabilization and prevention of migration of suspended API crystals,

(2) maintenance of product homogeneity throughout the shelf life,

(3) clean, no drip, no mess transfer of the product from the primary package to the skin surface and easy spreading and acceptable aesthetics.

1.4. Drug profile:

1.4.1. Curcumin:

Chemical formula: $C_{21}H_{20}O_6$

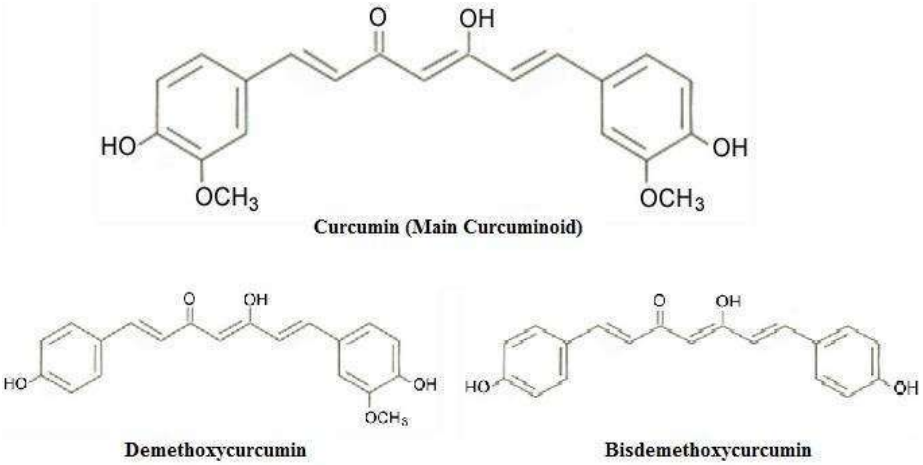
Molar mass: 368.385 $g.mol^{-1}$

Melting point: 183 ºC / 361 ºF

Curcumin, a bright yellow-orange powder, a polyphenol derived from Indian dietary spice turmeric (the common name for *Curcuma longa* L., Zingiberaceae family), has been widely used as a herbal remedy for centuries in indigenous medicine to treat a variety of inflammatory conditions and other diseases. The active medicinal ingredient of turmeric has been identified as curcuminoids, which includes an active component curcumin (diferuloylmethane)—(1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-hepadiene-3,5-dione) and is found to be beneficial in treating various disorders, including skin diseases. It has also been reported that curcumin possesses anti-inflammatory, antioxidant, and antiproliferative properties that are mediated through the regulation of several inflammatory cytokines, growth factors, protein kinases, transcription factors, and other enzymes.

1.4.1.1. Structure of Curcumin

The structure of curcumin, officially known as diferuloylmethane, (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-hepadiene-3,5-dione) is two ferulic acid moieties bound together with an additional carbon (methane) to abridge the carboxyl groups. It can exist in an enol form (pictured below) or a keto form, which is molecularily symmetrical with two ketone groups on the backbone.



(Modified from: Changtam C, et al. Curcuminoid analogs with potent activity against Trypanosoma and Leishmania species. Eur J Med Chem. (2010))

Fig 1.4.1.1: Chemical structure of curcumin

1.4.1.2. Isolation of Curcumin:

Curcumin is insoluble in water; an organic solvent has been used for its isolation. [26] developed a technique for isolating CUR from ground turmeric. They magnetically stirred the ground turmeric in dichloromethane and heated at reflux for 1 h. The mixture was suction-filtered, and the filtrate was concentrated in a hot-water bath maintaining at 50o C. The reddish-yellow oil residue was triturated with hexane and the resulting solid was collected by suction filtration. Further TLC analysis (3% methanol and 97% dichloromethane) showed the presence of all three components. Extraction of curcumin from turmeric powder is done with the use of a solvent consisting of a mixture of ethanol and acetone. Chemical analyses have shown that turmeric contains carbohydrates (69.4%), moisture (13.1%), protein (6.3%), fat (5.1%) and minerals (3.5%). The essential oil (5.8%) obtained by steam distillation of the rhizomes contains a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%), curcumin (3-6%) is responsible for the yellow color.

1.4.1.3. Chemistry of Curcumin:

Curcumin is a symmetric molecule, also known as diferuloylmethane. IUPAC name of this compound is (1E-6E)-1, 7-bis (4-hydroxy-3-methoxy phenyl)-1, 6- heptadiene-3, 5-dione. The chemical formula of curcumin is C21H20O6 and the molecular mass is

368.385g/mole. The structure of curcumin contains three chemical entities: two oxy-substituted aryl moieties containing ortho-methoxy phenolic OH– groups, connected through a seven carbon chain consisting of a α, βunsaturated β-diketone moiety. Curcumin is the most abundantly occurring natural analogue of a crude extract at 60%-70%, followed by demethoxycurcumin(DMC; 20%-30%) in which one methoxy group is absent, then bisdemethoxycurcumin(BDMC; 10%-15%) in which the methoxy group is absentfrom both the aryl rings, [30] along with numerous and less abundant secondary metabolites. Important chemical reactions associated with the biological activity of curcumin are the hydrogen-atom donation reactions leading to oxidation of curcumin, reversible and irreversible nucleophilic addition reactions, hydrolysis, degradation and enzymatic reactions. All these play important role in different biological activities of curcumin. Curcumin is a hydrophobic molecule with a calculated log P value is 3.43; however it is insoluble in aqueous physiologic media, which displays poor distribution and bioavailability. Curcumin is soluble in polar solvents like DMSO, methanol, acetone and ethanol. Thus, it tends to accumulate in hydrophobic regions, for example, the membrane of cells. Taken together, curcumin can perform as a hydrophobic reducing (antioxidant) agent and thereby scavenge various reactive oxygen species (ROS). It has also been demonstrated that curcumin was better than vitamin E in suppressing oxidative stress. The regeneration reaction of phenoxyl radicals by water-soluble antioxidants like Vitamin C restores curcumin for consecutive ROS elimination reactions. Curcumin is as efficient as intrinsic and lipid soluble antioxidants in the removal of superoxide radicals and stimulates the function of superoxide dismutase. The hydrogen donor site, α, β-unsaturated β-diketone moiety, is also considered the breakdown point in the curcumin structure, resulting in curcumin hydrolysis and degradation in water at room temperature and neutral pH. It has been reported that 90% of curcumin degrades within 30min in aqueous alkaline buffer, Being lipophilic in nature, the water solubility of curcumin could be enhanced when the diketo reaction site is binding in polymers, cyclodextrins, lipids, proteins and other macromolecular structures as the reaction site becomes protected from hydrolysis. It has been demonstrated that solvolysis is a minor pathway, and the primary pathway is autoxidation. Pharmacokinetic studies showed that after oral consumption, curcumin is metabolized to give sulfate and glucuronide derivatives. The chemical stability of curcumin can be enhanced by encapsulation with lipids or nanoparticles. Other methods

to enhancing stability have included synthetic manipulations to eliminate or protect the oxidation sites (phenolic-OH and enolic-OH) and derivatization of the β-diketone to decrease the activity of the enolate Michael acceptor. Besides, analogues of curcumin could be a more feasible way to for clinical application, further clinical studies are needed to evaluate and potentially confirm the beneficial effects of them.

1.4.1.4. Pharmacological Activity with Mode of Action/ Biological Activities:

ANTI-VIRAL ACTIVITY: It has been demonstrated that curcumin as a plant derivative has a wide range of antiviral activity against different viruses: papillomavirus virus (HPV), influenza virus, Hepatitis B virus (HBV), Hepatitis C virus (HCV), adenovirus, coxsackie virus, Human norovirus (HuNoV), Respiratory syncytial virus (RSV) and Herpes simplex 1 (HSV-1). Curcumin functionalized graphene oxide shown synergistic antiviral effect against respiratory syncytial virus infection. Respiratory syncytial virus (RSV), which is considered as the major viral pathogen of the lower respiratory tract of infants, has been implicated in severe lung disease. Developing a β-cyclodextrin (CD) functionalized graphene oxide (GO) composite, which displayed excellent antiviral activity and curcumin loading efficiently, showed that the composite could prevent RSV from infecting the host cells by directly inactivating virus and inhibiting the viral attachment, which possessed the prophylactic and therapeutic effects towards virus. The antiviral effect of curcumin was a dose-dependent manner. Curcumin inhibit activity of inosine-mono phosphatedehydrogenase (IMPDH) enzyme in either noncompetitive or competitive manner. By inhibition of IMPDH this led to reduce the level of intracellular guanine nucleotides which required for adequate RNA and DNA synthesis. Curcumin mechanism involve in viral entry or other life cycle stages rather than the replication of viral RNA. Therefore, by inhibition of IMPDH Curcumin have potential anti-proliferative, antiviral and antiparasitic effects.

ANTI-INFLAMMATORY ACTIVITY: Curcumin possesses significant anti-inflammatory activity in acute as well as in chronic models of inflammation. It is as potent as phenylbutazone in the carrageenan oedema test but only half as potent in chronic tests. Curcumin has been demonstrated to be safe in six human trials and has demonstrated anti-inflammatory activity. It may exert its anti-inflammatory activity by

inhibition of a number of different molecules that play a role in inflammation. Curcumin has been shown to regulate numerous transcription factors, cytokines, protein kinases, adhesion molecules, redox status and enzymes that have been linked to inflammation. Tumor necrosis factor α (TNF-α)is a major mediator of inflammation in most diseases, and this effect is regulated by the activation of a transcription factor, nuclear factor(NF)- κB. Whereas TNF-α is said to be the most potent NF-κB activator, the expression of TNF-α is also regulated by NF-κB. In addtion to TNF-α, NF-κB is also activated by most inflammatory cytokines; gramnegative bacteria; various disease-causing viruses; environmental pollutants; chemical, physical, mechanical, and psychological stress; high glucose; fatty acids; ultraviolet radiation; cigarette smoke; and other disease-causing factors. Therefore, agents that downregulate NF-κB and NF-κB– regulated gene products have potential efficacy against several of these diseases. Curcumin has been shown to block NF-κB activation increased by several different inflammatory stimuli. Curcumin has also been shown to suppress inflammation through many different mechanisms beyond the scope of this review, thereby supporting its mechanism of action as a potential anti-inflammatory agent.

ANTI-OXIDANT: Curcumin has been shown to improve systemic markers of oxidative stress it can modulate the activity of GSH, catalyses, and SOD enzymes active in the neutralization of free radicals. There is evidence that it can increase serum activities of antioxidants such as superoxide dismutase (SOD) A recent systematic review and meta-analysis of randomized control data related to the efficacy of supplementation with purified curcuminoids on oxidative stress parameters—indicated a significant effect of curcuminoids supplementation on all investigated parameters of oxidative stress including plasma activities of SOD and catalase, as well as serum concentrations of glutathione peroxidase (GSH) and lipid peroxides. It is noteworthy to point out that all of the studies included in the meta-analysis utilized some sort of formulation to overcome bioavailability challenges, and four out of the six used piperine. Curcumin's effect on free radicals is carried out by several different mechanisms. It can scavenge different forms of free radicals, such as reactive oxygen and nitrogen species (ROS and RNS, respectively) also, it can inhibit ROS-generating enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase. In addition, curcumin is a lipophilic compound, which makes it an efficient scavenger of

peroxyl radicals, therefore, like vitamin E, curcumin is also considered as a chain-breaking antioxidant.

ANTI-CANCER: One-fifth of the deaths worldwide annually are caused by various types of cancers. Cancer is a Result of successive genetic and epigenetic alterations resulting in apoptosis, uncontrolled cell Proliferation, metastasis, and angiogenesis. Anticancer activity of curcumin has been extensively investigated recently, and significant improvements in gastrointestinal, melanoma, genito-urinary, breast, and lung cancers have been seen. Many studies pointed out anticancer activities of curcumin alone or in combination with conventional chemotherapy drugs in treatment of cancer and its cancer-related complications. In-vitro and in-vivo studies have indicated that curcumin prevents carcinogenesis by affecting two primary processes: Angiogenesis and tumor growth Curcumin analogs S1- S3 containing sulfone strongly inhibited the growth of human prostate, colon, lung and pancreatic cancer cells. Scientific studies of plants used in various types of ethnic medicine have led to the discovery of many valuable drugs, including taxol, camptothecin, vincristine and Vinblastine.

1.4.1.5. Wound- Healing Activity of Curcumin: The ethosomal curcumin significantly recovered main aspects of wound repair including re-epithelization, neovascularization, collagen synthesis, granulation tissue formation. It also potentially inhibited growth of the burn bacterial flora including Pseudomonas aeruginosa as predominant bacteria among experimental isolations during 14 days treatment. It effciently fights against wound infection and promotes wound repair in burn injuries in rats. The growth factors are participated in wound healing process which stimulated by curcumin. The mechanisms of action of wound healing effect of curcumin include: immune-histochemical localization of transforming growth factor-β1 showed an increase in curcumin-treated wounds as compared with untreated wounds and modulating collagen and decreasing reactive oxygen species.

1.4.2. NEOMYCIN SULPHATE:

IUPAC : (2*R*,3*S*,4*R*,5*R*,6*R*)-5-amino-2-(aminomethyl)-6-[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-2-[(2*S*,3*R*,4*S*,5*R*)-4-[(2*R*,3*R*,4*R*,5*S*,6*S*)-3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3,4-diol;sulfuric acid

Molecular Formula: $C_{23}H_{52}N_6O_{25}S_3$

Molecular Weight: 908.9

Melting point: >187ºC

Synonyms: Fradiomycin, Kaomycine, Neomicina, Vonamycin

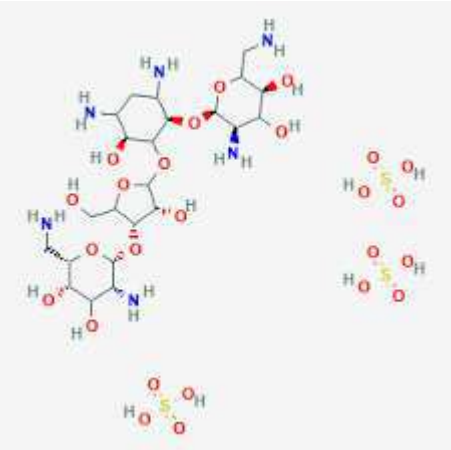


Fig 1.4.2.1: chemical structure of Neomycin Sulphate

Neomycin is a broad-spectrum aminoglycoside antibiotic drug that is derived from the metabolic products of *Streptomyces fradiae*. Neomycin is a complex comprised of three components, neomycin A, B, and C. Neomycin B, also known as framycetin, is the most active component of the complex and neomycin C is the isomer of neomycin B, making these two stereoisomers the active components of neomycin. Neomycin A, or neamine, is a moiety that conjoins two molecules of neomycin B and C together. Neomycin is active against both gram-positive and gram-negative organisms and mediates its pharmacological action by binding to bacterial ribosomes and inhibiting protein synthesis, which is crucial for the survival of bacteria.

Neomycin sulfate is the most common form for pharmaceutical preparations; because the compound is a complex, the amount of neomycin in products is measured in units. Neomycin sulfate as monotherapy is available in an oral solution for adjunct use in the treatment of hepatic coma. It is also used in combination with polymyxin B sulfates and hydrocortisone in otic suspensions for use in the treatment of bacterial infections in the external auditory canal, including infections caused by medical procedures in the ear. Neomycin is also used in combination with polymyxin B sulfates and dexamethasone in ophthalmic preparations for use in the treatment of inflammatory conditions and infections in the eye. Neomycin is also available in over-the-counter topical products to prevent minor skin infections.

Clinical uses: Neomycin is widely used in combination with other antibiotics, antifungal, and corticosteroids because of its availability, relatively low cost, and perceived efficacy. There are few well-controlled clinical trials documenting the efficacy and safety of topical neomycin. Neomycin has been shown to enhance re-epithelialization in wound healing. However, in view of its well-documented contact sensitivity, possible systemic toxicity, and cross-reactivity with other antibiotics and because of the emergence of resistance, it is difficult to recommend the use of topical neomycin in the treatment of superficial skin infections.

Adverse effects: Neomycin can cause irreversible deafness, which is a main reason it is no longer used parenterally. It is considerably more ototoxic than kanamycin. Sufficient absorption may occur after prolonged oral administration to cause ototoxicity, especially in the presence of renal impairment. Neomycin may also cause reversible renal damage if given parenterally. Like other amino glycosides, it can cause neuromuscular blockade, especially when given intraperitoneally. Large oral doses may cause vomiting or diarrhea.

Pharmacodynamics: Neomycin mediates its bactericidal action by inhibiting bacterial protein synthesis, thereby suppressing the growth and survival of susceptible bacteria. Following oral administration, the duration of bactericidal activity of neomycin ranged from 48 to 72 hours. By decreasing colonic bacteria that produce ammonia, neomycin was shown to be effective as an adjunctive therapy in hepatic coma to improve

neurologic symptoms. Neomycin is active against both gram positive and gram negative organisms, including the major *E. coli* species resident in the colon as well as the enteropathogenic forms of *E. coli*. It is also active against *Klebsiella-Enterobacter* group. Resistant strains of *E. coli*, *Klebsiella* and *Proteus spp*. may emerge from neomycin therapy. Neomycin has no antifungal activity and has some activity against some protozoa

Mechanism of action: Like other aminoglycoside antibiotic drugs, neomycin inhibits bacterial ribosomes by binding to the 30S ribosomal subunit of susceptible bacteria and disrupting the translational machinery of bacterial protein synthesis. Bacterial translation is normally initiated by the mRNA binding to the 30S ribosomal subunit and subsequent binding with 50S subunit for elongation.

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2. Literature Review:

1. **(Patel S. et al, 2019)** reviewed that wound management in diabetic patient is of an extreme clinical and social concern. The research on impaired healing process is proceeding hastily evident by new therapeutic approaches other than conventional such as single growth factor, dual growth factor, skin substitutes, cytokine stimulators, cytokine inhibitors, matrix metalloproteinase inhibitors, gene and stem cell therapy, extracellular matrix and angiogenesis stimulators. Although numerous studies are available that support delayed wound healing in diabetes but detailed mechanistic insight including factors involved and their role still needed to be revealed. Here the main focus is on the molecular cascades of cytokines (with growth factors) and factors responsible for delayed wound healing, molecular targets and recent advancements in complete healing and its cure.

2. **(Harold B. et al, 2007)** reviewed that Diabetic foot ulcers (DFUs),is a leading cause of amputations, affect 15% of people with diabetes. A series of multiple mechanisms, including decreased cell and growth factor response, lead to diminished peripheral blood flow and decreased local angiogenesis, all of which can contribute to lack of healing in persons with DFUs. Further demonstrated that in diabetic mice, hyperoxia enhances the mobilization of circulating endothelial progenitor cells (EPCs) from the bone marrow to the peripheral circulation (see the related article beginning on page 1249). Local injection of the chemokine stromal cell–derived factor–1α then recruits these EPCs to the cutaneous wound site, resulting in accelerated wound healing.

3. **(Uzoagu A.O. et al, 2017)** reviewed that Diabetes Mellitus Type II (DM2) is a growing international health concern with no end in sight. Complications of DM2 involve a myriad of co-morbidities including the serious complications of poor wound healing, chronic ulceration, and resultant limb amputation. In skin wound healing, which has definite, orderly phases, diabetes leads to improper function at all stages. While the etiology of chronic, non-healing diabetic wounds is multi-faceted, the progression to a non-healing phenotype is closely linked to poor

vascular networks. This review focuses on diabetic wound healing, paying special attention to the aberrations that have been described in the proliferative, remodeling, and maturation phases of wound angiogenesis. Additionally, this review considers therapeutics that may offer promise to better wound healing outcomes.

4. **(Nimberkar T.P. et al 2012)** characterized that the present investigation was to develop novel ointment formulation in combination of natural wound healing agent *Madhuca indica* inner bark extract, which is reported to possess wound healing and anti-bacterial activities. Combination of neomycin sulphate and *Madhuca indica* extract is good rational, where the extract produces synergistic wound healing effect with neomycin sulphate. Formulations containing fixed concentration (0.5 %) of neomycin sulphate and 3 %, 5 % and 7 % of bark extract were prepared. To assess the efficacy of formulations anti-bacterial activity, rheology, stability, spreadability and other physical characteristics were evaluated. The results obtained were encouraging and formulation containing neomycin sulphate (0.5%) with 5 % of Madhuca indica bark extract was found better than other formulations.

5. **(Akansha D. et al, 2009)** developed and characterized that the present investigation was to develop novel ointment formulation in combination of natural wound healing agent *curcuma longa*, which is reported to possess wound healing and anti-bacterial activities. Combination of neomycin sulphate and *curcuma longa* is good rational, where *curcuma longa* produces synergistic wound healing effect with neomycin sulphate. Formulations containing fixed concentration (0.5 %) of neomycin sulphate and 3 %, 4 % and 5 % of *curcuma longa* were prepared. To assess the efficacy of formulations anti-bacterial activity, rheology, stability, spreadability and other physical characteristics were evaluated. The results obtained were encouraging and formulation containing neomycin sulphate (0.5 %) with 5 % of *curcuma longa* was found better than other formulations.

6. **(Pathak A. K. et al, 2013)** developed and characterized the antibacterial patch that may stick to the wounds, providing protection against bacterial infection

without disturbing the healthy tissues present around the wound. As there is no external agent that can heal the wound, as body has its own wound healing mechanism, only thing that can be done is protecting the wounds against bacteria. So the film is prepared using neomycin antibacterial agent and using polymer gelatin that too has anti bacterial effect. The neomycin patch will be biocompatible material without given any side effect to the applied natural systems. The incorporation of PEG with gelatin has the aim of developing a material that would have good mechanical strength, be thermally stable on human body, and have good swelling property and effective water absorption capacity. The prepared product enhances both the rapidity of healing including reducing infection, pain, and scarring. An improved dressing also will reduce cost by improving the rate of wound healing and shorter the duration the treatment.

7. **(Jin S. G. et al, 2015)** characterized and developed a novel neomycin sulfate-loaded hydrogel dressing (HD), numerous neomycin sulfate-loaded HDs were prepared with various amounts of polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP) and sodium alginate (SA) using freeze-thawing technique, and their physical dressing properties, drug release, *in vivo* wound curing and histopathology in diabetic-induced rats were assessed. SA had a positive effect on a swelling capacity, but a negative effect on the physical dressing properties and drug release of HD. However, PVP did the opposite. In particular, the neomycin sulfate-loaded HD composed of drug, PVA, PVP and SA at the weight ratio of 1/10/0.8/0.8 had excellent swelling and bio-adhesive capacity, good elasticity and fast drug release. Moreover, this HD gave more improved wound curing effect compared to the commercial product, ensured the disappearance of granulation tissue and recovered the wound tissue to normal. Therefore, this novel neomycin sulfate-loaded HD could be an effective pharmaceutical product for the treatment of wounds.

8. **(Seyed N. M. et al, 2017)** reviewed that wound healing is a complex process that consists of several phases that range from coagulation, inflammation, accumulation of radical substances, to proliferation, formation of fibrous tissues

and collagen, contraction of wound with formation of granulation tissue and scar. Curcumin, the most active component of rhizome of Curcuma longa L. (common name: turmeric), has been studied for many years due to its bio-functional properties, especially antioxidant, radical scavenger, antimicrobial and anti-inflammatory activities, which play a crucial role in the wound healing process. Moreover, curcumin stimulates the production of the growth factors involved in the wound healing process, and so curcumin also accelerated the management of wound restoration. The aim of the present review is collecting and evaluating the literature data regarding curcumin properties potentially relevant for wound healing. Moreover, the investigations on the wound healing effects of curcumin are reported.

9. **(Menon V.P. et al, 2007)** reviewed the desirable preventive or putative therapeutic properties of curcumin, which have also been considered to be associated with its antioxidant and anti-inflammatory properties. Because free-radical-mediated peroxidation of membrane lipids and oxidative damage of DNA and proteins are believed to be associated with a variety of chronic pathological complications such as cancer, atherosclerosis, and neurodegenerative diseases, curcumin is thought to play a vital role against these pathological conditions. The anti-inflammatory effect of curcumin is most likely mediated through its ability to inhibit cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS). COX-2, LOX, and iNOS are important enzymes that mediate inflammatory processes. Improper up-regulation of COX-2 and/or iNOS has been associated with the pathophysiology of certain types of human cancer as well as inflammatory disorders. Because inflammation is closely linked to tumor promotion, curcumin with its potent anti-inflammatory property is anticipated to exert chemo-preventive effects on carcinogenesis. Hence, the past few decades have witnessed intense research devoted to the antioxidant and anti-inflammatory properties of curcumin. In this review, it was described that both antioxidant and anti-inflammatory properties of curcumin, the mode of action of curcumin, and its therapeutic usage against different pathological conditions.

10. **(Shan M. et al, 2018)** Designed and developed a gel based drug delivery system containing combinational drugs (ketoconazole, neomycin sulphate and diclofenac) for the effective treatment of Paronychia. The obtained results were analyzed and compared. All the test results were within the accepted limit. The physicochemical properties of the gels were assessed and it was found that the two formulations have enough gel consistency with good spreadability and extrudability. The drug content and drug release studies of the prepared gels were done and the results showed that the all the three drugs were properly loaded into the gel system, with good drug release profile. The antimicrobial activities of the formulated gels were proved by both in vitro antifungal and antibacterial studies. The in vivo antibacterial studies revealed a significant reduction in bacterial count in wistar rats treated with prepared gel when compared with standard drug solution. Later it was concluded that the drugs (ketoconazole, neomycin sulphate and diclofenac) were successfully incorporated into the different topical gel preparations with good physicochemical properties and antimicrobial activity. Therefore, it was concluded that our formulae could be very promising topical alternative for the treatment of Paronychia.

11. **(Miyanda P.M. et al, 2021)** characterized and prepared neomycin sulfate loaded ethosomes. Vesicle morphology revealed the presence of nanosized spherical or near spherical –shaped vesicular structures. Particle size analysis by Malvern zeta size was observed in nanometer range (500-2000 nm), high negative zeta potential values were also reported and polydispersity index within range 0.281-0.383. Further high entrapment efficiency from 55.63% to 85.84% was observed. The formulated ethosomes showed enhanced in-vitro drug release results of 65-80%. The Ethosomal formulae showed lower MIC (minimum inhibitory concentration) values (2.44 µg/ml) compared to Neosporin® Antibiotic ointment yielding at (4.88 ug/ml) and finally and high stability stamina after 45 days. The rationale behind this work was that a formulated permeation enhancing carrier could facilitate the transport of antibacterial molecules through the two biological barriers: stratum corneum of the skin and bacterial membrane/cell wall. Characterization results of

formulated ethosomes particle size, zeta potential, entrapment efficiency, *in vitro* drug release, microbiological assay and stability studies fully satisfy the rationale behind this work.

12. **(Dai X. et al, 2017)** characterized and prepared curcumin/gelatin-blended nanofibrous mats (NMs) by electrospinning to adequately enhance the bioavailability of the hydrophobic curcumin for wound repair. Curcumin was successfully formulated as an amorphous nanosolid dispersion and favorably released from gelatin-based biomimetic NMs that could be easily applied topically to experimental wounds. Further, synergistic signaling by the released curcumin during the healing process: (i) mobilization of wound site fibroblasts by activating the Wnt signaling pathway, partly mediated through Dickkopf-related protein-1, and (ii) persistent inhibition of the inflammatory response through decreased expression of monocyte chemoattractant protein-1 by fibroblasts. With a combination of these effects, the curcumin/gelatin-blended NMs enhanced the regenerative process in a rat model of acute wounds, providing a method for translating this ancient medicine for use in modern wound therapy.

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3. AIM AND OBJECTIVE:

Aim:

Design, development and evaluation of accelerated diabetic wound healing topical formulation of neomycin-sulphate and curcumin.

Objective:

- To formulate topical gel product with combination of neomycin sulphate and curcumin.
- To evaluate the in-vitro physicochemical properties of the prepared gel.
- To study the antimicrobial effectiveness of the prepared gel.
- To evaluate the wound healing potential of neomycin sulphate and curcumin combined topical drug product in diabetic rats.

4. PLAN OF WORK

4.1. Literature survey

4.2. Procurement of drugs and excipients

4.3. Physico-chemical /pre-formulation study

- 4.3.1. UV- visible spectrophotometer study
- 4.3.2. Fourier Transform Infrared Spectroscopy (FT-IR) Study
- 4.3.3. Differential Scanning Calorimetry (DSC) Analysis for melting point

4.4. Preparation of gel formulation

4.5. In-vitro analytical study

- 4.5.1. pH
- 4.5.2. Homogeneity
- 4.5.3. Viscosity
- 4.5.4. Spreadibility
- 4.5.5. Extrudability study
- 4.5.6. Stability study
- 4.5.7. Drug content study
- 4.5.8. Cell permeability study

4.6. In-vitro microbial study

- 4.6.1. Disc diffusion method

4.7. In-vivo study

- 4.7.1. Excision wound healing study on wistar albino rats

5. METHODOLOGY

5.1. PRE- FORMULATION STUDY:

5.1.1 Preparation of standard solution of Neomycin sulphate for UV Visible Spectroscopy

100 mg of the drug was dissolved in the 100 ml of the phosphate buffer ph 7.4 the concentration of the above solution was 1000 µg/ml and it is noted as stock 1. From the above stock 1 sample 1 ml of the sample was taken and makeup to the 100 ml in the volumetric flask and the concentration of the above solution was find to be 10 µg/ml and it is noted as the stock 2 from this series of the sample was taken such a 1 ml, 2 ml 3 ml, 4 ml, 5 ml and make it up to the 10 ml and the concentration of the above sample was found to be 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml and 5 µg/ ml.

5.1.2 Preparation of standard calibration curve of Neomycin sulphate by UV Visible Spectroscopy

The standard calibration curve of neomycin sulphate was obtained by measuring the absorbance of neomycin sulphate solution in concentration range (1-5µg/ml) prepared from stock solutions in phosphate buffer at 277 nm. Calibration curve of neomycin sulphate was then plotted with absorbance on y-axis and neomycin sulphate concentration on x-axis

5.1.3 Preparation of standard solution of Curcumin for UV Visible Spectroscopy

100 mg of curcumin was dissolved in the 100 ml of the phosphate buffer ph 7.4 the concentration of the above solution was 1000 µg/ml and it is noted as stock 1. From the above stock 1 sample 1 ml of the sample was taken and makeup to the 100 ml in the volumetric flask and the concentration of the above solution was find to be 10 µg/ml and it is noted as the stock 2 from this series of the sample was taken such a 1 ml, 2 ml 3 ml, 4 ml, 5 ml and make it up to the 10 ml and the concentration of the above sample was found to be 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml and 5 µg/ ml.

5.1.4 Preparation of standard calibration curve of Curcumin by UV Visible Spectroscopy

The standard calibration curve of curcumin was obtained by measuring the absorbance of curcumin solution in concentration range (1-5µg/ml) prepared from stock solutions in phosphate buffer at 424 nm in triplicate. Calibration curve of curcumin was then plotted with absorbance on y-axis and curcumin concentration on x-axis.

5.1.5. Differential Scanning Calorimeter (DSC) analysis for melting point of Neomycin sulphate:

Melting point of neomycin sulphate was studied by using differential scanning calorimeter (Perkin Elmer 4000). About 2mg of the sample were crimped in a standard aluminium pan and heated in a temperature range 10 ºC to 280 ºC at a heating rate of 10 ºC per minute in nitrogen atmosphere.

5.1.6. Differential Scanning Calorimeter (DSC) analysis for melting point of Curcumin:

Melting point of curcumin was studied by using differential scanning calorimeter (Perkin Elmer 4000). About 2mg of the sample were crimped in a standard aluminium pan and heated in a temperature range 10 ºC to 280 ºC at a heating rate of 10 ºC per minute in nitrogen atmosphere.

5.1.7 COMPATIBILITY STUDY:

5.1.7.1 Fourier Transform Infrared Spectroscopy (FT-IR) Study:

FT-IR studies were performed pure Neomycin sulphate, curcumin, physical mixture of Neomycin sulphate and curcumin (1:1), Neomycin sulphate + curcumin + carbopol 934 (1:1:1), Neomycin sulphate + curcumin + carbopol 934 + HPMC K4M (1:1:1:1) were performed in an Alpha FT-IR spectrophotometer (Bruker, Germany). A small quantity of sample was placed just below the probe on to which the probe was tightly fixed and scanned in wave number region 4000-500 cm^{-1}. The obtained IR spectra were interpreted for functional groups at their respective wave number (cm^{-1}).

Sl no.	Equipment	Company
1	Electronic balance	Model BS 124S, Labtronic
2	Magnetic stirrer	Rolex
3	pH meter	Systronics
4	FTIR spectrophotometer	Model ALPHA-E, Bruker, Germany
5	Viscometer	Brookfield LDV-E
6	UV Visible spectroscopy	Shimadzu
7	Homogenizer	Rolex

Table 5.1.7 : list of equipments and apparatus

5.2 Formulation of gel:

Working formula was for a quantity of 25 gm of gel. Various gel formulations were prepared using carbopol-934, Hydroxypropyl methylcellulose K4M (HPMC K4M) as gelling agents. Required quantity of gelling agents was weighed and dispersed in small quantity of distilled water to form a homogeneous dispersion. The drugs were dissolved in propylene glycol (PG) and added to the above solution. Other excipients Methyl paraben, Propyl paraben were also added with continuous stirring. The pH of the gel was brought to skin pH by adding required quantity of Triethanolamine (TEA). The final weight of the gel was adjusted to 25 grams with distilled water. The gels were stored in wide mouthed bottles. The composition of the various gel formulations is shown in (table .5.2.1)

Formulation code	F1	F2	F3	F4
Neomycin sulphate	0.125	0.125	0.125	0.125
curcumin	0.075	0.075	0.075	0.075
Carbopol 934	0.5	1	0.5	1
HPMC K4M	1	0.5	0.5	1
Propylene glycol	6	6	6	6
Methyl paraben	0.2	0.2	0.2	0.2
Propyl paraben	0.3	0.3	0.3	0.3
Triethanolamine	q.s	q.s	q.s	q.s
Ethanol	10	10	10	10
Distilled water	q.s	q.s	q.s	q.s

Table 5.2.1: different formulation codes of gel preparation

5.3 In-vitro formulation study:

5.3.1 Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in all containers. They were tested for their appearance and presence of any aggregates.

5.3.2 Measurement of pH

The pH of the gel formulations was measured by using pH meter by dipping the glass electrode completely into the gel system to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded.

5.3.3 Viscosity

Viscosity of gel should be measured by using the rotary viscometer at different rate and temperature. Gel have [low viscosity] Brookfield viscosity usually refers to a viscosity measurement performed with a Brookfield Viscometer. There are several models of viscometer available from Brookfield but the majority, operate in the same manner: the viscometer motor rotates the spindle at a defined speed (measured in rpm) or shear rate and the viscometer measures the resistance to rotation and reports a viscosity value. Various spindle designs can be employed, depending on the nature of the sample. Viscosity of the gel preparation was measured by using Brookfield viscometer with spindle-64.

5.3.4 Spreadibility

The gel formulation was placed in one of the glass slides; the other glass slide was placed on top of the gel such that the gel was sandwiched between the two slides. By this method spreadibility was measured on the basis of slip and drag method. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between the slides. 1kg weight was placed on the top of the slides for 5minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull off. With the help of string

attached to the hook & the time (in seconds) required by the top slide to cover a distance, which is to be noted later. A shorter interval indicates better spreadibility.

Spreadibility is calculated using the following formula:

$$S = M \times L / T$$

Where, S= Spreadibility, M= Weight in the glass slide, L= Length moved by the glass slide and T= Time (in sec.) taken to separate the slides completely from each other.

5.3.5 Extrudability study:

Measure the force required to extrude the material from tube. Extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminium collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of the extruded gel was to be collected and weighed.

5.3.6 Stability study:

Gel formulations are to be evaluated in terms of physical changes like phase separation and changes in color, odour, consistency etc., thereby affecting their stability and other desired properties. Samples of the gel formulations were kept at different temperature conditions like 25°C, 30°C and 40°C for 45 days. They were periodically observed for physical changes like phase separation and development of objectionable color and odour etc.

5.3.7 Drug content determination study:

The gel having weight of 500mg was weighed and solubilized in 50 ml of phosphate buffer solution pH 7.4 and the volumetric flask containing gel solution was shaken well in order to obtain complete solubility of the drug and filtered. The drug content was analyzed spectrophotometrically in 277 nm using phosphate buffer (ph 7.4)

5.3.8 Cell permeability study:

The diffusion studies of the prepared gels are to be carried out in Franz diffusion cell for studying the dissolution release of gels through cellulose membrane. Gel sample (0.5g) is taken in cellulose membrane and the diffusion studies are carried out at 37±1º using 250ml of phosphate buffer (pH 7.4) as the dissolution medium. 5ml of each sample is to be withdrawn periodically at 15, 30, 45, 60, 75 and 90 min and each sample is to be replaced with equal volume of fresh dissolution medium. Then the samples are to be analyzed for the drug content by using phosphate buffer as blank. The absorbance of diluted solution was measured 234 nm against the blank in UV spectrophotometer. Then the graph has to be plotted comparing the cumulative drug release of prepared gel with time.

5.4 In-vivo study:

5.4.1. WOUND HEALING STUDY:

It is already known that Neomycin sulphate is a broad spectrum aminoglycoside antibiotic which has a greater use in topical administration. Neomycin is used to reduce the risk of infection during surgery. Neomycin is most often used topically to treat superficial infections from staphylococci and gram-negative bacilli. Curcumin is found to be in turmeric which helps in wound healing by decreasing inflammation and oxidation. It also lowers the response of the body to cutaneous wounds which results in quicker wound healing.

Thus the combination of neomysin sulphate and curcumin in my project work is hypothetically assumed to promote wound healing activity in diabetic patients.

SELECTION OF ANIMALS:

Species and Strain: Wistar albino rats

Age and Weight: 2-3 months, 150-200g

Gender: either sex

Number of Wistar rats to be used: 30

INDUCTION OF DIABETES:

The animals are to be injected with a single dose of alloxan monohydrate (120 mg/kg) in cold normal saline solution (freshly prepared) in abdominal cavity by I.P. route to induce diabetes. Control animals were injected with normal saline solution. Fasting blood glucose levels were measured three days later to confirm the diabetic status of the animals. For blood glucose measurements blood samples were drawn from the tail vein & determined by glucometer strips.

PROCEDURE OF EXCISION WOUND CREATION:

Instruments such as forceps, surgical blade, pointed scissors, instrumental tray and gauze are (autoclaved at 121ºC for 15 mins) sterilized prior to surgery. New sterile pack of instruments will be used for each group of animals and in each group of animals the instruments will be wiped clean of blood and tissue with sterile gauze disinfected and rinsed in sterile saline or water.

Animals are to be anaesthetized before and during creation of the wounds. The dorsal furs of the animals are to be shaved and the anticipated area of the wound to be created has to be outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 500 mm2 and 0.2 cm depth has to be created along the markings using toothed forceps, a surgical blade, and pointed scissors. The entire wound has to be left open.

Animals are divided into three groups and each group consists of 10 rats:-

Controlled group: normal control applied

Test group: diabetic experimental study applied with test drug formulation

Standard group: diabetic experimental study applied with marketed formulation

Wounds area to be measured on different wound closure rate to be assessed by tracing the wound on days (0, 2, 4, 8, 12, 16, 18, 20). The wounds areas recorded are to be measured on graph paper.

No. of animals	Animal group	Type of preparation	Local anesthesia for less pain	Rehabilitation of animals
10	Group I	Controlled	NA	20 days
10	Group II	Test	2% w/v	20 days
10	Group III	Standard	2% w/v	20 days

Table 5.4.1.1: grouping of animals

Reference:

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9. Panwar S., Jain N.J., Gupta M.K. Wound healing potential of methanolic extract of flowers of *Butea monosperma* linn. in diabetic animals. **JDDT** (2018) 8(5):306-310
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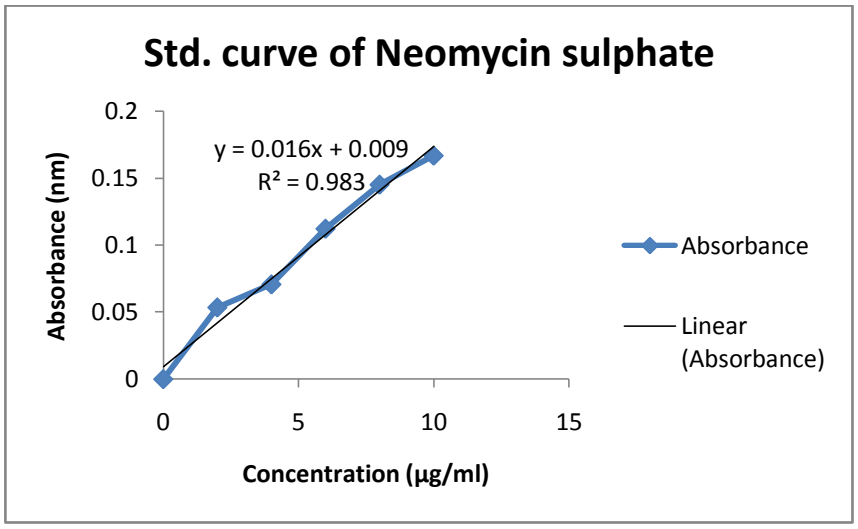
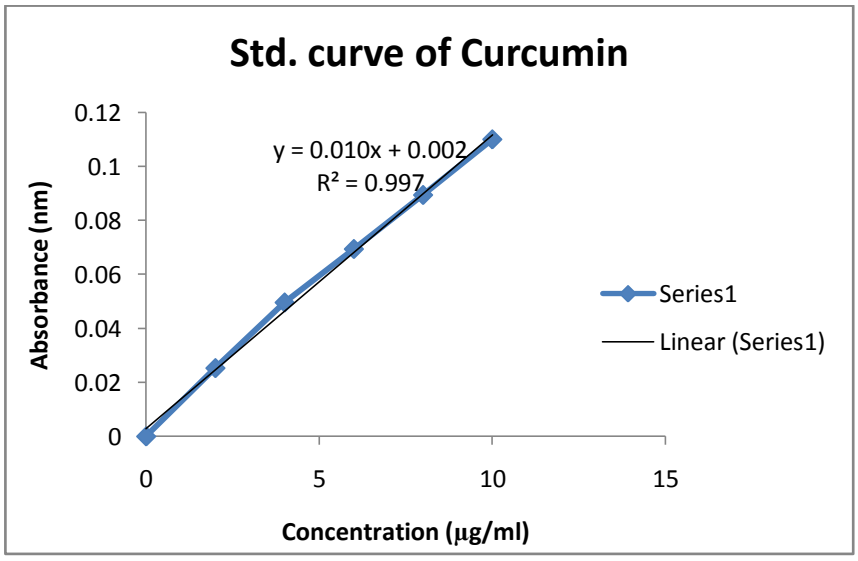
6. Result and Discussion

6.1. Pre-formulation study

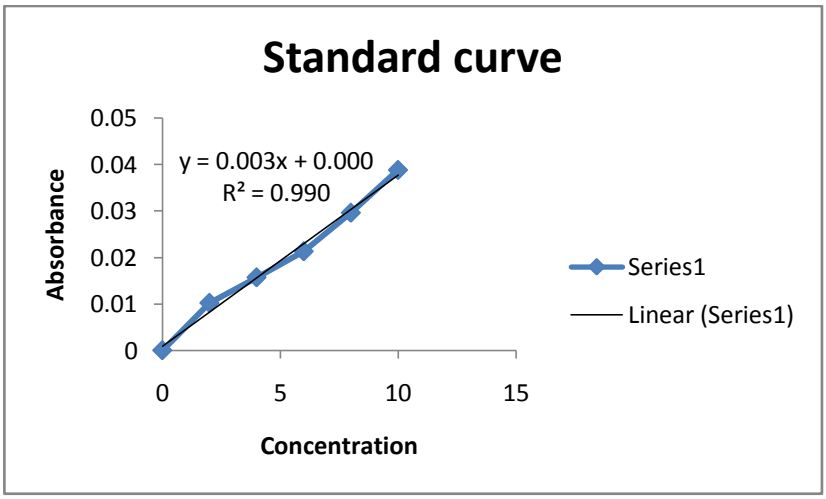
6.1.1. Standard calibration curve of Neomycin sulphate in phosphate buffer by UV Visible Spectroscopy at λ max 277nm



6.1.2. Standard calibration curve of Curcumin in phosphate buffer by UV Visible Spectroscopy at λ max 424nm



6.1.3. Standard calibration curve of Neomycin sulphate & curcumin in phosphate buffer by UV visible spectroscopy at λ max 265 nm



6.1.4. Differential Scanning Calorimeter (DSC) analysis for melting point of Neomycin sulphate:

After 2mg of neomycin sulphate was crimped in a standard aluminium pan and heated in a temperature range 10 ºC to 280 ºC at a heating rate of 10 ºC per minute in nitrogen atmosphere, the melting point of neomycin sulphate was found to be 221 ºC

6.1.5. Differential Scanning Calorimeter (DSC) analysis for melting point of Curcumin:

After 2mg of curcumin was crimped in a standard aluminium pan and heated in a temperature range 10 ºC to 280 ºC at a heating rate of 10 ºC per minute in nitrogen atmosphere, the melting point of curcumin was found to be 180 ºC.

6.2. COMPATIBILITY STUDY:

6.2.1 Fourier Transform Infrared Spectroscopy (FT-IR) Study analysis:

The important IR data are collected from the spectrum obtained are given in (table 6.2.1) & (table 6.2.2) below. Assignments of the frequencies were made on the basis of the literature values.

Functional Group	**Neomycin sulphate Range (cm^{-1})**
C-H (aromatic) stretching	2893.44
C-H (aromatic) bending	765.83
N-H stretching	3611.32
N-H bending	1513.56
C-O-C	1014.72
O-H (phenol) stretching	3611.32

Table 6.2.1: IR analysis of neomycin sulphate

Functional Group	**Curcumin Range (cm^{-1})**
C-H (aromatic) stretching	2919.39
C-H (aromatic) bending	857.39
C-H (alkene) stretching	2919.39
O-H (phenol) stretching	3588.37
O-H (phenol) bending	1206.27
C=O (ketone) stretching	1728.80
C=O=C stretching	2314
N-O stretching	1510
C=C bending	987.40

Table 6.2.2: IR analysis of curcumin

After the IR analysis interpretation of combination of neomycin sulphate and curcumin with excipients(gelling agents carbopol 934 and HPMC k4M) it was found that neomycin

sulphate has peaks of functional groups (C-O-C range 1017.48 cm-1, N-H bending range 1514.81 cm-1, C-H stretching range 2891.22 cm-1, O-H stretching range 3651.41cm-1, N-H stretching range 3651.41 cm-1) and curcumin has peaks of functional groups (C-H stretching range 2891.22 cm-1, C=O stretching range 1691.40 cm-1, C=C range 1017.48 cm-1, O-H bending range 1152.67 cm-1, N-O stretching 1514.81 cm-1, O-H stretching 3651.41cm-1). Thus, combination was found compatible as well as it was found no new peaks has emerged which means there is no degradation of the drugs after combining with the excipients.

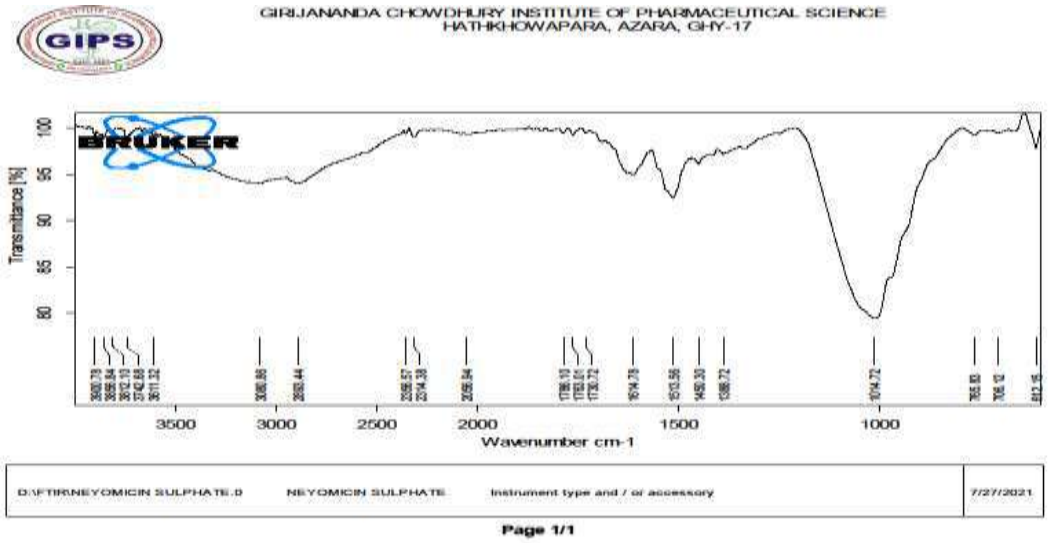


Figure 6.2.1: IR analysis graph of Neomycin sulphate

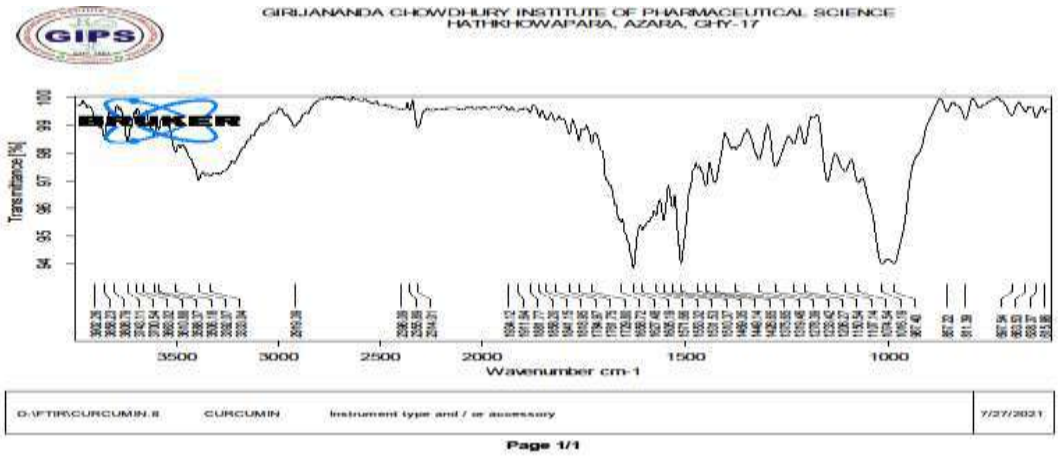


Figure 6.2.2: IR analysis graph of Curcumin

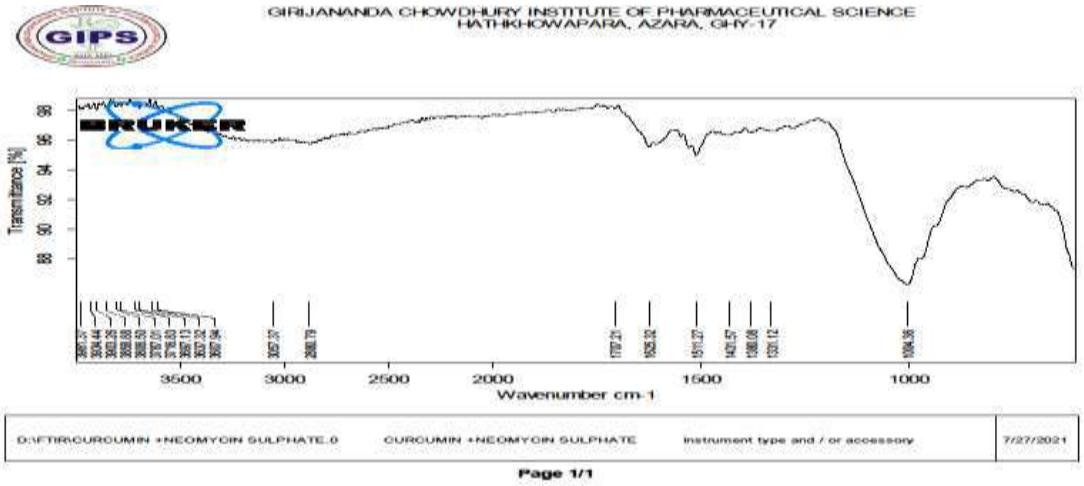


Figure 6.2.3: IR analysis graph of Neomycin sulphate & Curcumin (1:1)

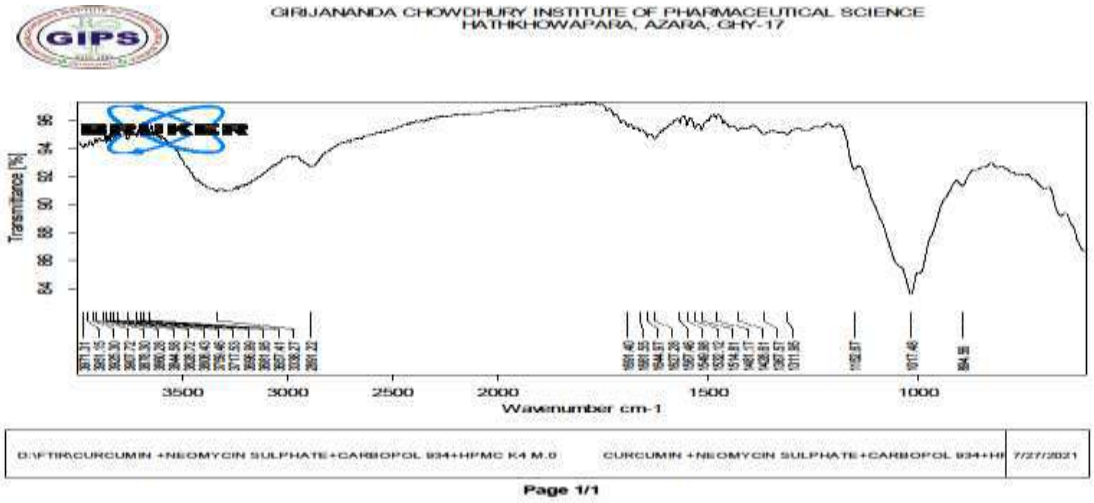


Figure 6.2.4: IR analysis graph of Neomycin sulphate+ curcumin+carbopol934+HPMCk4M (1:1:1:1)

6.3. In-vitro study:

6.3.1. Organoleptic Properties

The result of the visual observation of Neomycin sulphate was found as white powdery substance and curcumin was found to be yellowish and the final gel product was yellow in colour and odour was found characteristic and appearance was observed as smooth homogenous gel product.

6.3.2. Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in all containers. They were tested for their appearance and presence of any aggregates, discussed in (table 6.3.3.1)

6.3.3. Measurement of pH

The pH of the gel formulations were measured by using pH meter. (Table 6.3.4.1)

6.3.4. Spreadibility

The time (in seconds) required by the top slide to cover a distance of 7.5 cm was to be noted. A shorter interval indicates better spreadibility. (Table 6.3.4.1)

Spreadibility was calculated using the following formula:

$$S = M \times L / T$$

Where, S= Spreadibility, M= Weight in the glass slide, L= Length moved by the glass slide and T= Time (in sec.) taken to separate the slides completely from each other. The spreadibility of the different formulation decreases with the increase in its viscosity.

Table 6.3.4.1: Characterization properties of gel formulations

Formulation	Grittiness	Homogeneity	pH	Spreadibility
F1	+++	-	5.9	30
F2	+++	-	6.3	29
F3	++	-	6.4	31
F4	+++	-	6.4	27

+ Satisfactory, ++ good, +++ very good, - no grittiness

6.3.5. Viscosity

Viscosity of gel was measured by using the rotary viscometer at different rate and temperature. Gel have [low viscosity] Brookfield viscosity usually refers to a viscosity measurement performed with a Brookfield Viscometer. Various spindle designs can be employed, depending on the nature of the sample. Viscosity of the gel preparation was measured by using Brookfield viscometer with spindle-64. (Table 6.3.5.1)

Table 6.3.5.1: Viscosity of gel formulations in different shear rate (rpm)

Sl. no.	Speed (rpm)	Viscosity cps F1	Viscosity cps F2	Viscosity cps F3	Viscosity cps F4
1.	0	0	0	0	0
2.	1	39960	42720	29700	42920
3.	2	35920	37280	21790	38720
4.	3	21560	22920	15690	27300

6.3.6. Extrudability study:

This method was used to analyze the force required to squeeze the content out of the tube and the amount of content squeezed. All the formulations showed a good extrudability. There is no significance change in the extrudabilities of F1 & F2, but F4 has significantly slightly lower extrudability in compared to the other three formulation due to a bit higher viscosity. So, plug flow was limited in the formulations overall and was easy to squeeze

out the formulation from the collapsible tube minimum of 0.5 cm to maximum of 1.3 cm of the gel.

6.3.7. Drug content study: The percentage of drug content of the prepared gel formulations are discussed in the table below (6.3.7.1)

Table 6.3.7.1: % Drug content study

FORMULATION CODE	%DRUG CONTENT	STD. DEVIATION
F1	80.45784	±1.524828
F2	79.6845	±2.055343
F3	77.04117	±0.470886
F4	81.4845	±1.736212

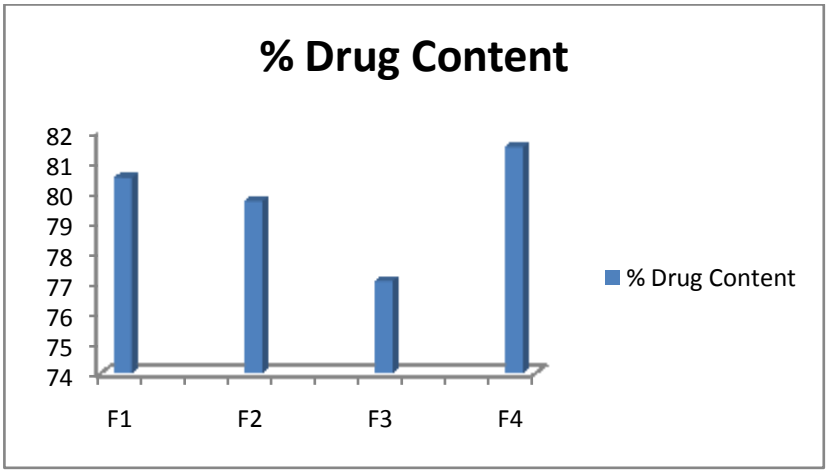


Figure 6.3.7: Graph of % drug content study

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7. Conclusion:

The present research work gives the demonstration of the development of diabetic accelerated wound healing gel through successful incorporation of mixture of the main drugs neomycin sulphate and curcumin. The development of the neomycin sulphate and curcumin combined gel was designed by varying the ratios of the gelling agents carbopol-934 and HPMC K4M and triethanolamine for maintaining the pH.

Neomycin sulphate and curcumin gel were formulated with the concentration (0.5% and 0.3%). Neomycin sulphate and curcumin combined gel were subjected to various evaluation parameters such as physical appearance, grittiness, homogeneity, pH measurement, viscosity study, spreadibility study, extrudability study and in-vitro drug content study. DSC analysis study was carried out as pre-formulation study for measuring the melting points of the drugs Neomycin sulphate and Curcumin. The organoleptic property of the gel formulation shows that the gel was yellow in colour and has a characteristic smell. The grittiness and homogeneity were found in good and acceptable condition. However, there was found to be in increase in viscosity where carbopol and HPMC concentration were increased on the other hand the spreadibility of the gel was found to vary depending on the viscosity of the gel, i.e, higher the viscosity lower the spreadibility. The pH of the formulations increased with increase in triethanolamine. The extrudability parameters were found in acceptable limits. The drug content test shows 77-82%. The FTIR spectral analysis confirmed the integrity and compatibility of the drugs Neomycin sulphate and Curcumin as well as the combination of drugs with the excipients. Thus, depending on in-vitro studies, formulation F4 is considered to be the ideal preparation in comparison to the other three formulations.

So, the preparation is hoped to provide treatment to diabetic patients with excision or incision wounds in accelerated diabetic wound healing depending on my literature survey which would be further carried out in future by continuing the research work in in-vivo study on diabetic rats.