CHAPTER ONE

1.0 GENERAL INTRODUCTION

Wound is an interruption in the continuity of the external surface of the body. Wound healing involves a well-orchestrated, complex process leading to repair of injured tissues. Wound healing can be delayed and this is more when an acute wound turns to chronic wound due to infection, non-ideal topical wound dressing preparation or underlying medical problems. Such chronic wound does not follow the normal pattern of repair due to physiological problems which lead to non-restoration of healthy granulation tissue in the wound bed associated with the loss of some physiological function (1). The final physiological strength of re-generated epidermis in wound healing process is about 80% of the original strength (2). Rusczak (3) reported that human collagen matrices treated dermal wound had 75% tensile strength after healing. The myofibroblast mediated collagen deposition on wound the weaker the tensile strength (4). White et al (5) reported that wound exposed to 100% hyperbaric oxygen had increased tensile strength in 8 days and Diegelmann et al (6) have reported that 30% collagen in wound strengthen the tissue repair. The composition of wound fluid can be used to determine the rate of the wound healing (1).

The knowledge of wound healing is important in formulating an ideal dressing preparation as suggested by Falcone and co-worker (7). An ideal wound medicament can ameliorate or prevent some complications of wound healing such as contracture, keloids, scar formation and various surgical operations Ramasastry (8,9). A lot of acute wounds turn into chronic wounds due to unavailability of ideal topical pharmaceutical formulation for wound dressing which should be able to facilitate the formation of healthy granulation tissue and optimize the efficiency of such wound medicaments which will ultimately reduce the time for
wound healing. Mackool et al (10) suggested that such medicament should be able to reduce scar and keloid formation. Mucin and honey have been shown in various reports to have wound healing effect. Adikwu and co-workers (11, 12, 13) have reported that on topical application of snail mucin to wounds superficial healing is accelerated. Subrahmanyam (14) showed that wounds dressed with honey showed shorter healing time than silver sulphadiazine. Molan (15) reported that at concentration of 58 % 345 samples of honey studied from 26 different floral sources showed antibacterial activity against Staphylococcus aureus as compared with phenol. Seven strains of bacteria found in wound have been reported to have their growth halted completely by gamma irradiated honey diluted to 5-10 % (16). Ghaderi et al (17) reported that ten-fold diluted honeys still completely halt the growth of all the major wound-infecting bacteria while Bergman et al (18) reported that topical application of undiluted honey is able to accelerate infected-wound healing. Efem et al (19) reported that 20 infected-wound cases treated with topical application of undiluted honey showed no pathogen after 1 week of treatment and Ali (20) reported that orally administered honey at the rate of 312 mg/kg twice daily was comparable to sucralfate (drug) in accelerating the healing of indomethacin induced gastric ulcers in rats. Others are Deinzer et al (21) who stated that honey contains pyrrolizidine alkaloids which have antibacterial activity while Gupta et al (22) reported that undiluted honey was efficacious in infected wounds of buffalo. Ndayisaba et al (23) reported that in 53 Burundian patients with wounds of diverse origin treated by daily topical honey application healing was successful in 29 patients within 5 weeks. This study evaluated the topical formulations of mucin and honey using standard pharmaceutical bases, wound healing effects, tissue re-epitheliazation and efficacy in reducing biolload in wounds are compared with silver sulphadiazine cream (SSD).
1.1 MUCIN

Mucins are mucoproteins secreted by cells. Mucins can raise the viscosity of the medium around them. Mucin is the major glycoprotein component of mucus (24). They are conjugated proteins in which protein is combined with a polysaccharide containing hexosamines or glycoproteins as reported by Adikwu et al (25). Mucins form a protective biofilm on the surface of epithelial cells, where they can provide a barrier to particulate matter and bind microorganisms. They have about 80% of their sugar glycosylated with large molecular weight glycoprotein \(2.14-14 \times 10^6\) Da. There are peptide cores that are rich in serine and threonines which are attached by O-glycosidic linkages composed of N-acetyl-glucosamine, N-acetyl-galactosamine, galactose, fructose and sialic acid. A lot of mucins are membrane bound due to the presence of a hydrophobic membrane-spanning domain that favours retention in the plasma membrane while some are secreted on mucosal surfaces and saliva (26).

Mucin can generally be defined as glycoproteins, which contribute to the mucus gel barrier and are part of the dynamic, interactive mucosal defensive system with protective, adhesive and lubricative functions. Mucin has a lot of biophysical properties that have made it a good candidate for pharmaceutical studies (27). Glycoproteins are now known to interact in various ways with many biologically important compounds such as enzymes, polymer, cations, drugs, viruses, particulate matters and bacteria. In the past 15-30 years several authors like Anosike (28), Ganon (29) and Pasternak (30) have written on mucus glycoproteins from different organs which have revealed that these macromolecules consist of sub-units held together by interchain disulphide bonds. Harding (27) in his work stated that these multiple crosslinks confer a kind of random gel network which confers mucus/mucin with visco-elasticity property. Ofakansi (31) reported that bioadhesiveness of gelatin/mucin increase with increase in
concentration of the admixture and Nnamani (32) reported also that the mucoadhesive force required to separate snail mucin applied to two surfaces increase as mucin concentration increases. Certain studies have indicated the healing property of mucin (11, 12). It can be used as medicament, or as biomaterial to be formulated as suitable delivery system for application in wound (13).

1.1.1 Classification of mucin

Young et al (33) observed that mucin being a major glycoprotein component of mucus is found in living systems such as egg white, plasma, connective tissues, blood and enzymes. It can be classified as a structural polysaccharide that has a high content of clustered oligosaccharides with O- glycosidically linked to polypeptides. Mucins can also be classified based on their sources, which may be snail, bovine, guinea pig, porcine, rat, rabbit and nematodes. It can also be classified based on the body part that secrets it, such as eye, ovary, saliva and gastro-intestinal tract (34 - 36).

1.1.2 Composition of mucin

In mucin, the protein unit is about 20 % w/w while the carbohydrate portion is about 80 % w/w oligosaccharide. The sugar unit of the glycoprotein, which may be branched, or straight chain contains short or long chain carbohydrates of 2 to 20 residues.

The carbohydrates that can be found in mucin include N-acetylgalactosamine, sialic acid, N-acetylglucosamine, mannose, L-fructose, xylose, galactose and arabinose. The protein portion of the mucin contains mostly amino acids, which form the linkage with the carbohydrates. Such amino acids include asparagines, threonine, serine, glycine, hydroxylysine, proline, phenylalanine, cysteine, alanine and valine. Threonine and serine are the most predominant amino acids in mucin (26, 28).
Acharan sulphate, a recently discovered glycoprotein isolated from snails of the species *Achatina fulica*, has a major disaccharide repeating unit of \( \text{--} \rangle \text{--4)} \text{--} \rangle \text{--2)} \text{--} \rangle \text{--acetyl,2-deoxy-alpha-D-glucopyranose(1--)\--4)} \text{--} \rangle \text{--2)} \text{--} \rangle \text{--sulfo-alpha-1-idopyranosyluronic acid making it structurally related to both heparin and heparin sulphate. Acharan sulphate is a main constituent of the mucus of snail (27).

1.1.3 **Physico-chemical properties of mucin**

The gel-like characteristic of mucin is due to the carbohydrate portion of the glycoprotein. Adikwu et al (13) reported that the presence of sialic acid gives the mucin its dense negative charge. This gives mucin a pKa value of about 2.6 and mucin molecule behaves eventually as anionic polyelectrolyte at pH values greater than 2.6. Blood (37) in his studies found that the amino acids in the glycoprotein confer amphipatic properties to mucin and as such can buffer small amounts of acid or alkali. The mucin gel is held together by primary (disulphide) or secondary (electrostatic or hydrophilic) bonds. In other words, the glycoprotein molecules are held in association with each other by means of non-covalent interaction to form a gel matrix that is responsible for the physiological and rheological properties of mucin (38). The flow of mucin is not proportional to the force applied due to increase in viscosity (27, 39).

Mucin has the ability to form self-assembly of drug-polymer or polymer-polymer complexes. In a study by Oliva *et al* (40) a spontaneous nanoencapsulation process (monitored by atomic force microscopy which is the force required to extract nano-particles from a polymer) occurred. The results demonstrate that polymer-polymer molecules can nanoencapsulate spontaneously, which offers possibility of controlling the release rate of a drug without the need of complex technological processes.
1.1.4 Mucin as a pharmaceutical material

Mucin has been widely investigated for a variety of microparticulate pharmaceutical forms. It also has potential applications in the delivery of radiopharmaceuticals, genes and peptides. It has also been used in mucoadhesive formulations for ocular, nasal, gastro-intestinal, buccal and vaginal drug administration (41, 42).

1.1.5 Assay of mucin

1.1.5.1 Immunoradiometric assay (IRMA)

In this assay, radiolabelled glucosamine is incorporated into the mucin. This radio-immunometric assay method was developed using monoclonal antibodies against epitopes which are associated with peptide core of gastric mucins (27). IRMA technique has been applied in supernatants of pancreatic cell culture to detect mucin in pancreatic cyst in order to diagnose the mucinous pancreatic cyst that is precancerous (13). The disadvantage of this technique is that it characterizes the high molecular weight glycoproteins containing glucosamine and does not detect mucins specifically.

1.1.5.2 Atomic force microscopic assay of mucin

In atomic force microscopic assay of mucin, quantitative measurements of biophysical characteristics of individual mucin molecules and molecular assemblies are measured (40). To enhance the characterization of human ocular mucins, purified mucins have been used to demonstrate the capabilities of this technique and derive biophysical properties unavailable through other techniques. The result showed that the antibodies bound to short polymers and longer polymers required longer reaction times. Influence of length and charge distribution on diffusion through gels is investigated by comparing the forces needed to extract mucin and DNA polymers from agarose gels (27).
1.1.5.3 Analytical ultracentrifuge assays

In this method, there are two principle approaches to assay mucin. The first approach is to use change in molecular weight using sedimentation equilibrium, but has disadvantage of having upper limit of about 50 MDa. Since complexes are large, a more efficient assay procedure is to use sedimentation velocity with change in sedimentation coefficient. There is a special procedure known as sedimentation fingerprinting where mucin is assayed for its effect on the mucoadhesion (27).

1.1.6 Current development and uses of mucin

1.1.6.1 Antibacterial activity

Snail mucin from *Archachatina marginata* (Family Arionidae) has been reported to have antimicrobial activity, while mucin in tears prevents infection and decrease in commensal bacterial load. Rudolph et al (43) in their work reported that purified ocular mucin inhibited bacterial growth while Adikwu et al (12) have suggested that due to its surfactant activity it prevents bacteria attaching to host cells.

1.1.6.2 Mucoadhesion

Blood (37) and Mortazavi et al (38) in their studies suggested that mucin as a polymer has a lot of interaction forces such as electrostatic interaction, van der Waals forces, hydrogen bonding, etc. Mucoadhesion can be explained considering interfacial energy theories as pointed out by Adamson (44). In aqueous solutions, mucin which is usually negatively charged interacts with cationic ions, drugs, or polymers that are positively charged. Non-ionic macromolecules, however, could interact with mucin mainly through hydrogen bonding.
1.1.6.3 Analgesic activity

Adikwu et al (13) reported that a new compound extracted from snail mucin is found to ease pain. The compound known as ACVI is more efficacious and has longer effect when compared to morphine. The compound does not have addictive effect and or side effect as morphine.

1.1.6.4 Tumour marker

Scientists have proved that mucin can be used as tumour marker. Ruldoph et al (43) in a study where dimethylhydrazine was used to induce tumours in rats reported that there was abnormal increase in expression of sialomucins (type of mucin in colon cancer of mammals). Similar sialomucins were detected in precancerous lesions and in the colon mucosa around the adenocarcinomas. No sialomucins were seen in normal colon mucosa. This implies that an alteration in mucin expression is an early event in colon cancerogenesis.

1.1.6.5 Wound healing property

In a study by Adikwu et al (12) it was reported that snail mucin from the giant African snail, *Archachatina marginata*, (Family Arionidae) has wound healing effect. The extract (mucin) remarkably increased the wound healing capacity of CicatrinR powder. King et al (45) in their study reported that mucin secretion in pig mucosa enhanced ulcer healing of the cell wall. Mucins from other sources have not been reported to have the same wound healing effect as snail mucin.

1.2 HONEY

Honey is carbohydrate-rich syrup produced by bees, primarily from floral nectars. The British Pharmacopoeia (46) defines purified honey as obtained by purification of the honey from the comb of the bee, *Apis mellifera L*, and other species of Apis. Honey has an extensive history
of traditional human medicinal use, in a number of societies. Molan (15) stated that it may be used alone or in combination with other substances, and has been administered both orally and topically.

1.2.1 Classification of honey

Generally, we have purified honey and natural honey. The purified honey is natural honey standardized to meet stipulated pharmacopoeia standardized. The natural honey is sugar syrup produced by worker bees from plant nectars, plant secretions and excretions of plant sucking insects (46).

1.2.2 Composition of honey

The two major sugars present in honey, are fructose (38 %w/w) and glucose (31 %w/w), sucrose (1 %w/w), other disaccharides and oligosaccharides. Gluconic acid, other acids and small amounts of proteins, enzymes (including glucose oxidase), amino acids and minerals may also be present. Potassium is the major mineral present.

The chemical composition of honey is highly variable because of the broad range of plants visited by honey bees when collecting the substance. Deinzer et al (21) reported that the plant species available in a geographic area determine the kinds and amounts of important compounds present in honey. Storage conditions may also influence the final composition of honey, with the proportion of disaccharides increasing over time. Molan and Allen (16) and Bergman et al (18) reported that there are a range of other, largely uncharacterized, substances present in some honeys that have antibacterial effects.

1.2.3 Physico-chemical properties of honey

Honey is yellow-amber coloured sticky viscous, translucent syrup. It has low moisture content (17%) and is mildly acidic with a pH of 3.2 and 4.5 (16, 18). The acidic pH is mostly due
to the presence of gluconic acid which is formed when bees secrete the enzyme glucose oxidase, that catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide. The low pH alone is inhibitory to many pathogenic bacteria.

1.2.4 Uses of honey

Many reports have indicated that honey is an effective remedy for stomach upsets. A report in the British Medical Journal (48) suggested that it shortened the duration of bacterial diarrhoea and was as effective as glucose at promoting the re-absorption of sodium and water from the intestine. Ali (20) reported that honey has been used to treat gastritis, duodenitis and duodenal ulcers.

Molan and co-worker (16) observed that honey has been used successfully as replacement for carbohydrate in oral rehydration therapy in acute diarrhoea. The use of honey in ophthalmic conditions have been reported in Egypt. Such conditions treated included chronic, non-specific conjunctivitis and persistent blepharitis (16).

Honey has a very long history of low-risk food use. Daily intake as a food could easily reach 100 g in some individuals, a dose far higher than is likely to be achieved when honey is consumed in therapeutic forms. It is often consumed alone, as a spread, or may be mixed with a wide range of other foods.

Deinzer et al (21), Gupta et al (22) and Ndayisaba et al (23) in various studies have shown that honey has antibacterial effects, attributed to its low pH, high osmolarity, hydrogen content and other uncharacterized compounds. The low water activity of honey is inhibitory to the growth of the majority of bacteria, and many moulds and yeasts (16,47). Honey is used in pharmaceutical preparations and cosmetics, as adjuvant, thickener, sweetener and vehicle (46).
Molan (15) in his study stated that honey is effective in treatment of wounds while Ghaderi et al (17) observed that it is effective in the treatment of skin wound in mice. There are other reports of the use of honey to treat wounds such as ulcers, burns, surgical wounds and gastric ulcers (18-20,21-23). There are many reports of the traditional medicinal use of honey in a large number of cultures. The Bible and Koran recommend its use. It has been used in a wide range of conditions, including gastrointestinal, respiratory, skin, measles and eye ailments (15).

1.3 WOUND

1.3.1 Classification of wound

Ramaswamy (8) and Nwome et al (9) reported that wounds can be classified by the duration of the wound repair. The short term wound healing is regarded as acute wound, while a long term wound healing (lasting more than 3 months) is called chronic wound. Wound can also be classified based on type of wound closure as either primary, secondary or tertiary. Primary wound closes with minimal intervention, while secondary wound closes by contraction and re-epithelialization. In tertiary wound, there is delayed primary closure, and it only closes when there is initial debridment and suture or surgical procedure.

1.3.2 Stages of wound healing

Wound healing is the body’s natural process of regenerating dermal and epidermal tissue. A set of events take place in a predictable fashion to repair the damaged tissue, and these events overlap with time. Although some authors (1,9) consider healing to take place in four phases, wound healing is generally grouped into three phases - inflammatory, proliferative, maturation/remodeling.
1.3.2.1 **Inflammatory phase**

The inflammatory phase includes the initial reaction to the injury in which a number of cells, including neutrophils, platelets and macrophages, migrate to the site. In the inflammatory phase, debris and bacteria are phagocytized and removed. At this stage some biological factors are released that cause the migration and division of cells involved in the proliferative phase (1, 9).

1.3.2.1.1 **Clotting cascade**

Clotting cascade is the first process of restoration of tissue integrity in inflammatory phase of wound healing. Coagulation is a rapid-fire response to initiate hemostasis and protect the host from excessive blood loss. This fibrin-fibronectin complex is the main structural support for the wound until collagen is deposited (1).

1.3.2.1.2 **Platelets**

Dasu *et al* (49) observed that platelets are the cells usually present in highest numbers shortly after injury occurs. The growth factors from platelets stimulate cells proliferation to facilitate wound healing.

1.3.2.1.3 **Vasoconstriction and vasodilation**

Inflammatory factors like thromboxanes and prostaglandins are released from ruptured cell membranes, and they cause the blood vessels to spasm to prevent blood loss. This causes vasoconstriction that lasts for 5-10 min (1, 9).

1.3.2.1.4 **Polymorphnuclear neutrophils**

Ovington (50) and Aschcroft *et al* (51) have pointed out that polymorphonuclear neutrophils (PMNs) are attracted to the wound site by fibronectin, growth factors, and substances
such as kinins and neutropeptides. Neutrophils clean the wound by secreting proteases that break down damaged tissue.

1.3.2.1.5  **Macrophages**

Macrophages are attracted to the wound site by growth factors released by platelets and other cells. Macrophages are stimulated by the low oxygen content of their environment to produce factors that induce and speed angiogenesis (50, 51).

1.3.2.2  **Proliferative phase**

The proliferative phase occurs when tissue reconstruction begins. This includes angiogenesis, epithelialization, and granulation. Fibroblasts begin to enter the wound site 2-3 days after the wound has occurred. With time the steps in this stage partially overlap as reported by Stadelmann et al (2) and Diegelmann et al (6).

1.3.2.2.1  **Angiogenesis**

This process is also called neovascularization. It occurs concurrently with fibroblast proliferation when endothelial cells migrate to the area of the wound. LaVan and co-worker (52) reported that angiogenesis is imperative for other stages of wound healing, like fibroblast and epidermal migration; as such cells require oxygen. Mulder et al (53) pointed out that endothelial cells are the stem cells that originate from parts of uninjured blood vessels which develop pseudopodia that push through the extracellular matrix into the wound site. Li and co-worker (54) observed that in a low-oxygen environment, macrophages and platelets produce angiogenic factors which attract endothelial cells chemotactically.
1.3.2.2 **Fibroplasia and granulation tissue formation**

Fibroblasts mainly proliferate and migrate in the first 2-3 days after injury. They are the main cells that lay down the collagen matrix in wound site, by migrating from normal tissue into the wound area from its margins. Granulation tissue begins to appear in the wound 2-5 days post injury.

1.3.2.3 **Epithelialization**

The re-epithelialization phase starts after formation of granulation tissue in an open wound. The epithelial cells migrate across the new tissue to form a barrier between the wound and the environment. Santoro and co-worker (55) observed that this occurs 17 times more than in normal tissue.

1.3.2.4 **Contraction**

Fibroblasts later differentiate into myofibroblasts to initiate wound contraction. Contraction continues even after the wound has completely reepithelialized.

1.3.3 **Maturation and remodelling phase**

The maturation phase of tissue repair starts when levels of collagen production and degradation are equal. The tensile strength of the wound increases up to 50 % - 80 % as strong as normal tissue at the end of this phase (1, 9).

1.3.3 **Factors that affect wound healing**

There are numerous factors that can affect wound healing. The size of wound is inversely proportional to the wound healing rate. The presence of an infectious agent in the wound can adversely affect the healing. Robson et al (56) observed that wound infection occurs when the bacterial count in the wound exceeds $10^5$ /g of tissue. The wound type determines the wound healing rate. Superficial (surface) wounds heal faster than deep or major wound. The wound in
normal nutritional patient heals faster than the wound in a nutritional deficient patient. Falcone and co-worker (7) suggested that nutritional support such as zinc, vitamin C, folate, iron, and copper are the key minerals and vitamins that can be given such patients. In a wound patient that has compromised immunity the rate of wound healing is usually slow. This leads to delay in the wound repair process as all other healing phases are equally delayed as observed by Zhu et al (57). Age and stress factors also lead to delay in healing. Sinclair et al (58) stated that in chronic wounds there is high level of protease activity that results in delayed wound healing caused by an increase in tissue destruction. Cocks et al (59) and Doumas et al (60) observed that leucocytes are up-regulated in such wounds. Grinnell and co-worker (61) also pointed out that protease can degrade growth factors which will lead to delay in wound healing. McDonad et al (62), Herrick et al (66) reported in their various studies that protease causes delay in wound healing. Other factors that can lead to delay in wound healing include: radiation, foreign body, chemotherapy agents, smoking, steroids and diabetes mellitus.

1.3.4 Histopathology of wound

Tissue disruption in higher vertebrates results in tissue regeneration. The disruption of the integrity of a tissue leads to histological imbalance which in turn results in pathological effect. This incapacitates the tissue from carrying out its normal physiological functions. The body has ability to commence repair process to restore the integrity of the tissue. Keswani et al (63) suggested that the aim of this process is to restore histological normality in the tissue.

1.3.4.1 Histological characteristics of wounds

The type of cells that appear in the wound depends on the stage of the healing. The healing cascade begins immediately following injury when the platelets come into contact with exposed collagen. Usually platelet aggregation and clotting factors are released, resulting in the
deposition of a fibrin clot at the site of injury. Hackam et al (64) in their studies pointed out that cytokines (endogenous peptides) role enhances fibroblast and smooth muscle cell chemotaxis and modulates collagen and collagenase expression. The result of this role is vigorous response of the matrix producing cells to ensure a rapid deposition of new connective tissue at the injury site during the proliferative phase that follows the inflammatory phase.

Cejkova (65) and Herrick et al (66) in their work observed that neutrophils are the predominant cells in the wound 24 hours post injury. The main function of the active amines released from the mast cells is to cause surrounding vessels to become leaky and allow the speedy passage of the mononuclear cells into the injury area.

Young et al (33) stipulated that within 48 hours post wound, fixed tissue monocytes become activated to turn into wound macrophages. The presence of wound macrophages is a sign that the inflammatory phase is nearing an end and that the proliferative phase is beginning. The phagocytic macrophages are responsible for removing nonfunctional host cells, damaged matrix, bacteria filled neutrophils, bacteria and foreign debris from the wound site.

In the proliferative phase of the wound, the predominant cell is the fibroblast. The fibroblast cell is of mesenchymal origin and is responsible for producing the new matrix needed to restore structure and function to the wounded tissue. Santoro and co-worker (55) stated that the final stage of the wound healing is characterized by proliferation of collagen cells for remodeling. The enzyme lysyl oxidase acts on collagen to form stable cross-links. As the collagen matures the intramolecular and intermolecular cross-links are formed, which give healed wound tissue its strength and stability over time.
1.3.4.2 **Wound scar**

Wound scar can be defined as the replacement of the normal structural elements of the tissue by distorted, nonfunctional and excessive accumulation of fibrotic tissue. For scar to form there is 2 - 3 times production of fibroblast in the wound from that of the normal skin. There is also increased density of mast cells that process procollagen into excessive collagen (8.9).

1.3.5 **Enzymology of wound**

Enzymes can be classified into six groups according to their mechanism of action namely: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (67, 68). Changes in pH affect the activity of the enzyme on the substrate (69, 70) with the enzyme-substrate interaction similar to kinetics reactions in physical chemistry (70).

1.3.6 **Matrix proteins and proteases in wound.**

Proteases are a family of enzymes that include the endopeptidases and exopeptidases, which catalyse the hydrolytic breakdown of proteins into peptides or amino acids. Ovington (50) pointed out that proteases are associated with the early inflammatory stage of wound healing in many ways. During angiogenesis, proteases are expressed significantly at the growing tip of blood vessels to facilitate vascular invasion. Aschcroft *et al* (51) reported that this class of enzymes also assists in wound debridement and cleansing of necrotic tissue, bacteria and foreign bodies. Proteases digest the extracellular matrix and assists in tissue remodeling during reconstructive and remodeling phase in normal wound healing.

Studies have shown that the biochemical environment of the non-healing wound is different from that of a healing wound. A chronic non-healing wound has a biochemical environment with evidence of excessive proteases and inflammatory cytokines and low levels of growth factors. The presence of a high level of bioburden in wound is prone to increase the
levels of proteases. Okada et al (71) indicated that there are higher protease activity level and endogenous enzyme inhibitors called tissue inhibitors of metalloproteases (TIMPs) in older patients.

Borregaard et al (72) reported that for a wound to heal, a balance is needed between the protein degrading activities of matrix metalloproteases (MMP’s) and other cellular activity that synthesizes and deposits protein components of granulation tissue.

In tissue remodelling and wound repair there are different types of proteases involved. Increase in the levels of these enzymes in the wound indicate tissue damage or tissue repair. The assay of such enzymes will indicate whether the wound healing rate will be slow or fast. Cullen et al (73) in their investigations observed that this assay can be used as a prognostic test to monitor wound healing.

1.3.7 The role of neutrophil elastase in wound healing

Neutrophil-derived elastase, plasmin and MMP’s are major proteases present in chronic wounds and have a role in delaying healing with the neutrophil-derived elastase being the predominant protease in chronic wounds. In their various studies, the view is collaborated by Aschroft et al (51), Jahovic et al (74).

1.3.8 Wound dressings

1.3.8.1 Categories of wound dressings

There are a lot of classes of wound dressings, some are which are described below:

1.3.8.1.1 Absorbent

Absorbents are the oldest class of dressings. Absorbent wound dressing medicament has attempted to maximize absorption based on fibre type, content and weave. The disadvantage of this type of wound dressing is adherence to wound.
1.3.8.1.2 Impregnated dressings

These types of dressings have been used for many centuries. They are usually paraffin gauze (tulle gras) which create non-adhesive or semi-occlusive surface. They equally include other fabrics impregnated with petrolatum or other substances that create non-adherent surfaces. Some are impregnated with antibiotic drug (e.g. neomycin) that minimally diffuses into the exuding wound (75).

1.3.8.1.3 Hydrocolloids

Hydrocolloids as wound dressings are extremely useful and versatile. They contain a pressure sensitive adhesive layer and a hydrophilic polymer. They are also available in paste. When in contact with the wound, the exudate is absorbed from the wound and a gel is formed that expand into the wound cavity. Because of their absorptive characteristics, they usually require less frequent dressing changes than conventional dressing materials (75, 76).

1.3.8.1.4 Foams

Foams are polymeric dressings that maximize absorbency and vapour permeability to provide optimal exudates handling. Foam dressings fit into deep wounds and expand as they absorb exudates. They create gentle pressure on the wound, which may contribute to reduction in periwound edema (73). This may enhance granulation tissue formation because reduced periwound edema may limit exudates production and improve periwound oxygenation.

1.3.8.1.5 Transparent films

These are transparent synthetic adhesive films that are semipermeable, and highly flexible. They have been used as dressings within the last two decades. Films reduce evaporative losses due to the skin stratum corneum, which can result in the loss of 3000 to 5000 g/m² of water over 24 hours (75).
1.3.8.2 Alternative dressings

A lot of substances known to man have been tried as wound dressings. The commonly used agents include vinegar, aloe vera, bleach, sugar and honey. It has been shown that sugar’s hypertonicity reduces periwound edema, which can improve tissue oxidation. The sugar may ferment within the wound, leading to antiseptic alcohol formation. The pH alterations of the wound encountered can have antiseptic effects (75).

Honey contains several proteins that have beneficial effects for wounds. Honey contains inhibine which is an enzyme that creates metabolic by products including hydrogen peroxide and gluconic acid that act as mild antiseptic.

1.4 THE USE OF ADJUVANTS IN DRUG FORMULATIONS

Adjuvants enhance the drug preparation, patient acceptability and the functioning of the dosage form as a drug delivery system and also enhance drug administration through the appropriate route and therapeutic efficacy (76).

1.5 THE USE OF RELEASE ENHANCERS IN DRUG FORMULATIONS

Release enhancers are additives that when added to drug in formulation can increase the rate of drug release. It has been shown that drugs formulated with release enhancers are better therapeutic products than those without them. Before drug absorption, distribution and excretion can take place, the drug must be released from its dosage form (25, 31).

1.6 DISSOLUTION AND ABSORPTION OF DRUGS

Dissolution is the process by which drugs solubilise in a medium. The medium can be blood or gastrointestinal fluid (76, 77). The drug particles are solubilised by physiological fluid before absorption can occur. A saturated layer called diffusion layer is formed by dissolved drug molecules. Then absorption occurs when the drug molecules pass through the diffusion layer and
make contact with the biological membrane. In ointments, creams and gels, the mode of
drug absorption is by passive diffusion. Theuwes et al (78) stated that the drug molecules that are
absorbed from the diffusion layer are replenished from the dissolved drug molecules from the
surface of the drug particle. This can be explained by considering the Noyes-Whitney equation
(Eqn. 8).

\[ \frac{dm}{dt} = KA (C_s - C) \] ..........Eqn 1.

\[ K = \frac{D}{h} \] ..........Eqn 2.

Where \( \frac{dm}{dt} \) represents the rate of dissolution, while K is dissolution rate constant that
incorporates diffusion coefficient D and membrane thickness h. A represents the surface area of
dissolving drug particles, while \( C_s \) is the concentration of drug in the saturated diffusion layer. \( C \) is
the concentration of drug in the dissolution medium at time t.

From the Noyes-Whitney equation, increase in value of K or surface area of the drug
results in increase of dissolution rate of a drug. This implies that any change in physico-chemical
parameter of a drug that enhances the drug dissolution will raise the absorption of such drug
(76,77).

1.7 ROUTES OF DRUG ADMINISTRATION

The most important factor in the selection of route of administration is absorption. In
drug therapy, effective dosage forms are selected based on rate of absorption in the route of
administration. Some major routes of drug administration include, oral, respiratory, topical,
rectal and parenteral.
1.7.1 Oral route

Drugs are most frequently taken by oral administration in the form of either tablets, capsules, suspensions, solutions, or emulsions. Some drugs are swallowed for their local action within the gastrointestinal tract. This effect is made possible by their insolubility and/or poor absorbability from this route.

1.7.2 Respiratory route

This involves the preparations that are usually small volume aqueous solutions or suspensions administered by drops or as a fine mist from a nasal spray container. Nasal drops are usually made isotonic with nasal secretions using sodium chloride. The viscosity can be varied using cellulose derivatives. Due to the fact that the buffering capacity of respiratory mucous is low, formulation at a pH of 6.8 is necessary (76,77).

1.7.3 Topical route

This involves drug administered topically or applied on the skin. Drug absorption via the skin is enhanced if the drug substance is in solution, and has a favourable lipid/water partition coefficient. The drug absorption is facilitated by drug application to abraded or broken skin. The pharmaceutical formulations applied to the skin are intended to serve some local effect. They provide prolonged local contact with minimal absorption (76).

1.7.4 Rectal route

It is the administration of drug through the rectum. Such drug is frequently administered rectally for its local effects and less frequently for its systemic effects. The drug given rectally is usually in form of solution, suppository or ointment. Drug absorbed through this route may not pass through the liver before entry into the systemic circulation. This is an important factor in the formulation of drugs that are destroyed in the liver (77).
1.7.5 Parenteral route

This route involves the administration of drug into the body through the hollow of a fine needle into the body at various sites and depths. The term parenteral is derived from the Greek words *para* (meaning beside) and *entero* (meaning intestine). It has three primary routes namely subcutaneous, intramuscular and intravenous. There are others such as intraspinal, intraperitoneal and intracardiac routes. The parenteral route is preferred when emergency treatment is required or when the drug is destroyed or inactivated in gastrointestinal tract or poorly absorbed for therapeutic response (76).

1.8 DRUG DELIVERY SYSTEMS

The two main objectives of drug delivery systems are to formulate drug product that is therapeutically predictable in terms of patient response, and is capable of being reproduced in large scale manufacturing with good product quality. Drug delivery systems can be divided into early and recent drug delivery systems.

1.8.1 Early drug delivery systems

. Some dosage forms the Egyptians used as early as 1550 BC are still relevant today. They include: gargles, inhalations, pills, lotions, trouches, ointments, plasters, suppositories and enemas. The rest include suspensions, solutions and powders and later followed by sachets, tablets and emulsions (79).

1.8.2 Recent drug delivery systems

The introduction of synthetic polymers in pharmaceutical formulation in the last quarter of the 20th century has accelerated efforts to move towards perfection in drug delivery. Some current drug delivery systems are as follows.
1.8.2.1 **Electromechanical systems (EMD)**

This technology uses electromechanical principle in which, when the device is planted into the body it monitors the patient drug requirement, and releases according to the therapeutic need, either minute-by-minute or second by second. A variation of EMD system is called System for Automatic Feedback Controlled Administration of Drugs (SAFCAD) as reported by Okhamafe (80). In this type of system, thiopentone has been administered in prolonged operation without risk of respiratory arrest.

1.8.2.2 **Floating dosage forms**

In this formulation the drug is dispersed in gel forming colloid or polymers, which are hydrophilic and as such, absorb moisture from the gastric fluid. Levodopa and bensarazide have been formulated as sustained release with this delivery system. This system enhances the residency time in the stomach without affecting the gastric emptying time or rate (76, 80).

1.8.2.3 **Ocular insert**

In this technology, soft contact lens is used as a drug reservoir.

1.8.2.4 **Micro-encapsulated systems**

The technology involved in this type of delivery is aimed at producing fine solid particles, solution or emulsion droplets into reproducible-coated form with polymeric films or shells. The polymers that are used are synthetic polymers and phospholipids (80).

1.8.2.5 **Niosomal drug delivery systems**

The liver and spleen uptake of niosomes makes this drug delivery system suitable for the targeting of diseases in these organs. Niosomes are non-ionic surfactant vesicles. Uchegbu (81) in her studies observed that this technology enhances the delivery of drug in tumour sites. In leishmaniasis, niosomal sodium stibogluconate has been used to improve parasite suppression in
the liver. This delivery system can be used as a depot for short acting peptide drugs on intramuscular or subcutaneous injection.

1.8.2.6 Implants

Implants are administered intramuscularly or subcutaneously with special injectors or by surgical incision. They are usually sterile, highly liquids, semi-solids or solids formulated to provide controlled and prolonged drug release over a long period. They are used as depot delivery either to provide sustained drug release for systemic therapy or to restrict high drug concentration to the immediate area surrounding the pathology.

1.8.2.7 Targeted systems

This drug delivery system has specificity and selectivity to the drug’s site of action as the paramount objective. It has the concept of “drug targeting” which is aimed at targeting the drug to its site of action instead of being distributed throughout the body. This enhances efficacy and reduce toxicity.

A tissue specific ligand, such as antibodies, sugar residue, apoproteins and hormones can be attached to a drug in form of nanoparticles or microspheres. A ligand is selected based on such characteristics as selectivity, recognition and specificity for the target. The drug is usually delivered to a tissue or cell region that ordinarily cannot be accessible to the free or untargeted drug. Ampicillin and gentamicin have been formulated as nanoparticles targeted systems to eradicate intracellular infection (80).

1.8.2.8 Muco-adhesive dosage systems (MAD)

These delivery systems have potential applications for oral, nasal, bladder and buccal delivery. This delivery system is formulated based on interfacial phenomenon that interaction between polymers and the mucus lining of tissue could keep a controlled release device within
the tissue for the desired time. The system is applied to appropriate mucosa for the treatment of both topical and systemic ailments. The polymers used in formulating MAD are water soluble and bioadhesive, with gelling properties. The system can be formulated as disc or powder. Bleomycin, a cytotoxic antibiotic is formulated as a compressed disc to treat cervical and uterine cancer. The drug release duration can be up to two weeks (80).

1.8.2.9 **Osmotic pump devices (OROS)**

The release of drug in this type of dosage form is independent of physiological factors such as pH and gastrointestinal mobility. This dosage form is designed with osmotically active substances like KCl, NaCl and glucose. A semi-permeable membrane that has a tiny orifice created by means of laser is used to coat the core containing the drug. The drug is released at zero order patterns through the orifice. This is achieved by the water from gastric fluid crossing the semi-permeable membrane by osmosis at a steady rate controlled by the solubility of the tablet core formulation. When the core gradually dissolves a saturated solution is formed. The hydrostatic pressure created by this process forces the saturated solution out through the orifice. Drug release can be sustained for up to 20 h and can be released at specified dosage intervals (80).

1.8.2.10 **Micro-encapsulation and tissue engineering**

Tissue engineering involves the use of living cells together with extracellular components usually natural or synthetic to formulate implantable part for tissue repair. The cells are cultured in biomaterial polymer scaffolds “growing” new tissue in bioreactors. In microencapsulated cell system, a living cell immobilization technique is used to prepare bioartificial organs for use in organ replacement therapy. In this technology, a viable cell from an organ rather than drug is microencapsulated. Islets of langerhans and human interferon have been microencapsulated (82).
1.8.2.11 Intra-vaginal ring

This is developed for systemic drug delivery. Oestradiol is delivered this way for treatment of menopausal symptoms. The ring is usually made from vulcanized silicon rubber. They are hydrophobic in nature, permeable, elastomeric, non-toxic, and biocompatible and are about 55 mm in diameter. They are designed in such a way that the drug is distributed in the matrix and a core system which acts as a reservoir and allows zero order controlled delivery. The major use of intravaginal ring is for contraceptive steroids and is designed to be in place for weeks or months. It is also used for local delivery of oestradiol in the treatment of vaginal atrophy (80, 82).

1.8.2.12 Powder injection

This is a drug delivery system that uses compressed gas to accelerate particles to a velocity sufficient enough to physically penetrate the stratum corneum. The drugs delivered using this system can act locally or diffuse into the blood stream to elicit systemic effect. This type of drug delivery has the advantages of simplicity, avoidance of the traditional barriers to transdermal delivery (81).

1.8.2.13 Transdermal delivery systems (TDS)

These are drug delivery systems that are applied topically through the skin. They can be targeted for local or systemic effect. Transdermal delivery systems can be formulated as patches, powder or creams. They have advantages of by passing hepatic first-pass metabolism, avoiding difficulties encountered in oral therapy (such as pH changes, interaction with food, and intestinal transit time) and termination of drug input at any time desired. In this type of technology, therapeutic blood levels of drug can be maintained for up to 24 h (80). Other current dermal deliveries as reported by Nanda et al (99) include; microporation, medicated tattoos, laser-
induced stress waves (LISWs), sonophoresis (phonophoresis), liposomes, magnetophoresis, iontophoresis and electrophoresis.

1.8.2.14 Pro-drug delivery

It involves the synthesis of a pro-drug molecule that is more lipophilic than the parent drug. This changes the physicochemical properties of the drug for better delivery (82). This technology enhances optimal dermal penetration of prodrug esters of NSAIDs (e.g. Ketorolac).

1.9 TOPICAL DRUG DELIVERY

The drug action is required at the site of application rather than for the systemic effect. This route can also be used for systemic drug delivery. Drug absorption via the skin is enhanced when the drug substance is in solution and has a favourable lipid/water partition coefficient and is nonelectrolyte (76).

In general, topical preparations are intended to serve some local action and are formulated to provide prolonged local contact with minimal absorption (83). A way of achieving this is through the use of drugs in powdered or solid form rather than in solution. Some systemic administration of drug can be achieved through topical delivery as pointed out by Shaw et al (84) in their investigation.

Topical formulations are mostly administered in form of ointments and related semisolid preparations like creams and gels. They can also be formulated as patches, pastes, powder, aerosol spray, solutions and lotions. Drugs to be applied to other topical surfaces like ear, eye and nose are usually formulated as ointments, suspensions and solutions.

1.10 OINTMENTS

Ointments contain dissolved or dispersed medicaments and are often greasy, semisolid and anhydrous. They are semisolid preparations intended for external application to the
epidermal surface of the skin or mucous membrane. They may contain drug substance (medicated ointment) or may not (non-medicated ointment). Due to their adhesiveness, which is due to their plastic rheological behaviour they continuously release medicament.

In this type of drug delivery system, Shaw et al (84) stipulated that systemic toxicity, patient non-compliance and absorption defects are eliminated.

1.10.1 Pharmaceutical attributes of ointments

Ointment base can be used as carrier for topical delivery of drug. The ointment bases are also used as emollient or for lubricating function. Ointments are easy to be applied to the skin surface and have ability to release drug for a prolonged time. When medicated ointment is applied to more skin area higher drug absorption occurs. Ointment acts as moisture barrier to the applied surface which enhances the hydration of such surface. Ointment base is non-toxic and inert. Ointments are good for percutaneous absorption and for local action of drug. The pharmaceutical attributes of ointments are spreadability, washability, wettability, pourability and attendant emollient property (85, 86,76,77,79).

1.10.2 Composition of ointments

Medicated or non-medicated ointments are made with various excipients to improve their aesthetic value and bioavailability. Some of such excipients include; vehicles, emulsifiers, preservatives, antioxidants and other adjuvants. Researchers have shown how characteristics of excipients affect release kinetics of drugs from tablets, suppositories, ointments and suspensions. Barr (85) reported that excipients can act as drug vehicles and have ability to affect drug penetration by modifying the permeability of the skin barrier phase. Mctaggart and co-worker (87) stated that antipyrine has higher concentration in polymerized gel and Gibaldi et al (88) in their studies reported that surfactants increase dissolution kinetics in miceller solution under non-
sink conditions. Aikwa et al (89) in their investigations asserted that drug release in hydrogel polymer is optimized in a particular pH while Tomita and co-workers (90) suggested in their report that addition of EDTA in drug delivery enhances absorption. In other words adjuvants in drug formulation are significant in the overall drug formulation characteristics.

1.10.3 Ointment bases

Ointment base is an excipient which acts as a vehicle in the formulation of semi-solid preparation. There are various substances that can be used as ointment bases in the formulation of drug for topical delivery. In the selection of ointment base some factors that can be considered include (i) desired release of drug from the ointment base, (ii) desirability of occlusion of moisture from the skin by the base, (iii) enhancement of percutaneous absorption of the drug by the base, (iv) stability of the drug in the ointment base and (v) the influence of the drug on the consistency or other features of the ointment.

There is no ideal ointment base in pharmaceutical preparation, but a selected base should possess most of the above stipulated properties (79). There are four classes of ointment bases recognized by United States Pharmacopoeia (USP) (91), namely: hydrocarbon bases, water soluble bases, water-removable bases and absorption bases.

1.10.4 Classification of ointments

They can be classified based on the site of application (91) as follows: otic (aural), ophthalmic, topical (dermal), rectal and vaginal ointments.

1.10.5 Formulation of ointments

The method for a particular preparation of ointment depends primarily upon the nature of the ingredients and scale of production. The aim of such preparation is to disperse drug substance uniformly throughout the vehicle, a finely subdivided or dissolved medicament. Two methods
are generally used in ointment preparations. They are trituration (levigation) and fusion (76, 79, 86).

1.10.5.1 Levigation (trituration)

This is the act of incorporating drug into the ointment base by using mortar, pestle, spatula and ointment tile. This method is used in the preparation of small quantities of ointment. The finely powdered drug material is levigated thoroughly with small quantity of the base to form a concentrated medicated ointment. It is eventually diluted geometrically with the base to form the required medicated ointment of the desired concentration.

Small hand operated mills with two porcelain rollers are available for dispensing prescription of small quantities. In pilot scale manufacturing, roller mill is usually used to reduce solid particles and disperse them in the base. When the base is large, mechanical mixers are normally used.

1.10.5.2 Fusion

In this method, all or some of the components of an ointment are combined by melting together and cooling with constant stirring until congealed. When it is on a small scale, the fusion process may be conducted in a porcelain dish or glass beaker. But when on a large scale, it is usually carried out in large steam-jacketed kettles. In preparation of ointments that have emulsion type of formula, the fusion process will involve melting process and an emulsion process (76, 79). In other words, it involves the fusion of two-phase system of oil phase and aqueous phase. The melting temperature of the fusion process is usually 70 °C - 75 °C.

1.10.6 Evaluation of ointments

Evaluation techniques include the following, skin irritancy or allergy test, stability of the active ingredients, rheological properties, phase separation, stability of adjuvants and apparent
pH (76). Others are microbial and particulate contamination, loss of water and other volatile components, particle size and particle size distribution of dispersed phase (86).

### 1.10.7 Skin allergy tests

A number of test procedures have been devised to test for irritancy level both in animals and human skins (92, 93). The positive results of the test ointments may not out rightly disqualify the products as there are other factors that are considered. Such tests include: Draize, Draize-Shelanski-repeat-insult, Kligman-maximization and 21-cumulative irritancy tests.

### 1.10.8 Rheological behaviour of ointments

Ointment being semisolids exhibit non-newtonian flow when being applied to the skin. Ointment shows a pseudo-plastic behaviour and does not significantly delay drug absorption (76).

### 1.11 CREAMS

Creams are semisolid emulsions with either oil or water as dispersing phase for external application. They are usually employed as emollients or as medicated applications to the skin.

#### 1.11.1 Pharmaceutical attributes of creams

Creams as emulsions contain two immiscible liquids in which one is dispersed in the other as minute globules. This is achieved with the aid of emulsifying agent. In cosmetology, creams can be applied to the skin for their cooling, bleaching, emollient, moisturizing, sun screening or deodorant effects.

When drug is incorporated into a cream base, the product formed is regarded as medicated cream. They are applied to the skin for localized effects and treatment of skin conditions of various natures.
1.11.2 Classification of creams

Creams can generally be classified into two basic types - oil-in-water (o/w) and water-in-oil (w/o).

1.11.3 Components of creams

Creams are emulsions that are complex systems with various components that are homogenous. Pharmaceutical creams contain suspensions or solutions of medicinal agents and other excipients such as, antioxidants, colorants, preservatives, emulsifiers, thickeners and perfumes. In formulation, it is necessary to blend all these components to achieve a stable dosage form (76).

1.11.3.1 Cream bases

They can be either hydrophobic or hydrophilic in nature and are responsible for viscous consistency of creams. Cream bases are similar to those used in ointments (86). They include, hydrocarbon, water soluble, water removable, absorption bases.

1.11.3.2 Emulsifying agents

The essence of including emulsifying agent in the formulation of creams is to facilitate actual emulsification and to ensure stability. The choice of emulgent depends on its emulsifying characteristics, route of administration and toxicity (76).

Emulsifying agents can further be classified based on their composition such as; synthetic or semi-synthetic surface active agents, naturally occurring materials and their derivatives and finely divided solids (94).

Other additives that can be incorporated in cream include; antioxidant, preservatives, colourant and fragrance.
1.11.4 Properties of creams

Creams are generally less viscid and lighter than ointments. They are considered to have greater aesthetic appeal as a result of their non-greasy characteristic and their ability to leave a film on the skin upon rubbing (79). Creams that contain stearic acid and cetyl alcohol produce non-tacky films while those that contain synthetic or natural polymers leave a tacky coating on the skin. It is advisable to use the minimum amount of polymers in cream formulations (76).

The physicochemical properties of the creams such as, pourability, spreadability and washability affect the patients’ choice of creams. Other factors that can affect patients’ acceptability of cream include the colour and fragrance. The consistency and homogeneity and some properties of cream depends on the technological procedures used in the manufacturing of the cream (77).

1.11.4.1 Rheological behaviour of creams

The rheological properties of creams are determined by the structural association of components of the creams and the storage time. The Rheological behaviour on the other hand affects the therapeutic usefulness of creams (76,77,79). Creams generally are non-newtonian in their flow characteristics.

Creams are viscoelastic which implies that they exhibit both elastic properties of solids as well as viscous characteristics of fluids. The rheology of creams is very important as it affects the cream consistency, pourability and spreadability, adherence to the skin, drug release from the base and its permeation into the skin. The viscosity of the cream is affected by the degree of homogenization of the cream, the dispersed volume, nature and concentration of the emulgent. It can be controlled by changes in the viscosity of continous phase and globule size (dispersed phase) (39,76).
1.11.4.2 Stability of creams

Stability is undoubtfully an integral part of dosage form design. It may be affected by temperature and properties of the adjuvants (25). An important function of semi-solid dosage form base is the control it exerts over the release and hence the therapeutic activity of the drug incorporated. The stability of cream preparations can be evaluated on the basis of changes in pharmacological, microbiological, viscosities, relative densities and other physicochemical characteristics.

A pharmaceutical liquid or semi-solid product is regarded to be physically unstable when there is sedimentation creaming, cracking, coagulation or flocculation of particles (86). Creaming occurs when dispersed globules migrate upward through the continuous phase. The globules may coalescence due to the rupture of the interfacial film, thereby leading to cracking of the cream. Coagulation or sedimentation in a cream involves downward movement of globules and obeys the Stoke’s law. The application of shearing force can lead to redispersibility of sediments in the cream. The dispersed phase of a cream should not exceed 74 % of the total volume of the system as this may lead to phase inversion. Increase in temperature results in physical instability of the cream as phase separation may occur.

Creams are conducive for the growth of micro-organism especially the oil-in-water emulsion types. Microbial degradation of cream leads to development of odour and cloudy appearance. Preservatives are usually incorporated in the cream formulation to prevent microbial growth and degradation of the product.

Chemical instability in creams occurs mostly by hydrolytic, photolysis, complexation and oxidative reactions (95). Antioxidant is usually incorporated to prevent oxidation. The chemical reaction is usually caused by the chemical incompatibilities of the constituents of the
cream. The anionic and cationic emulgents are chemically incompatible and should not be used together in cream formulation. This can result in therapeutic failure as the pharmacological properties of the cream are altered. Such chemical reactions can lead to colour changes and odour production.

1.11.5 Formulation of creams

Creams are emulsified systems that contain two immiscible phases. Various emulgents produce emulsification by different mechanisms. The immiscibility of the lipid and aqueous phases is as a result of the cohesive forces between molecules of each phase being stronger than the adhesion forces of the two phases (86, 95).

During the formulation of creams, the oil phase is separated from the aqueous phase. Each of the two phases is heated to 70 °C - 76 °C and eventually mixed together with continuous stirring. At the elevated temperature the kinetic energy of the emulgent at the oil-water interface is increased (77,79, 86, 95). In considering the micromeritics of emulsion, globule diameter (0.5-2.5 μm) gives optimal size in terms of physical stability and texture of the cream.

1.12 GELS

Gelling agent forms semi-solid gels as a result of aggregation of colloidal particles that are interlaced and are considered colloidal dispersions as they contain particles of colloidal dimension. The continuous phase is held within meshes in the network with only a small percentage of disperse phase required to give rigidity to the gel. A gel with liquid is called a jelly while xerogel contains no liquid (75, 76). Gels are aggregates of many molecules and an increase in temperature can cause some gels to assume liquid state.
1.12.1 Composition of gels

Gel as a semi-solid system contains components which include continuous phase, disperse phase, gelling agents, preservatives, antioxidants, colourants and fragrance. The continuous phase of a gel is the dispersion medium which may be aqueous or non-aqueous. The disperse phase consists either small inorganic particles or large organic molecules that are interpenetrated by a continuous phase (liquid). Generally most gels are produced in aqueous medium. Examples of gelling agents include acacia, gelatin, tragacanth, aluminum hydroxide and magnesium hydroxide. Gels can be attacked by microbial organisms and preservatives like sorbic acid, benzoic acid etc are used to preserve them. Gels may be incorporated fragrance and colourant to enhance the aesthetic property.

1.12.2 Classification of gels

There are three types of gel formations.

Flocculated lyophobic gels are regarded as continuous floccule. The flocculation is formed by special mechanism through interaction between crystal structure and di- or tri-valent molecules. This interaction is due to electrostatic attraction between the face and edge of different particles, which forms a gel structure (76). Examples of such gels are Aluminum hydroxide and Magnesium hydroxide gels. Such gels are not solvent loving but are lyophobic gels. The forces (electrostatic and van der Waals) that hold the particles together in this type of gel are weak. The bonds formed are weak which makes such gels thixotropic when little shear force is applied. This phenomenon is a non-chemical isothermal gel-sol-gel transformation (76).

In lyophilic-thermal gels particles form gels in this group by macromolecules forming a network simply by entanglement or attraction by hydrogen bonds between molecules or by van der Waals’ forces. The gels have characteristic liquefaction when an increase in temperature is
applied. This is due to the break of weak bonds. Examples of such gels are polyacrylamide, agar, gelatin and other polymers. They form three dimensional network of hydrophilic polymer gel. This class of gel often contract spontaneously and exudates some of the fluid medium. This process is regarded as syneresis. Here the gelling process is irreversible as primary valency bonds are formed.

Hydrogel-xerogel hybrid gels are formed by the grouping or association of molecules that exhibit both lyophilic and lyophobic properties (79).

1.12.3 Formulation of gels

Gels are formulated by incorporating some quantities of gelling agents into a cold or warm liquid usually aqueous and allow to stand until required gel is formed. The drug and other adjuvants like preservatives, colurorants, fragrance and antioxidants are then incorporated using fusion method in the right quantities.

1.12.4 Evaluation of gels

Gels can be evaluated by determining some of the parameters that include microbial stability, physical and chemical stability, pharmacological and particle size.

1.12.5 Rheology of gels

Flocculation in gels is the reason for their anomalous rheological behaviour. The rheology of gels can be characterized as having pseudoplastic property exhibiting non-Newtonian flow when shear force is applied. Thermal gels often contract spontaneously and exudate some of the fluid. This process is called syneresis. It is due to increase in the number of bonding points which consequently leads to coarsening of the matrix structure that results in the expression of liquid from the gel.
Some gels like clays, aluminum hydroxide exhibit thixotropy in their rheological pattern. This involves a non-chemical isothermal gel-sol-gel transformation. It is as a result of the weak bonds in the gel that are broken when the gel is sheared and reform when the gel is unsheared. The particles collide leading to flocculation and the gel is reformed.

Xerogels change into a gel-like substance when rehydrated. This group includes alginites and dextranomers. Alginites are derived from the *laminaria* seaweed and are composed of mannuronic and guluronic acid. The guluronic acid reacts with calcium to form a gel. This type of xerogel has been used as wound dressing as it absorbs large amount of wound exudate and then acts as a hydrogel in facilitating moist wound healing (76, 79). The spreadability, wetability, washability and pourability are some of the rheological properties that affect patients’ acceptability of gel product.

1.13 GENERAL THEORETICAL CONSIDERATION OF DRUG RELEASE MECHANISMS

The release pattern of a drug from a formulation can be affected by the physicochemical properties of the drug and it’s dissolution rate. Diffusion according to Fick’s first law is the major process by which drug is released. Different drug release time profiles are obtained according to drug formulation design (22). There are three major drug release patterns based on their kinetics (79) namely: zero order, first order and where release rate decrease exponentially with time. Examples of drug release that decreases exponentially with time can be explained by some models like: Wagner (96) explained that Wagner’s plot tends to produce straight lines and can be used for comparison of drug $t_{50}$ release time. Langenbucher cube root model as explained by Nakano *et al* (97) that a straight line is obtained when the cube root of the relative undissolved drug is plotted against time in semi-solid formulations. Higuchi (98,99) and
Thongborisute *et al* (100) have reported that Higuchi square root law was originally developed to explain the release of drugs from matrices in slow rate; others are Baker-Lonsdale model and Hixon-Crowell cube root law as pointed out by Okore (101).

1.13.1 **Surface phenomenon**

Prakongpan *et al* (102) in their work had explained how surface phenomenon involves solubilization kinetics in the diffusion layer to the overall kinetics of drug release profile. This process starts with the dissolution mechanism for a nonionic (unionized), non-reactive drug solid, which will undergo three basic processes, contact of biological fluid with the drug particle interface; physical reaction and the transport of drug solutes away from the interface into the bulk solution (sink condition).

It is assumed here that the second and third steps are the rate limiting steps in drug release. In a modified Nernst diffusion layer theory (102), most dissolution or drug release conditions both convection and diffusion are expected to be significant. The Nernst theory can be written as,

\[
\frac{dg}{dt} = F = \frac{A(C_s - C_b)}{h + \frac{1}{D} + \frac{1}{P}}
\]

\[\text{Eqn. 3}\]

Where \(g\) is the total amount of drug particle dissolved into biological fluid at time \(t\), \(A\) is the surface area of drug particle exposed to biological fluid, \(F\) is the dissolution rate or rate of drug release, \(D\) is the diffusion coefficient of drug solute in the biological fluid, \(C_s\) is the concentration of drug solute in the bulk (sink condition), \(h\) is the Nernst effective diffusion layer thickness, \(P\) is the effective interfacial permeability coefficient. \(C_b\) is the saturated concentration of drug solute.

When the surface equilibrium is immediate, \(1/P\) is negligible then,
\[ F = \frac{AD(C_s - C_b)}{h} \]  

Eqn 4

This implies that the drug release is determined by bulk transport only (102). But when the equilibrium at the surface is slow then \( \frac{1}{P} \) is much bigger than \( \frac{h}{D} \)

\[ F = AP(C_s - C_b) \]  

Eqn 5

This shows that the rate of drug dissolution or rate of drug release is controlled by the rate of drug solute transfer at or near the drug particle-solution interface as such it is interfacially controlled.

1.13.2 **Zero order drug release mechanism**

In zero-order drug release mechanism, the rate of drug release is constant irrespective of time. It is dependent on the saturation concentration of the drug under sink condition. In zero-order release, it is expected that the physicochemical parameters will remain constant without affecting the solubility of the drug. In this condition most drugs will have zero-order mechanism of release up to about 95 percent and drug salts that are of moderate solubility will have about 50 percent release. In other words, there are two conditions required for a drug preparation to have zero-order release pattern. Firstly the drug moiety of the product will be totally soluble and its internal concentration decreases with time. Secondly, the sink condition should not be fixed. In osmotic pump delivery system, it is designed to have zero-release pattern (77,79).

1.13.3 **First order drug release mechanism**

Drug release and gastrointestinal absorption could follow first order kinetics in which the release rate is dependent on drug concentration permeability of the membrane to the particular drug and diffusion coefficient of the drug membrane thickness (79).

The equation of first order model can be written as,
In \( C = \ln C_0 - K_t \) ...............................................................Eqn 6

\( C \) is the amount of drug undissolved at the time \( t \), \( C_0 \) is the amount of drug undissolved at \( t = 0 \) and \( K \) is rate constant.

1.13.4 The Higuchi square root law

Higuchi (98) proposed a theoretical equation describing the release of drugs from homogenous ointments, creams and gels. For practical applications for drug release in these dosage designs he stated the equation as,

\[
Q = 2C_0 \sqrt{\frac{D}{\pi}} \sqrt{t} \quad \text{..........................................................Eqn 7}
\]

The diffusion coefficient of the drug in the semi-solid preparation is found by rearranging Eqn. 7 to,

\[
D = \left( \frac{Q}{\sqrt{t}} \right)^2 \left( \frac{\sqrt{\pi}}{2C_0} \right)^2 \quad \text{..........................................................Eqn 8}
\]

In replacing \( \frac{Q}{\sqrt{t}} \) by \( \text{tg} \alpha \) (calculated slope of the line of the release) equation 16 is obtained as,

\[
D = \left( \text{tg} \alpha \frac{\sqrt{\pi}}{2C_0} \right)^2 \quad \text{..........................................................Eqn 9}
\]

When a releasing surface area is introduced the diffusion coefficient of the drug becomes independent of the apparatus.

\[
D = \left( \frac{\text{tg} \alpha}{A} \frac{\sqrt{\pi}}{2C_0} \right)^2 \quad \text{..........................................................Eqn 10}
\]
Q = amount of drug release per unit area at time t (g cm\(^{-2}\)), D is diffusion coefficient of drug in the ointment (cm\(^2\) s\(^{-1}\)), \(C_o\) is the unit concentration of dissolved drug in the ointment or cream or gel (g cm\(^{-3}\)), t is time (s) and A is releasing surface area.

When A and \(C_o\) are kept constant in equation 10. The diffusion coefficient is dependent only on the slope of the releasing line (\(tg\alpha\)). This therefore implies that the diffusion coefficient of a drug in ointment base can be calculated from the slope of the function of the release rate of the drug as determined from drug release graph and from equation 10. This theory applies to a wide range of preparations.

**1.13.5 Hixon-Crowell cube root law**

This theory was first developed in relation to the dissolution of solid particles. The equation of this law can be expressed as (101),

\[
\frac{1}{D_t^{\frac{1}{3}}} = \frac{1}{D_0^{\frac{1}{3}}} = K_t \quad \text{.................................................................Eqn 11}
\]

Where \(D_0\) and \(D_t\) are the weights of the particles initially and at time t respectively. \(K\) is the rate constant. For this theory to hold the drug release of dosage form is governed by dissolution rate of the drug particle. It is also expected that the surface area of the dissolving particles decreases as dissolution progresses (101).

**1.14 FACTORS ENHANCING THE RELEASE AND ABSORPTION OF DRUGS FROM TOPICAL WOUND FORMULATIONS**

The release of drugs from semi-solid topical formulations (ointments, creams, gels) involve melting and or dissolution of drug particles and finally absorption of the drug molecules. The factors that affect this process are physiological factors, drug-related and formulation related factors.
1.14.1 Drug substance related factors

1.14.1.1 Surface properties

From the biopharmaceutical point of view, the surface energy is vital in drug particle interaction between the ointment base and wound fluid. This interaction is detected by the surface properties of the drug. The smaller the contact angles the better the immersion of the drug particles in the wound fluid, which will increase the rate of dissolution. In order to improve surface characteristics like surface tension of vehicle and drugs, surfactants are added to some ointment (cream and gel) formulations to increase wettability and rate of dissolution of the drug, which will enhance absorption.

1.14.1.2 Particle size

The particle sizes range 50 - 150 µm gives better product uniformity of content, bioavailability and good dissolution rate. The sizes can be manipulated to reduce agglomeration of drug particles due to van der Waal forces. The drugs that have low solubility are formulated in micronized form for better biopharmaceutical result (76).

1.14.1.3 Solubility

The lipid-water solubility (partition coefficient) of the active drug is an important factor in considering the release and absorption from topical formulations (ointments, creams and gels). There is proportionality between drug release rate and water solubility. A lipophilic drug that is distributed in fatty base will have less tendency to escape to the surrounding fluid, than would a hydrophilic drug in a fatty base. Macrogol (water soluble) bases easily release both hydrophilic and lipophilic drugs for absorption (76,79). The ability of the wound fluid to dissolve drug determines the attainable drug concentration, which is the driving force for drug absorption (76).
1.14.1.4 pKa

pKa is the ionization or dissolution constant of drug. In wound environment the pH of the wound is about 6.6 - 7.2. About 75 % of all drugs are weak bases while 20 % are weak acids, and 5 % are either non-ionic, amphoteric or alcoholic (76). Most drugs are therefore subject to ionization, which affects their permeability across biological membranes. Changes in physiochemical parameters of the drug and formulation composition can affect drug dissolution rate. In ointment, cream, gel formulations the permeation rate is affected by such factors as size, relative aqueous and lipid solubility and ionic charge on the drug molecules.

1.14.2 Physiological factors

1.14.2.1 Volume of wound fluid

The volume of wound fluid varies with the size and type of the wound as increase in wound size leads to increase in wound fluid (103,104). Stenn and co-worker (105) reported that hydrophilic drugs dissolve in the wound secretion and through osmotic effects, water is then attracted for complete dissolution of the drug. The characteristics of biological secretion such as surface tension, viscosity and composition can affect the rate of dissolution and absorption of drug particles (106).

1.14.2.2 pH and buffer capacity of the wound fluid

Kaufman et al (107) reported that the wound fluid has a pH of between 6.6 - 7.2. It is therefore essentially neutral and does not have buffering capacity on wound formulations e.g. ointments, creams and gels as to affect the potency of the drug.
1.14.3 Vehicle or base related factors

1.14.3.1 Composition of the base

The composition of the ointment base will affect the release of incorporated drug. Drugs that are hydrophilic have better bioavailability when formulated with lipophilic vehicles while those that are lipophilic are better formulated in hydrophilic base so as to have better release and absorption in the wound surface. When the drug is highly soluble in the vehicle the release rate into the wound fluid will be slow and this tends to give poor absorption. Baichwal et al (108) stated that the vehicle that tends to discourage the active drugs from remaining in the base is used in semi-solid formulations. The base should have good lipid-water partition coefficient that is favourable for the release of drug from the ointment (cream or gel) base (109, 110).

In topical preparation the semi-solid base should have rheological behaviour that encourages the spreading of the medicament on the wound surface to maximize therapeutic effect.

1.14 RELEASE OF DRUGS FROM OINTMENTS, CREAMS AND GELS IN WOUND

Drug release for ointments, creams and gels, whether lipophilic or hydrophilic-based, involves a series of steps (76). It includes the dissolving in wound fluid by the hydrophilic base which allows the drug particles to be solubilized by the wound fluid to have contact with cell membrane through which the drug is absorbed (104).

In ointments, creams and gels, the drug release is by the process of passive diffusion and through osmotic effects the base draws wound aqueous liquid for complete dissolution (76).
1.16 **RATIONALE FOR THE STUDY**

The existing topical medicaments for wound healing have their shortcomings especially chronic and diabetic wounds that do not heal easily. Some wounds form fibrotic scar or keloids on healing, requiring surgical intervention. In addition, delayed wound healing has shown that recent remarkable advances in understanding the cellular and molecular mechanisms of wound repair have not yet led to similar advancements in wound care. These led Jahovic et al (74) to report that despite many advanced medical treatments, cutaneous thermal wound is still a great problem. They also observed that mucin (glycoprotein) accelerated epidermal regeneration. Subrahmanyam (14) reported that in 25 randomly allocated partial thickness burn patients treated with honey dressing, 84% showed satisfactory epithelization by the 7th day and 100% by the 21st day. Wound healing is a physiologically and biochemically complex process. Mucin and honey individually have wound healing properties.

This study aims at investigating the effect of mucin and honey combination in treating wounds and to ascertain whether the combination of both in standard topical formulations (ointments, creams and gels) can be an effective wound repair-promoting agent.

1.17 **OBJECTIVES OF THE STUDY**

The objectives of this study are;

- To evaluate the wound healing effect of honey and mucin
- To evaluate the wound healing effect of standard topical formulations of mucin and honey.
- To determine the histopathological and morphometric parameters in the wound healing effect of the formulations.
- To determine the effect of mucin, honey or their combination on wound protease enzyme expression and determine concentration of the neutrophil elastase in wound fluid during the process of wound tissue regeneration and healing.

1.18 AIMS OF STUDY

The aims of the study are as follows:

- To develop an effective wound medicament.
- To formulate mucinated-honey into standard pharmaceutical preparation.
- To ascertain the synergism of mucin and honey on wound healing.
- To minimize wound hypertrophic scar.
- To prevent acute wound becoming chronic wound.
- To elucidate the enzymological status of mucinated-honey dressed wounds.
CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

Purified mucin type 111 (Sigma U.K). Honey was procured locally from Nsukka central market. The following chemicals and materials were used as obtained from their manufacturers: succinyl alanyl-alanyl-alanyl-p-nitroanilide N3050, Folin Ciocalteu’s phenol reagent (Sigma-Aldrich, Steinhein, Germany). Gelatin A powder (Fairlawn, New Jersey, USA), Mueller Hinton agar (Lancashire, U.K), emulsifying wax, white soft paraffin, liquid paraffin (BDH Chemicals, England), flamazine cream (Hoechst, Germany), salicylic acid BP (Lewes, Sussex), sodium hydroxide, monobasic potassium phosphate (Bergoyne, India), disodium hydrogen phosphate anhydrous, sorbic acid, chlorocresol (BDH, Poole, England). Rabbits and albino rats were obtained from the Animal house of the Department of Pharmacology, University of Nigeria, Nsukka. Bacterial cultures sourced from Pharmaceutical microbiology laboratory of University of Nigeria, Nsukka.

2.2 Methods

2.2.1 Standardization of honey

The honey used for the study was standardized to Pharmaceutical Codex (PC) by diluting the honey with sterile distilled water to a weight of 1.36 g/ml at room temperature (29 ± 1°C).

2.2.2 Determination of antimicrobial activity of honey

Various dilutions (1:1, 1:2, 1:3 and 1:4) of the standardized honey were prepared using sterile distilled water. The antibacterial activity of each dilution obtained against three species of bacteria usually implicated in wound contamination (15) namely: *Staphylococcus aureus*, *Pseudomonas aureginousa* and *Escherichia coli* were determined. Cultures of the bacteria were
collected with inoculum loop and seeded in 1 ml sterile distilled water and then transferred into 9 ml, sterile double strength nutrient broth and incubated at 37 °C for 24 h in an incubator (Gallenkamp, Model 1H-150). The bacterial suspension was made by transferring 1 ml of the seeded nutrient broth to 99 ml of sterile molten Mueller Hinton agar.

38 g of Mueller Hinton agar powder was soaked in 0.5 litre of deionized water for 10 min., mixed using a vortex mixer and sterilized by autoclaving (Haereus Type KKB500) for 15 min. at 121 °C. It was allowed to cool to 47 °C and 20 ml was poured into petri dish and allowed to solidify at 29 ± 1 °C. A sterile glass borer was used to make 6 mm holes in the solidified agar. Two (2) drops of each dilution of honey was then placed in each of the holes and incubated at 37 °C for 48 h. The zone of inhibition was measured in mm using a calibrated ruler. This was repeated for all the three organisms tested.

2.2.3 Determination of antimicrobial activity of mucin

The antibacterial activity of mucin was evaluated using the same procedure described above for honey. Various dilutions (1:1, 1:2, 1:3 and 1:4) of mucin were prepared with sterile distilled water giving concentrations of 200 mg/ml, 100 mg/ml, 66.67 mg/ml, 50 mg/ml and 40 mg/ml respectively.

2.2.4 Determination of antimicrobial activity of mucin-honey mixture

The determination of antibacterial activity of the combination of mucin and honey was done using the same procedure described for honey. The mucin:honey mixtures were in the ratios of 1:1, 1:2, 1:3, 1:4, 2:1, 2:3, 3:1 and 4:1 by weights. The minimum inhibitory concentration (MIC) was calculated from the x-intercept of the plot of inhibition zone diameter squared against log concentration.
2.2.5 Preparations of ointments, creams and gels

Calculations for the various formulations were done based on the initial evaluation of antibacterial activity of mucin, honey and 1257.14 mm$^2$ area of the circular wound to be inflicted on the rats. A piece of tracing paper was used to trace 20 mm diameter circle with the aid of compass. The paper was then dressed with ointment base and weighed. This was repeated with mucin and honey. A 10 mg and 20 mg weight of mucin and honey was required to cover the wound area while 30 mg of the mucin and honey mixture was required. This combination has efficient antimicrobial activity against the three tested bacteria in the preliminary studies.

2.2.5.1 Formulation of ointment batches

The ointment (batches 1; 23.5 % honey, 2; 11.75 % mucin, 3; 11.75 % mucin: 23.5 % honey and 4; ointment base) were prepared using British Pharmacopoeia standard (46). Emulsifying ointment base was prepared by fusion method using the formula:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsifying wax</td>
<td>30 g</td>
</tr>
<tr>
<td>White soft paraffin</td>
<td>50 g</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>20 g</td>
</tr>
</tbody>
</table>

100 g

The various ingredients were weighed and put in 500 ml beaker, heated in bath water to 75 $^\circ$C to melt and stirred to mix till cooled.

The batch 1 ointment was formulated using the formula:

Honey 23.5 g

Chlorocresol 1 g

Emulsifying ointment base 75.5 g

100 g
The emulsifying ointment base prepared was used in each ointment batch and various quantities of mucin and honey were incorporated using fusion method to give the required formulations. 1 g of chlorocresol was melted in 0.5 ml of water and incorporated into the respective batches as preservative. 0.5 g of salicylic acid was added to 25 g of each formulation intended for release studies. The ointments were stored in wide mouth ointment glass jars and covered with caps at ambient temperature of 29 ± 1 °C.

2.2.5.2 **Formulation of cream batches**

The standard Aqueous cream BP (52) was prepared using fusion method according to the formula used is as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsifying ointment</td>
<td>300 g</td>
</tr>
<tr>
<td>Chlorocresol</td>
<td>1 g</td>
</tr>
<tr>
<td>Distilled water, freshly boiled and cooled</td>
<td>699 g</td>
</tr>
<tr>
<td></td>
<td>1000 g</td>
</tr>
</tbody>
</table>

The weighed quantity of chlorocresol was dissolved in water with the aid of gentle heat. The weighed emulsifying ointment was melted by heating in 500 ml beaker on a water bath at 75 ± 1 °C. The aqueous phase and oil phase were mixed by gradual and steady addition of the aqueous phase into oil phase with constant stirring using a glass rod. The stirring was continued until the emulsion cooled to 35 ± 1 °C. In each of the cream (batches 5; 23.5 % honey, 6; 11.75 % mucin, 7; 11.75 % mucin: 23.5 % honey and 8; cream base). The various quantities of mucin and honey were incorporated into 100 mg cream base using fusion method to give the required formulations. 0.5 g of salicylic acid was added to the 25 g of each cream preparation intended for
release studies. The cream formulations were transferred into wide mouthed glass jars, covered with screw caps and stored at room temperature.

2.2.5.3 **Formulation of gels**

To prepare the gels (batches 9; 23.5 % honey, 10; 11.75 % mucin, 11; 11.75 % mucin: 23.5 % honey and 12; 15 % gel base), the gel base was prepared by adding hot distilled water to 15 g of gelatin powder, this was then stirred to give a uniform mixture which was allowed to cool. The various quantities of mucin and honey (as in cream batches) were incorporated by trituration into the gel base respectively to give the required gel batch. 0.5 g of salicylic acid was incorporated into 25 g of the gels using fusion method for release studies. They were stored in wide mouthed glass jars. The standard (batch 13; 1 % silver sulphadiazine cream) used in this study was Flamazine cream\(^R\) (Hoechst, Germany) to which 0.5 g of salicylic acid was added for release studies. Formula for all the formulations are shown in Table 1.

2.2.6 **Evaluation of ointment, cream and gel batches**

2.2.6.1 **Absolute drug content**

The absolute drug content of the ointments was determined 30 min after formulation by assaying the quantity of salicylic acid in each batch of the ointment at 37 ± 1 °C with phosphate buffer solution pH 7.6 as the dissolution medium. In each batch 0.1 g of the ointment was weighed and dissolved in the phosphate buffer solution. The volume was made up to 100 ml with the phosphate buffer solution. The solution was allowed to stand for about 30 min before the salicylic acid content was assayed at a wave length of 298 nm using a U.V. spectrophotometer (Coleman, U.V. Model 6/20A). This procedure was repeated five times for each batch and the average taken. The absolute salicylic acid content of each batch of the ointment was determined using Beer’s plot. This was repeated for the standard.
Table 1. Formula for all the formulations

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
<th>Batch 5</th>
<th>Batch 6</th>
<th>Batch 7</th>
<th>Batch 8</th>
<th>Batch 9</th>
<th>Batch 10</th>
<th>Batch 11</th>
<th>Batch 12</th>
<th>Batch 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honey</td>
<td>23.5 g</td>
<td>-</td>
<td>23.5 g</td>
<td>-</td>
<td>23.5 g</td>
<td>-</td>
<td>23.5 g</td>
<td>-</td>
<td>23.5 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucin</td>
<td>-</td>
<td>11.75 g</td>
<td>11.75 g</td>
<td>-</td>
<td>11.75 g</td>
<td>11.75 g</td>
<td>-</td>
<td>11.75 g</td>
<td>11.75 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Emulsifying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ointment base</td>
<td>76.5 g</td>
<td>88.25 g</td>
<td>64.75 g</td>
<td>100 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cream base</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>76.5 g</td>
<td>88.25 g</td>
<td>64.75 g</td>
<td>100 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gel base</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>76.5 g</td>
<td>88.25 g</td>
<td>64.75 g</td>
<td>100 g</td>
<td>-</td>
</tr>
<tr>
<td>1 % Silver sulp</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adizaine cream</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 g</td>
</tr>
<tr>
<td>Total</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
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<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
</tr>
</tbody>
</table>
2.2.6.2 Absolute drug content evaluation in creams

In determining absolute salicylic acid content of creams the same procedure described for ointment batches was adopted (section 2.2.6.1).

2.2.6.3 Absolute drug content evaluation in gels

The absolute salicylic acid content of the gels was determined using the same procedure described for creams in section 2.2.6.1.

2.2.6.4 Skin allergy tests

14 rabbits of both sexes, aged between 3 - 4 months weighing between 1.2 kg - 1.52 kg were used for this study. The animals which had not been previously exposed to laboratory use were housed under standard conditions with a 12 h light and dark cycle with free access to water and food. A new razor blade (Eagle, Shanghai, China.) was used to shave off the hair to area of 2828.57 mm² on their body. The three shaved areas on each rabbit were in the anterior region of the: cervical, thoracic and sacral regions. The areas were washed with distilled water and allowed to dry.

Each of the formulated batches of ointments creams or gels was respectively applied with sterilized cotton bud over shaved area every 24 h for 7 days. The animal skin was observed for any skin reaction.

2.2.7 Drug release studies

2.2.7.1 Drug release studies for the formulations

A modified drug release apparatus (Magnetic stirrer assembly) described by Billups and Patel (111) shown in Fig.1 was used for release studies. A cellophane membrane (D) was fixed to the base of the beaker. Each preparation (0.5 g) was weighed and placed into the cellophane membrane. 500 ml of phosphate buffer solution of pH 7.6 was introduced into the beaker (C) as
release medium. This evaluation was carried out at 37 ± 1 °C (thermostat regulated). The magnetic stirrer rod (F) was set at a speed of 120 rpm. At predetermined time intervals, 5-ml aliquots were withdrawn and replaced with equal volume of release medium. The withdrawn aliquots were analyzed spectrophotometrically at a wave length of 298 nm. The above procedure was repeated for all the batches. This process was carried out under sink condition.

2.2.7.2 Agar diffusion method

Ferric chloride agar solution was prepared by adding 80 g of agar powder to 400 ml of distilled water in a round bottomed flask. This was heated for 15 min in a steam bath with constant shaking while adding 5 drops of 0.1M ferric chloride to the agar solution. Agar solutions (20 ml each) were transferred into Petri dishes and sterilized in an autoclave (Type KKB500, F-Nr 7500778, Heraeus, Holland) at 121° C for 15 min.

The molten agar solution was poured into ten sterile agar plates and allowed to set after which four holes of 6 mm in diameter were bored. A medium sized metal spatula was used to press 0.5 g of ointments, creams and gels into the holes. The diffusion rate of salicylic acid from all the batches formulated was measured by the diameter of the purple colour produced as a result of its reaction with ferric chloride using calibrated ruler at predetermined intervals of 15, 30, 45, 60, 90 and 120 min. The diameters were plotted against corresponding time intervals to produce distance-time graphs. The slope of these graphs which correspond to the rate of diffusion was determined.

2.2.8 In vitro skin permeability studies

2.2.8.1 In vitro skin permeability studies for ointment batches

The skin permeability of salicylic acid was determined in vitro on all the ointment batches using porcine ear skin as the membrane. This model is a modified version of Kaur et al
Figure 1: Apparatus used for the release study.
(112) procedure. The diffusion cell was maintained at constant temperature (37 ± 1 °C) using a magnetic stirrer. The ear skin of a freshly slaughtered pig was excised with surgical blade placed in phosphate buffer solution and frozen until used. The thickness of the skin was measured with Vernier calipers and scrubbed until the thickness was about 1 mm. A 0.1 g quantity of the ointment to be tested was placed on the porcine membrane. The diffusion medium used was phosphate buffer solution of pH 7.6. Aliquots of the medium were withdrawn from the sampling port after a fixed time interval and were replaced with an equal quantity of the buffer solution. Samples were analyzed in a spectrophotometer (Coleman, U.V. Model 6/20A, UK.) at 298 nm. Sink conditions were maintained throughout the study.

The total amount of drug permeating across a unit diffusion surface and into the receptor was calculated and plotted as a function of time. The flux was calculated from the slope of the linear portion of cumulative amount-time plots and expressed as the mass of drug passing across 1 mm² of skin over time. Apparent permeability coefficient (P_{app}) was calculated by using the formula below (Eqn. 12):

\[
P_{app} = \frac{\Delta Q}{\Delta t \times 60 \times A \times C_0} \text{ (cm}^2\text{s})\] ……………………………………….Eqn 12

where \(\Delta Q\) is quantity of drug permeated, \(C_0\) is initial concentration of drug, \(A\) is exposed surface area, \(\Delta t\) is the steady state slope of the linear portion of the plots drug permeated versus time.

2.2.8.2 **In vitro skin permeability studies for cream batches**

The *in vitro* skin permeability studies were carried out in the cream batches using the same procedure described for ointment batches (section 2.2.8.1).
2.2.8.3 **In vitro skin permeability studies for gel batches**

These studies were done on the gel batches as described for ointments in section 2.2.8.1.

2.2.9 **Physical stability of the ointments, creams and gels**

All the batches formulated were evaluated by observing changes in their organoleptic properties, such as colour, odour, texture, microbial growth, cracking of creams, presence of air bubbles and particulate matters. Such changes in properties were visually observed at various time intervals from day zero to week 14 of storage at temperature of 29 ± 1 °C. The formulations were examined for phase separation, colour change, pH, odour and absolute drug content on alternate days.

2.2.10 **Stability studies on the ointments, creams and gels**

All the formulations were stored at 29 ± 1 °C, 40 ± 1 °C and 45 ± 1 °C respectively for 14 weeks in an oven (Gallenkamp, model 1H-150). The salicylic acid content of each formulation was determined after 3, 6, 9, 12 and 14 weeks.

2.3 **Pharmacodynamic studies**

2.3.1 **Animal studies**

Albino rats of 252 of both sexes, aged 3 - 4 months that had not been used for any studies and weighing between 168 g to 210 g were used for this study. They were housed in the animal house of the Department of Pharmacology, University of Nigeria, Nsukka. The animals were exposed to 12 h light and dark cycle with free access to water and food.

2.3.2 **Conditioning and anaesthetizing of the rats**

The rats were allowed free access to food and water before the commencement of the studies. They were anaesthetized with ketamine hydrochloride as the base anaesthetic at a dose of 6 mg/kg body weight intra-peritonealy and maintained with 0.04 mg/kg diazepam. Six rats were used for each batch of the formulations.
2.3.3 **Surgical infliction of wounds**

After the rat was anaesthetized, about 30 mm diameter circle was shaved with surgical blade on the pelvic anterior region. The area was washed with sterile distilled water and allowed to dry. An area of 1257.14 mm$^2$ was marked in the shaved region. Sterile surgical forceps were used to lift the rat skin, which was then incised and excised, following the marked area. The rat was put into cage and allowed to recover from the anaesthesia. The dressing of the wound commenced 24 hours post wounding. The temperature of the rat was monitored rectally daily for 2 weeks. This procedure was repeated six times for each batch of the preparations.

2.3.4 **Wound healing studies**

Each of the preparations was applied to cover the surface of the wound on alternate days after washing the wound surface with sterile distilled water. The initial diameter of the circular wound was measured and monitored to evaluate the rate of healing. The diameter of the wound was the average of the vertical and horizontal diameters of the wound area.

2.3.5 **Wound bioload studies**

The surface viable count method was employed in this bioload study. The base line microbial load value was determined 24 h post-incision. A sterile swab stick was used to mop the surface of the wound and rinsed in 10 ml sterile distilled water. A Petri dish incubated for 24h with seed bacteria in Mullen-Hinton agar was placed in a digital bacteria colony counter for bacteria count (model B1107 Jurgen, England). The wound bioload was determined for all the batches on days 2, 4, 7 and 9.

2.3.6 **Wound enzymological studies**

In this study, the modified Barisoni et al (113) model was used to determine the neutrophil elastase concentration in the wound area. This was determined by using succinyl
alanyl-alanyl-alanyl-p-nitroanilide as substrate. The assay medium was 0.2 M Tris-HCl in a phosphate buffer solution of pH 7.6. Wound wash was collected by washing the surface of the wound with 5 ml sterile water. The substrate (1 ml) was mixed with various volumes (100-300 micromoles) of the wound wash and incubated at 37 ± 1 °C for different lengths of time (0-150 min). The reaction was stopped at intervals by adding 0.2 ml of acetic acid and the absorbance measured at 410 nm and that of protein at 750 nm using U.V. spectrophotometer. The concentration of neutrophile elatase was calculated by using the slope of pre-determined standard curve of p-Nitrophenol while that of protein was calculated using the slope of pre-determined standard curve of protein (114).

This procedure was repeated in all the wounds until healed, except for those wounds that could not heal after 21 days. The wound wash was collected before the wound was dressed with the particular batch.

2.3.7 Histological studies on the wounds

160 rats were used for this study. The base line histopathological value of the wound was determined one day post-incision using modified methods of Subrahmanyam (14) and Ghaderi et al (17). The wounded area and the edge tissue were excised and put in phosphate buffer solution (pH 7.6) in a glass container. Ten animals were sacrificed each day after collecting wound biopsy. This was repeated in all the batches at 1, 4, 9 and 13 days post-incision. 16 animals treated with base batches 4 (ointment base), 8 (cream base) and 12 (15% gelatin gel base) died on day 3 as a result of pyrexia and are not included in the study. Histological examination coupled with visual scoring allowed the assessment of changes to the wounded area. The histological score method was a modified procedure of Lee et al (115) under light microscopic examination. Numbers 0 to 5 were ascribed to the characteristics (oedema, angiogenesis, pus
cells, granulation, fibroblast, collagen, epithelia cells) of the regenerated tissue repair in wounds. The percentage of the score is plotted against time in days.

Each specimen was placed in pH 7.6, buffered phosphate solution for at least 48 h. The transverse section of the specimen against the skin surface was dehydrated with ethanol, embedded in paraffin wax, and stained with haematoxylin and eosin. Each wound sample was examined and evaluated under light microscope (Leica Diastar). Photomicrographs of the three randomly selected sites of each wounded sample were taken with a digital camera (Moticam 1000 1.3m Pixel). Histological changes in the wound tissue were evaluated.

2.3.8 Statistical analysis

The data on salicylic acid releases, and percentage skin permeation were compared using the analysis of variance (ANOVA). Reductions in wound diameter, neutrophil elastase and bioload were tested using Student’s t-tests and SPSS ver. 10.
CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 Standardization of honey

The honey used was standardized to Pharmaceutical codex standard of 1.36 mg/ml. It was observed for 14 months with no visible change in organoleptic properties as there was no foul odour or discolouration and no change in consistency of the standardized honey.

3.2 Antibacterial activity of mucin and honey

The results of the antibacterial properties of mucin and honey presented in Tables 2 show that mucin had the highest inhibition zone diameter (IZD) against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *Escherichia coli*. The same ratios of honey did not exhibit antibacterial activity against *Staphylococcus aureus* but showed activity against *Pseudomonas aeruginosa* and *Escherichia coli*. The inhibition zone diameter decreased with decrease in concentration of mucin or honey.

Various strains of *Staphylococcus aureus* have been reported to be inhibited by 5-10 % honey in an infected wound by Cooper *et al* (116). In similar studies done by Obasieki-Ebor *et al* (117) undiluted honey distillate had antimicrobial activity against *Escherichia coli*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* wound bacteria. Honey has been used to treat neonatal post operative wound infection (118) and while the antimicrobial activity of Sudanese honey in wounds (119) has been reported. Ali *et al* (120) in his investigation observed the inhibitory effect of honey on *Helicobacter pylori*. Human salivary mucin has also been reported to have antifungal activity (121). Bilbruck *et al* (122) reported that mucin enhances the bactericidal activity of chlorhexidine against *E. coli* in wound dressing.
Table 2: Inhibition zone diameter (cm) of mucin and honey against *Escherichia coli* 
*Staphylococcus aureus* and *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Ratios (MHW)</th>
<th>1:0</th>
<th>1:1</th>
<th>1:2</th>
<th>1:3</th>
<th>1:4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>2.06 ± 0.08</td>
<td>1.74 ± 0.06</td>
<td>1.45 ± 0.03</td>
<td>1.02 ± 0.07</td>
<td>0.62 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>(1.65 ± 0.08)</td>
<td>(1.08 ± 0.04)</td>
<td>(0.56 ± 0.12)</td>
<td>( - )</td>
<td>( - )</td>
</tr>
</tbody>
</table>

| **Staphylococcus aureus** | 2.48 ± 0.04 | 2.4 ± 0.04 | 2.15 ± 0.05 | 1.84 ± 0.11 | 1.22 ± 0.09 |
|                          | ( - ) | ( - ) | ( - ) | ( - ) | ( - ) |

| **Pseudomonas aeruginosa** | 2.15 ± 0.05 | 1.85 ± 0.07 | 1.5 ± 0.02 | 1.06 ± 0.06 | 0.85 ± 0.03 |
|                          | (1.82 ± 0.06) | (1.18 ± 0.03) | (0.75 ± 0.04) | ( - ) | ( - ) |

MHW is mucin or honey: water ratio. Values in brackets are inhibition diameters (cm) for honey while values not in brackets are for mucin (cm) against tested bacteria. - means no inhibition, every value is group mean while ± is standard deviation.
A combination of a number of factors may be implicated in the mechanism of antimicrobial activity of honey. The humectant property of honey is likely to play a major role when applied topically (117). Vardi and co-workers (118) reported that the enzyme glucose oxidase derived hydrogen peroxide has known antimicrobial activity. Deinzer et al (21) reported that other phenolic compounds like benzoic acid, caffeic acid, cinnamic acid, cinnamyl alcohol, vanillin, benzyl alcohol, flavonoids (examples galangin, kaempferol, quercetin etc), fatty acids, essential oils may contribute to antimicrobial activity. Mucin ability for particle adhesion can lead to bacteria adhesion. This may be due to surfactant activity of mucin which can affect the integrity of bacterial cell wall thereby making it susceptible to cytoplasmic leakage (12, 43). Brid et al (36) indicated that the structural versatility of the mucin glycoproteins can also affect bacterial cell wall through hydrogen bonding as such incapacitating the bacterial activity.

3.3 Antimicrobial activity of mucin-honey mixture

The various combination ratios of mucin and honey (1:1, 1:2, 1:3, 1:4 and 2:3) showed enhanced activity against the three bacteria tested (Table 3). The 2:3 combination showed highest activity against all the test organisms. The activities in decreasing order of magnitude were as follows: *Staphylococcus aureus* > *E. coli* > *Pseudomonas aeruginosa*. The high antibacterial activity of the mixture of mucin and honey may be due to synergy in the two polymers different antibacterial mechanisms of action. Mucin exerts antimicrobial action by surface activity of bacterial adhesion and surfactant activity as reported by Adikwu et al (12) while honey exerts its antimicrobial action by hydrogen peroxide, gluconic acid and denaturation of bacterial cell wall (22,23).
Table 3: The bacteria inhibition diameter (cm) of mucin : honey combination against *Escherichia coli* *Staphylococcus aureus* and *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Ratios: (MH)</th>
<th>2:3</th>
<th>1:1</th>
<th>1:2</th>
<th>1:3</th>
<th>1:4</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>3.23 ± 0.06</td>
<td>2.82 ± 0.05</td>
<td>2.21 ± 0.06</td>
<td>1.61 ± 0.06</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>3.42 ± 0.09</td>
<td>3.11 ± 0.02</td>
<td>2.61 ± 0.01</td>
<td>2.22 ± 0.03</td>
<td>1.4 ± 0.04</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>2.82 ± 0.04</td>
<td>2.31 ± 0.06</td>
<td>1.72 ± 0.06</td>
<td>1.2 ± 0.04</td>
<td>0.93 ± 0.03</td>
</tr>
</tbody>
</table>

Each value is group mean while ± is standard deviation. MH is mucin: honey ratio.
3.4 **Minimum inhibitory concentration (MIC) of mucin, honey and mucin-honey combinations**

The MIC against the three test organisms (Table 4) showed that mucin had higher MIC against *Staphylococcus aureus* while honey had no activity. In the combination of mucin:honey (1:4) there was higher MIC against *Staphylococcus aureus* and lower MIC against *E. coli* and against *Pseudomonas aeruginosa*. Figs. 2-4 are the plots of inhibition zone diameter (IZD) squared against log concentration of mucin, honey or their combinations against the three tested bacteria. The mucin: honey combination had higher slopes than either mucin or honey alone (Table 5) indicating enhanced antibacterial activity of the mixture. It also implies that mucin: honey combination had higher antibacterial activity on the three tested bacteria than mucin or honey alone. Mucin had higher activity compared with honey and the higher the mucin ratio to honey the lower the MIC.

3.5 **Organoleptic properties for ointment, cream and gel batches**

After 14 weeks of storage at ambient temperature 29 ± 1 °C, there was no foul odour, discolouration or change in consistency and pH of all the batches of ointments and creams (Tables 6-8). There was however, change in the consistency and pH of the gel batches. The pH of the gels decreased by 1.2 thereby becoming more acidic (Table 8). The observed instability of the gel batches may be due to the nature of gel that can undergo hydrolytic degradation under aqueous state (76). There were no air bubbles or particles noticed during the period of storage in all the batches formulated. Those formulations that contained mucin (batches 2, 3, 6, 7, 10 and 11) adhesive unlike those that contained only honey (batches 1, 5 and 9). The adhesive effect of the formulations that contained mucin is as a result of the mucoadhesive property of the mucin molecules.
Table 4: The minimum inhibitory concentration (MIC) of mucin and honey against the three tested bacteria

<table>
<thead>
<tr>
<th></th>
<th>Escherichia coli</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucin</td>
<td>34.67 ± 1.63 mg/ml</td>
<td>36.98± 0.73 mg/ml</td>
<td>36.39 ± 0.19 mg/ml</td>
</tr>
<tr>
<td>Honey</td>
<td>50 mg</td>
<td>NA</td>
<td>50 mg/ml</td>
</tr>
<tr>
<td>Mucin:honey combination (1:4)</td>
<td>33.5 ± 0.52 mg</td>
<td>33.13 ± 0.17 mg</td>
<td>33.88 ± 1.81 mg</td>
</tr>
</tbody>
</table>

Table 5: Slope values of graphs of bacteria inhibition zone diameter of mucin honey and their admixture against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Type</th>
<th>Mucin-honey combination (1:4)</th>
<th>Mucin</th>
<th>Honey</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.686</td>
<td>0.308</td>
<td>0.2</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.974</td>
<td>0.634</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.892</td>
<td>0.603</td>
<td>0.339</td>
</tr>
</tbody>
</table>

- = no anti-bacterial activity against *staphylococcus aureus*
Fig. 2: Inhibition zone diameter of mucin, honey and their admixture against *Pseudomonas aeruginosa*.

Fig. 3: Inhibition zone diameter of mucin, honey and their admixture against *Staphylococcus aureus*.

Fig. 4: Inhibition zone diameter of mucin, honey and their admixture against *Pseudomonas aeruginosa*.
### Table 6: Physical properties of ointment formulations

<table>
<thead>
<tr>
<th>Batches</th>
<th>Minimum deviation (MID) mg</th>
<th>Maximum deviation (MAD) mg</th>
<th>Drug content (mg)</th>
<th>Colour</th>
<th>pH</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (23.5% honey)</td>
<td>(0.10)</td>
<td>(0.65)</td>
<td>9.45 ± 0.023</td>
<td>+</td>
<td>0.35</td>
<td>nonadhesive</td>
</tr>
<tr>
<td>2 (11.75% mucin:)</td>
<td>(0.09)</td>
<td>(0.71)</td>
<td>9.48 ± 0.024</td>
<td>+</td>
<td>0.25</td>
<td>adhesive</td>
</tr>
<tr>
<td>3 (11.75% mucin:</td>
<td>(0.49)</td>
<td>(0.88)</td>
<td>9.66 ± 0.024</td>
<td>+</td>
<td>0.4</td>
<td>adhesive</td>
</tr>
<tr>
<td>4 (emulsifying</td>
<td>(0.44)</td>
<td>(3.36)</td>
<td>9.39 ± 0.10</td>
<td>++</td>
<td>0.3</td>
<td>nonadhesive</td>
</tr>
<tr>
<td>ointment base)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (1% silver sulphadiazine cream)</td>
<td>(0.20)</td>
<td>(1.52)</td>
<td>9.59 ± 0.045</td>
<td>+++</td>
<td>0.1</td>
<td>nonadhesive</td>
</tr>
</tbody>
</table>

MID is minimum deviation in mg, MAD is maximum deviation in mg, ± is standard deviation, + is less white in colour, ++ is moderate white in colour and +++ is highly white in colour. Nonadhesive means not sticky by touch while adhesive means sticky by touch.
**Table 7: Physical properties of cream formulations**

<table>
<thead>
<tr>
<th>Batches</th>
<th>Minimum deviation (MID) mg</th>
<th>Maximum deviation (MAD) mg</th>
<th>Drug content (mg)</th>
<th>Colour</th>
<th>pH</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (23.5% honey)</td>
<td>(0.08)</td>
<td>(2.24)</td>
<td>9.6 ± 0.07</td>
<td>+</td>
<td>0.1</td>
<td>nonadhesive</td>
</tr>
<tr>
<td>6 (11.75% mucin)</td>
<td>(0.22)</td>
<td>(1.6)</td>
<td>9.79 ± 0.06</td>
<td>++</td>
<td>0.15</td>
<td>adhesive</td>
</tr>
<tr>
<td>7 (11.75% mucin: 23.5% honey)</td>
<td>(0.04)</td>
<td>(0.14)</td>
<td>9.91 ± 0.01</td>
<td>+</td>
<td>0.2</td>
<td>adhesive</td>
</tr>
<tr>
<td>8 (aqueous cream base)</td>
<td>(0.06)</td>
<td>(0.76)</td>
<td>9.49 ± 0.02</td>
<td>+++</td>
<td>0.12</td>
<td>nonadhesive</td>
</tr>
<tr>
<td>13 (SSD)</td>
<td>(0.20)</td>
<td>(01.5)</td>
<td>9.59 ± 0.05</td>
<td>+++</td>
<td>0.1</td>
<td>nonadhesive</td>
</tr>
</tbody>
</table>

MID is minimum deviation in mg, MAD is maximum deviation in mg, ± is standard deviation, + is less white in colour, ++ is moderate white in colour and +++ is highly white in colour. Nonadhesive means not sticky by touch while adhesive means sticky by touch.
Table 8: Physical properties of gel formulations

<table>
<thead>
<tr>
<th>Batches</th>
<th>Minimum deviation (MID) mg</th>
<th>Maximum deviation (MAD) mg</th>
<th>Drug content (mg)</th>
<th>Colour amber</th>
<th>pH change</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (23.5% honey)</td>
<td>(0.62)</td>
<td>(1.7)</td>
<td>9.65 ± 0.06</td>
<td>+</td>
<td>0.9</td>
<td>adhesive</td>
</tr>
<tr>
<td>10 (11.75% mucin)</td>
<td>(0.07)</td>
<td>(0.32)</td>
<td>9.68 ± 0.03</td>
<td>+</td>
<td>0.9</td>
<td>adhesive</td>
</tr>
<tr>
<td>11 (11.75% mucin: 23.5% honey)</td>
<td>(0.04)</td>
<td>(0.95)</td>
<td>9.84 ± 0.03</td>
<td>++</td>
<td>1.1</td>
<td>adhesive</td>
</tr>
<tr>
<td>12 (15% gelatin gel)</td>
<td>(0.11)</td>
<td>(1.3)</td>
<td>9.55 ± 0.04</td>
<td>++</td>
<td>1.2</td>
<td>adhesive</td>
</tr>
<tr>
<td>13 (SSD)</td>
<td>(0.20)</td>
<td>(1.5)</td>
<td>9.59 ± 0.04</td>
<td>white+++</td>
<td>0.1</td>
<td>nonadhesive</td>
</tr>
</tbody>
</table>

MID is minimum deviation in mg, MAD is maximum deviation in mg, ± is standard deviation, + is less amber colour, ++ is moderate amber colour and +++ is highly white. Nonadhesive means not sticky by touch while adhesive means sticky by touch.
3.6 **Absolute drug content**

3.6.1 **Absolute content of salicylic acid in the ointments, creams and gels**

Tables 6-8 show that the salicylic acid content of all the batches complied with British Pharmacopoeia (46) requirement with 0.5 g quantity of the formulations assessed containing approximately 10 mg of salicylic acid.

3.7 **Skin allergy test**

During the 7 days of observation of the animal skin, there was no noticeable skin allergy such as erythema, oedema or redness on the shaved surface of the skin tested. This shows that all the ointments, creams and gels preparations conformed to the European Pharmacopoeial standard (92), for allergy of topical formulations.

3.8 **Release studies**

3.8.1 **Release of salicylic acid in the ointments, creams and gels**

In the release studies, it was observed that formulations that contain mucin showed faster and higher initial release which is similar to the results obtained by Adikwu and Okafor (25). The preparations containing only honey as additive showed lower release of salicylic acid. The addition of mucin to honey in the formulation of ointments, creams and gels enhanced the release profile of honey. This will encourage a kind of sustained release of honey and mucin on the wound for an efficient wound healing. It will confer wound dressing by the patients.

3.8.1.1 **Stirrer beaker method**

The formulations containing mucinated-honey (MH) had better salicylic acid release than the ones containing mucin or honey alone. The MH formulations using analysis of variance (ANOVA) showed higher drug release in ointment batches than the rest of the preparations. The same pattern was observed in the cream and gel batches. The salicylic acid release followed the
pattern cream > gel > ointment > SSD (p < 0.05). Figs. 5-7 represent the release profile for the ointments, creams and gels respectively. In all the dosage forms, the presence of MH resulted in the highest drug released followed by mucin preparations, honey formulations and the standard (SSD). The enhanced release of the salicylic acid in the MH ointment (batch 3), cream (batch 7) and gel (batch 11) may be as a result of enhanced absorption of the release medium. Earlier work by Adikwu and Okafor (25) indicated that mucin (glycoprotein) enhances adsorption, absorption, sorption and release processes which has enhanced in honey combination. Sorption process can also be facilitated by emulsifying wax (76,79).

All the formulations showed first order kinetics (Figs. 8-10) which indicates that diffusion is the major mechanism of drug release in the ointment, cream and gel preparations which suggests that MH preparation enhances drug release by diffusion. This is consistent with the possibility of a rapid process of drug exhaustion from the semi-solid preparations as reported by Billupis and co-worker (111).

The release rate and the resulting diffusion coefficients of salicylic acid in all the formulations are shown in Tables 9-11. The formulations containing MH showed higher drug release rate function and corresponding diffusion coefficient than SSD, mucin, honey and bases (p < 0.05).

Weert et al (123) stated that since a semi-solid has properties in between those of a solid and a liquid, drug compounds can be loaded into the vehicle or polymer by a simple physical mixing of two polymers without the need to use solvents or chemical method. Physical mixing of polymers may involve the cross linking of their molecules that determine subsequent properties. In this class of pharmaceutical dosage forms, the drug release pattern can be modified by the balancing of hydrophobic and hydrophilic moieties in polymers to induce rapid drug release.
Fig. 6: Release profile of salicylic acid from creams compared with BP aqueous cream base and silver sulphanilamide cream as standards.

Fig. 7: Release profile of salicylic acid from gelatin gels compared with 15% gelatin gel base and 1% silver sulphanilamide cream as standards.
Fig. 8: Release of salicylic acid from ointments in phosphate buffer pH 7.6 plotted according to first-order kinetics.

- **Batch 1**: 23.5% honey
- **Batch 2**: 11.75% mucin
- **Batch 3**: (1:2) 11.75% mucin to 23.5 mg honey
- **Batch 4**: BP ointment base
- **Batch 13**: 1% Silversulphadiazine cream
Fig. 9: Release of salicylic acid from creams in phosphate buffer pH 7.6 plotted according to first-order kinetics

- Batch 5: 23.5% honey
- Batch 6: 11.75% mucin
- Batch 7(1:2) 11.75% mucin to 23.5% honey
- Batch 8: BP aqueous cream base
- Batch 13: 1% silver sulphadiazine cream (standard)
Fig. 10: Release of salicylic acid from gelatin gels in phosphate buffer pH 7.6 plotted according to first-order kinetics.

- Batch 9: 23.5% honey
- Batch 10: 11.755% mucin
- Batch 11: (1:2) 11.75% mucin to 23.5% honey
- Batch 12: 15% gelatin base
- Batch 13: 1% silver sulphurdiazine cream
Table 9: Characteristics of drug release for the ointment batches 1-4 and SSD

<table>
<thead>
<tr>
<th>Batches</th>
<th>Function for release rate (y)</th>
<th>Diffusion coefficient x 10^4 (cm^2 s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (23.5 % honey)</td>
<td>0.288 + 0.516x</td>
<td>2.3712</td>
</tr>
<tr>
<td>2 (11.75 % mucin)</td>
<td>0.45 + 0.554x</td>
<td>2.6148</td>
</tr>
<tr>
<td>3 (11.75 % mucin: 23.5 % honey)</td>
<td>0.834 + 0.561x</td>
<td>2.7835</td>
</tr>
<tr>
<td>4 (emulsifying ointment base)</td>
<td>0.284 + 0.475x</td>
<td>2.036</td>
</tr>
<tr>
<td>SSD</td>
<td>0.401 + 0.468x</td>
<td>1.8948</td>
</tr>
</tbody>
</table>

SSD is 1% silver sulphadiazine cream
### Table 10: Characteristics of drug release for cream batches 5-8 and SSD

<table>
<thead>
<tr>
<th>Batches</th>
<th>Function for release rate (y)</th>
<th>Diffusion coefficient x $10^{-4}$ (cm$^2$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (23.5 % honey)</td>
<td>$0.879 + 0.562x$</td>
<td>2.7265</td>
</tr>
<tr>
<td>6 (11.75 % mucin)</td>
<td>$1.179 + 0.58x$</td>
<td>2.7927</td>
</tr>
<tr>
<td>7 (11.75 % mucin: 23.5 % honey)</td>
<td>$2.132 + 0.611x$</td>
<td>3.027</td>
</tr>
<tr>
<td>8 (aqueous cream base)</td>
<td>$0.575 + 0.521x$</td>
<td>2.3953</td>
</tr>
<tr>
<td>SSD</td>
<td>$0.401 + 0.468x$</td>
<td>1.8948</td>
</tr>
</tbody>
</table>

SSD is 1 % silver sulphadiazine cream
Table 11: Characteristics of drug release for gel batches 9-12 and SSD

<table>
<thead>
<tr>
<th>Batches</th>
<th>Function for release rate (y)</th>
<th>Diffusion coefficient x 10^{-4}(cm^2.s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (23.5 % honey)</td>
<td>0.383 + 0.543x</td>
<td>2.5174</td>
</tr>
<tr>
<td>10 (11.75 % mucin)</td>
<td>0.597 + 0.567x</td>
<td>2.7271</td>
</tr>
<tr>
<td>11 (11.75 % mucin: 23.5 % honey)</td>
<td>0.582 + 0.598x</td>
<td>2.9368</td>
</tr>
<tr>
<td>12 (15 % gelatin gel)</td>
<td>0.337 + 0.508x</td>
<td>2.2523</td>
</tr>
<tr>
<td>SSD</td>
<td>0.401 + 0.468x</td>
<td>1.8948</td>
</tr>
</tbody>
</table>

SSD is 1 % silver sulphadiazine cream
The rate of drug release will be reduced with increase in time. The combination of mucin and honey in MH preparations did not cause retardation of salicylic acid release. The drug release involves penetration of the wound fluid into the semi-solid preparation which will subsequently dissolve the salicylic acid molecules for diffusion into the wound area. This partly accounts for the enhanced wound healing observed in full thickness experimental wounds in rats dressed with MH preparations.

The hydrophilic nature of mucin and honey will also contribute to the microenvironment of the wound as the two polymers will have better physico-chemical properties than each of the polymers. The enhanced adhesion property of the MH preparations will increase their adhesion on wound, thereby allowing more contact time when the wound is dressed with such preparation. This also partially explains the observed enhanced healing of wounds dressed with MH preparations. The enhanced salicylic acid release from MH preparations can be said to be as a result of the mixture of mucin and honey, (two hydrophilic polymers) to cause an initial fast release and
later slow release as stated by Aikawa et al (89). Honey is gel like and mucin has a gel forming property. The physical homogenous mixture of the two polymers will optimize the effect of each on wound healing. Miscible interaction of honey and mucin with biological membrane gives enhanced drug delivery and increase in permeability of the two polymers. McTaggart et al (87) in their studies comparing release rate of different gels reported that the mixture of hydrogels results in cross-linking of their molecules which affects the release rate. Other release studies (124,125,126,127) have also shown that drug release in semi-solid preparations is diffusion dependent. The wound fluid can diffuse into the MH preparation which consequently will lead to localized delivery of active constituents of the polymers within the wound cells as to facilitate cell regeneration, granulation, re-epithelization, increase in cell density and final keratinization (healing) of the wound.

3.8.1.2 Agar diffusion method

In drug release studies using agar diffusion technique (Table 12), diffusion diameter (ADD) of MH preparations were higher than those containing mucin or honey alone or SSD. The diffusion diameters of MH preparations were in the following rank order: MH cream > MH gel > MH ointment > SSD (silver sulphadiazine cream) (p < 0.05). In mucin preparations (M) the same pattern was exhibited; cream > gelatin gel > ointment, while in honey preparations (H) the diffusion diameter of gel preparation was higher than that of cream and ointment. In comparison with SSD, all the preparations that contained MH, mucin or honey alone showed higher ADD than the standard (SSD).

The results of the agar diffusion study on all the formulations are shown in Figs. 11-13. In the ointment batches MH had the highest slope of 1.051 followed by mucin with 0.89, honey with 0.689, SSD 0.779 and ointment base with 0.545 (Fig. 11). In cream batches, (Fig.12) the
Table 12: Agar diffusion diameter (mm) of salicylic acid release (120 minutes) in the formulations

<table>
<thead>
<tr>
<th>Type</th>
<th>Mucinated-honey</th>
<th>11.75% mucin</th>
<th>23.5% honey</th>
<th>Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(11.75% mucin:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.5% honey)</td>
<td></td>
<td></td>
<td>SSD = 13 ± 0.14, ± is standard deviation.</td>
</tr>
<tr>
<td>Ointment</td>
<td>17.5 ± 0.71</td>
<td>15 ± 0.28</td>
<td>13.51 ± 0.37</td>
<td>9.8 ± 0.141</td>
</tr>
<tr>
<td>Cream</td>
<td>21.5 ± 0.32</td>
<td>18 ± 0.245</td>
<td>14 ± 0.28</td>
<td>12.5 ± 0.37</td>
</tr>
<tr>
<td>Gel</td>
<td>18.5 ± 0.14</td>
<td>17 ± 0.42</td>
<td>15 ± 0.74</td>
<td>12 ± 0.25</td>
</tr>
</tbody>
</table>
order was MH > mucin > honey > SSD > aqueous cream base. The order was the same for gel formulations (Fig. 13).

All the MH formulations release salicylic acid faster than other formulations. Higuchi (98) has suggested that the release of drug from semi-solid formulation increases with square root of time. The diffusion rate is related to release rate and hence the results are in line with the Higuchi theory. The results also suggest that the release of salicylic acid from the ointment, cream and gel preparations are largely diffusion controlled. In other words, drug release in MH formulation is a surface phenomenon that involves diffusion, partition of drug across the interface medium and dissolution of the drug in the base. This is in conformity with reports of similar studies (109,110,111). The relatively higher drug release from MH formulations may be due to enhanced diffusion ability of the preparations as they contain homogenous mixture of honey and mucin which are both polymers. Such polymers exert osmotic pressure when in solution. Martin et al (128) stated that potential pressure of each solution is expressed as chemical potential of the different molecules and ions present in the solution. The amount of chemical potential of each type of molecule or ion is directly proportional to its fractional concentration in the solution. Gibald et al (88) observed that polymer particles interact with each other by either chemical or physical bonds that result in osmotic pressure change. This causes diffusion which may lead to membrane potentials that are explainable by Nernst equation (76).

3.9 \textit{In vitro} skin permeability studies

The results of the permeation studies in terms of percentage permeation, steady-state flux (SSF) and apparent permeability coefficient ($P_{\text{app}}$) are shown in Tables 13-15. The results indicated that MH formulations had higher skin permeation compared to the other agents with
Table 13: Comparison of ointment formulations and SSD in terms of \% permeation, steady-state flux (SSF) and apparent permeability coefficient (P_{app}) from in vitro permeation studies using pig skin

<table>
<thead>
<tr>
<th>Batches</th>
<th>drug permeation in 5 h (mg)</th>
<th>% drug permeation</th>
<th>SSF (\mu g/min/cm^2)</th>
<th>\times 10^{-4}(cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (23.5 % honey)</td>
<td>1.32 ± 0.159</td>
<td>13.98</td>
<td>11.0092 ± 1.16</td>
<td>4.4606 ± 0.005</td>
</tr>
<tr>
<td>2 (11.75 % mucin)</td>
<td>1.48 ± 0.046</td>
<td>15.59</td>
<td>12.325 ± .385</td>
<td>4.8006 ± 0.15</td>
</tr>
<tr>
<td>3 (11.75 % mucin: 23.5 % honey)</td>
<td>1.67 ± 0.196</td>
<td>17.32</td>
<td>13.9475 ± 1.64</td>
<td>4.8533 ± 0.57</td>
</tr>
<tr>
<td>4 (emulsifying ointment base)</td>
<td>1.22 ± 0.208</td>
<td>12.95</td>
<td>10.1317 ± 1.73</td>
<td>4.228 ± 0.77</td>
</tr>
<tr>
<td>SSD</td>
<td>1.4 ± 0.127</td>
<td>14.6</td>
<td>11.6667 ± .994</td>
<td>4.5755 ± 0.46</td>
</tr>
</tbody>
</table>

± is standard deviation. SSD is 1 \% silver sulphadiazine cream
Table 14: Comparison of cream formulations and SSD in terms of % permeation, steady-state flux (SSF) and apparent permeability coefficient (P<sub>app</sub>) from in vitro permeation studies using pig skin

<table>
<thead>
<tr>
<th>Batches</th>
<th>drug permeated in 5 h (mg)</th>
<th>% drug permeation</th>
<th>SSF (µg/min/cm²)</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; x10&lt;sup&gt;-4&lt;/sup&gt;(cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (23.5% honey)</td>
<td>1.32 ± 0.104</td>
<td>13.76</td>
<td>11.0092 ± 1.16</td>
<td>4.7447 ± 0.37</td>
</tr>
<tr>
<td>6 (11.75% mucin)</td>
<td>1.7 ± 0.346</td>
<td>17.37</td>
<td>14.1667 ± 0.346</td>
<td>5.0195 ± 1.02</td>
</tr>
<tr>
<td>7 (11.75% mucin:</td>
<td>1.83 ± 0.11</td>
<td>18.44</td>
<td>15.2500 ± 3.11</td>
<td>5.2963 ± 0.46</td>
</tr>
<tr>
<td>23.5% honey)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (aqueous cream base)</td>
<td>1.26 ± 0.104</td>
<td>13.25</td>
<td>10.5010 ± 3.78</td>
<td>4.4493 ± 1.85</td>
</tr>
<tr>
<td>SSD</td>
<td>1.4 ± 0.127</td>
<td>14.6</td>
<td>11.6667 ± 0.994</td>
<td>4.5755 ± 0.46</td>
</tr>
</tbody>
</table>

± is standard deviation. SSD is 1% silver sulphadiazine cream
Table 15: Comparison of gel formulations and SSD in terms of % permeation, steady-state flux (SSF) and apparent permeability coefficient ($P_{app}$) from in vitro permeation studies using pig skin

<table>
<thead>
<tr>
<th>Batches</th>
<th>Drug permeated in 5 h (mg)</th>
<th>% drug permeation</th>
<th>SSF ($\mu g/\text{min/cm}^2$)</th>
<th>$P_{app} \times 10^{-4}$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (23.5% honey)</td>
<td>1.49 ± 0.208</td>
<td>15.43</td>
<td>12.4125 ± 1.73</td>
<td>4.6909 ± 0.66</td>
</tr>
<tr>
<td>10 (11.75% mucin)</td>
<td>1.65 ± 0.15</td>
<td>17.01</td>
<td>13.7283 ± 1.25</td>
<td>4.7017 ± 0.43</td>
</tr>
<tr>
<td>11 (11.75% mucin: 23.5% honey)</td>
<td>1.69 ± 0.058</td>
<td>17.17</td>
<td>14.0792 ± 0.048</td>
<td>5.1089 ± 0.18</td>
</tr>
<tr>
<td>12 (15% gelatin gel)</td>
<td>1.25 ± 0.069</td>
<td>13.07</td>
<td>10.4167 ± 0.577</td>
<td>4.382 ± 0.3</td>
</tr>
<tr>
<td>SSD</td>
<td>1.4 ± 0.127</td>
<td>14.6</td>
<td>11.6667 ± 0.994</td>
<td>4.5755 ± 0.46</td>
</tr>
</tbody>
</table>

± is standard deviation. SSD is 1% silver sulphadiazine cream
the order being MH > Mucin > Honey > SSD. In ointment batches (Table 13) similar pattern was observed.

In the cream formulations (Table 14) MH showed highest SSF with $P_{app}$, followed by mucin, honey, SSD and aqueous cream base. The gel formulations (Table 15) exhibited the same pattern as ointments and creams with higher values than ointment batches. The permeation results showed that MH preparations showed a more enhanced permeation of salicylic acid across pig skin than the standard (SSD).

Tang et al (129) using the two-parameter, Fickian diffusion model and the developed skin porous-pathway theory, have shown that hydration leads to induction of new pores/reduction of the tortuosity of existing pores within an excised pig skin. The permeabilities of drugs across pig skin may be due to structural changes in the skin although the exact mechanism is unclear. The apparent permeability coefficients of formulations that contained MH compared well with SSD in all the preparations (ointments, creams and gels). Radu et al (130) observed that drug release from collagen matrices is in most cases governed by diffusion from swollen matrices but may also involve enzymatic matrix degradation or hydrophobic drug-matrix or polymer interactions. This results when a hydrophilic polymer takes up some quantity of aqueous liquid when in contact with physiological fluids and swells. They further observed that drug release is achieved by counter current diffusion through a penetrating solvent with the release rate being determined by the diffusion rate of the solvent in the polymer. An adequate dissolution rate is important to maintain a steady concentration in the formulation. This is because dissolution behaviour is an important parameter affecting the drug permeation flux through the stratum corneum from a suspension. As drug dissolution rate is lower than permeation flux, drug concentration in the suspension decreases causing a decrease in permeation itself.
**In vitro** permeation studies of the various formulations (Tables 13-15) show that the increase in salicylic acid permeation can be primarily attributed to the increase in salicylic acid solubility in the formulation containing MH (batches 3, 7 and 11). Nakano *et al* (97) in their studies of percutaneous absorption pointed out that there should be balance between lipid and aqueous solubility of drug to optimize permeation. This may imply that MH has such characteristics. It therefore can be said, that the conventional pharmaceutical bases used for ointment, cream and gel formulations did not act as enhancer modifying the permeation coefficient of the drug in stratum corneum. The observed increase in skin permeability may also be as a result of the hydrophilic nature of MH combination. The higher permeation flux may also be attributed to the presence of a diffusion layer at the skin surface where the MH acts as a carrier, carrying the drug from the donor phase to lipophilic part of the skin. This has been reported by Cross and co-worker (131) who stated that the concentration gradient over the diffusion layer is the main driving force for the drug molecules to be delivered from the base to the surface of the barrier (skin). The dissolved drug availability is crucial for effective drug delivery. Ceschel *et al* (132) observed that complexation of two polymers can increase drug flux in percutaneous permeation. The formulations containing MH improved permeation flux of salicylic acid across porcine skin more than mucin, honey or SSD.

3.10 **Physical stability of the ointments, creams and gels**

There was no foul odour, discolouration or change in consistency of all batches of ointments and creams, after 14 weeks of storage at ambient temperature. There was change in the consistency of the gel batches as they became less viscous after three weeks of storage.
3.11 **Stability studies on ointments, creams and gels**

The results of stability tests on the formulations are shown in Tables 16-18. There was reduction in drug content for all the formulations when stored at 29 °C, 40 °C and 45 °C for 14 weeks although this was very slight. The decrease, as expected increased with increase in temperature. Plots of log concentration versus time of MH formulations at 29 °C indicate that none of the batches had below 95 % of drug content after 14 weeks Figs. (14 – 16). The results showed that the MH formulations are stable under ambient temperature. The data obtained was in line with Arrhenius equation (76,79)

3.12 **Pharmacodynamic studies**

3.12.1 **Wound healing rate**

The average cumulative % reduction of wound diameter after 3 – 9 days of treatment with ointment batches (Table 19) showed that MH preparation had the highest reduction followed by mucin, honey and SSD. In the cream batches MH showed the highest % wound reduction followed by honey, mucin and SSD. The wounds treated with the gel batches showed that the MH formulation also had the highest % reduction in wound diameter, followed by formulations containing honey, mucin and SSD.

The results showed that after 9 days of wound dressing, MH formulations exhibited superior wound healing ability to those of honey, mucin or standard (SSD). In comparative consideration of 9-15 days (Table 19) as to 3-9 days of wound dressing with the formulated products there was observed increase in the % wound reduction with time. For wounds treated with ointment formulations the MH showed highest % average wound reduction followed by mucin, honey and SSD. The same descending order of magnitude was observed in cream batches. MH had the highest % cumulative wound reduction followed by mucin, honey and SSD.
Table 16. Degradation of salicylic acid (mg) in ointment batches stored for 14 weeks at three storage temperature

<table>
<thead>
<tr>
<th>Batches</th>
<th>29°C</th>
<th>40°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (23.5% honey)</td>
<td>0.007 mg (0.08 ± 0.012%)</td>
<td>0.089 mg (0.95 ± 0.23%)</td>
<td>0.113 mg (1.19 ± 0.015%)</td>
</tr>
<tr>
<td>2 (11.75% mucin)</td>
<td>0.032 mg (0.33 ± 0.211%)</td>
<td>0.127 mg (1.34 ± 0.71%)</td>
<td>0.132 mg (1.39 ± 1.33%)</td>
</tr>
<tr>
<td>3 (11.75% mucin, 23.5% honey)</td>
<td>0.017 mg (0.18 ± 0.03%)</td>
<td>0.082 mg (0.85 ± 0.112%)</td>
<td>0.098 mg (1.01 ± 0.71%)</td>
</tr>
<tr>
<td>4 (ointment base)</td>
<td>0.066 mg (0.71 ± 0.17%)</td>
<td>0.079 mg (1.1 ± 0.11%)</td>
<td>0.136 mg (1.45 ± 0.21%)</td>
</tr>
<tr>
<td>SSD (1% silver sulphadiazine)</td>
<td>0.022 mg (0.23 ± 0.017%)</td>
<td>0.115 mg (1.2 ± 0.172%)</td>
<td>0.151 mg (1.57 ± 0.061%)</td>
</tr>
</tbody>
</table>

Each value is the average of determinations from three replicate samples (mg). The figures in brackets are percentage averages for the actual amounts of drug degraded. ± is standard deviation.
Table 17. Degradation of salicylic acid (mg) in ointment batches stored for 14 weeks at three storage temperatures

<table>
<thead>
<tr>
<th>Batches</th>
<th>29°C</th>
<th>40°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (23.5% honey)</td>
<td>0.013 mg (0.13 ± 0.111%)</td>
<td>0.102 mg (1.06 ± 1.01%)</td>
<td>0.107 mg (1.12 mg ± 0.116%)</td>
</tr>
<tr>
<td>6 (11.75% mucin)</td>
<td>0.011 mg (0.11 ± 0.061%)</td>
<td>0.073 mg (0.74 ± 0.005%)</td>
<td>0.104 mg (1.06 ± 0.91%)</td>
</tr>
<tr>
<td>7 (11.75% mucin: 23.5% honey)</td>
<td>0.022 mg (0.12 ± 0.031%)</td>
<td>0.096 mg (0.97 ± 0.22%)</td>
<td>0.109 mg (1.1 ± 0.023%)</td>
</tr>
<tr>
<td>8 cream base (BP)</td>
<td>0.011 mg (0.11 ± 0.034%)</td>
<td>0.104 mg (1.1 ± 0.04%)</td>
<td>0.101 mg (1.06 ± 0.005%)</td>
</tr>
<tr>
<td>SSD (1% silver sulphadiazine cream)</td>
<td>0.022 mg (0.23 ± 0.017%)</td>
<td>0.115 mg (1.2 ± 0.172%)</td>
<td>0.151 mg (1.57 ± 0.061%)</td>
</tr>
</tbody>
</table>

Each value is the average of determinations from three replicate samples (mg). The figures in brackets are percentage averages for the actual amounts of drug degraded. ± is standard deviation.
Table 18. Degradation of salicylic acid (mg) in ointment batches stored for 14 weeks at three storage temperature

<table>
<thead>
<tr>
<th>Batches</th>
<th>29oC (mg)</th>
<th>40 oC (mg)</th>
<th>45 oC (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (23.5 % honey)</td>
<td>0.013 mg (0.13 ± 0.005 %)</td>
<td>0.08 mg (0.83 ± 0.67 %)</td>
<td>0.1 mg (1.04 ± 0.31 %)</td>
</tr>
<tr>
<td>10 (11.75 % mucin)</td>
<td>0.025 mg (0.26 ± 0.121 %)</td>
<td>0.113 mg (1.16 ± 0.103 %)</td>
<td>0.152 % (1.6 ± 0.71%)</td>
</tr>
<tr>
<td>11 (11.75 % mucin: 23.5 % honey)</td>
<td>0.014 mg (0.15 ± 0.076 %)</td>
<td>0.083 mg (0.85 ± 0.15 %)</td>
<td>0.104 mg (1.06 ± 0.25 %)</td>
</tr>
<tr>
<td>12 (gel base)</td>
<td>0.011 mg (0.13 ± 0.012 %)</td>
<td>0.113 mg (1.8 ± 0.171 %)</td>
<td>0.103 mg (1.5 ± 0.115 %)</td>
</tr>
<tr>
<td>SSD (1 % silver sulphadiazine cream)</td>
<td>0.022 mg (0.23 ± 0.017 %)</td>
<td>0.115 mg (1.2 ± 0.172 %)</td>
<td>0.151 mg (1.57 ± 0.061 %)</td>
</tr>
</tbody>
</table>

Each value is the average of determinations from three replicate samples (mg). The figures in brackets are percentage averages for the actual amounts of drug degraded. ± is standard deviation.
Fig. 14: Plot of log conc. salicylic acid against time for mucinated-honey ointment for batch 3 (11.75% mucin: 23.5% honey) at 29 °C.

Fig. 15: Plot of log conc. salicylic acid release against time for mucinated-honey cream for batch 7 (11.75% mucin: 23.5% honey) at 29 °C.

Fig. 16: Plot of log salicylic acid conc. against time for mucinated-honey gel batch 11 (11.75% mucin: 23.5% honey) at 29 °C.
Table 19. Percentage wound reduction at 3-15 days post dressing

<table>
<thead>
<tr>
<th>Batches</th>
<th>3-9 days post dressing</th>
<th>9-15 days post dressing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ointments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (23.5 % honey)</td>
<td>36.63 ± 12.8 %</td>
<td>75.38 ± 15.8 %</td>
</tr>
<tr>
<td>2 (11.75 % mucin)</td>
<td>45.63 ± 11.2 %</td>
<td>81.5 ± 15.1 %</td>
</tr>
<tr>
<td>3 (11.75 % mucin: 23.5 % honey)</td>
<td>53.88 ± 11.2 %</td>
<td>89.25 ± 8.7 %</td>
</tr>
<tr>
<td><strong>Creams</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (23.5 % honey)</td>
<td>43 ± 15 %</td>
<td>81.5 ± 10.1 %</td>
</tr>
<tr>
<td>6 (11.75 % mucin)</td>
<td>41.88 ± 17.3 %</td>
<td>84 ± 12.4 %</td>
</tr>
<tr>
<td>7 (11.75 % mucin: 23.5 % honey)</td>
<td>56.88 ± 18.5 %</td>
<td>88.63 ± 10 %</td>
</tr>
<tr>
<td><strong>Gels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (23.5 % honey)</td>
<td>44.75 ±12.8 %</td>
<td>73.23 ± 10.1 %</td>
</tr>
<tr>
<td>10 (11.75 % mucin)</td>
<td>41.88 ± 13.2 %</td>
<td>74.75 ± 11 %</td>
</tr>
<tr>
<td>11 (11.75 % mucin: 23.5 % honey)</td>
<td>47.75 ± 12.6 %</td>
<td>80.88 ± 12.5 %</td>
</tr>
<tr>
<td><strong>SSD (1 % silver sulphadiazine cream)</strong></td>
<td>23.25 ± 13.5 %</td>
<td>50.5 ± 7.8 %</td>
</tr>
</tbody>
</table>
In the wounds dressed with gel preparations, the same descending order of wound reduction was observed. The MH equally showed the highest % reduction in wound followed by mucin, honey and SSD.

Fig. 20a shows some pictures of freshly surgically excised 1257.14 mm² full thickness wounds (a, b, c and d) in rats. Fig. 20b shows the pictures (a, b, c and d) of ointment treated wounds in day 9. Picture c is wound treated with MH ointment which had accelerated wound healing as at day 9, while picture b is wound treated with mucin ointment which had higher reduction of wound diameter than wound treated with honey (picture a). Picture d is wound treated with standard (SSD). At this stage, MH exhibited a faster rate of healing than the other preparations. By day 15, in the ointment treated wounds, MH had complete healing unlike mucin, honey and SSD treated wounds (Fig. 20c).

The pictures in Fig. 21a indicate that the MH cream treated wounds at 9 days healed faster than mucin, honey or SSD treated wounds. The order was the same after 15 days (Fig. 21b). Results of gel treated wounds (Figs. 22a and 22b) also indicated that MH had the highest rate of wound reduction relative to the other agents after 9 and 15 days treatment.

At 50% reduction in wound diameter, the MH showed shorter days in wound healing than mucin, honey or SSD. In terms of 90% reduction in wound diameter MH was also better than the others.

The observed enhanced wound healing in the formulations containing MH formulations may be due to the ability of mucin to adhere to wound surface as reported by Fogelson (35) and increase the bioavailability of the honey in the wound area. The acceleration in wound healing by MH is consistent with studies reported by Adikwu et al (11) that snail mucin dispersed in detarium gum gel accelerated wound healing in rat. Honey accelerates wound healing (15) and
Fig. 20a. Photographs (a,b,c and d ) of freshly excised wounds in rats
Fig. 20b. Post-wound treatment for ointment batches after 9 days, Photographs, a, b, c and d are pictures of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively.
Fig. 20c. Post-wound treatment for ointment batches after 15 days. Photographs a, b, c and d are for wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively.
Fig. 21a. Post-wound treatment for cream batches after 9 days. Photographs a, b, c and d are wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively.
Fig. 21b. Post-wound treatment for cream batches after 15 days. Photographs a, b, c and d are pictures of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively.
Fig. 22a. Post-wound treatment for gel batches after 9 days. Photographs a, b, c and d are wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively.
Fig. 22b. Post-wound treatment for gel batches after 15 days. Photographs a, b, c and d are wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively.
facilitates the regeneration of cells in wounds (17), and is reported (14) to heal wounds in superficial burns faster than SSD. The microenvironment in wound bed preparation is significant in overall wound healing rate and any topical agent that maintains favourable wound microenvironment will help in wound healing (133).

The observed effect of the MH may be attributed to the amphiphilic nature of mucin and honey. These co-polymers enhanced drug permeation which may imply that MH preparation gives favourable wound microenvironment for accelerated wound healing (75).

3.12.2 **Wound bioload studies**

Table 20 shows percentage bacterial reduction in wounds dressed with the formulated products. From this result, the MH formulation showed a better wound bacterial reduction than mucin, honey or SSD. It was equally observed that the rate of wound healing increases with wound bioload reduction.

Wound bacteria bioload reduction is presented in Figs. 17-22. Watson (134) showed that antibacterial activity rate of substance could be expressed in the same form as a first order chemical reaction. The interpretations of such rates are based on theoretical mechanisms that are called mechanistic theories. This can be expressed as:

\[ K = \log \left( \frac{P_0}{P^{-x}} \right) \]  

Where \( K \) is the rate constant, \( t \) is the time of contact with wound, \( P_0 \) is the initial number of bacteria in the wound and \( P^{-x} \) is the number of bacteria cells after exposure to time \( t \).

For convenience, Eqn.13 shows the plots drawn as % survivors against log time of exposure and log % survivors against log time of exposure. The shape of the curve depends on the rate of bacteria reduction. When the bacteria reduction rate is fast a straight line is obtained (Figs. 17-19) while if it is slow, the shape becomes sigmoidal (Figs. 20-22). The MH did not
Table 20: Percentage bacterial reduction in wound swabs from day 3-11 post-treatment for batches

<table>
<thead>
<tr>
<th>Type of formulation</th>
<th>MH (11.75% mucin 23.5% honey)</th>
<th>M (11.75% mucin)</th>
<th>H (23.5% honey)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ointments</td>
<td>93.31 ± 4.61</td>
<td>90.31 ± 2.38</td>
<td>88.64 ± 2</td>
</tr>
<tr>
<td>Creams</td>
<td>95.52 ± 4.7</td>
<td>94.74 ± 1.07</td>
<td>91.81 ± 3.02</td>
</tr>
<tr>
<td>Gels</td>
<td>87.54 ± 2.61</td>
<td>77.69 ± 8.94</td>
<td>78.09 ± 2.26</td>
</tr>
</tbody>
</table>

SSD = 78.09 ± 2.27. SSD is 1 % silver sulphadiazine cream, ± is standard deviation. MH = mucinated:honey, M= mucin and H= honey preparations.
Fig. 17. Plot of percent bacteria survived against log time for ointment batches

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3(1:2): 11.75% mucin to 23.5% honey
- Batch 13: 1% silver sulphadiazine cream (standard)
Fig. 18. Plot of percent bacteria survived against Log time for cream batches

- Batch 5: 23.5% honey
- Batch 6: 11.75% mucin
- Batch 7(1:2) 11.75% mucin to 23.5% honey
- Batch 13: 1% silver sulphadiazine cream (standard)
Fig. 19. Plot of percent bacteria survived against Log time for gelatin gel batches

- Batch 9: 23.5% honey
- Batch 10: 11.75% mucin
- Batch 11:(1:2) 11.75% mucin to 23.5% honey
- Batch 13: 1% silver sulphadiazine cream
Fig. 20: Plot of percent bacteria survived against Log time for ointment batches

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3(1:2): 11.75% mucin to 23.5% honey
- Batch 13: 1% silver sulphadiazine cream (standard)
Fig. 21: Plot of percent bacteria survived against Log time for cream batches

- Batch 5: 23.5% honey
- Batch 6: 11.75% mucin
- Batch 7(1:2) 11.75% mucin to 23.5% honey
- Batch 13: 1% silver sulphadiazine cream (standard)
Fig. 22: Plot of percent bacteria survived against Log time for gelatin gel batches

- **Batch 9**: 23.5% honey
- **Batch 10**: 11.75% mucin
- **Batch 11**: (1:2) 11.75% mucin to 23.5% honey
- **Batch 13**: 1% silver sulphadiazine cream
completely complied with the staright lines and sigmoid shapes. The bioload reduction increased the overall therapeutic efficiency of MH preparations in wound healing. MH induced early resolution of inflammatory stage of the wound and probably served as a chemoattractant for the neutrophils while it prevents the destructive effect of neutrophil derived elastase. The concentration of bioload in a wound determines the degree of damage it will have on such wound. Some authors (8,9) indicated that wound bioload of less than $10^5 /g$ has no clinical defect as the body defense mechanism will be able to control the bioload. From our studies however, the bioloads were higher than $10^5 /g$ but all the formulations including the control reduced the bioload to lower concentrations.

Wounds are altered by the presence of infection which leads to delay in wound healing and any agent that eliminates or reduces wound infection will enhance wound healing (56). The antibacterial activity of mucin in accelerating wound healing has been reported (121).

The enhanced wound bioload reduction observed in MH wound dressing may be as a result of combination of antibacterial actions of mucin and honey. This leads to early resolution of infection which in turn accelerates wound healing.

3.12.3 Characteristics of wounds treated with formulated products

Tables 21 - 23 indicate some of the characteristics of wounds treated with the formulated products. The wounds that were treated with ointment preparations of MH had the fastest resolution of the inflammatory, proliferative and maturation phases of wound healing (Table 21). The wounds showed early resolution of oedema, fever, minimal scar, early establishment of angiogenesis, fibroblast cells and high density keratinocytes than mucin, honey or SSD formulations. The wounds that were treated with the creams and gels respectively followed the same characteristics as the ointment batches (Tables 22 and 23).
**Table 21: Some characteristics of wounds treated with ointment batches and SSD on day 13**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Oedema</th>
<th>Fever</th>
<th>Angio-</th>
<th>Fibroblast</th>
<th>Granulation</th>
<th>Keratinocytes</th>
<th>Scar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(days)</td>
<td>(days)</td>
<td>(days)</td>
<td>(days)</td>
<td>(days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (23.5% honey)</td>
<td>&lt; 4 ± 1.15 &lt; 5 ± 2.3 &lt; 5 ± 1.73 &lt; 7 ± 1.3 &lt; 7 ± 1.7</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (11.75% mucin)</td>
<td>&lt; 3 ± 1.73 &lt; 3 ± 0.58 &lt; 5 ± 1.8 &lt; 5 ± 0.31 &lt; 7 ± 1.15</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (11.75% mucin, 23.5% honey)</td>
<td>&lt; 2 ± 0.47 &lt; 2 ± 1.64 &lt; 3 ± 1.5 &lt; 4 ± 1.1 &lt; 5 ± 0.58</td>
<td>+++</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSD</td>
<td>&lt; 8 ± 2.31 &lt; 7 ± 1.1 &lt; 9 ± 2.89 &lt; 11 ± 3.46 &lt; 12 ± 0.61</td>
<td>++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SSD is 1 % silver sulphadiazine cream, ± is standard deviation, < is less than, - means minimal + is low, ++ means moderate and +++ means high.
Table 22: Some characteristics of wounds treated with cream batches and SSD on day 13

<table>
<thead>
<tr>
<th>Batch</th>
<th>Oedema (days)</th>
<th>Fever (days)</th>
<th>Angio-Granulation (days)</th>
<th>Fibroblast (days)</th>
<th>Keratinocytes (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (23.5% honey)</td>
<td>&lt; 4 ± 0.58</td>
<td>&lt; 4 ± 2.1</td>
<td>&lt; 5 ± 1.3</td>
<td>&lt; 7 ± 1.73</td>
<td>++</td>
</tr>
<tr>
<td>6 (11.75% mucin)</td>
<td>&lt; 3 ± 1.1</td>
<td>&lt; 3 ± 1.15</td>
<td>&lt; 5 ± 0.58</td>
<td>&lt; 5 ± 1.2</td>
<td>++</td>
</tr>
<tr>
<td>7 (11.75% mucin: 23.5% honey)</td>
<td>&lt; 2 ± 1.73</td>
<td>&lt; 3 ± 1.1</td>
<td>&lt; 3 ± 0.58</td>
<td>&lt; 5 ± 1.3</td>
<td>+++</td>
</tr>
<tr>
<td>SSD</td>
<td>&lt; 8 ± 2.31</td>
<td>&lt; 7 ± 1.1</td>
<td>&lt; 9 ± 2.89</td>
<td>&lt; 11 ± 3.46</td>
<td>++</td>
</tr>
</tbody>
</table>

SSD is 1% silver sulphadiazine cream, ± is standard deviation, < is less than, - means minimal + is low, ++ means moderate and +++ means high.
Table 23: Some characteristics of wounds treated with gel batches 9-11 and SSD on day 13

<table>
<thead>
<tr>
<th>Batch</th>
<th>Oedema</th>
<th>Fever</th>
<th>Angio-</th>
<th>Fibroblast</th>
<th>Granulation</th>
<th>Kerat-</th>
<th>Scar</th>
<th>Inocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(days)</td>
<td>(days)</td>
<td>(days)</td>
<td>(days)</td>
<td>(days)</td>
<td>(days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (23.5% honey)</td>
<td>&lt; 5 ± 1.7</td>
<td>&lt; 5 ± 1.1</td>
<td>&lt; 5 ± 0.58</td>
<td>&lt; 7 ± 1.5</td>
<td>&lt; 9 ± 3.46</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>10 (11.75% mucin)</td>
<td>&lt; 3 ± 2.31</td>
<td>&lt; 4 ± 1.3</td>
<td>&lt; 5 ± 2.89</td>
<td>&lt; 7 ± 3.11</td>
<td>&lt; 7 ± 0.74</td>
<td>++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>11 (11.75% mucin: 23.5% honey)</td>
<td>&lt; 3 ± 1.2</td>
<td>&lt; 3 ± 0.58</td>
<td>&lt; 5 ± 1.15</td>
<td>&lt; 5 ± 0.63</td>
<td>&lt; 5 ± 2.31</td>
<td>+++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SSD</td>
<td>&lt; 8 ± 2.31</td>
<td>&lt; 7 ± 1.1</td>
<td>&lt; 9 ± 2.89</td>
<td>&lt; 11 ± 3.46</td>
<td>&lt; 110 ± 61</td>
<td>++</td>
<td>+++</td>
<td></td>
</tr>
</tbody>
</table>

SSD is 1 % silver sulphadiazine cream, ± is standard deviation, < is less than, - means minimal + is low, ++ means moderate and +++ means high.
The MH wound healing is able to show early resolution of oedema and fever. This resulted to early establishment of angiogenesis, fibroblast and granulation in wounds treated. Mucin (135) is reported to moderate neutrophil elastase resulting in early wound healing. Honey is also known to have enhanced wound healing characteristics (136). The presence of the SH group which decreases enzyme activity on the mucin explain its ability to reduce the neutrophil elastase activity in wounds.

Fibroblasts play multivariable role in wound tissue differentiation and granulation. Young et al (33) indicated that fibroblast is the most common support cell and is responsible for secreting the extracellular matrix in most tissues. One of the main functions of fibroblast is to maintain the integrity of supporting tissues by continuous slow turnover of the extracellular matrix constituents. Fibroblasts in form of myofibroblasts also play an important role in contraction and shrinkage of the resultant scar tissue as in MH treated wounds. Therefore the early appearance of fibroblast cells in the wound treated with MH preparations would partly account for the accelerated wound healing observed in MH treated wounds.

The granulation substance (supporting tissue) fills the wound space for early wound healing as suggested by Herrick et al (66). Elastin cells found in supporting tissues confer elasticity to enable recovery of tissue shape following normal physiological deformation. After granulation, re-epithelialization occurs and high density of keratinocyte cells indicates the maturation and healing of the wound as observed in the MH treated wounds.

The MH treated wounds also showed enhanced epithelialization and keratinocytes (+++ and a markedly diminished inflammatory response (<2days) unlike SSD that had low keratinocytes density (+) and reduced oedema in 8±2.31 days (Tables 21-23). Early resolution of inflammation during healing minimizes scar formation (10) as observed in MH treated wounds.
The studies on honey by Ghaderi et al (17), Bergman et al (28), Efem (19) and Ali (20) also reported such wound characteristics but at less intensity compared to MH treated wounds.

3.12.4 **Histological studies of the wound healing process using the various formulations**

The histopathological evaluation results showed that MH healed wounds faster than honey, mucin or SSD when inflammatory reactions, pus cells, regenerated cells density and angiogenesis are considered.

Fig. 23 shows the histological scores of wounds treated with ointment batches (1-3) in comparison to SSD in 13 days. The MH treated wound had drastic reduction in histological score within the 13 days followed by mucin, then honey and SSD (standard). Fig. 23a shows the micrographs of the transverse sections of wounds 4 days post-treatment for ointment batches and SSD. It shows that for MH (c) within 4 days of dressing, the oedema (i) had resolved, granulation had reached an advanced stage (ii) and a high degree of angiogenesis had occurred (iii). The same was applicable to wounds treated with mucin but with less cell regeneration. Honey treated wounds still had the oedema present (i) while the granulation was less than the mucin treated wounds. The SSD had diffused oedema, less granulation and angiogenesis within the period under study. The micrographs in Figure 23b shows that the MH had reached the proliferative stage by day 9 as is characterized by high protein synthesis activity and blood vessels i, ii and iii, while mucin had high angiogenesis ( i), squamous epithelial cells (ii) and blood cells (iii). The (micrograph a) indicates honey treated wounds showed fewer angiogenesis and squamous epithelial cells than mucin. The SSD treated wounds (Fig 23b, d) were still trapped in the inflammatory stage of the wound healing as there were fewer squamous cells (ii) and less re-establishment of revascularization (i, iii). Fig. 23c shows that on the 13th day of dressing, the MH treated wounds had more defined squamous epithelial cells formed (ii), less
Fig. 23. Plot of histological scores of ointment batches against time

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3: (1:2) 11.75% mucin to 23.5% honey
- Batch 13: 1% silver sulphadiazine cream
Fig. 23a. Micrographs (a, b and c) of treated wounds for ointment batches 1, 2 and 3 respectively and SSD (d) in 4 days. a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
Fig. 23b. Micrographs (a, b and c) of treated wounds for ointment batches 1, 2 and 3 respectively and SSD (d) in 9 days. a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively.
(Hematoxylin and Eosin stain)x200
Fig. 23c. Micrographs (a, b and c) of treated wounds for ointment batches 1, 2 and 3 respectively and SSD (d) in 13 days. a, b, c and d are transverse sections of wounds treated with 23.5\% honey, 11.75\% mucin, mucinated-honey (11.75\% mucin:23.5\% honey) and 1\% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
revascularization (i) and less protein synthesis (iii). This is followed by mucin treated wounds with re-epithelialization (iii), high protein synthesis (ii) and blood vessels (i). The honey treated wounds had re-epithelialization (iii), high collagen formation (i) and high protein synthesis (ii). SSD treated wounds showed less re-epithelialized cells (iii), less protein synthesis at the centre of the transverse section ii and high protein activity at the basal membrane (i). This suggests that the SSD wounds were still at the proliferative stage of the wound healing at day 13, while the wounds dressed with the other batches had passed this stage.

Figs. 24 and 25 show that plot of the histological scores of the cream and gel batches followed the same pattern as for the ointment batches. Figure 24a shows the micrographs of wounds treated with cream batches day 4. It shows that for MH the oedema had fully resolved (i) and granulation (ii) and high protein synthesis (iii) already taken place. This was followed by mucin treated wounds as seen in micrograph b with little oedema (i), granulation (ii), and revascularization (iii), while honey treated wounds had little oedema, granulation (ii), and revascularization (iii). The standard (micrograph d) is still in the inflammatory stage as can be seen from more oedema (i) and less revascularization (ii). Fig. 24b shows that after 9 days of wound treatment, the MH treated wound (micrograph c) had more evidence of reaching the maturation stage than the mucin and honey treated wounds (micrograph b, a). The standard showed less revascularization (i) with inflammation (iii) and less healing (ii). Fig. 24c shows that by day 13 of treatment, MH treated wound (micrograph c) had high density squamous re-epithelization (ii), less blood vessels (iii) and protein synthesis (i), followed by mucin treated wounds (micrograph b), re-epithelization cells (ii), basement membrane (i) and high collagen density (iii). The honey treated wounds (micrograph a) still showed high density of revascularization (ii), re-epithelialized tissue (iii) and blood vessel (i). This indicates less healing
Batch 1: 23.5% honey
Batch 2: 11.75% mucin
Batch 3: (1:2) 11.75% mucin to 23.5% honey
Batch 13: 1% silversulphadiazine cream

Fig. 24. Plot of histological scores against time for cream batches.
Fig. 25. Plot of histological scores against time for gel batches.

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3: (1:2) 11.75% mucin to 23.5% honey
- Batch 13: 1% silversulphadiazine cream
Fig. 24a. Micrographs (a, b and c) of treated wounds for cream batches 5, 6 and 7 respectively and SSD (d) in day 4. a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
Fig. 24b. Micrograph (a, b and c) of treated wounds for cream batches 5, 6 and 7 respectively and SSD (d) in 9 days. a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
Fig. 24c. Micrographs (a, b and c) of treated wounds for cream batches (5, 6 and 7) respectively and SSD (d) in 13 days. a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
than mucin treated wounds. In Fig. 24c the MH and mucin treated wounds had fully re-
epithelialized and entered maturation stage at day 13 unlike the standard that was still in 
proliferative stage. 

In the gel treated wounds (Fig. 25a) at 4 days, the oedema had not yet resolved in honey 
treated wounds (i) (micrograph a) but there was good angiogenesis (ii) and (iii). The mucin 
group still had oedema (i), (ii) with good vascularization (iii) (micrograph b). In the MH wounds, 
the oedema had resolved (ii) with good granulation (i) and angiogenesis (iii) (micrograph c). 
Micrograph d of wound treated with SSD depicted by generalized oedema. By day 9 (Fig. 25b) 
MH wounds showed clear superiority with high density of collagen (ii) and elastin fibres (iii) and 
(i) (micrograph c), unlike the honey wounds (micrograph a) with less collagen (iii), less elastin 
fibres (i) and less vascularization (ii). Mucin treated wounds (micrograph c) had less collagen 
(ii) and elastin fibres (i) and still in early proliferative stage of wound healing (revascularization) 
(iii) (micrograph b). The SSD treated wound was still trapped in inflammatory/proliferative 
stages (micrograph d). By day 13 of treatment with MH gel formulation products (Fig. 25c), the 
wound showed higher degree of maturation of as depicted by stratified epithelia (i), moderate 
vasculature (ii) and collagen (iii) (micrograph c) while mucin treated wound had less elastin (i), 
high angiogenesis (ii) and high collagen density (iii) (micrograph b). The same features with 
higher densities were applicable in honey (micrograph a) treated wound. The SSD (micrograph 
d) treated wound was still at early proliferative stage of healing as depicted by high angiogenesis 
and collagen cells. 

Fig. 25d, shows a comparison of MH treated wounds using different dosage forms 
(micrographs a, b and c) and SSD treated wound (micrograph d). The MH treated wounds 
showed coarse, closely packed, bundles of collagen in the dermis interwoven by elastin fibres
Fig. 25a. Micrographs (a, b and c) of treated wounds for gel batches (9, 10 and 11) respectively and SSD (d) in 4 days. a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
Fig. 25b. Micrographs (a, b and c) of treated wounds for gel batches (9, 10 and 11) respectively and SSD (d) in 9 days. a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
Fig. 25c. Micrograph (a, b and c) of treated wounds for gel batches (9, 10 and 11) respectively and SSD (d). a, b, c and d are transverse sections of wounds treated with 23.5% honey, 11.75% mucin, mucinated-honey (11.75% mucin:23.5% honey) and 1% silver sulphadiazine cream respectively. Hematoxylin and Eosin stain x200
Fig. 25d. Micrographs (a, b and c) of mucinated-honey formulations treated wounds for batches 3 (ointment), 7 (cream), 11 (gel) respectively, compared to SSD (d) in 13 days.
while SSD had loose granulation cells indicative of slow healing. The epithelia in MH treated wounds are closely packed squamous cells (see Fig. 25d) (micrograph b) with minimal intercellular material between them. The basement membrane provides metabolic support and binding the epithelium to the underlying support tissue. This is unlike SSD still in proliferative stage as at day 13 post treatment.

The thickness of regenerating epidermis is uneven which a characteristic of regenerating skin (33). This shows that new epidermis emanates from surviving islets of epithelial cells in the basal layer. Mucin in submucosa is known to enhance epidermal cell differentiation (138). Gore et al (139) reported that topical delivery of wound medicament influences wound microenvironment. This favours rapid cell regeneration. Selby (140) observed that the histological examination of cells differentiation in wound healing shows the effect the medicament has on the wound tissue. The newly regenerated cells move upward before moving sideways. The enhanced wound healing by MH implies that MH confers faster keratinocytes, endothelial cells, fibroblasts and inflammatory cells proliferation and migration to the site of injury and regeneration of cells.

MH treated wounds healed faster than mucin, honey and SSD treated wounds. The descending order of magnitude for all the formulations is as follows: MH > Mucin > Honey > SSD (p > 0.05). The relative deficiency of SSD on wound healing has also been reported (141). The stratum corneum/epidermal regeneration and histological scores showed differences in rate of tissue regeneration in full thickness wounded rats when MH topical preparations were applied on alternate days in wound dressing in comparison to SSD (Fig. 25d). This may be due to the ability of MH to cause cellular and biochemical processes such as increase in cellular metabolism that facilitate cell regeneration. Work done by Hackman et al (64) reported that any
A topical agent that causes early cellular and biochemical processes in the wound area facilitates wound healing. Stadelmann et al. (2) stated that extra cellular matrix (ECM) is a passive mechanical support system with an integral signaling medium that coordinates maintenance of a normal skin structure and events of wound healing. The functions of matrix metalloproteases (MMPs) and serine proteases in normal wound healing process include promoting cell migration and activating growth factors. Excessive protease activity can damage the newly-formed extra-cellular matrix (ECM) and prevent the migration of undamaged epidermal cells across its surface from the edge of the wound. Normally protease activity levels rise in a wound as part of the body’s natural defence against infection. Neutrophil elastase (NE) is one of the proteases that do not discriminate and can degrade the essential components of the newly formed ECM. The level of NE shows whether an acute wound turns to a chronic wound (73,74). This is due to the fact that high concentration of NE in a wound prevents it from early healing. But protease activity can be modified by inhibitors, pH and temperature. MH formulations have the ability to restore the correct balance of neutrophil elastase activity in wound and help protect the ECM, promote cell migration and thereby stimulate wound healing. Considering both the rate of wound healing and histological results, it implies that the application of MH in wound dressing enhanced early establishment of ECM. This in turn accelerates the biochemical cascades that are involved in wound healing.

As tissue cells differentiate into a stratified squamous epithelium above a newly generated basement membrane, the granulation tissue forms below the epithelium and are composed of inflammatory cells, fibroblasts and newly formed and forming vessels. Angiogenesis (i.e. the generation of new capillary blood vessels from pre-existing vasculature to provide nutrients and oxygen to granulate tissue) is potentiated in MH treated wounds, earlier
than mucin, honey or SSD treated wounds (Fig. 25d). The reason for MH induced accelerated wound healing may be due to the of physico-chemical compatibility of mucin and honey. This is due to the fact that honey is acidic while mucin is negatively charged as a result of carboxyl and sulphate side groups on the dissacharide units. Young et al (33) pointed out that under physiological environment mucins (glycoprotein) appear to be involved in cell recognition phenomenon which may lead to the formation of intracellular adhesion and adsorption of molecules to the cell surface. In some situations it provides mechanical and chemical protection for the plasma membrane. The implication of this is that there will be early establishment of angiogenesis as seen in MH treated wounds. Li et al (54) reported that any exogenous agent that has the ability to stimulate angiogenesis accelerates wound healing. Santoro and Gaudino (55) observed that mucin (intergrin) molecules have the ability to facilitate the keratinocytes invasion of the wound bed for early wound healing while Falanga (133) explained that cells can only migrate over living tissue and that keratinocytes migration is best enhanced by moist wound environment compared to dry environment, MH being a co-polymer can enhance wound moisture thereby facilitating cell migration for early wound healing (141).

With respect to wound healing rate, othe order of the MH preparations were MH cream < MH ointment < MH gel < SSD (P < 0.05).

3.12.5 Phases of wound protease enzyme studies

The enzyme neutrophil elastase basal concentration for ointment, cream and gel batches are presented in Figs. 26 - 28 respectively as concentration of neutrophil elastase (NE) against time pre-treated wounds. There was significant reduction in neutrophil elastase (NE) activity in wounds treated with the formulated medicaments as days of treatment increases. Figs. 29 and 32 show the NE % reduction in wounds treated with ointments in 7 and 11 days respectively, MH
Fig. 28: Plot of wound neutrophil elastase basal conc. against time in enzyme-substrate reaction for ointment batches.

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3: (1:2) 11.75% mucin to 23.5% honey
- Batch 13: 1% silver sulphadiazine cream (SSD)
Fig 27: Plot of wound neutrophil elastase basal conc. against time for cream batches and SSD

- Batch 5: 23.5% honey
- Batch 6: 11.75% mucin
- Batch 7: (1:2) 11.75% mucine to 23.5% honey
- Batch 13 (SSD)
Fig 28: Plot of neutrophil elastase basal conc. against time for gel batches

- Batch 9: 23.5% honey
- Batch 10: 11.75% mucin
- Batch 11: (1:2) 11.75% mucin to 23.5% honey
- Batch 13 (SSD)
Fig. 29: Plot of neutrophil elastase conc. against time for ointment batches and SSD at day 7.

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3(1:2) 11.75% to 23.5% honey
- Batch 13 (SSD)
had the highest reduction in wound NE enzyme, followed by mucin, SSD and honey except on day 3. Cream batches also showed the same pattern of NE reduction in the wounds (Figs. 30 and 33). In gel treated wounds, MH had the highest NE reduction, followed by mucin, SSD and honey (Figs. 31 and 34). All batches irrespective of dosage form followed the same descending order of intensity % NE wound reduction; MH > mucin > SSD > honey (p < 0.05). This shows that MH preparations were better at reducing neutrophil elastase enzyme proteolytic activity on the wound regenerated tissue. The order may be due to the ability of MH to cause conformational changes in NE moiety to inactivate the enzyme thereby facilitating wound healing as the inflammatory stage is reduced. Enzyme conformational changes impacted by exogenous agents as process of inhibition in enzyme activity have been reported by a number of workers (137,142,143,144).

In the wound wash substrate and enzyme reaction studies, increase in substrate concentration resulted in increased neutrophil enzyme concentration due to the substrate specificity to neutrophil elastase enzyme. The kinetics of the neutrophil elastase concentration with increase in substrate concentrations suggest that the NE reaction increases with increase substrates but the overall NE concentration decreases with time. The same order was observed in wounds dressed with cream batches and gel batches. It has been reported (82) that the accumulation of wound neutrophil elastase suggests its role as the source of mediators involved in delayed epithelial regeneration and hence delayed in wound healing.

Figs. 32-34 also show the total % wound neutrophil elastase reduction in 11 days post treatment with formulated products. It was in the order MH > mucin > SSD > honey (p < 0.05) for all the dosage forms.
Fig. 30: Plot of neutrophil elastase conc. against time for cream batches and SSD at day 7.

- **Batch 5**: 23.5% honey
- **Batch 6**: 11.75% mucin
- **Batch 7**: 11.75% mucin to 23.5% honey
- **Batch 13**: SSD
Fig. 31: Plot of neutrophil elastase conc. against time for gel batches and SSD at day 7.

- Batch 9: 23.5% honey
- Batch 10: 11.75% mucin
- Batch 11(1:2): 11.75% mucin to 23.5% honey
- Batch 13 (SSD)
Fig. 32: Plot of final neutrophil elastase conc. against various days for ointment batches

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3(1:2) 11.75% mucin to 23.5% honey
- Batch 13

Neutrophil elastase conc. at 410 nm

Time (days)
FIG 33: Plot of final neutrophil elastase conc. against various days of wound dressing for cream batches

- Batch 5: 23.5% honey
- Batch 6: 11.75% mucin
- Batch 7(1:2) 11.75% mucin to 23.5% honey
- Batch 13
FIG 34: Plot of final neutrophil elastase conc. against various days of wound treatment for gel batches

- Batch 9: 23.5% honey
- Batch 10: 11.75% mucin
- Batch 11(1:2) 11.75% mucin to 23.5% honey
- Batch 13
The results show that MH had the best ability to reduce NE in wounds, which suggests that reduction of wound neutrophil elastase enzyme is one of its mechanisms of accelerating wound healing. This is probably mediated by the mucin component of the MH as NE reduction in wounds dressed with mucin preparations has earlier been reported. Vasson et al (145) reported that mucin has the ability to suppress polymorphonuclear neutrophil and accelerates regeneration of cells in wounds. Zhu et al (57) revealed that mucous secretions from different tissues have identical proteins that have strong affinity for neutrophil elastase. The low % NE reduction of honey preparations is expected since this is not the major mechanism of wound healing by honey. Early reduction of NE in wounds leads to early inflammatory phase resolution, early wound granulation and maturation since growth factors which are important in the wound healing process (146) are not digested by proteolytic enzymes in the wound.

Honey contains glucose oxidase enzyme that initiates antibacterial activity on wound and could possibly be digested by NE in wound exudates but the antineutrophile elastase activity of mucin will help in preventing this, thereby causing enhanced wound healing when MH preparation is applied on wound. Human epithelial mucin has anti neutrophil elastase activity (147) while guinea pig mucin’s anti-inflammatory effect (148) and its modulating effect of inflammatory cells (149) have been reported. Since hydrogel polymers in aqueous environment allow water molecules to penetrate their structures, the hydrophilic nature of mucin and honey chains would be expected to allow water molecules to penetrate into their cross-linked net work polymer molecules. This can facilitate hydration in wound microenvironment which in turn accelerates wound healing. Barisoni et al (113) found that in burn patients, there are primary and secondary leucocytosis and that over 80 % of the leucocytes are neutrophils. Since neutrophil elastase has the ability to solubilize elastin, collagen, fibronectin and proteoglycan, it might be
involved in the degenerative processes that locally affect the dermal tissues. The antineutrophil elastase activity of MH preparation is considered to be induced by the mucin moiety as suggested by Fournier et al (144) which may afford the enzyme alternate proteoglycan (glycoprotein) to digest instead of digesting new tissue re-generated. Akazawa et al (150) reported that mucin increases regeneration of cultured hepatocytes. This underlines the ability of mucin to stimulate cell regeneration and differentiation which is additive to honey cell regeneration in wounds as found in MH treated wounds.

Wounds treated with formulated preparations also showed reduction in the total protein content of wound wash. The highest % reduction was from days 3-11 in ointment batches in the wound dressed with MH, followed by mucin with, SSD and honey (Fig. 35). The cream and gel batches also showed that MH cream had the highest % reduction in total protein concentration followed by mucin and honey while SSD had the least (Figs. 36 and 37).

The damage to connective tissue is primarily mediated by neutrophil elastase thus a decrease in the concentration of this enzyme will lead to decrease in proteolytic activity in the wound and a protection of matrix formed in the process of healing. Groutas et al (151) suggest a synergistic involvement of other serine, metalloproteinase and cysteine enzymes that are released by phagocytic cells. Diegelmann et al (6) observed that neutrophil elastase enzyme has been identified as the major protease responsible for fibronectin degradation in both chronic and acute wound tissues. Thus the ability of MH formulations to inhibit the neutrophil elastase in wounds results in accelerated wound healing observed.

3.13 Probable mechanisms of Mucinated-Honey enhanced wound healing effect

Topical MH significantly accelerated full thickness skin wound healing compared to mucin or honey alone, as shown in Figs. 21b and 25d; Tables 21-23. This is associated with
Fig. 35: Plot of total protein concentration (µg/ml) of wound wash in various days of treatment for ointment batches

- Batch 1: 23.5% honey
- Batch 2: 11.75% mucin
- Batch 3(1:2) 11.75% mucin to 23.5% honey
- Batch 13
Fig. 36: Plot of total protein concentration (µg/ml) of wound wash in various days of treatment for cream batches

- Batch 5: 23.5% honey
- Batch 6: 11.75% mucin
- Batch 7(1:2) 11.75% mucin to 23.5% honey
- Batch 13
Fig. 37: Plot of total protein concentration (μg/ml) of wound wash in various days of treatment for gel batches

- Batch 9: 23.5% honey
- Batch 10: 11.75% mucin
- Batch 11: 11.75% mucin to 23.5% honey
- Batch 13
reduction in neutrophil elastase concentration (Figs. 32-34) which facilitates early regeneration of wound cells and significant reduction in bioload of the wounded tissue (Table 20). There is enhanced wound healing effect when mucin and honey are combined in topical wound treatment, compared to mucin, honey or SSD alone.

Some other probable mechanisms of MH wound healing include cell adhesion which can occur by recognition of cell-surface glycoprotein and carbohydrates on circulating cells by the molecules whose expression has been enhanced on resident cells. Nwome et al (9) pointed out that cells attach to surrounding extracellular protein matrix via structural receptors called integrins. This observation is consistent with studies done by Rudolph et al (43), Stadelmann et al (2) and Falcone et al (7). Many of these receptors attach to a specific amino acid sequence in structural proteins, such as that of arginine-glycine-aspartic acid. Mucin contains a lot of amino acids while honey being a carbohydrate may facilitate the cell regeneration and adhesion in wound healing process.

The surface activity of mucin can be as a result of amphiphilic structure of mucin polymer. The over-expression of p-glycoprotein (mucin) in cancerous tissues results in reduction in the accumulation of therapeutic agents at tumour sites. This is due to the inability of the drug to be attached to the site because of the surfactant activity of p-glycoprotein (mucin) which inhibits surface adsorption of the drug molecules. The mucin-honey complex used in this study has the ability to prevent the adhesion of microbes on the surface of the wound thereby reducing the bioload of the wound (12). This will facilitate the healing of the wound.

Fibronectin, a glycoprotein, when applied on wound is reported to be able to stimulate rapid synthesis of fibroblasts, endothelial cells and monocytes in soft tissue wound sites (138). This promotes attachment and migration of epidermal cells, fibroblasts, endothelial cells and
monocytes. It also promotes basement membrane assembly, thereby rapidly facilitating wound healing.

The nature of honey and mucin interaction can be discerned from the report by Brid et al (36) who observed that polymer chains with a higher flexibility might create more depth of interfacial region for contact and subsequently provide a better environment for entanglement between the polymers. The two polymers, on contact, allow their chains diffuse across the interface as a result of concentration gradient. Human saliva mucin has 357 amino acid residues which make the glycoprotein chains diffuse across a polymer interface (74). This explains why the physical homogenous mixture of mucin and honey as in MH will lead to polymer flexibility and diffusion in a pattern of interpenetration of polymers across interface to form a mixture that has different physicochemical characteristics from mucin or honey alone. The expanded network of both polymers permits both mechanical entanglement and provides a contact surface for hydrogen bonding. These unique properties make physical mixture of mucin and honey an excellent material for the development of new wound biomedical applications.
CHAPTER FOUR
SUMMARY AND CONCLUSION

This study had examined the standard topical formulations of mucin, honey and their admixtures and their effects on wound healing. On the basis of stability all the preparations were stable at temperatures less than 45 °C after 14 weeks of storage. The following major findings were observed; MH preparations had the highest % salicylic acid release, permeation, steady state flux, apparent permeability coefficient, highest % wound bioload reduction and highest % wound reduction compared to mucin, honey or SSD formulations.

The histological observation of the wounds showed that MH performed better than SSD and others. This is as a result of the ability of MH formulations (ointment, cream and gel) to stimulate proliferation and promote survival of cells in the epidermis and dermis of wounded rat skin. It was also observed that MH formulations had the highest % wound neutrophil elastase enzyme reduction compared to mucin, honey or SSD.

In summary, MH can be characterized as controlling proteolytic activity of neutrophil elastase and establishing early re-vascularization, granulation, re-epithelialization and decrease in wound bioload. All these factors led to accelerated enhanced healing observed in the full thickness experimental wounds in rats. The MH formulations dressed wounds exhibited faster wound healing than those of mucin, honey or control (SSD). In conclusion, from the findings of the study MH preparations had accomplished most of the aims and objectives of this study. Thus, new wound biochemical application of mucin and honey can be achieved by delivering such in standard topical pharmaceutical base as MH formulations.
REFERENCES


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