ENHANCEMENT OF WOUND HEALING ACTIVITY OF PAPAIN UREA FILM FORMING HYDROGEL USING CHITOSAN

A Dissertation submitted to

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In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

BRANCH- I (PHARMACEUTICS)

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled "**ENHANCEMENT OF WOUND HEALING ACTIVITY OF PAPAIN UREA FILM FORMING HYDROGEL USING CHITOSAN"** submitted by **R. SRUTHI [Reg. No: 261910205]** for the award of the degree of **"MASTER OF PHARMACY IN PHARMACEUTICS"** is a bonafide research work done by her in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences, Tiruchirappalli during the academic year 2019-2021 under my guidance and supervision.

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This dissertation is submitted for acceptance as project for partial fulfillment of the degree of **"MASTER OF PHARMACY"** in Pharmaceutics, of The Tamil Nadu Dr. M.G.R. Medical University, Chennai – 32 during October 2021

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CONTENT

LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATIONS	EXPANSION
FFH	Film Forming Hydrogel
MTT	3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide
DMSO	Dimethyl Sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
PBS	Phosphate Buffered Saline
OD	Optical Density
NCCS	National Centre for Cell Science
ICH	International Council of Harmonisation
IC50	Half Maximal Inhibitory Concentration
RH	Relative Humidity
UV	Ultraviolet
FTIR	Fourier Transform Infrared Spectroscopy
SD	Standard Deviations
m^2	Square Metre
cm^2	Square Centimetre
g	Gram
mg	Milligram
ml	Millilitre
sec	Second
^oC	Degree Centigrade
µg	Microgram
λ max	Absorption Maxima
rpm	Revolutions Per Minute
nm	Nanometre
hrs	Hours
min	Minutes
cps	Centipoise
IP	Indian Pharmacopoeia
A.R.	Analytical Reagent

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INTRODUCTION

1. INTRODUCTION

The skin is the most easily accessible component of the body and provides a barrier against environmental micro and macromolecules [1]. The adult human body's skin has a surface area of about $2m^2$ and receives about one-third of the total blood circulating all through the body [2]. The stratum corneum is the primary site of drug absorption through the skin. The stratum corneum is composed of dead, keratinized epidermal cells with a thickness of 10m and serves as a barrier to drug permeation. As a result, drug molecule transport across the skin is difficult [3].

The purpose of drug delivery through the skin is either topical therapy of skin conditions or transdermal absorption of drugs into the systemic circulation. The topical route provides a vast and varied surface in addition to the simplicity of application by self administration and serves as an option to oral drug delivery as well as hypodermic injection [4]. The pace and degree of medication absorption through skin are determined by skin physiology, drug physicochemical qualities, and the delivery mechanism. The present dosage forms, such as patches, ointments, creams, and so on, have a number of drawbacks. Patches have a number of drawbacks, the most common of which is skin irritation[5], due to their occlusive properties, which cause sweat duct obstruction, preventing water vapour loss from the skin's surface, difficulty in applying on curved surfaces, pain while removing off, and poor aesthetic appeal. Semisolid preparations, such as creams and ointments, eliminate some of these disadvantages but have additional constraints. These do not maintain consistent touch with the skin's surface and are quickly washed away by the patient's clothing [6]. As a result, in the event of chronic infections such as athlete's foot, ringworm, and candidiasis, frequent application is needed [7]. Furthermore, these leave a sticky and greasy feeling after application, which leads to poor patient compliance [8, 9]. As a result, there is a need for the design of a dosage form that allows for less frequent administration by maintaining intimate contact with the skin for an extended period of time, hence improving patient compliance.

The film forming system (FFS) is a revolutionary technique that can be utilised in place of traditional topical and transdermal formulations. It is described as a non-solid dose form that forms a film *in situ*, i.e. after being applied to the skin or any other bodily surface. These systems comprise the drug and film-forming excipients in a vehicle that, when in contact with the skin, evaporates the solvent, leaving behind a film of excipients and the drug. The generated film might be a solid polymeric material that acts as a matrix for prolonged drug

release into the skin or a residual liquid film that is swiftly absorbed in the stratum corneum [10].

1.1. Skin [11]

The skin encases the body and is interconnected by membranes that line the orifices of the body.

- It protects the underlying structures from damage and microbial invasion.
- It has sensory (somatic) nerve endings for pain, temperature, and touch.
- It helps to regulate body temperature.

1.1.1. Structure of the skin

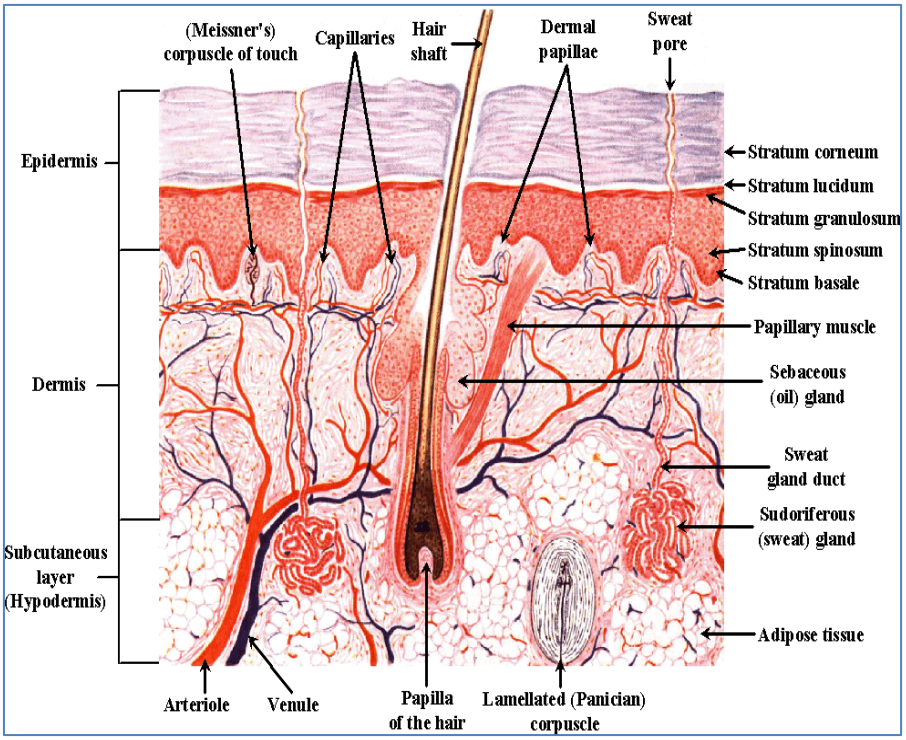


FIG.1: STRUCTURE OF SKIN

The skin is the human body's greatest organ, accounting for around 15% of total adult body weight. The human skin is one of the easiest parts of the body to administer topical medications. Molecules primarily penetrate the skin through three pathways: the intact stratum corneum, the sebaceous follicle, as well as the sweat ducts. The topical drug delivery approach is used to administer drugs to specific areas of the body via the skin, ophthalmic, rectal, and vaginal route.

Skin provides various critical functions, including:

- Physical, biological, and chemical protection
- Prevention of excess water loss from the body
- Plays an important role in thermoregulation

- Drugs can be destroyed by skin enzymes

The 3 components of skin are the epidermis, dermis, and subcutaneous tissue. Every cm^2 of human skin has 40-70 hair follicles and 200-300 sweat ducts. The skin's pH fluctuates from 4-5.6. The typical adult's skin has a surface area of about $2m^2$and receives roughly one-third of the blood circulating through the body.

a) Epidermis

It is a stratified squamous epithelial layer composed of dendritic and keratinocyte cells. Melanocytes, Merkel cells, and Langerhans cells are also found in the epidermal layer. Keratinocytes, on the other hand, make up the vast majority of cells.

- Stratum germinativum (base layer, sometimes referred to as the rowing layer): It has columnar keratinocytes with a long axis perpendicular to the dermis that adhere towards the basement membrane zone.
- The stratum spinosum (prickly cell layer or squamous cell layer) has a thickness of 5 to 10 cells. To increase epidermal cell coupling and tolerance to physical pressures, abundant desmosomes (adhering spot) are bridged in cross intercellular spaces between spinous cells.
- Stratum granulosum (granular layer): This layer is composed of live cells that are responsible for the continued synthesis and modification of the proteins involved in keratinization. It has 1-3 cells of layer thickness.
- Stratum corneum (horny layer): Corneocytes are wrapped by a continuous extracellular lipid matrix, which is protein-rich but low in lipid content (owing to their hydrophilic nature).
- Malpighian layer (pigment layer): This is the layer where the protoplasm has not yet converted into horny material.

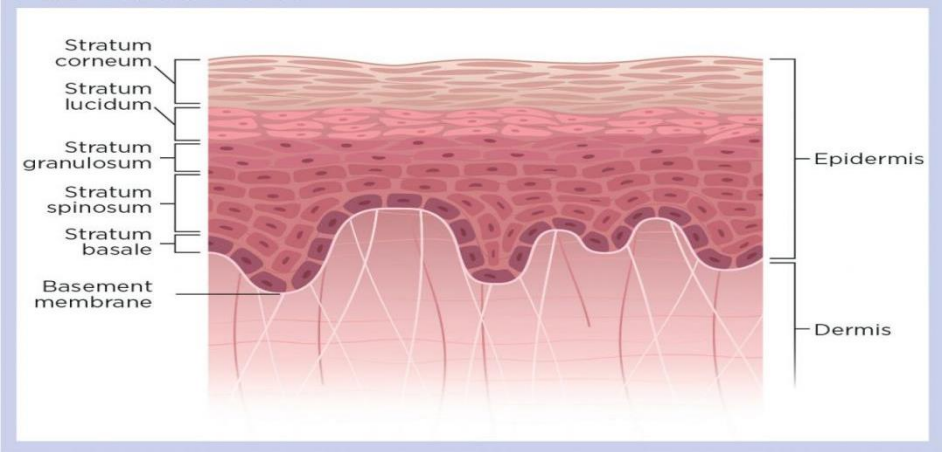


FIG.2: STRUCTURE OF SKIN LAYERS

b) Dermis

It has a thickness of 1.5-4 mm and is located beneath the epidermis (thickest of the three layers of the skin). The majority of the skin's structures, comprises of sweat and oil glands, hair follicles, nerve endings, blood and lymph vessels. The dermis is made up primarily of collagen and elastin. It holds a significant amount of the body's water supply. The dermis also contains scavenger cells from the immune system. If a foreign organism attempts to enter through the epidermis, these cells will both infiltrate and destroy it. It is an integrated system of fibrous, amorphous, and filamentous connective tissue that permits neurons, circulatory networks, fibroblasts, appendages, and mast cells to enter in response to stimuli. It has a thickness ranging from 2000 to 3000 m. Collagen is the major dermal component, accounting for 70% of the skin's dry weight.

c) Subcutaneous Layer (Connective Tissue)

Even though fatty tissue can serve as a drug depot, the subcutaneous tissue, or hypodermis, is not considered an appropriate part of the structured connective tissue, which comprised of loose textured, fibrous, white connective tissue containing blood and lymph vessels, sweat gland skin reaches the circulatory system prior to actually reaching the hypodermis.

d) Blood and Lymph Vessels

Arterioles connect to capillary branches that nourish sweat glands, sebaceous glands, hair follicles, and the dermis. A network of lymph veins runs through the dermis.

e) Sweat Glands

Sensory receptors (specialised nerve ends) which is densely packed in the dermis, respond to touch, temperature, pressure, and pain. Incoming inputs activate many types of sensory receptors. The Pacinian corpuscle feels intense pressure. The skin is an important sensory organ that allows people to receive information about their environment. Nerve impulses are created in the dermal sensory receptors and then transported to the cerebrum's sensory area, where feelings are perceived.

1.1.2. Functions of the Skin [12]

a) Protection

The epithelium provides a quite water-resistant layer to the skin, which protects the deeper and more delicate components. As a key non-specific defense mechanism, it acts as a barrier against the following threats:

- Invasion of microorganisms
- Toxic substances
- Hypo hydration

- Agents of physical nature

Langerhans cells, a type of microphage, are specialized immune cells located in the epidermis. Because the skin contains sensory nerve endings, the body responds to unpleasant or painful stimuli with reflex action, preventing from further injury.

b) Body Temperature Regulation:

The body temperature maintains fairly consistent at roughly 36.8°C across a wide variety of external temperatures, ensuring that the optimal range for enzyme activity required for metabolism is maintained. Health variations are often confined to 0.5°C to 0.75°C, though they rise somewhat in the evening, during activity, and in women shortly after ovulation.

c) Heat Production:

When the metabolic rate increases, the body temperature increases; when it decreases, the body temperature decreases. Some of the energy lost to the environment during metabolic activity comes in the form of heat, which is produced by the body's most active organs. Skeletal muscle contraction generates a significant amount of heat, and the more severe the muscular exercise, the greater the amount of heat produced. Heat is created as a byproduct of the liver's metabolic activity. The metabolic rate and heat generation increase after eating. Heat is generated by the digestive organs as a result of peristalsis and the chemical reactions that occur during digestion.

d) Heat loss:

The majority of heat loss from the body is caused by the skin. Only heat loss through the skin is under your control. The heat loss via the outside pathways is unregulated. The difference in temperature between body and the environment, the quantity of body surface exposed, and the type of clothes worn, all influence heat loss via the skin. Air insulates against heat loss when trapped between layers of clothing and between the skin and clothing. As a result, numerous layers of light-weight clothes provide more effective cold-weather insulation than a single heavy garment.

e) Drug Transport across Skin

The epidermis and dermis are the two primary layers of the skin. Blood vessels are thickly packed in the skin's subcutaneous layer. The two basic pathways for medication absorption through the skin are as follows:

• Intercellular

• Trans-cellular

The second most prevalent way of delivery is the pilosebaceous channel. Permeation is normally accomplished via the intercellular matrix, although it has been established that the

trans-cellular channel provides a faster alternative route for highly polar compounds.

The keratinized corneocytes and the horny layer's vast non-polar lipid intercellular cement are regarded to be the fundamental components involved in the preservation of efficient drug barriers in normal undamaged skin. Organic solvents like propylene glycol, Di Methyl Sulphoxides (DMSO), and surfactants can aid in drug penetration through the skin.

Permeation enhancers alter the barrier qualities of the stratum corneum by a variety of methods, including enhancing solubility, partitioning the stratum corneum, and fluidizing the crystalline structure of the stratum corneum.

Because of technological improvements, several medications can now be absorbed through the skin. These can be utilized to treat not only the afflicted skin areas, but also the entire body via a systemic way. As indicated by approximately identical rates of chemical penetration through the isolated stratum corneum and the entire skin, the barrier is located in the outer layer of the epidermis and the stratum corneum.

1.2. MECHANISM OF FILM FORMATION AND PERMEATION

As shown in Fig. 3, the film forming system is applied directly to the skin and creates a thin, transparent film in situ upon solvent evaporation. The composition of the film producing system changes dramatically after application to the skin due to the loss of the volatile components of the vehicle, resulting in the creation of residual film on the skin surface. During this process, the concentration of the medication increases, reaching saturation and maybe super saturation on the skin's surface. By enhancing the thermodynamic activity of the formulation without compromising the skin's barrier, super saturation results in increased drug flux through the skin, decreasing side effects or irritation [12, 13].

The concept of super saturation can be explained by the modified version of Fick's law of diffusion. Fick's law of diffusion is given by Eq.

$$J = \frac{DKC_v}{h}$$

Where

J = rate of drug permeation per unit area of skin per unit time (flux)

D = diffusion coefficient of drug

C_v= concentration of drug

h = thickness of barrier to diffusion

This equation shows that the rate of drug penetration over the skin is proportional to the drug concentration. This is true, however, when the full drug is dispersed in the vehicle.

Modified form of Fick's law of diffusion:

$$J = \frac{\alpha D}{\gamma H}$$

Where

α = thermodynamic activity of drug within formulation

γ = thermodynamic activity of drug within membrane. According to this equation, the drug flux is exactly proportional to the system's thermodynamic activity, which is connected to saturation. Increasing the super saturation, on the other hand, promotes thermodynamic instability [14].

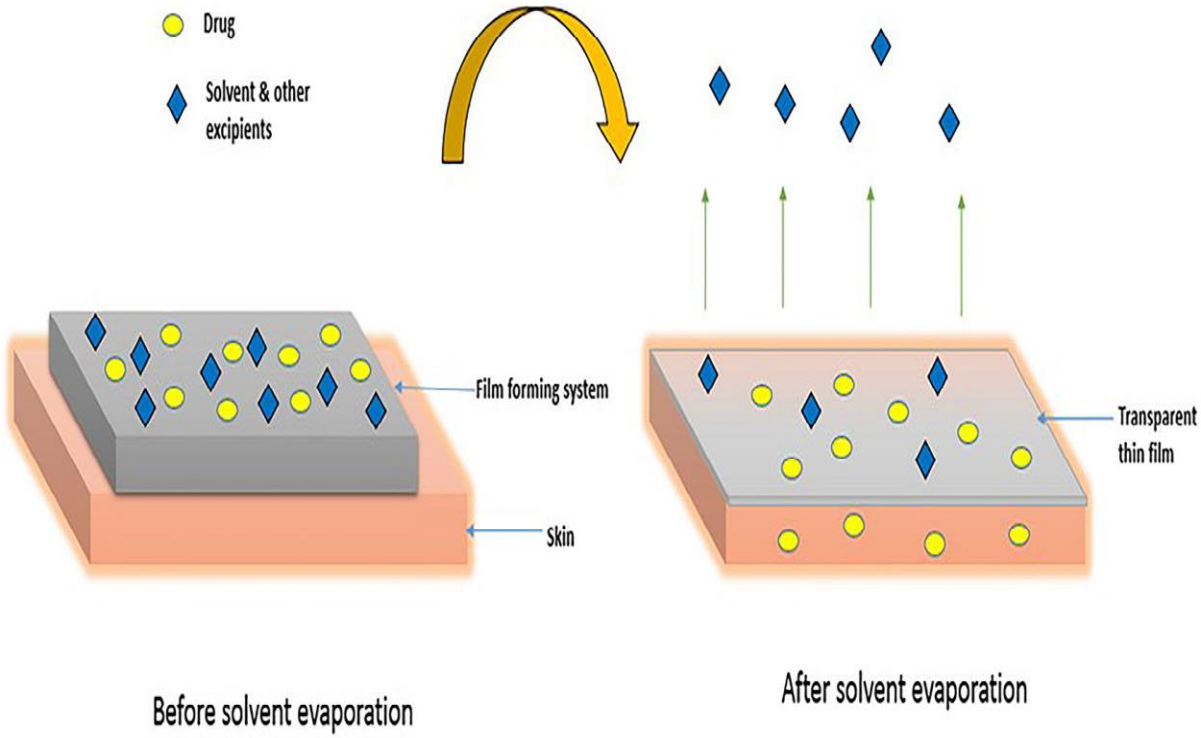


FIG.3: MECHANISM OF FILM FORMATION

FFS produces supersaturated solutions immediately after application to the skin, hence solving the issue of instability. As a result, it improves medication penetration through the skin as compared to other transdermal dosing forms. The distribution efficiency of ethinylestradiol film forming solutions was examined. *In vitro* ethinylestradiol permeation from the film forming solution with or without enhancer was compared to the penetration from the commercially available patch (EVRA®) through human epidermis. The penetration of the film producing formulations was greater than that of the commercial patch. Without the enhancer, the formulation delivered more than twice as much ethinylestradiol as the

commercial patch. With enhancer, the formulation supplied approximately seven times the amount of ethinylestradiol as the commercial patch. As a result, these systems are beneficial in terms of increasing drug permeability [15].

1.3. COMPARISON OF TOPICAL DRUG DELIVERY SYSTEMS

The comparison profiles are shown in Table 1.

TABLE 1: COMPARISON OF TOPICAL DRUG DELIVERY SYSTEMS

PROPERTIES	PATCHES	FILM FORMING SYSTEMS	SEMISOLIDS
Visual appearance	Highly visible	Visible	Visible
Skin feel	Non sticky, non greasy	Non- sticky, non - greasy	Sometimes sticky and greasy
Administration	Convenient	Convenient	Sometimes messy
Dose adjustment	Low	High	High
Dosing frequency	1 – 7 d	1- 2 d	1d or less
Sustained release	Yes	Yes	No
Occlusive properties	Yes	No	No
Wipe of resistance	Yes	Yes	No
Residual remains	Possible	No	No

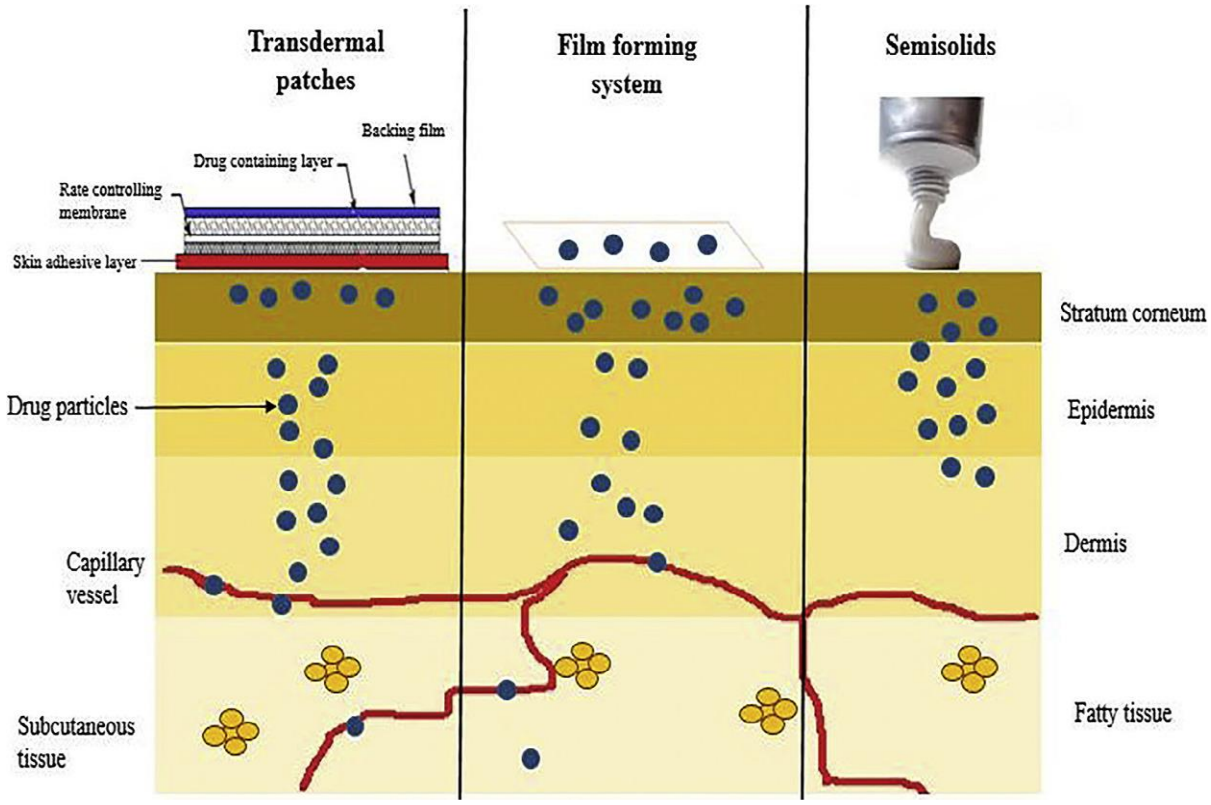


FIG.4: RELEASE PROFILE OF TOPICAL AND TRANSDERMAL DRUG DELIVERY SYSTEMS

1.4. APPLICATION OF FILM FORMING SYSTEMS:

Initially, film forming technologies were mostly utilised in surgery or wound care. Tissue glues made from film-forming solutions or gels have been used to close surgical incisions. Film formers used for this purpose can be natural, such as fibrin, or synthetic, such as cyanoacrylates. These wound care treatments can be drug-free or contain antibacterial medicines to prevent wound infections [16]. It can also be utilised for non-medical purposes, such as the delivery of active chemicals in beauty products, such as silicone film forming technology used in the preparation of cosmetic creams and ointments [17].It can also be utilised as a transparent peel off mask technology for skin nourishment, acne treatment, and other purposes [18]. The film forming process may also be employed as a substrate for various barrier membranes used in industry. Barrier membranes are frequently used to protect workers from detergents, acids, bases, as well as other hazardous chemicals, infrared heat, UV radiation, and other hazards, such as hydrophilic and hydrophobic lotions and ointments [19]. Film-forming polymers are sprayed on the soil, generating a membrane film and enhancing soil integrity and temperature, which is important in crop protection [20].

1.5. FILM FORMING HYDROGELS

Gels are semisolid dosage forms that comprise both solid and liquid components. The liquid component, which is immobilised in a three-dimensional network of interconnected solid components, can be hydrophobic or hydrophilic in nature [21]. Aqueous gels comprising hydrophilic polymers that form three-dimensional networks in water are known as hydrogels [22]. Film forming gel is applied to the arms, shoulders, interior portions of the thighs, or abdomen to generate a thin bio adhesive film on the skin [23]. The drug material is dissolved in the film producing vehicle and hence integrated into the skin film. The film can act as an external reservoir or limit the delivery of drug substance to the skin, managing drug release [24]. Complete skin contact throughout the application is required; thus, the formulation requires high flexibility to respond to skin movement, as well as high substantively, strong adherence to the skin for consistent drug administration and absorption. As a result, along with gelling agents, film forming agents, plasticizers, preservatives, and other ingredients, it is utilised in the formulation. In comparison to previous forms, these systems are easier to use and apply, have a good consistency and adhesiveness, good flexibility and elasticity, and are easy to manufacture [25]. Hydrogels that form films are commonly utilised in wound healing. The formulation applied to the point of injury provides a film that is resistant to physiological stress induced by the mobility of skin.

Hydrogels are three-dimensional network structures composed of natural and synthetic polymers. Because of their porous structure, they may absorb and transmit a considerable amount of water [26]. Hydrogels are made up of polymeric matrices that swell in water but do not dissolve [27]. Hydrogels can have a water content of up to 99%.

Hydrogels are typically dried and have the ability to absorb water in the following ways:

- Primary Bound Water: Primary bound water is created when a dry hydrogel engrosses water via hydrophilic group attachment.
- Secondary Bound Water: When the hydrogels consume more water after the primary bound water, it requires extra hydrophilic group interaction and is referred to as secondary bound water.
- Total Bound Water: Total bound water was the product of the combination of primary and secondary bound water.
- Free Water: After all of the other classes have been soaked, the remaining water absorption is referred to as free water, which takes up all or most of the available space [28].

Hydrogels activate natural living tissue more than practically any other type of synthetic biomaterial due to their high moisture content, porosity, and smooth texture. Hydrogels may be chemically stable or deteriorate and dissolve with time [29].

Hydrogel's ability to hold water is due to the hydrophilic functional group connected to the polymeric backbone. Only a few tiny molecules, such as oxygen, metabolites, and nutrients, may travel through the hydrogel structure due to the high moisture penetration. Because of their ability to hold water, hydrogels are attractive candidates for use in controlled dosage forms. Adequate design and experimental methodologies can yield benefits for the treatment of diseases needing selective medication delivery. Hydrogels are particularly essential due to their high drug affinity and stability. Light, temperature, magnetic field, electrical impulses, pH, and ionic strength are some of the stimuli used to induce drug release from hydrogel networks. They can be administered orally, nasally, ocularly, subcutaneously, or transdermally, among other methods.

Hydrogels are now widely used as wound dressings. Hydrogel is an excellent candidate for topical application due to its distinct and substantial features such as cooling, cushioning, and clarity. Hydrogels' high water content adds greatly to skin elasticity and moisturization [30].

Hydrogels of various sorts have been produced and evaluated for biomedical field as wound healing materials [31]. They have the ability to repair and regenerate damaged tissue due to their high water holding capacity, biocompatibility, compressive modulus, strong elasticity, near structure to biological tissue, non-adhesion with soft tissue, and regulated biodegradability [32]. The hydrogel's water absorbing ability was confirmed to solve the problem of ablation and bloating of healing wounds, and also maintaining a moist environment all around wounds for better vascularisation [33].

Hydrogels are easy to apply and adaptable for wound healing, having a pain-relieving impact on the injured tissue. Hydrogels, due to their high moisture content, lower wound temperature and calm the wounded area [34]. The usage of hydrogel, in particular, is beneficial in the treatment of dry wounds. Hydrogels can be used to treat wounds at all phases of wound healing, including inflammation, cell proliferation, maturation, and homeostasis. They are metabolite permeable, non-reactive, as well as non-irritant towards biological tissue [35]. The various varieties of hydrogel dressing are best suited for wound treatments given to a specific wound. These distinguishing features of hydrogels impede their rapid adoption in the dressing sector, where hydrogels are offered as soaked gauze, gels, or sheets [36].

1.6. COMPONENTS OF FILM FORMING SYSTEMS

- **Drug**

The medications must have acceptable qualities that are independent of the dose form for use in film forming systems. In general, medications that are relevant to these systems are highly powerful, infiltrate the skin quickly, produce no skin irritation, and are reasonably stable to the enzymes found in the epidermis. Other pharmacological qualities, such as the partition coefficient, determine the route a drug will go through the skin. Second, the drug's molecular weight is a crucial element in drug penetration since small molecules pass human skin more easily than large molecules.

- **Polymers**

Polymers constitute the FFS's base, and a wide range of polymers are accessible for their preparation. These polymers can be employed alone or in conjunction with other film forming polymers to produce the necessary film characteristics [37]. At skin temperature, these polymers should create a transparent, flexible film. The list of polymers along with their molecular weight and properties are mentioned below Table 2.

TABLE 2: LIST OF POLYMERS

POLYMER	APPLICATIONS
Chitosan	• Excellent film forming ability • Opens the tight junctions of mucosal membrane, thereby enhancing the paracellular permeability and penetration of drug [38] • Controls drug release [39]
Hyaluronic acid	• It is used in the preparation of hydrogels and has the benefit of delayed drug release and a longer time of action [40].
Carrageenan	• It has an advantage of controlled drug release and tissue engineering applications [41].
Alginic acid	• Used as co – transplantation on rat spinal injury [42].
Collagen	• It has the potential to regulate drug release from hydrogels as well as stimulate cell

	development [43].
Gelatin	• The dressing, which is dependent on sodium alginate and gelatin, was created by cross-linking sodium chloride and glutaraldehyde [44].
Polyethylene glycol	• Capable of forming controlled gelation as a result of a protoinitiator or by combining with cross linkers used in the enzyme immobilization [45].
Polyvinyl pyrrolidone	• Solubility in water and other solvents • Adhesive and binding property • Acts as a bioavailability enhancer [46]
Polyvinyl alcohol	• Water soluble • Excellent film forming and adhesive properties • Nontoxic and biocompatible [47]
Polycaprolactone	• Used in drug delivery and tissue engineering.
Eudragit	• Transparent, elastic, self-adhesive • Good adhesion to the skin [48]
Hydroxypropyl cellulose	• Nonionic, pH insensitive polymer • Water soluble [49]
Hydroxypropylmethyl cellulose	• Produce a light, non-greasy uniform film with good texture • Do not interact significantly with other ingredients • Comfort feel in occlusive state on application to skin [50]
Ethyl cellulose	• Nontoxic, non irritating, non allergic material • Good film forming properties that form tougher films [51]

- **Solvents**

Solvents play a crucial role in film formation. The solvent employed in film forming systems aids in drug solubilisation and has an effect on drug permeability [52]. Solvents widely used for topical and transdermal applications are:

Eg:

Glycols: Propylene glycols, polyethylene glycols

Alcohols: Ethanol, butanol, isopropanol, benzyl alcohol, lanolin alcohols, fatty alcohols

Other solvents: Ethyl acetate, oleic acid, isopropyl myristate

- **Plasticizers**

Plasticizers are used in film forming systems to make the film more flexible and to increase the tensile strength of the formed film. The plasticizer must be compatible with the polymers and also have low skin permeability. Plasticizers that are commonly utilised include glycerine, polyethylene glycol, sorbitol, dibutyl phthalate, propylene glycol, triethyl citrate, and many others [53].

1.7. TOPICAL APPLICATION FOR WOUND HEALING:

Topical drug delivery is one of the easiest ways of administration, and it is used to eliminate side effects and localize large amounts of medicine at the target site. Because of their low toxicity and long-term drug release, hydrogels are regarded good carriers for topical drug delivery. Furthermore, hydrogels feature biocompatibility, smoothness, and water-holding capacity, which can mimic natural tissue qualities and, due to their swelling and hydrating capacity, can avoid pain to enclosed tissues. Another important advantage of hydrogels is their capacity to protect drugs from extreme environmental conditions [54].

A wound is defined as any sort of skin damage or breakdown produced by a medical, physiological condition, or trauma [56].

When an injured or wounded tissue is entirely healed, it is returned to its previous or natural anatomical form, structure, and function within an acceptable time frame. Wounds are divided into the following groups based on the number of skin layers and the defective skin component.

- Superficial wounds: Epidermis is affected.
- Partial thickness wounds include the epidermal as well as deeper dermal layers.
- Full thickness wounds contain deeper tissues including subcutaneous layers.

Wounds are also classified as:

a. Acute wounds

b. Chronic wounds

c. Complicated wound

Most wounds heal on their own thus treatment for these wounds are limited to protecting them from the environment. However, for nonhealing wounds, a more proactive healing-promoting treatment is required. As a result, wound care is determined by the type of wound as well as the objective of therapy. While acute and nonhealing wounds are classed based on time to healing, burn wounds are classified based on the extent of the burn. Only the epidermis is damaged in a superficial burn, the epidermis and papillary dermis is damaged in a superficial partial thickness burn, while the reticular dermis in a deep partial-thickness burn, and the subcutaneous fat in a full-thickness burn. Cleaning and debridement of the site, as well as antibiotics if necessary, are the basic treatments for superficial burns. Deep partial-thickness and full-thickness burn wounds are typically treated with topical antibacterial treatments, but for larger areas, skin replacements or skin grafts such as autologous split thickness graft are the standard method around the world. The current standard of therapy for nonhealing wounds involves frequent dressing changes, wound debridement, medications to combat infections, and avoidance of increasing pressure at the wounded location.

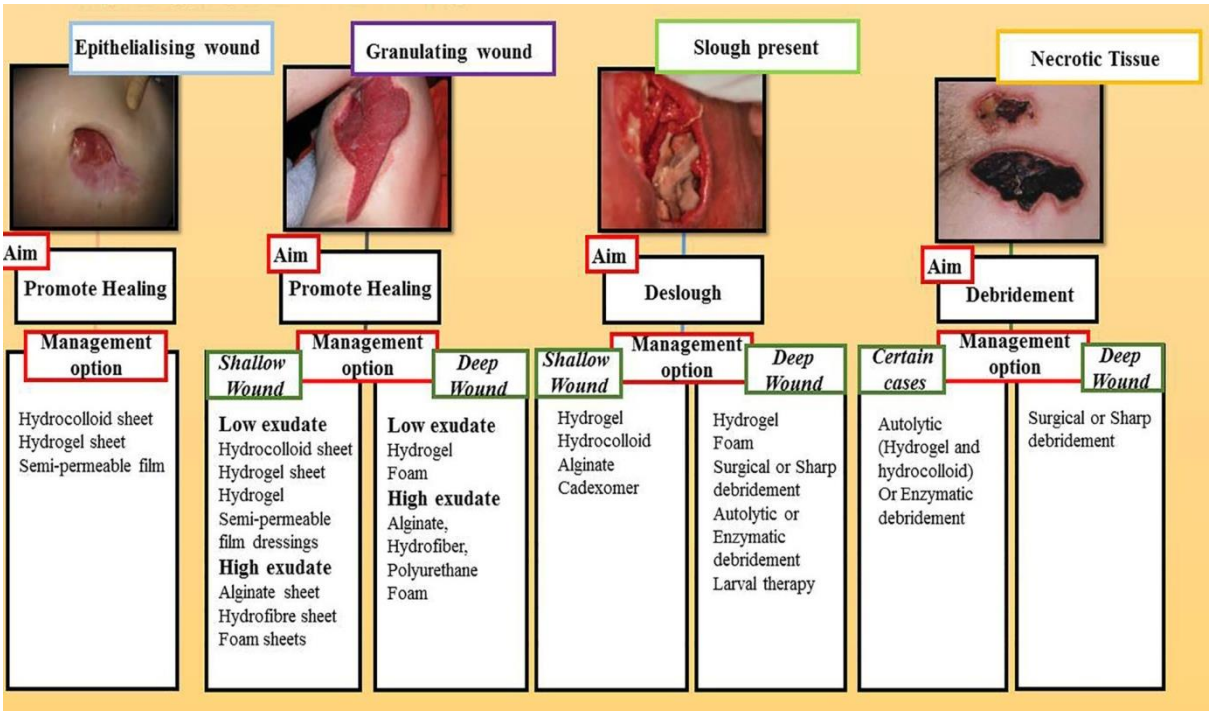
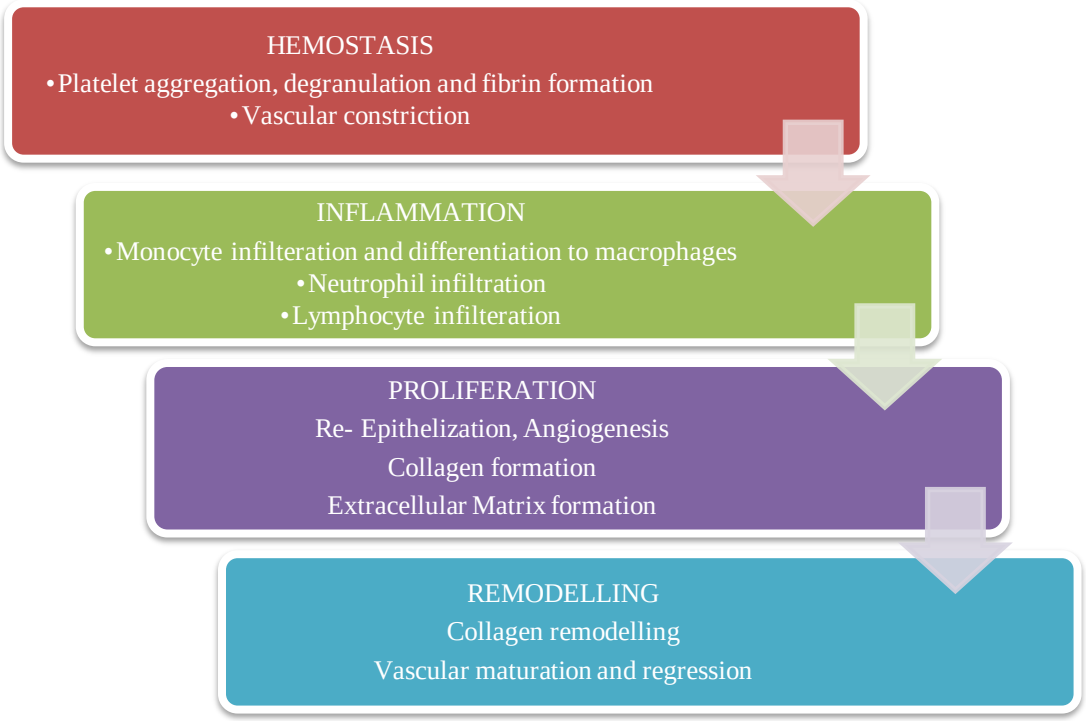


FIG.5: THE DIFFERENT LEVELS OF WOUND CARE APPLIED FOR DISTINCT WOUND CATEGORIES.

1.7.1. Wound healing process: [56]



- **Haemostasis phase:** The fibrin clot forms the provisional wound matrix at the time of damage, and platelets release several growth factors that commence the repair process.

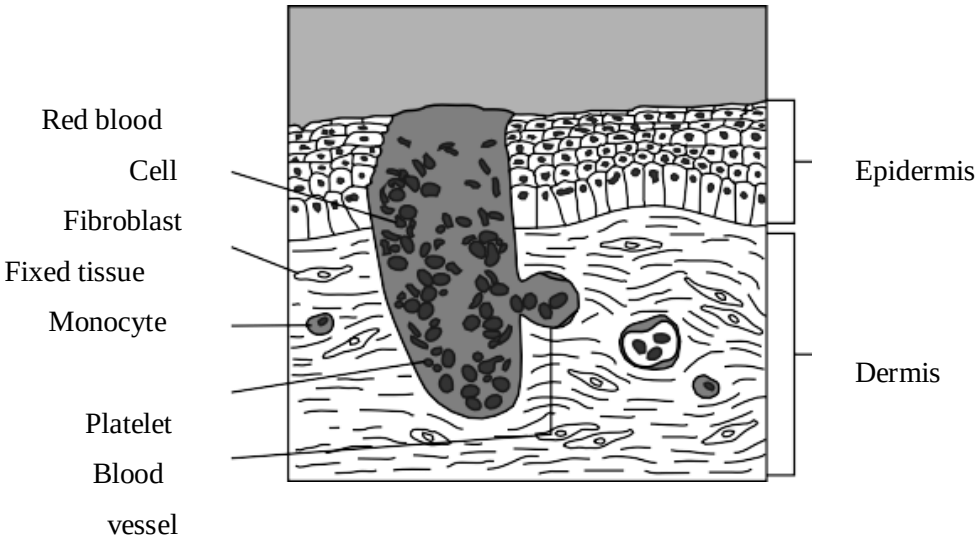


FIG. 6: HAEMOSTASIS PHASE

- **Inflammation phase**: Within a day of injury, neutrophils connect to endothelial cells in the vessel walls surrounding the wound (margination), change the shape and travel through cell junctions (diapedesis), then migrate towards the wound site (chemotaxis)

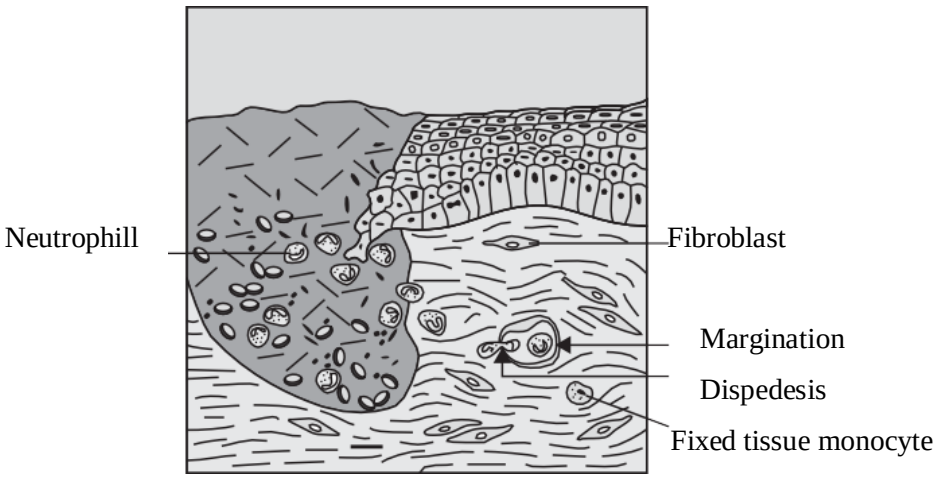


FIG 7: INFLAMMATION PHASE

- **Proliferation phase:** Fixed tissue monocytes become activated and migrate to the site of injury. They stimulate wound macrophages, which destroy germs and release proteases to remove denatured extracellular matrix. They also secrete growth factors, which encourage the proliferation of fibroblasts, epidermal cells, and endothelial cells, as well as the formation of scar tissue.

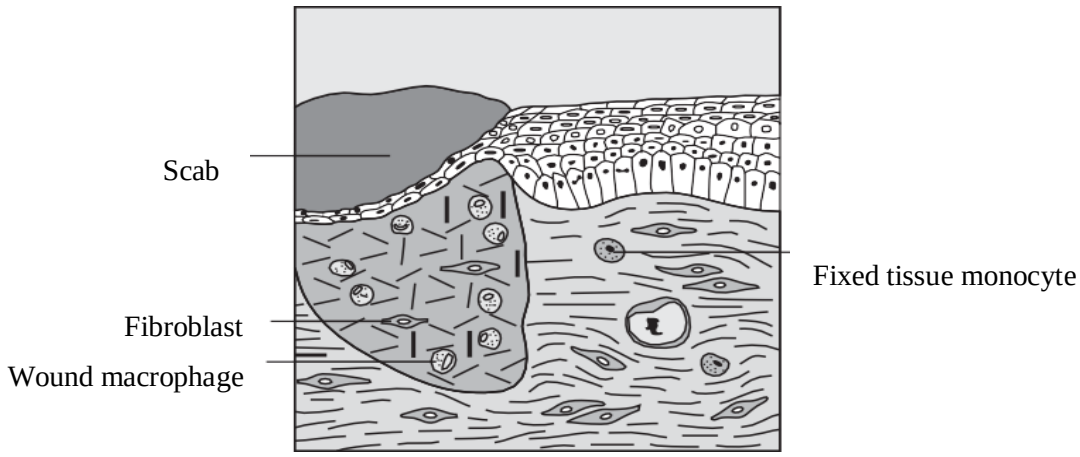


FIG 8: PROLIFERATIVE PHASE

- **Remodelling phase:** This initial, disorganised scar tissue is gradually replaced by a matrix that more closely resembles normal skin's organised extracellular matrix.

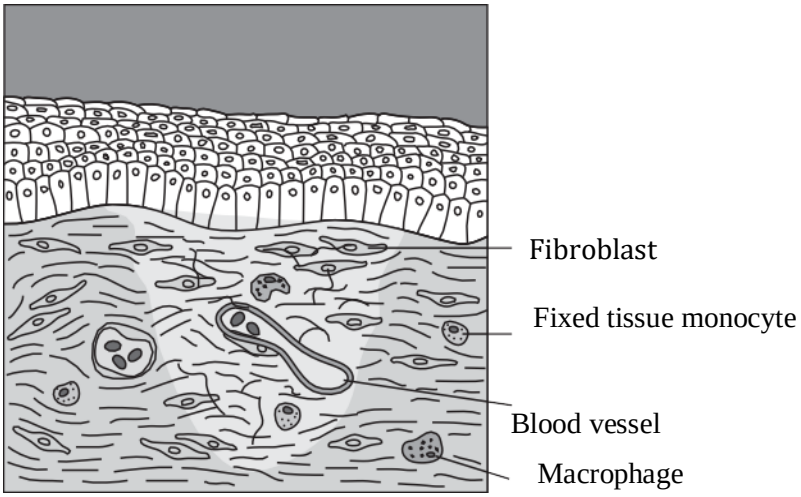


FIG 9: REMODELLING PHASE

Wound healing is a four-step process with four overarching, specified, and automated phases. Each process must be regulated in a precise and efficient manner. Any disruption or continuation of these processes may cause the wound to heal more slowly, and there is a danger that the wound will become chronic [56].

The majority of wound dressings are polymers in the form of gauzes, gels, hydrogels, hydrocolloids, and so on. Hydrogels have proven to be the most effective wound healing treatment. Hydrogels are suitable wound dressings because they can create a moist environment at the wound site, aid in the removal of wound exudates, prevent infection, and promote tissue regeneration [57]. A wound is an injury to human tissues that happens either inside or outside of the body, primarily in the skin, as a result of a fracture, cut, or other damage. As a result of the wound, normal tissue structure and function can be altered [58].

Wounds are classified into two types: acute and chronic. Acute wounds heal completely in a shorter period of time, whereas chronic wounds take longer. Wound healing involves hemostasis, inflammation, replication, and remodeling [59]. Wound infection, wound depth, foreign body contact, stress, strain, age, illnesses, and other factors all influence wound healing. Scarring formed during natural healing as a result of collagen fibre deposition, and it will last for a long period. Scarless healing occurs with the assistance of wound dressings [60].

In wound dressing applications, anti-bacterial and anti-inflammatory hydrogels have a favourable effect [61]. A versatile wound healing and pH-responsive hybrid hydrogel consisting of carboxylated agarose and tannic acid that is ionically crosslinked with zinc salts. Tannic acid's antimicrobial, anti-inflammatory, and anti-oxidant characteristics, along with carboxylated cellulose's pH-responsive feature, resulted in increased strength and anti-bacterial activity [62]. Another polysaccharide utilized in the creation of hydrogel is dextran. It aids in situ gelation and regulated release of immobilized growth factor via chitosan microparticles, which enhances wound healing *in vivo* [63].

Chitosan is an excellent wound healing substance due to its haemostatic characteristics. A physically cross-linked chitosan hydrogel can repair the skin of a pig with third-degree burns in the dorsal region [64]. An injectable hydrogel was created using a disulphide bond cross-linking of thiolated polyethylene glycol and silver nitrate. This hydrogel contains desferrioxamine, an angiogenic medication that, due to its angiogenic action, has the potential to cure diabetic wounds. To aid in the prevention of wound-related illnesses and scarring, a wide range of hydrogel-related wound care treatments are commercially available [65]. Amorphous gel, hydrogel pads, hydrogel film, and hydrogel impregnated gauze are some of the different types of hydrogel wound treatment currently on the market [66]. This hydrogel aids wound healing by promoting autolytic debridement and moisture distribution to the wound site. The humid environment encourages wound granulation and epithelisation. The main advantages are that it relieves wound pain and is simple to apply. It is used to overlay hydrogel in conjunction with secondary film dressing [67].

Wound healing is a biological process that restores the structure and function of injured skin to its natural state. Wound dressing compositions must serve the following functions for desirable wound care: (a) provision of moisture and occlusion, (b) protection from pollutants and infections, and (c) ease of application and withdrawal with the avoidance of dressing-related stress. Many hydrophilic polymers, such as polyvinylalcohol (PVA), chitosan, sodium carboxymethyl cellulose, and sodium alginate, have been used to construct wound dressing formulations, resulting in their great performance in wound treatment. However, wound dressing formulations have drawbacks such as a difficult manufacturing process and administration. The film-forming hydrogel (FFH) is a hydrogel dosage form that, following administration to the wound site, transforms from hydrogel to film-type by solvent evaporation. This formulation combines the benefits of both hydrogel and film formulations. When compared to other wound dressing forms, it is easier to use and apply, as well as

simpler to make. Furthermore, even if the wound is curved or contoured, the FFH system can be flexibly applied to any wound location.

Chitosan, a polysaccharide produced from chitin that is composed of copolymers of glucosamine and N-acetylglucosamine, was used as the film forming agent in this study. Chitosan was chosen for its outstanding biocompatibility and biodegradability. Because of its haemostatic, antibacterial, and healing stimulating qualities, chitosan has also been utilized as a topical dressing or hydrogel for wound healing. Chitosan mode of action in tissue engineering has been reported to be simulation of polymorphonuclear and mononuclear cell migration, along with stimulation of re-epithelization and normal skin regeneration. Based on this knowledge, it is believed that combining Papain Urea and chitosan, both of which have similar properties, in tissue repair can speed up recovery.

Papain is a nonspecific cysteine protease generated from the *Carica papaya* fruit that is capable of degrading a wide range of necrotic tissue substrates. Papain, a proteolytic enzyme derived from *Carica papaya* fruit, has a strong affinity for denatured (nonviable) protein. Simultaneously, clinical and laboratory research have revealed that the enzyme has no negative effects on the live tissue surrounding the lesion. When Papain and urea are mixed, they create a powerful debriding agent. Papain is a nonselective protein that degrades any protein containing cysteine residues (which are present in most proteins, including growth factors). For centuries, Papain-urea formulations have been utilized in clinical settings, particularly for pressure ulcers, and current evidence indicates that these debriding systems are successful when administered properly. Papain is active at pH levels ranging from 3 to 12. It is ineffectual as a debriding agent when used alone and requires the addition of activators to boost its digestive effectiveness. The combination of Papain and urea stimulates two additional chemical reactions. First, it exposes the Papain activators via solvent action. Second, it denatures nonviable protein content in lesions, making it more amenable to enzymatic breakdown. In pharmacologic tests, the combination of Papain and urea resulted in twice as much digestive action as Papain alone.

Enzymatic debridement is a highly selective and active technique of debridement that is widely utilized in conjunction with a variety of other therapies, most commonly following sharp debridement in conjunction with moisture balancing dressings. It is a topical treatment that makes use of naturally occurring proteolytic enzymes or proteinases, which are necessary for wound healing. Proteinase activity in chronic wounds benefits not just debridement and also more fundamental features of cell motility essential for epithelialization. However, the specific proteases available for clinical use are determined by the location of the practitioner.

In theory, topical enzymes were utilized in this therapy to eliminate necrotic tissue in the wound site by digesting and dissolving the damaged tissue.

As a result, in this study, multiple FFH formulations are created using chitosan in order to generate a novel Papain urea - loaded film-forming hydrogel (FFH) for effective wound healing, and their film-forming times, drug release, and *in vitro* wound healing activities are assessed [68].

1.8. ADVANTAGES OF FILM FORMING DRUG DELIVERY SYSTEMS

- Avoidance of first pass metabolism
- Convenient and simple to use
- Avoidance of consequences and inconveniences of intravenous therapy and of diverse conditions of absorption such as pH changes, the presence of enzymes, and gastric emptying time
- Easily discontinue medications when needed
- Deliver drug more selectively to a targeted place
- Prevention of gastro-intestinal incompatibility
- Improved patient compliance
- Make it feasible for self-medication.
- Continuous drug input allows for greater efficacy with a lower total daily dose of medicine.
- Providing medicines with a short biological half-life and a small therapeutic window for use
- Avoid drug level fluctuations, as well as inter- and intra-patient variances.
- The ability to administer medications to a specific site more selectively.

1.9. DISADVANTAGES OF FILM FORMING DRUG DELIVERY SYSTEMS

- Skin irritation or dermatitis might occur as a result of the drug or excipients.
- Some medications have poor skin permeability.
- Drugs with larger particle sizes cannot be effectively absorbed through the skin.
- Allergic responses are possible.

LITERATURE SURVEY

2. LITERATURE SURVEY:

Shima Tavakoli *et al.*, 2020[69] the most current discoveries in the field of hydrogel-based skin replacements for skin replacement were reviewed. In recent years, tremendous progress has been achieved in the field of skin tissue engineering to generate new skin substitutes. Because of their porous and hydrated molecular structure, hydrogels are one of the possibilities with the greatest potential to imitate the original skin microenvironment. They can be used as a permanent or temporary dressing for various wounds to help the wounded epidermis, dermis, or both regenerate and heal. Hydrogels are classified into two types based on the substance used to make them: natural and synthetic. Furthermore, by adding nanoparticles into hydrogels, "*in situ*" hybrid hydrogels with improved characteristics and customised functionality can be obtained.

Sibusiso Alven *et al.*, 2020[70] Hydrogels made from two biopolymers—chitosan and cellulose for enhanced wound care were reviewed. Wound care remains a difficulty around the world, despite the fact that numerous wound dressing materials have been developed for the treatment of acute and chronic wounds. Hydrogels, films, wafers, nanofibers, foams, topical formulations, transdermal patches, sponges, and bandages are among the wound dressings currently in use. Hydrogels have special properties that make them acceptable wound dressings, such as providing a moist environment for wound healing, having high moisture content, or forming a barrier against bacterial infections, and they are appropriate for the management of oozing and granulating wounds. Because of their non-toxic, biodegradable, and biocompatible qualities, biopolymers have been used in their development. Hydrogels were created by crosslinking biopolymers like cellulose and chitosan with chosen synthetic polymers, resulting in better mechanical, biological, and physicochemical properties. They were beneficial by hastening wound re-epithelialization and mimicking skin structure, encouraging skin regeneration. Antibacterial drugs were loaded into them to inhibit bacterial invasion of wounds.

S. Peers *et al.*, 2020[71] Chitosan hydrogels have been used in a variety of ways as drug delivery systems to control and/or delay the release of medications incorporated into these polymeric matrices. A large amount of study has been conducted on the sustainable and controlled distribution of medications. The capacity of hydrogels, which have a high water content, to provide local and delayed delivery has already been established for a wide range of medicinal chemicals and polymer types. Because of its unique features, chitosan, a polysaccharide, stands out as a first-choice polymer for the creation of hydrogels in biomedical, cosmetic, and health-related applications (as harmlessness, biodegradability

antimicrobial capacity and mucoadhesivity). Furthermore, chitosan facilitates drug passage via biological barriers.

Thao T. D. Tran *et al.*, 2019[72] reviewed recent breakthroughs in film-forming systems in order to offer the principles and methodologies of these systems as applied to controlled drug release Advancement in film-forming system advent a new generation of such systems. Despite the several techniques for transdermal drug administration that are available, patient compliance and drug targeting at the desired concentration remain issues for effective therapy. With the advantages of films and hydrogels combined, precise and efficient film-forming technologies hold considerable promise for managing drug delivery through the skin. In film-forming systems, the shortcomings of both systems (films and hydrogels) will be addressed. To modulate medication release via the skin, several approaches have been developed, including adjustments to film-forming polymers, plasticizers, additives, or maybe even model drugs in formulations.

Abeer H. Khasraghi *et al.*, 2019[73] Using a patient-friendly medication delivery strategy, researchers developed a lornoxicam topical film-forming gel for the treatment of rheumatic illnesses. Lornoxicam film-forming gels were created utilizing a polyvinyl alcohol and polyvinylpyrrolidone polymeric blend in various ratios, a plasticizer, polyethylene glycol 400 (PEG 400), ethanol, and water as solvents. The created film-forming gels and films generated following solvent evaporation were examined, and the effect of polymeric blend concentration and ratio, along with plasticizer and ethanol concentrations, was investigated. All of the created film-forming gels had acceptable qualities in terms of homogeneity, consistency, spreadability, and pH value. Variations in polymer, ethanol, and PEG 400 concentrations had a varying effect on drying time and drug release rate from film-forming gels. All gel formulations fit Higuchi's model, with Korsmeyer and Peppas release exponent values (n) in the 0.5850–0.9485 range, indicating a diffusion-controlled mechanism. The optimised formulation F5 was determined to have better characteristics than others, such as a drying time of 5 minutes and 55 seconds, a sustained release profile of 34.44 percent drug release after 6 hours, and fairly good results in other tests.

Nurcan Bektas *et al.*, 2019[74] Wound whether acute or chronic, are one of the most common health problems globally, and pharmaceutical medications or traditional remedies are frequently used to treat them. More research into wound care is being conducted at a quick pace. Vitexin is a phenolic molecule found in many medicinal plants that has a variety of pharmacological actions including anti-inflammatory, analgesic, and antioxidant properties. The current work aims to evaluate the wound healing efficacy of a chitosan-based gel

formulation with vitexin *in vivo* and *in vitro*. *In vitro* experiments were conducted using cytotoxicity and wound healing assays, whereas *in vivo* research were conducted using an excisional wound model. Tissues extracted from the wound area were histologically analysed. On the 7th, 14th, and 21stdays, the wound healing progress was monitored. When wound construction was examined, a chitosan-based gel formulation containing vitexin surpassed the control group. Histological investigations revealed that the vitexin formulation aided skin regeneration. The wound healing assay with NIH 3T3 and HaCaT cells revealed significant cell proliferation with vitexin/chitosan dispersion. Finally, both *in vivo* and *in vitro*, our test ingredient, a chitosan-based gel formulation containing vitexin, speeded up wound healing.

Zahra Shariatinia *et al.*, 2018[75] Chitosan (CS), the second most abundant natural polysaccharide after cellulose, has beneficial properties such as biocompatibility, nontoxicity, and biodegradability. In the human body, CS is degraded to harmless byproducts (amino sugars). Hydrogels are polymeric materials having three-dimensional networks that hold a large amount of water within their structures and are widely used in biomedical and environmental applications. Injectable hydrogels typically have functional groups that are sensitive to pH, temperature, or irradiation stimuli. Injectable scaffolds can be created *in situ* via a stimuli-responsive effect, overcoming the disadvantage of standard scaffolds, which require surgery to be implanted upon that desired tissue. The antimicrobial property of chitosan-based hydrogels will be discussed, as well as their applicability in controlled drug delivery/release systems, tissue engineering, injectable hydrogel synthesis, and water treatment (removal of heavy/toxic metals and colours). Furthermore, a molecular dynamics (MD) simulation was executed on the delivery of the anticancer chlorambucil (CB) drug utilising three silica filled polymeric nanocomposites based on chitosan (CS), polylactic acid (PLA), and polyethylene glycol (PEG), and it was demonstrated that among three drug delivery systems (DDSs), the CS nanocomposite was the most effectual DDS due to its lower drug diffusion was assessed for the CS system that lead to the most sustained/controlled drug delivery.

Kashmira Kathe *et al.*, 2017[76] the main types of film forming systems (sprays/solutions, gels, and emulsions) and their assessment parameters were reviewed. The skin is regarded as an essential channel of medication administration for both local and systemic effects. The efficiency of topical therapy is determined by the medication's physicochemical qualities, the patient's commitment to the treatment plan, and the system's capacity to cling to the skin during therapy in order to increase drug penetration through the skin barrier. Traditional

formulations for topical and dermatological drug administration have drawbacks such as poor skin adhesion, poor permeability, and decreased patient compliance. For the treatment of diseases of bodily tissues and wounds, the medicine must be kept at the treatment site for an extended period of time. Topical film forming systems are such evolving drug delivery methods that are intended for topical application to the skin and stick to the body, generating a thin transparent film and delivering active ingredients to human tissue. These are designed for skin application as a moisturizing or protective agent, as well as for local action or transdermal penetration of a systemic medication. Transparency is a notable property of this polymeric system that has a significant impact on patient acceptance.

Reham F. El-Kased *et al.*, 2017[77] Honey has been used to cure wounds since ancient times and continues to be used now. The current study intended to create a honey-based hydrogel and test its antibacterial and wound healing characteristics *in vitro* and *in vivo*. Three honey concentrations were combined with gelling agents, chitosan and carbopol 934, to create topical honey hydrogel compositions. The pH, spreadability, swelling index, in-vitro release, and antibacterial activity of the produced formulations were all examined. pH and spreadability were in the 4.3–6.8 and 5.7–8.6 cm ranges, respectively. In-vitro honey release from chitosan-based hydrogels was greater, with diffusional exponent 'n' 0.5 indicating Fickian diffusion process. Hydrogel formulas were tested for antibacterial activity *in-vitro* against *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, and Streptococcus pyogenes* using the Disc Diffusion antibiotic sensitivity test. The honey-chitosan hydrogel with 75% has the most antimicrobial effect. This solution was tested for *in-vivo* burn healing in mice using burn-induced wounds. In comparison to a commercial solution, the formulation was examined for burn healing and antibacterial activity.

Priyanka Chhabra *et al.*, 2017 [78]At the molecular level, the effectiveness of an innovative 5 % chitosan gel formulation in healing burn wounds was compared to traditional silver sulfadiazine (SSD) treatment. Various concentrations of chitosan gel were developed and optimised in terms of spreadability, extrudability, and viscosity. Burn wounds were inflicted on 18 rats divided into three groups of six. Group I acted as the control group, Group II received regular SSD ointment, and Group III received optimised chitosan gel for 16 days. The percentage of wound contraction was used to compare wound healing efficacy, and any differences in wound healing activity at the molecular level were determined by the amounts of hydroxyproline, hexosamine, collagen, and antioxidant analyses.The optimal test formulation was 5 % chitosan gel based on viscosity, spreadability, and extrudability. In comparison to the control and standard treatment groups, Group III animals treated with

chitosan gel demonstrated quicker wound contraction. At the completion of the 16th day, healing rates of 95.5 ± 2.4 %, 84.1± 4 2.1 %, and 34.9 ± 2.1 % were found in the cases of chitosan gel, standard, and control treatments, respectively. Topical administration of 5% chitosan gel boosted collagen synthesis & stability at the wound site, as demonstrated by higher hydroxyproline and hexosamine levels and up-regulated collagen Type I expression. In addition, there was a considerable rise in endogenous enzymatic and nonenzymatic antioxidant levels, as well as a decrease in lipid peroxide levels, in chitosan gel treated burn wound granulation tissue. Histological investigation further revealed that the 5 % chitosan gel had superior healing efficiency when compared to standard approach. In compared to existing treatment options, the results show that 5% chitosan gel has the potential for the development as an efficient burn wound healing agent.

Dong-Won Oh *et al.*, 2017[79] It was reported that a chitosan-based film forming gel containing ketoprofen was successfully prepared, with outstanding mechanical properties, skin penetration, and anti-inflammatory and analgesic effects. The film forming gel, which attached to skin surfaces and created a film upon application, has a benefit onto skin in that it provides protection and continuous drug release to the application site. The purpose of this work was to create a chitosan-based film forming gel containing ketoprofen (CbFG) and to assess the CbFG and film from CbFG (CbFG-film). Chitosan, lactic acid, and other skin permeability enhancers were used to create CbFG. Texture analysis; viscometry, SEM, DSC, XRD, and FT-IR were used to assess the physicochemical properties. An *in vitro* skin permeation investigation was carried out with a Franz diffusion cell and excised SD-rat and hairless mouse dorsal skin to investigate the process of skin permeation. *In vivo* efficacy testing was also performed in a mono-iodoacetate (MIA)-induced rheumatoid arthritis animal model. Excellent anti inflammatory and analgesic benefits were also shown in the *in vivo* efficacy trial.

M. C. Gunde *et al.*, 2016[80] Plants and their extracts offer enormous potential for managing and treating burn wounds. Multiple strategies are used by the plant enzyme to promote wound healing and tissue regeneration. Several studies have found that chitosan is beneficial at accelerating wound healing. The study's goal was to create an effective topical gel formulation with plant enzymes (papain and bromelain) and chitosan as a gelling agent. Animals in *in vivo* investigations were separated into six groups, as follows: Group I (control) received no therapy, Group II received a blank chitosan gel, Group III, IV, and V received Chitosan gel with papain, bromelain, and a papain-bromelain combination, respectively, and Group VI (positive control) received the conventional drug (Framycetin Sulphate IP).The rate

of wound contraction as well as the period of epithelization was used to assess healing. According to the findings, chitosan gel containing plant enzymes has potential effects in burn healing process.

J. Barry Wright *et al.*, 2016[81] for some wound types, enzymatic debridement of nonviable tissue remains a useful debridement strategy. Several enzymes have already been, are, or will be beneficial as debriding agents. One of these enzymes is Papain, a plant-derived enzyme with a long tradition of clinical effectiveness. Furthermore, this enzyme, which is available as a commercial product called Accuzyme, has been investigated in a range of laboratory and clinical settings to determine its safety and effectiveness. The findings of these investigations contribute to the clinical safety profile of Accuzyme as a topical debriding agent for wounds. Accuzyme® (Healthpoint, Ltd., Fort Worth, Texas) seems to be a white, hydrophilic ointment containing papain (8.3 x 105 USP units of activity per gram, based on the USP lot H reference standard) and 100mg urea per gram.

M.Y.H. Porsani *et al.*, 2016[82] Papain is a proteolytic enzyme extracted from green papaya leaves and/or latex. This enzyme is well-known as a medicinal fruit that is frequently utilized in human medication to cure wounds of various etiologies. However, there are few research and reports in veterinary medicine. Sunflower oil is another herbal medicine that is commonly utilized in wound healing (*Helianthus annus*). It possesses antibacterial and inflammatory effects that promote local neovascularization by stimulating tissue granulation, cell migration, fibroblast proliferation, and differentiation. Three dogs were treated for infected necrosis wounds that were large, extensive, and severe, with a variety of etiologies**.** Except for one dog, who received sunflower oil at the end of the treatment, all instances were treated with Papain gel. Papain gel has been shown to be beneficial in the treatment of wounds, particularly in wound debridement and necrotic tissue removal. Furthermore, the healing time was reduced as compared to the sunflower oil treatment. Finally, herbal medications are inexpensive and widely available. This study facilitates the development of newer research on the usage of this medication in animal wound healing.

Sneha Paul *et al.*, 2015 [83]Chitosan, a substance synthesized from the natural polymer chitin then cast into a transdermal patch with bio-silver nanoparticles obtained from *Ganoderma lucidum*, has been investigated for wound healing activities. Chitosan has been prepared with an 85 % degree of deactyelyation and revealed by FTIR (Fourier transform Infrared spectroscopy) and XRD (Xray diffraction spectroscopy); *Ganoderma lucidum* has been used to synthesize silver nanoparticles and proved by TEM (Transmission electron microscope), EDAX (Energy dispersion x-ray spectroscopy), & XRD (x-ray diffraction (x-ray diffraction

spectroscopy). An approach was made out here to formulate transdermal patches. Chitosan was included for flexibility and permeability enhancement, Glycerol was utilized as a plasticizer, Gelatin was used to provide moisturising content, and silver nanoparticles served as a cross linking agent. The produced transdermal patch was then tested for biomedical characteristics such bioadhesion strength, heamocompability, *in vitro* degradeability, and microbial penetration. The results demonstrated that the transdermal patch could satisfy the necessary criteria for an acceptable wound dressing material with desirable properties, as well as the requirements for obtaining the transdermal patch for wound care.

Ju-Young Kim *et al.*, 2014[84] The researchers looked at the healing benefits of a chitosan-based, film-forming gel containing tyrothricin (TYR) in rat wound models such as burns, abrasions, incisions, and excisions. The chitosan film layer successfully covered and healed a variety of wounds after solidification. Wound size was evaluated at predefined time intervals following wound induction, and the results of the film-forming gel were compared to the negative (no treatment) and positive control groups (commercially available sodium fusidate ointment and TYR gel). When compared to the negative control, the film-forming gel dramatically improved healing of wounds from 1 to 6 days after wound induction in burn, abrasion, and excision wound models. Importantly, as compared to the positive control treatments, the film-forming gel facilitated considerably greater recovery.In the incision wound model, the breaking strength of wound strips from the film-forming gel group was considerably higher than that of the negative and positive control groups. In wounds treated with the film-forming gel, histological investigations demonstrated advanced granulation tissue development and epithelialization. According to the findings, this film-forming gel could be effective in healing a variety of wounds, including burns, abrasions, incisions, and excisions.

Sneha Ranade *et al.*, 2014[85] A topical formulation combining the benefits of polymeric films and gels for medication delivery was reported. Film-forming gels were created utilising cellulose polymers; when applied to the skin, the gel forms a continuous film that releases the medicine at a controlled rate. The concentrations of cellulose polymers and humectants were changed, and the effects on the physicochemical properties of the gels were evaluated. The pH, clarity, drying time, viscosity, drug content, spreadability, textural qualities, as well as *in vitro* and *ex-vivo* drug diffusion profiles of the gels were all assessed. In order to examine the anti-nociceptive potential of the local anaesthetic film forming gel, the optimised formulations were exposed to hot-plate and tail-flick tests in rats. The optimised gel, made with a polysaccharide polymer and ethanol as the solvent, was clear, transparent, and

lustrous, with a short drying time. Aesthetically pleasing films developed over the skin. The gels demonstrated a high rate of drug diffusion over the pig ear membrane, with a flux value of 211μg/hr/cm^2. The anti-nociceptive activity of the film forming gels revealed that they could significantly ($P \leq 0.05$) reduce pain perception. Local anaesthetic film-forming gels have the capacity to block peripheral pain and thus may be considered to be acceptable delivery strategies for pain management.

Ximing Liu *et al.*, 2014[86] Using a ternary phase diagram and the Box-Behnken design, researchers created a transparent film-forming hydrogel formulation for tolterodine (BBD). Carbopol 980 (neutralised by triethanolamine), hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), and Tween 80 were utilised as matrices. The solvent was a combination of water and ethyl alcohol. In mice, the measured cumulative drug release rate (86.02 %) was similar with the predicted value (85.42 %).Tolterodine steady-state flow (J) across rat entire skin, epidermal, dermal, and subcutaneous tissue was 15.83, 18.55, 37.15, and 81.82 mg /cm. The results of Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) revealed that the hydrogels may influence lipid status in SC, that was consistent with Ea (8.638 kcal/mol) of tolterodine from optimal formulation in rats. In pharmacokinetic investigations, the hydrogels had a better sustained-release over 24 hours and absolute bioavailability (24.53 %) than tolterodine tablets (15.16 %) in rats. The hydrogels were appropriate for systemic tolterodine administration for the treatment of hyperactive bladder.

Julia Hurler *et al.*, 2012[87] Chitosan is currently regarded as among the most potential polymers in the development of wound dressings. The study focuses on its usefulness as a vehicle for nano-delivery systems for burn therapy. The bioadhesion of wound dressing to the injured site is one of most important characteristics. The bioadhesive characteristics of chitosan were compared to those of Carbopol, a synthetic origin polymer. Texture analysis was used to assess gel cohesiveness, adhesiveness, and hardness in chitosan-based hydrogels of varying molecular weights. *In vitro* release experiments revealed no differences in model antibacterial drug release from the various hydrogel compositions. Bioadhesion tests on pig ear skin were done, and the detachment force needed to remove the die from the skin, as well as the amount of leftover formulation on the skin, was evaluated. Although there was no big variation in detachment force between Carbopol-based and chitosan-based formulations, nearly twice as much chitosan formulation retained on the skin as Carbopol formulations. The results confirmed the significant potential of chitosan-based delivery methods in advanced wound care. Furthermore, the results imply that formulation retention on *ex vivo* skin

samples could provide more information on formulation bioadhesiveness than detachment force assessment.

Ezekiel Amri *et al.*, 2012[88] the review discusses papain's biological relevance, characteristics, and structural elements that are significant for understanding their biological activity. Its manufacturing capabilities and market opportunities are also examined. Papain is a plant proteolytic enzyme for the cysteine proteinase family cysteine protease enzyme that has made tremendous progress in understanding its roles. Papain is naturally found in papaya (*Carica papaya L.)* made from raw papaya fruit latex. The enzyme may degrade polypeptides, which are organic polymers composed of amino acids, and so plays an important part in a variety of biological processes in physiological and pathological states, drug creation, and industrial applications and pharmaceutical preparations.The functionality of papain is due to its unusual structure, which helps illuminate how proteolytic enzymes work and makes it useful for a variety of functions.

Alistair Young *et al.*, 2011[89] Wound healing is a versatile biological process that results in tissue integrity restoration. It can be divided into four different phases: haemostasis, inflammation, proliferation, and tissue remodelling. This article discusses the biological foundation of wound healing as well as the extracellular signalling systems that regulate it. Platelets, neutrophils, macrophages, and fibroblasts are all discussed in depth. The idea of healing through primary and secondary intention is explained. Malnutrition, hypoxia, immunosuppression, chronic disease, and surgery are all known to have a negative impact on healing. In order tominimise patient mortality from delayed healing, surgeons must understand the key physiological mechanisms involved in healing.

Hosamath Vijaykumar *et al.*, 2011[90]The goals of this study are to examine the efficacy of collagenase vs. papain–urea for debridement of chronic non-healing ulcers/wounds, as well as to assess their involvement in encouraging ulcer healing through granulation and reducing ulcer/wound size. From November 2007 to August 2009, 100 patients were studied in a comparative study at M.S. Ramaiah Hospitals in India. The patients were chosen, randomised, and put into two groups of 50 each. Group 1 was given collagenase, while Group 2 was given Papain–urea. Patients were assessed at 0, 1, 2, 3, and 4 weeks for ulcer size reduction, granulation, discharge, and overall treatment response.The papain-urea group outperformed the collagenase group in the second, third, and fourth weeks (p value 0.05), with a substantial improvement of 28% in the Papain-urea group and 12% in the collagenase group. The average follow-up period was 7.28- 8.14 weeks. Papain-urea and collagenase have been shown to be effective in promoting enzymatic wound debridement. When

compared to collagenase, Papain-urea is a better enzymatic debriding agent that facilitates quicker granulation.

Mohamed Mahmoud Nasef *et al.*, 2011[91] Physical solution blending was used to create hydrogel films from chitosan (Cs) and agar blends in varied amounts. A few of the films were ionically cross-linked after being treated with calcium chloride solution. Fourier transform infrared (FTIR) spectroscopy, thermogravimetry analysis (TGA), differential scanning calorimetry (DSC), and a universal mechanical tester were used to characterise the films. The formation of films from crosslinked and non-crosslinked pure Cs and agar, as well as their mixes in varying ratios, was studied.The water swelling of the Cs/agar blended films were found to be decreased by the addition of agar and this reduction is proportional to the amount of agar in the blend (5-25 percent). The overall results of this research indicate that blending Cs with agar produces hydrogel films with enhanced stability related properties, such as lower swelling and high melting temperatures, both of which were strengthened further by ionic crosslinking of the blended films using calcium chloride. Cs/agar blend films were found to be stiffer and far less elastic than pure Cs films, and this behaviour is increased by ionic crosslinking while being a function of agar content.

Ibrahim A. Alsarra *et al.*, 2009[92] The wound healing capabilities of chitosan with varying molecular weight and degree of deacetylation have been investigated. Chitosan, FucidinR ointment, and to blank were used to assess the macroscopic image and histopathology. The rate of contraction was determined by measuring the unclosed area as a function of the time. When compared to untreated, treated, and FucidinR ointment-treated rats, wounds treated with high molecular weight–high degree of deacetylation chitosan contracted at the fastest rate. Wounds treated with high molecular weight chitosan had considerably more epithelial tissue ($p< 0.05$) than wounds treated with some other treatment, as well as the high molecular weight chitosan treatment group had the best re-epithelization and wound closure. In wounds treated with high molecular weight chitosan, histological examination and collagenase activity studies demonstrated advanced granulation tissue development and epithelialization ($p<0.05$). Chitosan samples with a high molecular weight and a high degree of deacetylation show promise as a therapy system for cutaneous burns.

Theddeus O.H. Prasetyono *et al.*, 2009 [93] developed a step-by-step conceptual framework for healing intentions that encompassed all acute and chronic wounds. In terms of the "hierarchy" of healing intention, main intention is the best choice, followed by tertiary intention, and finally secondary intention. Wound healing is a physiological transition that is also considered as one of the most complicated in human physiology. In the wound healing

process, a complex set of reactions and interactions between cells and mediators occur, involving cellular and molecular activities. The inflammatory phase is designed to eliminate devitalized tissue and protect against invasive infection. The proliferative phase is distinguished by the production of granulation tissue within the wound bed, which is made up of new capillary networks, fibroblasts, and macrophages in a loose arrangement of supportive structure. This second phase, which lasts from day 8 to day 21 after the injury, is also the epithelialisation phase. The natural period of proliferative phase is a reflection for us in treating wounds to achieve the objective of a closed wound. The ultimate maturation phase is also distinguished by the proper balance of collagen deposition and breakdown. There are at least three prerequisites that are ideal local conditions for the nature of the wound to go through a normal healing process, namely: 1) all tissue associated in the wound and neighbouring should be vital, 2) no foreign bodies in the wound, and 3) free from excessive contamination/infection.

Benjamin Goldman *et al.*, 1959[94] 2,917,433, Topical aqueous Papain that is stable, United States Patent and Trademark Office, This invention pertains to unique and effective therapeutic formulations, particularly those suited for topical treatments to infected human tissue, particularly in the treatment of wounds requiring debridement. This invention is more specifically concerned with novel stable proteolytic compositions containing Papain as an active substance, said Papain being embedded in the composition in such a manner that it retains full activity in storage and becomes instantly active upon contact with tissue requiring dissolution.

Hall G. Holder *et al.*, 1938[95]The usefulness of carbamide (urea) as an adjuvant to wound healing is established once again, owing principally to the lytic action of strong aqueous solutions on necrotic tissue and other debris. Diluted urea solutions are used to increase cell growth in wound healing.

AIM AND OBJECTIVE

3. AIM AND OBJECTIVE:

3.1. AIM

Enhancement of Wound Healing Activity of Papain Urea Film Forming Hydrogel Using Chitosan

3.2. OBJECTIVES

- To develop and evaluate film forming hydrogel for Papain Urea using Chitosan
- To transform film forming hydrogel from the hydrogel to film-type by solvent evaporation after application to the wound site.
- In Papain Urea hydrogel, Papain is an enzyme used to heal wounds; Urea is an adjunct to wound therapy, the polymer chitosan also has wound healing properties.
- To overcome the limitation of typical topical formulation such as easy removal, this often leads to limited wound exposure and ineffective activity.
- To prolong the contact time of active substances to the skin and thereby reducing the application frequency
- To offer easier use and application, and simpler manufacture
- To improve patient compliance by decreasing dosing frequency.
- To provide sustained release drug for longer periods of time.
- To prepare biodegradable and eco friendly formulation

PLAN OF THE WORK

4. PLAN OF THE WORK:

- Literature review
  - ✓ Drug
  - ✓ Disease
  - ✓ Excipient
  - ✓ Dosage form
- Drug and Excipient profile
- Preformulation study
  - ✓ Identification of physiochemical characteristics of drug
  - ✓ Determination of solubility in different in different solvent
  - ✓ Compatibility studies with polymer using FTIR
- Trial formulation of Papain Urea Film Forming Hydrogel
- Evaluation
  - ➢ Physiochemical evaluation of film forming gels
    - o Clarity
    - o pH
    - o Homogeneity
    - o Extrudability
    - o Spreadability
    - o Viscosity
    - o Drug content
    - o Drying time
  - ➢ *In vitro* diffusion studies
  - ➢ *In vitro* wound healing activity
  - ➢ Stability studies
- Result and discussion
- Summary and conclusion
- Bibliography

DRUG AND EXCIPIENT PROFILE

5. DRUG AND EXCIPIENT PROFILE

5.1. DRUG PROFILE: [96, 97, 98]

5.1.1. PAPAIN

Papain is a proteolytic enzyme derived from the fruit Carica papaya. It is of natural origin used to heal wounds

Structure:

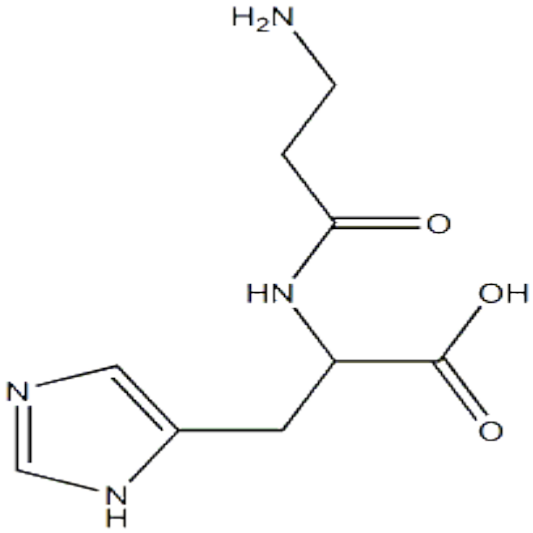


FIG 10: STRUCTURE OF PAPAIN

Chemical name: Beta Alanyl L-Histidine

Chemical formula: $C_9H_{14}N_4O_3$

Molecular weight: 226.23

Appearance (Colour): Yellowish white

Appearance (Form): Fine powder

Category: Enzymatic debridement

Solubility: Soluble in water, insoluble in most organic solvents

Bulk density: 800Kg/m^3

pH: 4-7

Pharmacology/Pharmacokinetics

Papain is active at pH levels ranging from 3 to 12. It is ineffectual as a debriding agent when used alone and requires the addition of activators to boost its digestive effectiveness. The combination of Papain and urea stimulates two additional chemical reactions. First, it exposes the Papain activators via solvent action. Second, it denatures nonviable protein content in lesions, making it more amenable to enzymatic breakdown. In pharmacologic tests, the combination of Papain and urea resulted in twice as much digestive action as Papain alone.

Activity: > 6000 USP units/mg

Dose: 830,000units/g, 1or 2 times daily

Adverse Effects:

In general, Papain-urea is well tolerated and non-irritating. When using Papain-Urea, a limited number of patients may feel a transient "burning" sensation. The exudates from enzymatic digestion can irritate the skin on occasion. More frequent dressing replacements will help to reduce this discomfort until the amount of exudates diminishes.

Precautions/Contraindications:

Papain-Urea is contraindicated in patients who have shown sensitivity to Papain or any other components of this preparation.

Drug Interactions:

The Papain may be inactivated by a solution of hydrogen peroxide. The manufacturer's labeling instructions contain precautions to prevent hydrogen peroxide throughout the wound washing process. Heavy metal salts such as lead, silver, and mercury may inactivate Papain. As a result, on the wound treated with Papain-Urea, contact with topical treatments containing these metals should be avoided.

Storage: 2^{o}C to 8^{o}C, Keep container tightly closed in a dry and ventilated place

5.1.2. UREA

Urea is a keratolytic emollient used to treat hyperkeratic lesions and moisturize the skin

Structure:

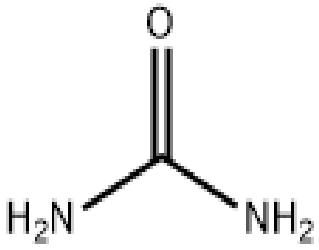


FIG 11: STRUCTURE OF UREA

Chemical Name: Carbamide

Chemical Formula: CH_4N_2O

Molecular weight: 60.056

Appearance: White crystalline powder

Category: Humectant, moisturizing

Solubility: Very soluble in water, soluble in ethanol

Density: 1.32 g /cm^3

Bulk density: 720 – 760 kg/m^3

Melting point: 134ºC

pH: 7.5 -9.5

Pharmacology:

- 10% hydrate skin
- 15% accelerate fibrin degradation
- 40% proteolytic

Mechanism of Action:

Urea is a humectant at low concentrations, but at high concentrations (>20 percent), it induces protein breakdown in the epidermis. Urea dissolves the intercellular matrix of stratum corneum cells, facilitating desquamation of scaly skin and finally softening hyperkeratotic areas. Because of the effect of the powerful aqueous solution on necrotic tissues and other debris, it is used as an adjunct to wound healing.

Dose: 100mg/g

Side effects:

Local irritation, Rashes, Temporary stinging

Warning:

Keep out of reach of children. Not for use near eye. Ischemic skin necrosis reported with high concentration or irrigation

Contraindications: Hypersensitivity, Viral skin disease

Storage: 15ºC to 25ºC, Keep container tightly closed in a dry and ventilated place

5.2. EXCIPIENTS PROFILE: [99]

5.2.1. CHITOSAN

Deacetylated chitin, a linear polysaccharide of deacetylated beta-1, 4-d-glucosamine. It is used in hydrogel and to treat wounds.

Structure:

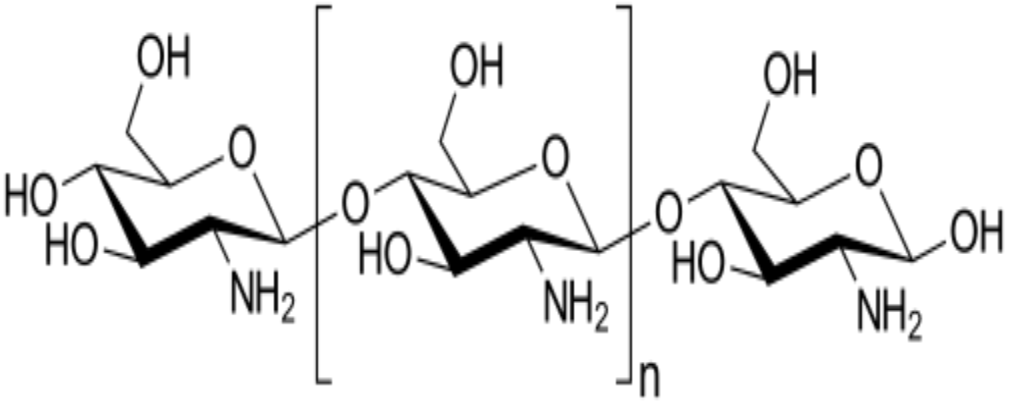


FIG 12: STRUCTURE OF CHITOSAN

Chemical name: (1, 4)-2-Amino-2-desoxy- beta-D-glucan

Chemical formula: $(C_6H_{11}NO_4)_n$

Molecular weight: 1526.5

Appearance (Colour): White to pale yellow

Appearance (Form): Powder

Category: Wound healing natural polymer

Solubility: 1% solution in 1% Acetic acid, Colourless to pale yellow

pH: 6 – 6.5.

Side Effects

Side effects of chitosan supplements may include constipation, nausea, and an upset stomach. When applied to the skin: Chitosan is possibly safe for most people when applied to the skin for a short time. Chitosan can cause irritation.

Precautions/Contraindications

Shellfish allergy: Chitosan is taken from the outer skeleton of shellfish. There is a concern that people with allergies to shellfish might also be allergic to chitosan.

Drug Interactions

Warfarin (Coumadin) interacts with chitosan

Warfarin is a blood thinner. There is some concern that taking chitosan might increase the blood thinning effects of warfarin (Coumadin). Taking chitosan with warfarin (Coumadin) could increase the chance of bruising or bleeding. If you take warfarin, avoid taking chitosan.

Storage:

Room temperature. Keep container tightly closed in a dry and well-ventilated place.

5.2.2. ACETIC ACID

Acetic Acid is a synthetic carboxylic acid with antibacterial and antifungal properties. Although its mechanism of action is not fully known, undissociated acetic acid may enhance lipid solubility allowing increased fatty acid accumulation on the cell membrane or in other cell wall structures. Acetic acid, as a weak acid, can inhibit carbohydrate metabolism resulting in subsequent death of the organism.

Structure:

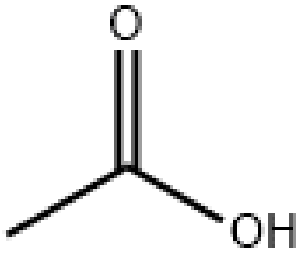


FIG 13: STRUCTURE OF ACETIC ACID

Chemical Formula: **CH_3COOH**

Molecular weight: 60.05

Appearance: clear colourless liquid with strong odour

Solubility: Miscible with ethanol, ethyl ether, acetone, benzene; soluble in carbon tetrachloride, carbon disulfide

Density: 1.051 g /cm^3

Viscosity: 1.056 m Pa-s at 25 °C

Melting point: 16.6 °C

pH: Aqueous solution 1.0 molar = 2.4; 0.1 molar = 2.9; 0.01 molar = 3.4

Side effects:

Common side effects may include mild stinging or burning with the first use.

Warning:

Discontinue promptly if sensitization or irritation occurs

Storage: Store acetic acid in a cool, well-ventilated area in a tightly sealed container.

Uses:

Topically applied dilute acetic acid, which is cheap and easily available, has been found to be effective in chronic wounds. Acetic acid has antibacterial and antifungal properties. These properties could help clean the skin and prevent infections from bacteria or fungi.

5.2.3. PROPYLENE GLYCOL

Structure:

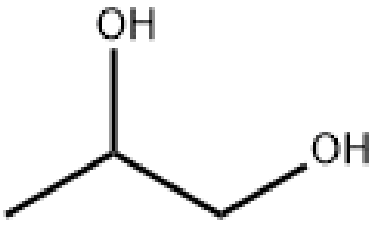


FIG 14: STRUCTURE OF PROPYLENE GLYCOL

Chemical Name: 1, 2-propanediol

Chemical Formula: $C_3H_8O_2$

Molecular weight: 76.09

Appearance: Clear, colourless, hygroscopic, viscous liquid

Solubility: Soluble in water, ethanol and acetone

Density: 1.04g /cm^3

Viscosity: 1.056 m Pa-s at 25 °C

Melting point: 16.6 °C

pH: 6 – 8

Safety:

Propylene glycol is used in a wide variety of pharmaceutical formulation and is regarded as nontoxic material

Storage: Store acetic acid in a cool, well-ventilated area in a tightly sealed container.

Uses:

Since propylene glycol has great viscosity and hygroscopicity and is non-toxic, it is widely utilised in the food, pharmaceutical, and cosmetic industries as a hygroscopic agent, antifreeze, lubricants, and solvents. Propylene glycol is extensively used in the pharmaceutical business as a solvent, plasticizer, softener, and excipient, among other things, in the production of many types of ointments and salves. Because of its high mutual solubility with many spices, propylene glycol is also utilised as a cosmetic solvent and softener. Propylene glycol is also used as a tobacco moisturising agent, an antifungal agent, lubricant

for food processing equipment, and a solvent for food marking ink. Propylene glycol aqueous solution is a good anti-freeze agent.

5.2.4. SODIUM BENZOATE:

Sodium salt of benzoic acid.

Structure:

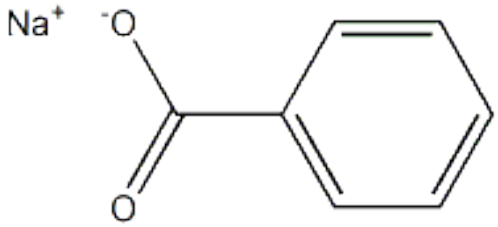


FIG 15: STRUCTURE OF SODIUM BENZOATE

Chemical Name: 1, 2-propanediol

Chemical Formula: $C_7H_5O_2Na$

Molecular weight: 144.10

Appearance: White hygroscopic crystalline powder or granules

Solubility: Freely soluble in water, sparingly soluble in ethanol

Density: 1.50 g /cm^3

Melting point: 16.6 ℃

pH: 8

Safety:

Sodium benzoate is one of the most reliable ingredients on the market and very safe for consumers.

Storage:

Store acetic acid in a cool, well-ventilated area in a tightly sealed container.

Uses:

It is used as a preservative in pharmaceutical preparations and foods. It may also be used as a test for liver function.

MATERIALS AND METHODS

6. MATERIALS AND METHODS

6.1. List of Materials:

TABLE 3: LIST OF MATERIALS

SNO	MATERIALS NAME	CATEGORY	COMPANY NAME
1.	Papain	Drug	Sisco Research Laboratories Pvt Ltd, Mumbai
2.	Urea	Drug	Sisco Research Laboratories Pvt Ltd, Mumbai
3.	Chitosan (Medium molecular weight)	Polymer	Sisco Research Laboratories Pvt Ltd, Mumbai
4.	Chitosan (High molecular weight)	Polymer	Sisco Research Laboratories Pvt Ltd, Mumbai
5.	Glacial acetic acid	Solvent	Spectrum laboratories, Mumbai
6.	Propylene glycol	Plasticizer	Spectrum laboratories, Mumbai
7.	Sodium benzoate	Preservative	Spectrum laboratories, Mumbai

(All are A.R. Grade)

6.2. List of Equipments:

TABLE 4: LIST OF EQUIPMENTS

S.No.	EQUIPMENTS	MAKE	PURPOSE
1.	Electronic balance	Precision Balance	For weighing purpose
2.	Digital pH meter	Elico L 1120	Surface pH study
3.	Fourier transform- IR	Perkin-Elmer	Compatibility studies
4.	Magnetic stirrer	Rotek	Diffusion Studies
5.	UV spectrometer	Shimadzu	Determination of Absorption maxima & concentration of active substances
6.	Environment test Chamber	Heco	Stability studies
7.	Brookfield viscometer	Brookfield	Determine the viscosity of the gel
8.	Melting point apparatus	Digital electronic melting point apparatus	To measure the melting point of drug

PREFORMULATION STUDIES

7. PREFORMULATION STUDIES

Preformulation is a stage in the development process in which researchers characterize the physical, chemical, and mechanical properties of the drug material in order to create an effective, stable, and safe dosage form. As a result, preformulation studies are required to characterize the medicine in order to properly develop the drug delivery system. This project's preformulation studies included the following:

- Description
- Melting point
- Solubility
- Identification of drug sample
- Drug – excipient compatibility studies

7.1. Description

Organoleptic characters of drug was observed and recorded by using descriptive terminology.

1.2.Melting point

Capillary tube, which is sealed at one end is charged with sufficient amount of dry powder to form a column in the bottom of the tube 2.5mm to 3.5mm, and packed down as closely as possible by moderate tapping on a solid surface. The apparatus is operated according to the standard operating procedure. The block is heated until the temperature is about 30^{o} C below the expected melting point. The capillary tube is inserted into the heating block, and the heating is continued at a rate of temperature increased of about 1^{o} C to 2^{o} C per minute until melting is completed.

The temperature at which the detector signal first leaves its initial value is defined as the beginning of melting, and the temperature at which the detector signal reaches its final value is defined as the end of melting, or the melting point. The two temperatures fall within the limits of the melting range.

1.3.Solubility Studies

The spontaneous interaction of two or more substance to form a homogenous molecular dispersion is called as solubility.

Procedure

Spontaneous interaction of two or more substance to form a homogenous molecular dispersion is called as solubility. 10 mg of drug was a suspended separately in 10 ml of different solvents at room temperature in tightly closed tubes and shaken. The solubility profiles of drugs in various solvents are shown in the table.

TABLE 5: GENERAL SOLUBILITY PROFILEI.P.1996

DESCRIPTIVE TERM	PARTS OF SOLVENT REQUIRED FOR 1 PART OF SOLUTE.
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10, 000
Practically insoluble	Greater than or equal to 10,000

1.4.Hygroscopic nature:

2gm of the test specimens were weighed accurately in petridish and the weight were noted down. Thus the test substance was exposed to 75%RH at 40ºC in environmental stability testing chamber and other was kept in room temperature for 7 days period. The specimen was weighed after 7 days and the difference in weight was noted down

1.5.Identification of Drug sample

Finding absorption maxima (λ max)

The absorption maxima were found for drug identification. Ultraviolet visible spectrometry has been used to obtain specific information on the chromophoric part of the molecules. Organic molecules in solutions when exposed to light in the visible/ultraviolet region of the spectrum absorb light of particular wavelength on the type of electronic transition associated with the absorption.

1.6.Standard curve:

Preparation of standard plot for Papain in phosphate buffer pH 7.4

Accurately weighed amount of Papain (100mg) was dissolved in small quantity of Phosphate buffer pH 7.4 and then diluted to 100ml. Each ml of stock solution contains 1000µg of Papain. From this stock different standard of working standard solution i.e. 10, 20, 30, 40, 50, 60µg/ml were made up with phosphate buffer pH 7.4 and absorbance was measured at 277.4nm using phosphate buffer pH 7.4 as blank by UV spectrometric method. A graph is plotted by using concentration at X- axis and absorbance at Y- axis**.**

1.7.Fourier transforms infrared (FTIR) spectral analysis

FTIR is used to identify group in the molecule. The drug is mixed with KBr disk was scanned at 4mm/s at a resolution of 2cm over a wave number region of 400 to 400$cm^{-1.}$ The characteristic peaks were recorded.

Drug – Excipient compatibility studies by FT –IR analysis

Infrared spectrum of any compound or drug gives information about the group present in that particular compound. The IR absorption spectra of the pure drug and physical admixtures of drug with various excipients were taken in the range of 4000 – 400cm^{-1} using KBr disc method and observed for characteristics peaks of drug.

Drug – Excipient compatibility was carried out by FT – IR analysis. Initially the IR spectrums of pure drug, Papain, Urea, Chitosan, Glacial acetic acid, Propylene glycol, Sodium benzoate were obtained. After that admixtures of drug with other excipients were prepared and IR spectra were obtained. The obtained spectra of physical admixtures was observed for major peaks and recorded. The results of this observation were concluded that there is no interaction between the drug and other excipients.

FORMULATION AND EVALUATION

8. FORMULATION AND EVALUATION

8.1. FORMULATION DEVELOPMENT

Pharmaceutical development studies must be carried out in order to choose the best dosage form and a stable formulation. These studies provide a full account of all of the steps involved in the development of the finalized procedure. Such details are designed to highlight crucial process characteristics that must be regulated in order to provide a dependable and reproducible quality product.

SIMPLE AGITATION TECHNIQUE:

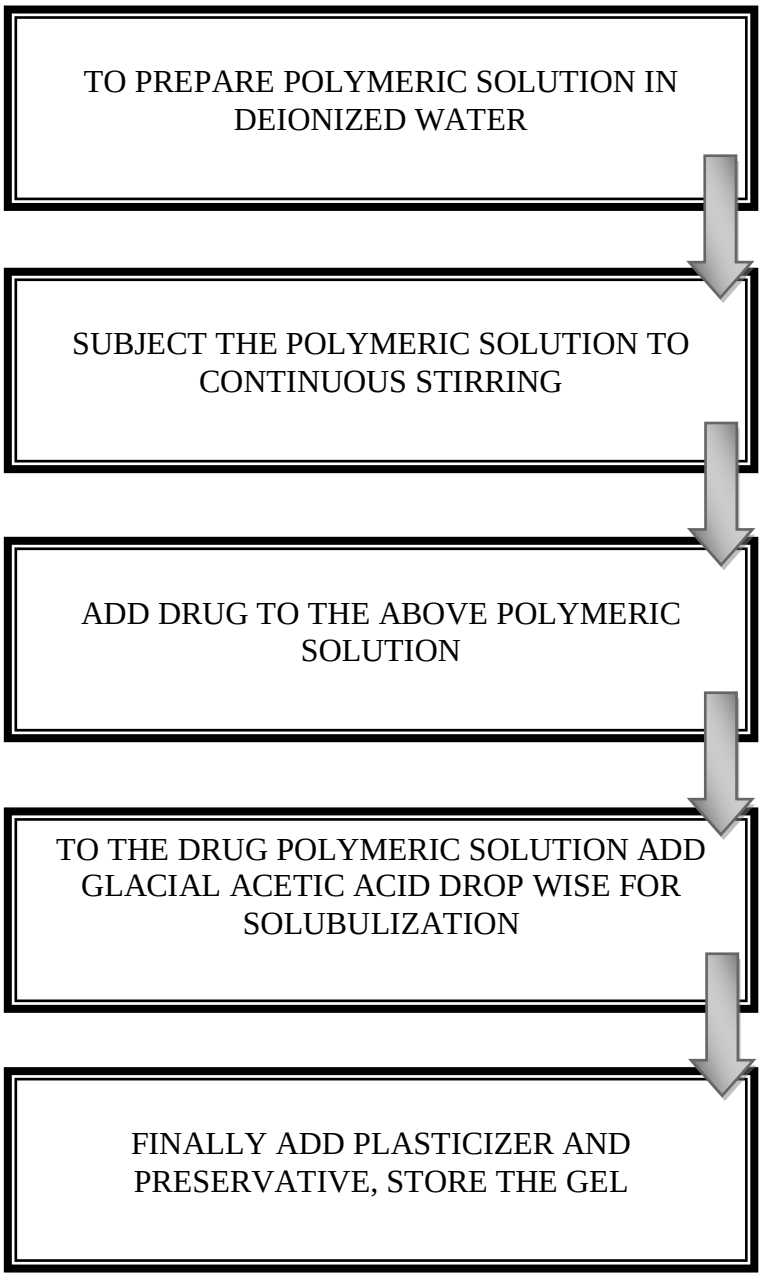


FIG 16: PROCESS FLOW CHART FOR FILM FORMING HYDROGEL

PREPARATION OF PAPAIN UREA FILM FORMING HYDROGEL:

The formulation was prepared using simple agitation method. Briefly, the weighed amount of chitosan was added, and dispersed slowly in distilled water with continuous magnetic stirring. Calculated quantity of Papain and urea was mixed together and added to the above solution. Glacial acetic acid was added drop wise for chitosan solubilisation and mixed by agitation. Finally a plasticizer propylene glycol and preservative sodium benzoate is added and mixed well. The formulation is subjected to homogenization for uniform dispersion of particles. All procedures were conducted at room temperature.

FORMULA:

TABLE 6: FORMULATION OF PAPAIN UREA FILM FORMING HYDROGEL

SNO	INGREDIENTS	F1	F2	F3	F4	F5	F6
1	Papain	6.9	6.9	6.9	6.9	6.9	6.9
2	Urea	5	5	5	5	5	5
3	Chitosan – Medium molecular weight	1	1.5	2	-	-	-
4	Chitosan –High molecular weight	-	-	-	1	1.5	2
5	Glacial acetic acid	q.s	q.s	q.s	q.s	q.s	q.s
6	Propylene glycol	2	2	2	2	2	2
7	Sodium benzoate	0.25	0.25	0.25	0.25	0.25	0.25
8	Distilled water (Up to 50g)	50	50	50	50	50	50

1.2.CHARACTERIZATION OF FILM FORMING HYDROGEL:

a) Clarity [100]

The clarity of all the formulations was measured visually against a black and white background and was categorised as turbid, clear, or very clear.

b) pH [101]

2.5 g of gel were precisely weighed and dissolved in 25 ml of distilled water. A digital pH meter was used to measure the pH of the mixture.

c) Homogeneity [102-104]

After the gels had been placed in the container, all prepared gels were visually inspected for their appearance and the presence of any aggregates.

d) Spreadability [102-104]

The spreadability of the formulation was determined by measuring the spreading diameter of 0.5 g of gel formulation placed between two horizontal smooth surface glass plates (20 cm × 20 cm). The initial diameter in centimeters formed by placing the gel on the glass plate was noted. Another glass plate (weighing 200 g) with the same dimensions was placed over the gel for 1 min until no more expansion of the gel was observed. The upper plate was gradually removed and diameter of the circle formed after spreading of the gel was measured in centimeters.

e) Extrudability [102-104]

In this method the formulated gel were filled in standard capped collapsible aluminium tube and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and clamped. 500g was placed over the slide and the cap was removed. The amount of the extruded gel was collected and weighed. The percent of extruded gel was calculated (> 90% extrudability excellent, >80% extrudability satisfactory).

f) Viscosity measurement [102-104]

A viscometer was used to assess the gel's viscosities (Brookfield DV-II). The spindle (6) was revolved at 100 revolutions per minute. The formed gel samples were allowed to settle for 30 minutes before the measurements were taken. The mean of the three samples was calculated.

g) Drug content [105]

A precisely weighed amount of gel (1g) was placed in a 100 ml volumetric flask containing 50 ml phosphate buffer solution (pH 7.4), and the volume was filled to the desired level with phosphate buffer. To achieve complete drug solubility, the volumetric flask containing gel was sonicated for 15 minutes. The mixture was filtered using a membrane filter with a pore

size of 0.45 m. Using an ultraviolet (UV)/visible spectrophotometer, the absorbance of the produced solution was measured at a λ max of 277.4nm against phosphate buffer (pH 7.4) as a blank.

h) Drying time

The drying time of the films obtained after the gels were applied was measured. On the glass slide, 1 g of the gel was placed. The time required for the gel to dry completely and develop a film was noted. Another glass slide was lightly pressed over the films to ensure complete drying. If no gel remained visible on the glass slide upon removal, the film was considered dry.

i) *In vitro* diffusion study

1g of the formulation was placed in the donar compartment over an egg membrane that had been washed and immersed in the diffusion medium for 24hrs. The donar compartment is dipped in the receptor compartment, which contains 400ml of pH 7.4 phosphate buffer, and the beaker holding diffusion medium (receptor compartment) is kept at 37° C with constant stirring at 22 rpm using a magnetic stirrer. Every hour for 12 hours, 5 ml aliquots are removed from the diffusion medium and replaced with the same amount of new, pre-warmed diffusion medium. The materials were tested spectrophotometrically for Papain at 277.4 nm using a Shimazdu Double beam UV-Visible spectrophotometer.

TABLE 7: PARAMETERS FOR *IN-VITRO* DIFFUSION STUDY

PARAMETERS	**PAPAIN UREA FILM FORMING HYDROGEL**
Diffusion medium	Phosphate buffer pH 7.4
Volume	400 ml
Rpm	22
Temperature	37º C ± 1º C
UV Absorbance Measurement	277.4 nm

The diffusion study was carried out for all the fair formulations and the release profiles were compared using kinetic model and statistical method

j) Drug release kinetics [106]

Several theories and kinetic models describe the diffusion of drug release dosage forms. There are several models to represent the drug dissolution profiles where f (t) is a function of time related to the amount of drug dissolved from the pharmaceutical dosage form.

The quantitative interpretation of the values obtained in the diffusion assay is facilitated by the usage of a generic equation that mathematically translates the diffusion curve function of some parameters related with the pharmaceutical dosage forms. Drug diffusion from liquid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time't' or Q (t). Some analytical definitions of the Q (t) function are commonly used, such as zero order, first order, Higuchi, Korsmeyer - Peppas, Hixson - Crowell models, Weibull models. These models are used to characterize drug dissolution/release profiles.

- **Zero Order Kinetics**

This model represents an ideal release profile in order to achieve the pharmacological prolonged action. Zero order release constitutes drug release from the dosage form that is independent of the amount of drug in the delivery system (that is, a constant release rate). The following equation is used to express the model:

$$Q_t = Q_o + K_o t$$

Where,

Q_t is the amount of drug dissolved in time t

Q_o is the initial amount of drug in the solution

K_o is the zero order release constant

For practical purposes the equation is rearranged:

$$\text{Percent drug released} = Kt$$

This is applicable to dosage forms like transdermal systems, coated dosage forms, osmotic systems as well as matrix tablets with low soluble drugs.

- **First Order Kinetics**

First order release constitutes drug release in a way that is proportional to the amount of drug remaining in its interior; in such a way that amount of drug released by unit time diminish. The following equation is used to express the model:

$$\log Q_t = \log Q_o + Kt/2.303$$

Where,

Q_t is the amount of drug dissolved in time t

Q_o is the initial amount of drug in the solution

K is the first order release constant

For practical purposes the equation is rearranged:

Log % of drug unreleased = Kt/2.303

This model is applicable to dosage forms such as those containing water-soluble drugs in porous matrices.

- **Higuchi Model**

Higuchi describes drug release as a diffusion process based in Fick's law, square root dependent. The following equation is used to express the model:

$$Q_t = K_h t^{1/2}$$

Where,

Q_t is the amount of drug dissolved in time t

K_h is the first order release constant

For practical purposes the equation is rearranged:

Percent drug released = $Kt^{1/2}$

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drugs.

- **Peppas-Korsmeyer Model**

This model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved

The following equation is used to express the mode

$$Q_t/Q_\infty = Kt^n$$

Where,

Q_t is the amount of drug dissolved in time t

Q_∞ is the amount of drug dissolved in infinite time

n is the release exponent indicative of drug release mechanism

K is the kinetic constant

For practical purposes the equation is rearranged:

Log percent drug released = log k +n log t

Peppas used n value in order to characterize different release mechanism concluding for values of n = 0.5 for Fickian diffusion and values of n, between 0.5 to 1.0 for anomalous transport (corresponds to diffusion, erosion and swelling mechanism or mixed order kinetics)

and higher values of n, n=1 or n>1 for case-II transport (corresponds to erosion and relaxation of swollen polymer layer).

The diffusion study was carried out for all the fair formulations and the release profiles were compared using kinetic model and statistical methods.

k) MTT assay for cell cytotoxicity [107-108]

Principle

The MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay is based on the capacity of a viable cell's mitochondrial dehydrogenase enzyme to cleave the tertrazolium rings of the pale yellow MTT and form a dark blue coloured formazan crystal that is largely impermeable to cell membranes, likely to result in its accumulation within healthy cells. Solubilization of cells with detergents (DMSO) results in the liberation of solubilized crystals. The number of surviving cells is proportional to the amount of formazan product produced. A multi-well plate reader can be used to quantify the colour.

Materials required

DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), 1X PBS was from Himedia, (India), 96 well tissue culture plate and wash beaker were from Tarson (India).

Procedure

Cell culture

Vero cells (African green monkey kidney cells) cell line was purchased from NCCS, Pune and cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin and maintained under an atmosphere of 5% CO_2 at 37^oC.

MTT Assay

Cell culture

Vero cells (African green monkey kidney cells) cell line was purchased from NCCS, Pune and cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37^oC.

MTT Assay

Using Vero cells, the formulation was tested for *in vitro* cytotoxicity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, trypsinization was used to harvest Vero cells, which were then pooled in a 15 ml tube. The cells were then

plated at a density of 1105 cells/ml cells/well (200 L) into a 96-well tissue culture plate in DMEM medium with 10% FBS and 1% antibiotic solution for 24-48 hours at 37°C. In a serum-free DMEM medium, the wells were washed with sterile PBS and treated with various concentrations of the formulation. Each sample was replicated three times, and the cells were incubated for 24 hrs at 37°C in a humidified 5% CO_2 incubator.

After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 hrs until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value was calculated using GraphPad Prism 6.0 software (USA).

l) Wound healing assay [107-108]

Migration is an important property of living cells that is required for normal development, immune response, and disease processes such as cancer metastasis and inflammation. Methods for studying cell migration are extremely necessary and beneficial in many areas of biomedical research, including cancer biology, immunology, vascular biology, cell biology, and developmental biology.

Materials required

DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), 1X PBS was from Himedia, (India). 6 well tissue culture plate and wash beaker were from Tarson (India).

Procedure

Cell culture

Vero cells (African Green Monkey Kidney cells) cell line was purchased from National centre for cellular sciences, Pune, and cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37ºC.

Wound healing assay

The wound healing assay was used to assess cell migration after treatment in both cancer and non-cancer cell lines. Vero cells were seeded into a six-well tissue culture dish and grown in complete medium to 90 percent confluency. As outlined, cell monolayers were injured by a plastic tip (1 mm) that touched the plate. Wounded monolayers were then washed with

medium four times to remove cell debris before being incubated in 1 percent FBS medium. The cells were incubated for 24 hrs after being treated with 130µg/ml of formulation. Cells were observed using an inverted microscope with a camera. Image-J software was used to measure the wound area (NIH, Bethesda, MD, USA). The wound area percentage was calculated as the wound area of control and treated sample.

m) Stability study [109]

Officially, stability is defined as the amount of time that a drug product retains the same characteristics and qualities that it had at the time of manufacturing. This process begins throughout the early stages of development. Instabilities in current formulations are frequently found only after long durations of storage under normal conditions. Various tests involving the storage of items under conditions that promote decomposition have been introduced to minimize the time required to gather information.

Objectives of accelerated stability studies

- The detection of degradation in multiple initial formulations of the same product in a timely manner. This is useful when deciding on the optimal formulation from a set of options.
- The prediction of shelf life, which is the amount of time a product, will last when stored under predicted or specified storage conditions.
- The provision of a timely means of quality contact to ensure that no unanticipated changes in the stored product have happened.

TABLE 8: STABILITY STORAGE CONDITIONS

S.NO.	STUDY PERIOD	STORAGE CONDITION	MINIMUM DURATION
1	Longer	25 ±2 ° C 60 ± 5% RH	6 Months
2	Intermediate	30 ± 2 ° C 65 ± 5% RH	3 Months
3	Accelerated	40 ± 2° C75 ± 5% RH	3 Months

The optimized formulation were packed in containers and kept in stability chamber at 40 ° C/75% RH. After specific period of storage for stability, the formulation was evaluated for physical parameters, *in-vitro* drug release and assay.

RESULT AND DISCUSSION

9. RESULT AND DISCUSSION

9.1. RESULTS

PREFORMULATION STUDIES

DESCRIPTION

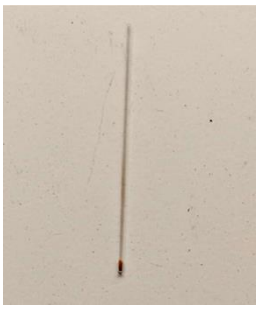
Color: White

Odor: Pungent

Form: Fine powder

MELTING POINT

TABLE 9: MELTING POINT DETERMINATION

DRUG	MELTING POINT
Papain	Decomposes  **FIG 17: MELTING POINT**

SOLUBILITY

TABLE 10: SOLUBILITY PROFILE OF PAPAIN

S.NO	SOLVENT	SOLUBILITY
1	Distilled water	Freely soluble
2	Phosphate buffer	Very soluble
3	Ethanol	Practically insoluble
4	Chloroform and ether	Insoluble
5	Propylene glycol	Soluble

HYGROSCOPIC NATURE

TABLE 11: HYGROSCOPIC NATURE OF PAPAIN

AT ROOM TEMPERATURE	75% RH AT 40^{O}C
Sample No – 1	Sample No - 2
Weight gain observed nil	Weight gain observed nil

IDENTIFICATION OF DRUG SAMPLE:

TABLE 12: ABSORPTION MAXIMA OF PAPAIN IN PHOSPHATE BUFFER pH 7.4

SOLVENT	λ MAX	ABSORBANCE
Phosphate buffer pH 7.4	277.40	0.488

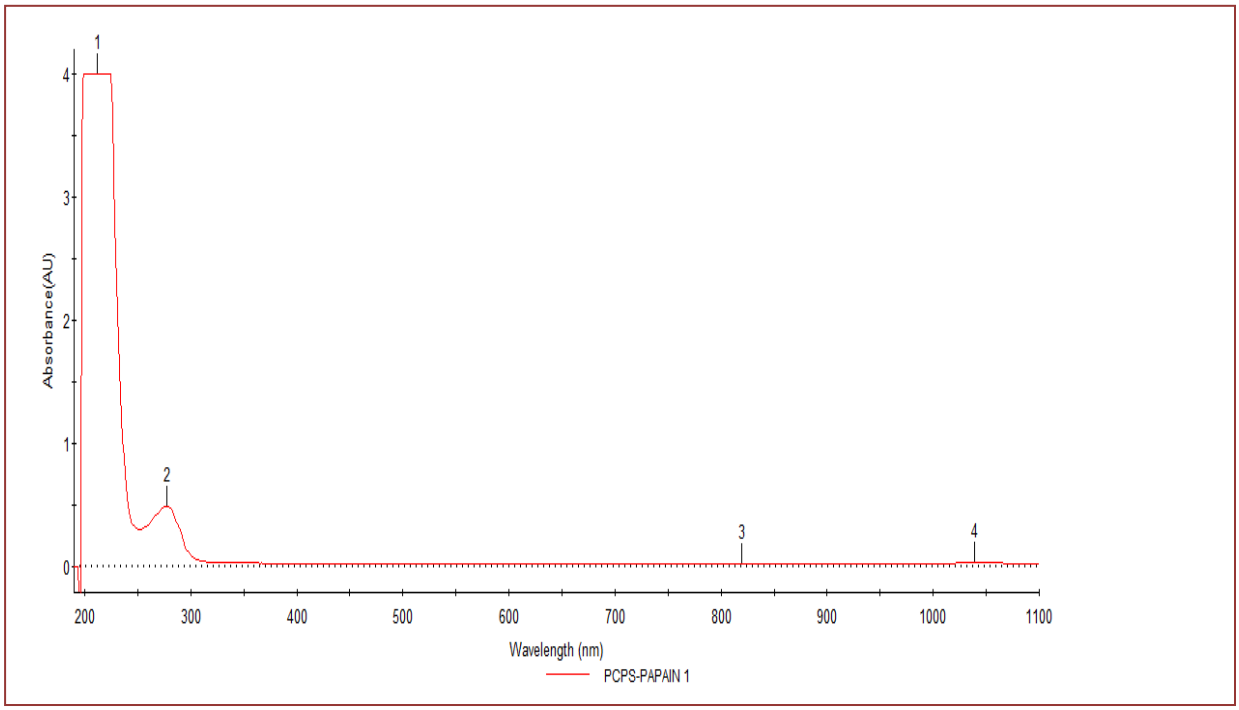


FIG 18: UV SPECTRUM OF PAPAIN IN PHOSPHATE BUFFER pH 7.4

STANDARD PLOT OF PAPAIN IN PHOSPHATE BUFFER pH 7.4

TABLE 13: UV ABSORBANCE PAPAIN IN PHOSPHATE BUFFER pH 7.4

S.NO	CONCENTRATION µg/ml	ABSORBANCE AT 277.4nm
1	10	0.021
2	20	0.038
3	30	0.051
4	40	0.065
5	50	0.080
6	60	0.105

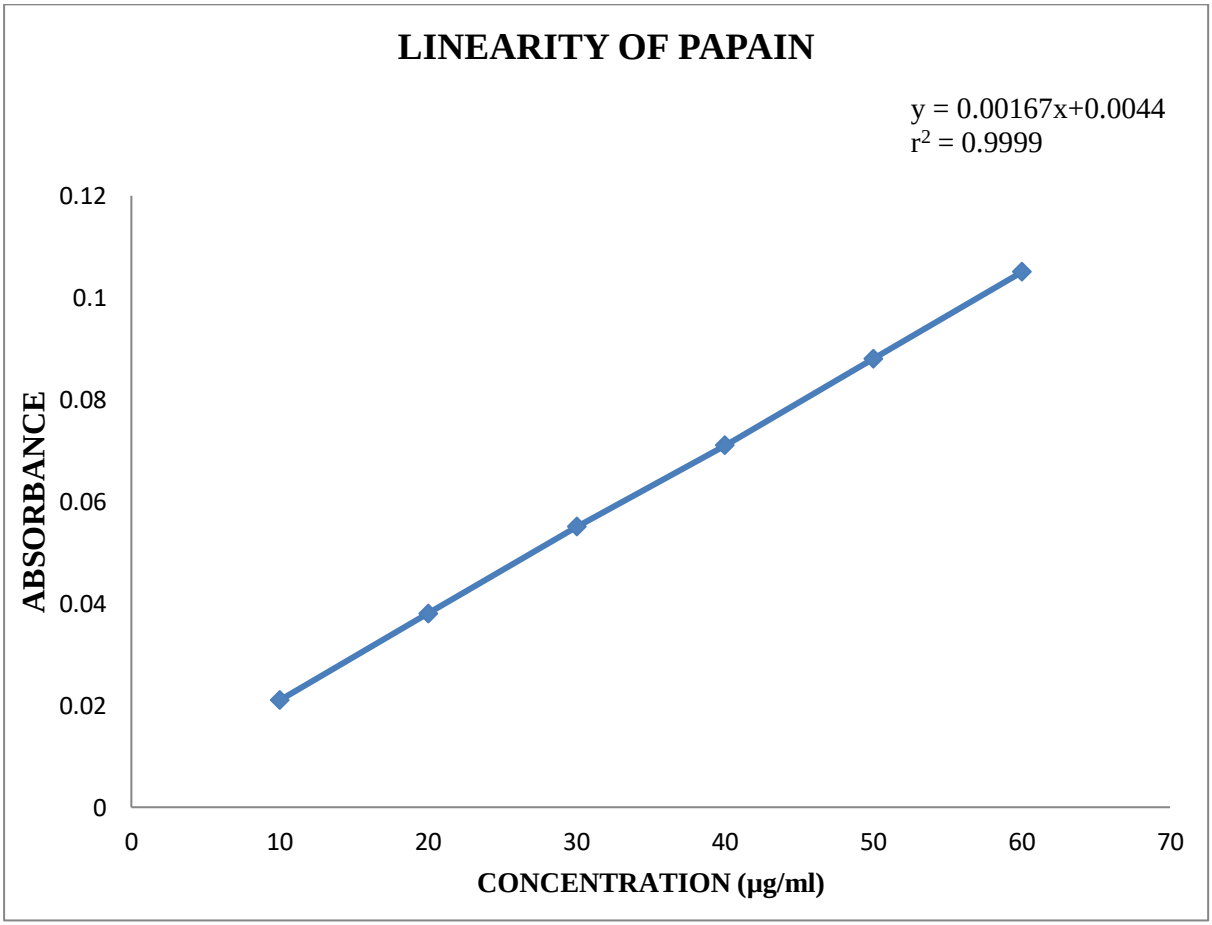


FIG 19: STANDARD PLOT OF PAPAIN

FTIR STUDIES

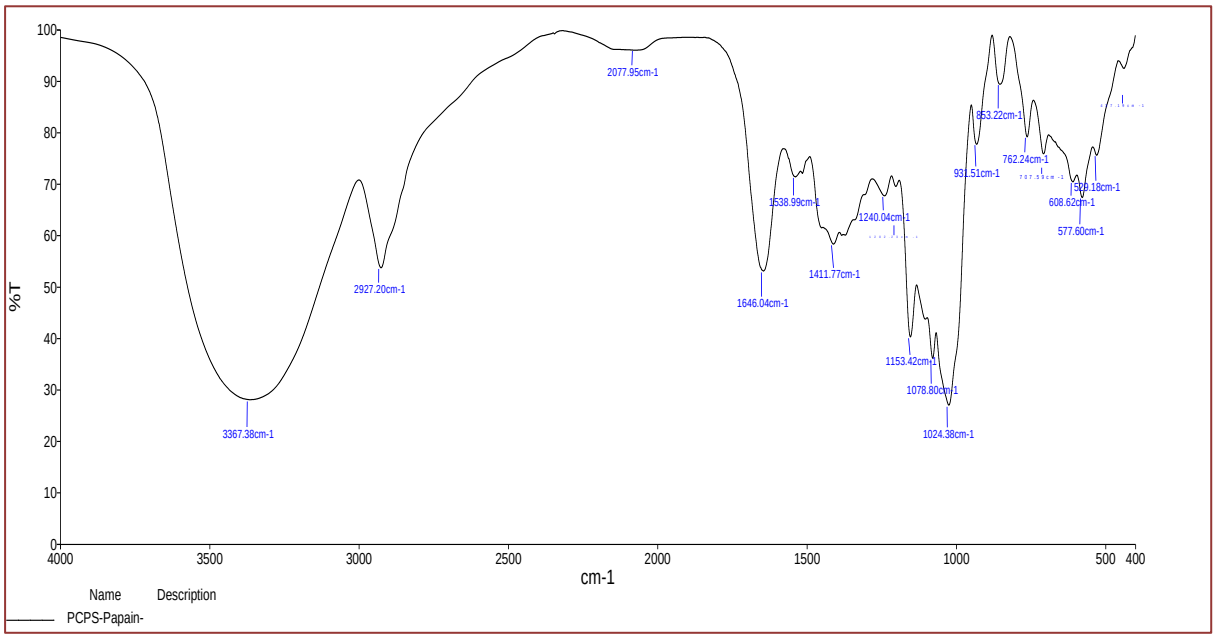


FIG 20: FTIR OF PAPAIN

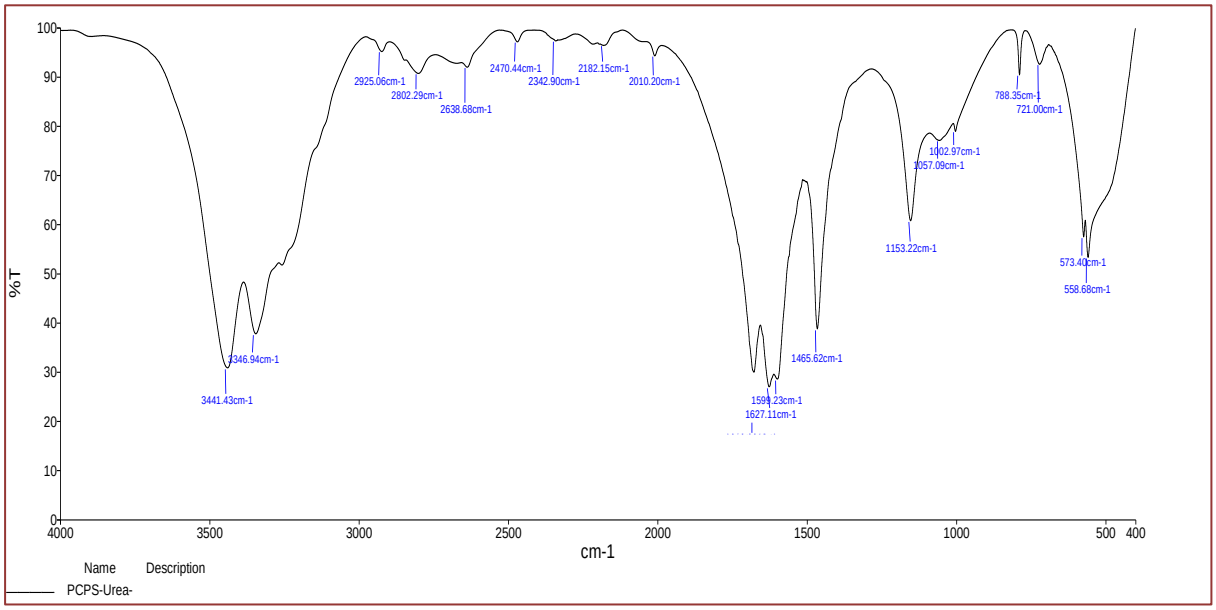


FIG21: FTIR OF UREA

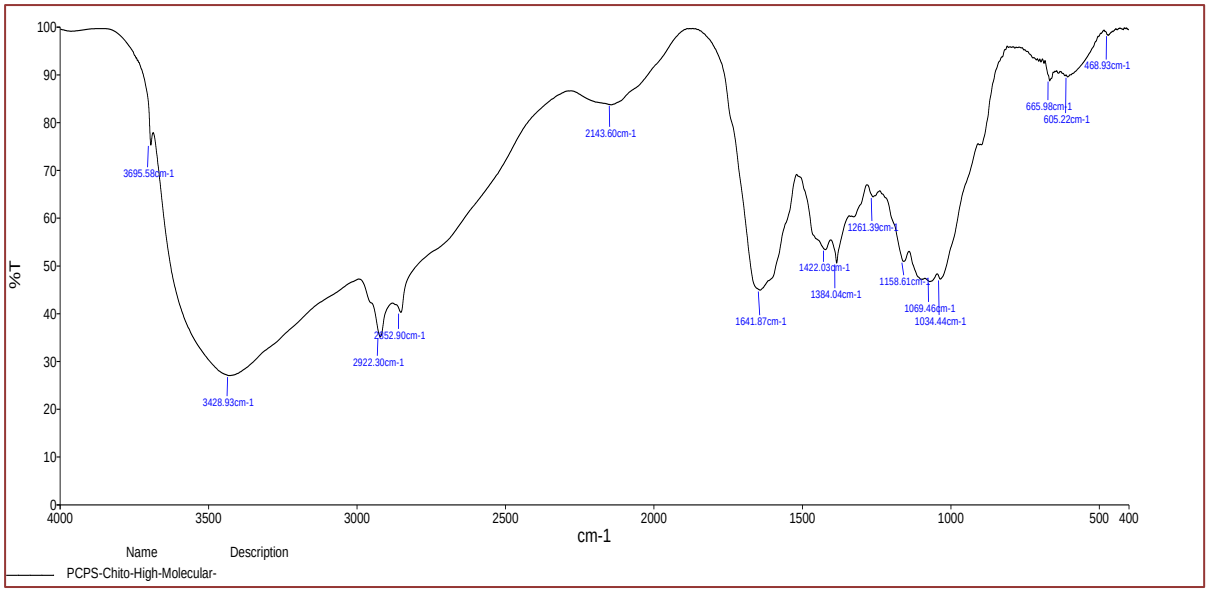


FIG 22: FTIR OF CHITOSAN HIGH MOLECULAR WEIGHT

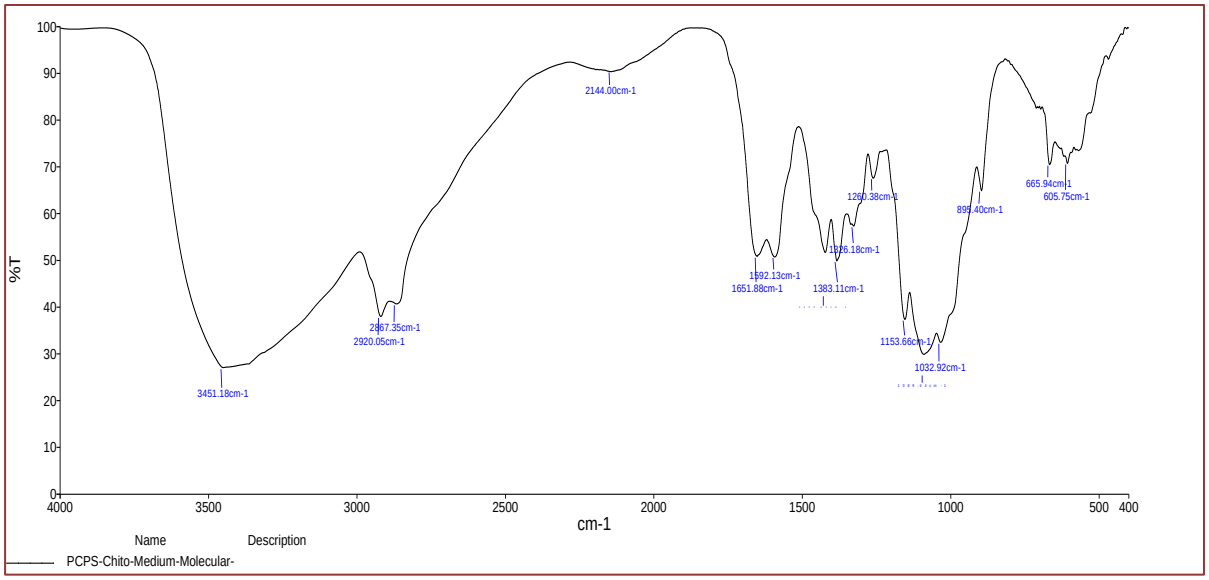


FIG 23: FTIR OF CHITOSAN LOW MOLECULAR WEIGHT

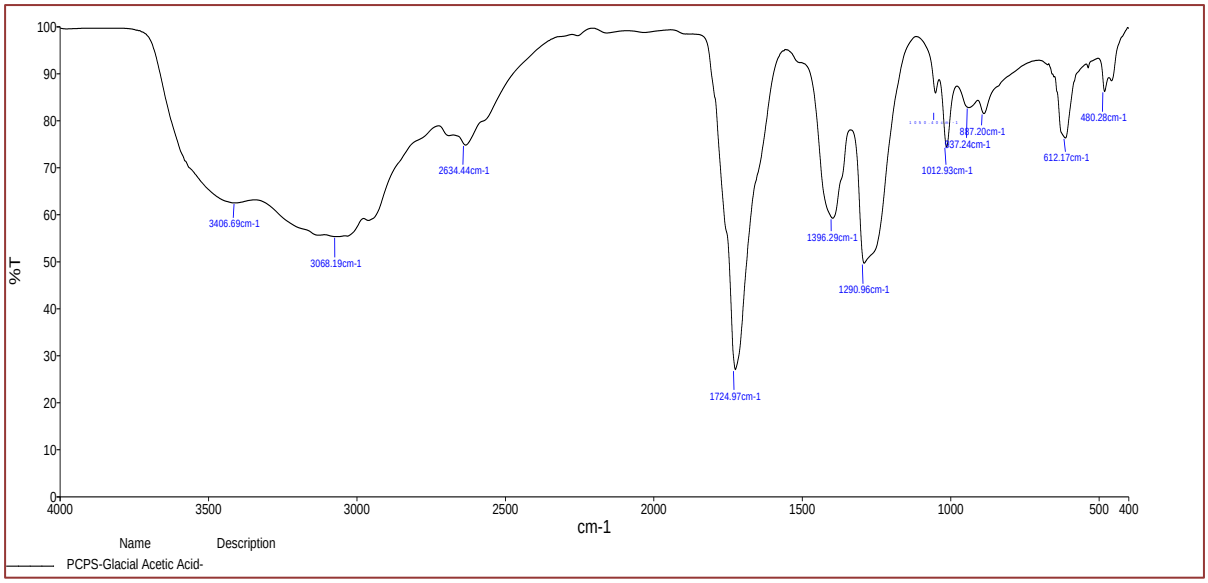


FIG 24: FTIR OF GLACIAL ACETIC ACID

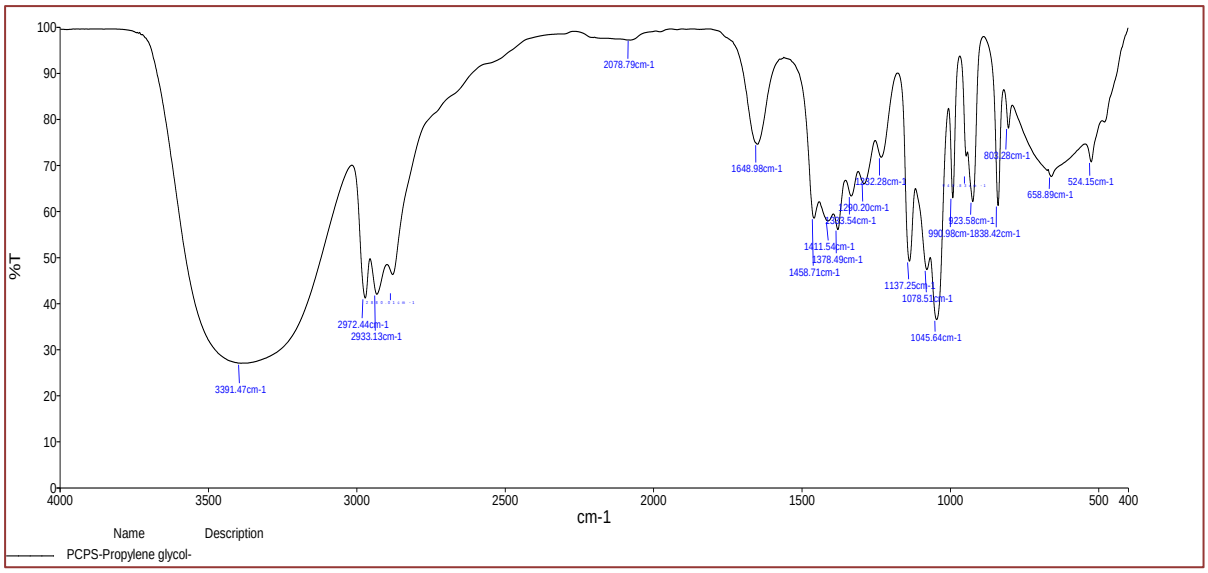


FIG 25: FTIR OF PROPYLENE GLYCOL

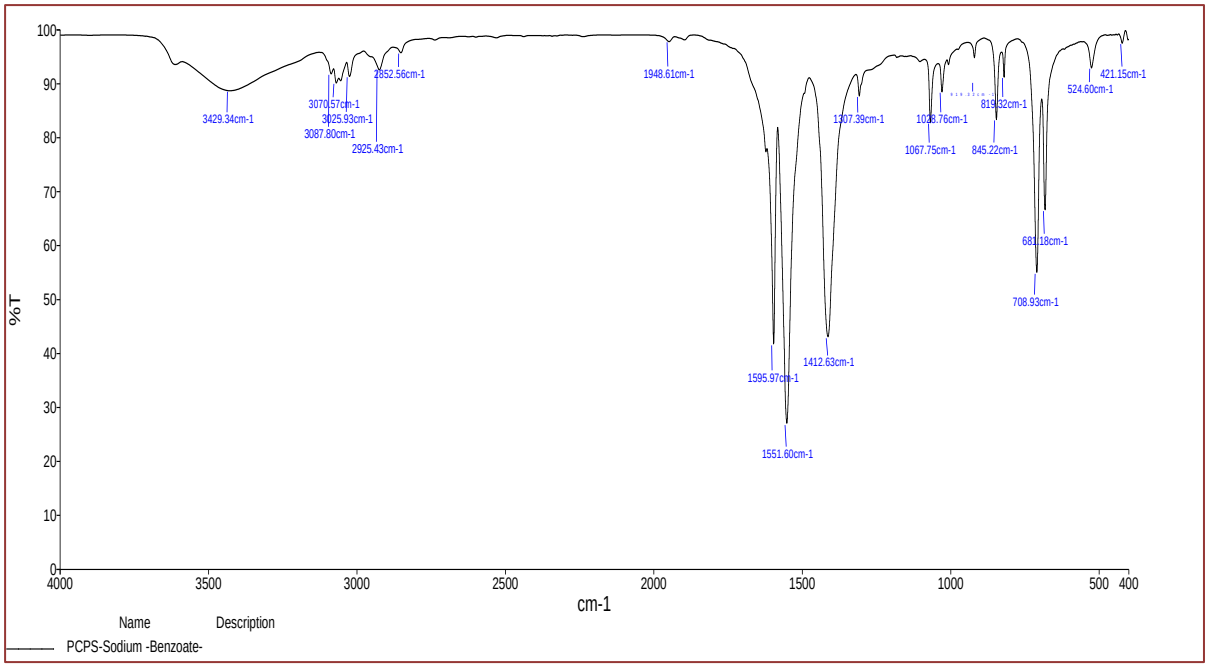


FIG 26: FTIR OF SODIUM BENZOATE

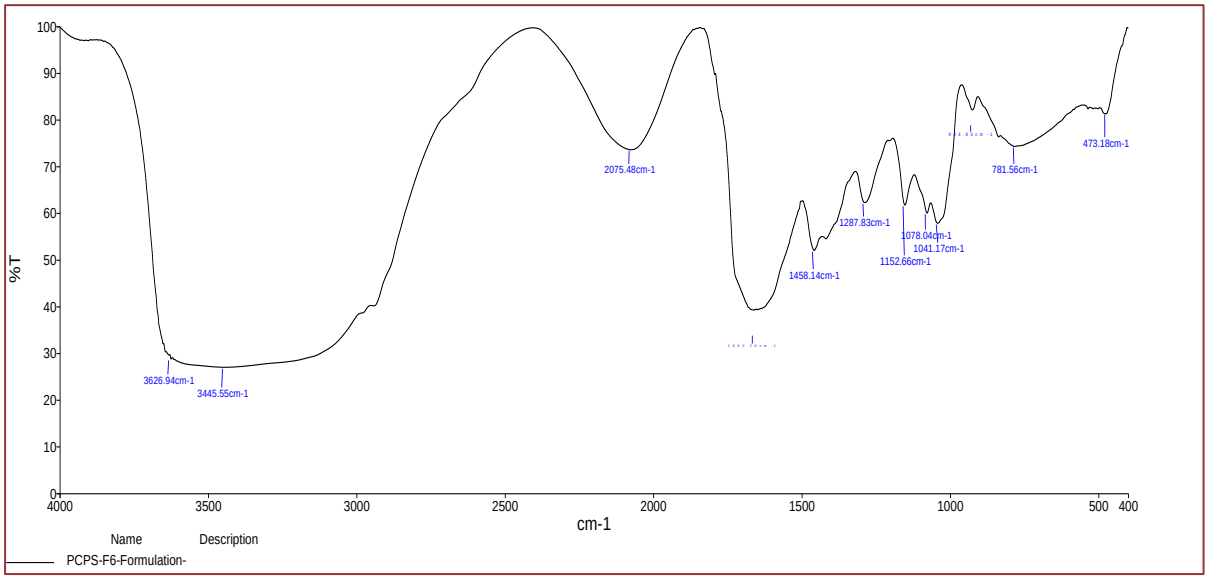


FIG 27: FTIR OF PHYSICAL MIXTURE

TABLE 14: PRINCIPLE PEAKS AND CHEMICAL GROUP PRESENT IN IR STUDY

SNo	Functional groups	Peak Values in Cm^{-1}							
		Papain	Urea	Chitosan High M.wt.	Chitosan Low M.wt.	Glacial Acetic Acid	Propylene Glycol	Sodium benzoate	Physical Mixture
1	N- H Stretching	3367.38	3346.94	3428.93	-	-	-	-	-
2	C- H Stretching	2927.20	-	2852.90	2920.05	-	8972.44	3087.80	-
3	C=C Stretching	1646.04	1641.87	-	-	-	1648.98	1595.97	-
4	N- O Stretching	1538.99	-	-	-	-	-	-	-
5	O-H Bending	1411.77	-	1422.03	1328.18	1396.29	1411	1412.63	-
6	C-N Stretching	1240	-	-	1153.66	-	-	-	1287.83
7	C-O Stretching	1153.42	-	1158.61	-	1290.96	-	1307.39	1152.66
8	O-H Stretching	-	3441.43	3695.53	3451.18	3406.61	3391.47	3429.34	3624.94
9	N-H Bending	-	1627.11	1641.87	-	-	-	-	-
10	C-H Bending	-	-	1384.04	1383	887.20	-	-	1660.28
11	C=O Stretching	-	-	-	1260.38	1724.97	-	-	-
12	C=C Bending	-	-	665.98	895.40	-	-	-	-

There are no extra peaks seen other than the normal peal in the spectra of the mixture of the drug and excipients and so there is no interaction with the drug and excipients and they are compatible with each other.

The IR spectra of the drug and polymer combination were compared with the spectra of the pure drug and individual excipients in which no shifting of peaks was significantly found, indicating the stability of drug during film forming hydrogel development

CHARACTERIZATION

CLARITY

TABLE 15: FORMULATION APPEARANCE

SNO	FORMULATION	APPEARANCE AND TRANSPARENCY
1	F1	Transparent and clear
2	F2	Transparent and clear
3	F3	Transparent and clear
4	F4	Transparent and clear
5	F5	Transparent and clear
6	F6	Transparent and clear

FIG 28: FORMULATION F6

pH DETERMINATION

TABLE 16: COMPARATIVE pH OF FORMULATIONS

SNO	FORMULATION	pH
1	F1	6.51± 0.03
2	F2	6.68± 0.08
3	F3	6.70± 0.04
4	F4	6.41± 0.06
5	F5	6.52± 0.01
6	F6	6.70± 0.04

All values are expressed as mean ± SD, n=3

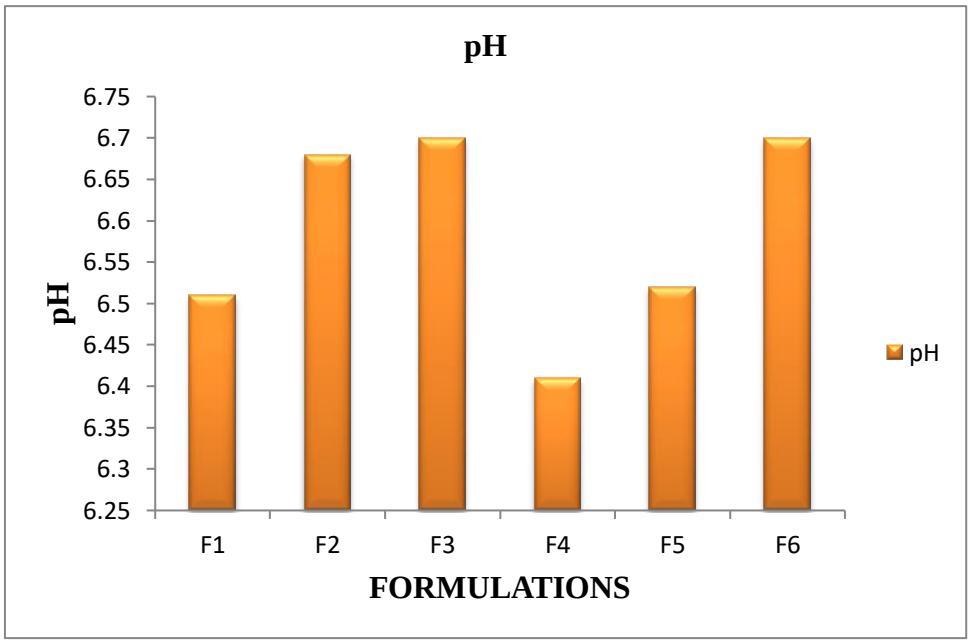


FIG 29: COMPARATIVE pH OF FORMULATIONS

HOMOGENEITY

TABLE 17: COMPARATIVE HOMOGENEITY OF FORMULATION

SNO	FORMULATION	HOMOGENEITY
1	F1	Homogeneous
2	F2	Homogeneous
3	F3	Homogeneous
4	F4	Homogeneous
5	F5	Homogeneous
6	F6	Homogeneous

EXTRUDABILITY

TABLE 18: COMPARATIVE EXTRUDABILITY OF FORMULATIONS

SNO	FORMULATION	EXTRUDABILITY
1	F1	+
2	F2	++
3	F3	++
4	F4	+
5	F5	++
6	F6	++

+ - Satisfactory **++ - Excellent**

SPREADABILITY

TABLE 19: COMPARATIVE SPREADABILITY OF FORMULATIONS

SNO	FORMULATION	SPREADABILITY (cm)
1	F1	7.00± 0.08
2	F2	6.21± 0.09
3	F3	5.70± 0.06
4	F4	7.34± 0.05
5	F5	6.76± 0.08
6	F6	5.50± 0.05

All values are expressed as mean ± SD, n=3

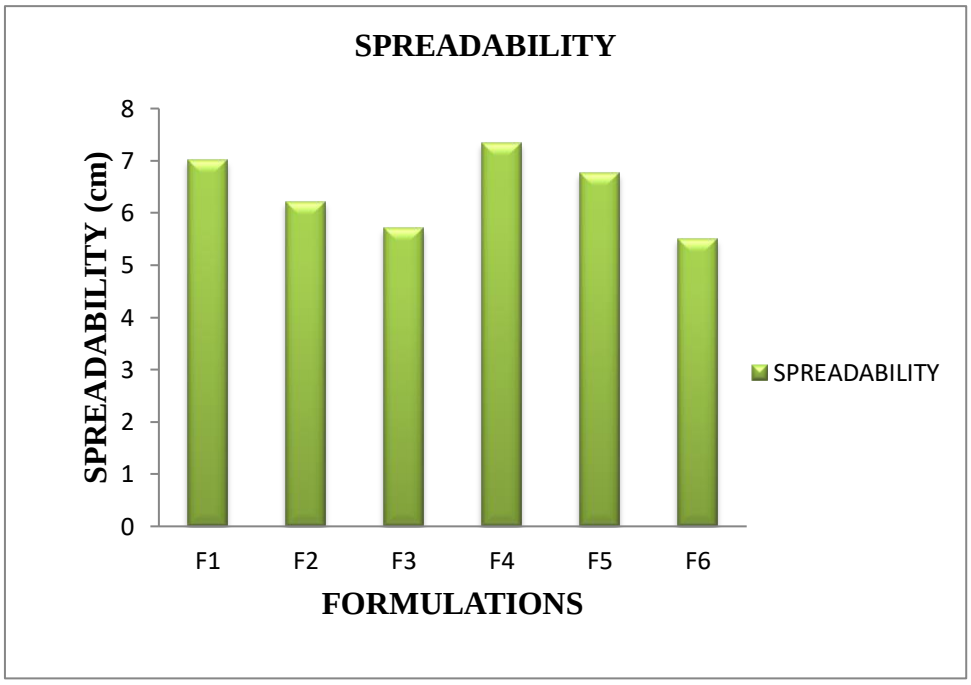


FIG 30: COMPARATIVE SPREADABILITY OF FORMULATIONS

VISCOSITY

TABLE 20: COMPARATIVE VISCOSITY OF FORMULATIONS

SNO	FORMULATION	VISCOSITY (cps)
1	F1	2500±60.27
2	F2	3600±136.16
3	F3	4500±56.34
4	F4	2900±55.12
5	F5	3800±152.54
6	F6	4900±67.05

All values are expressed as mean ± SD, n=3

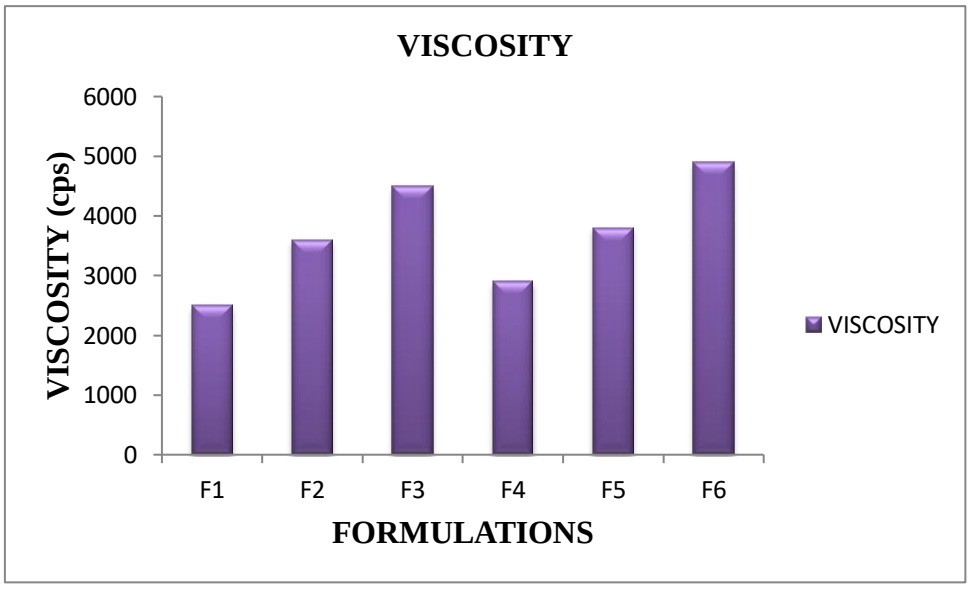


FIG 31: COMPARATIVE VISCOSITY OF FORMULATIONS

DRUG CONTENT

TABLE 21: COMPARATIVE CONTENT UNIFORMITY OF FORMULATIONS

SNO	FORMULATION	CONTENT UNIFORMITY (%)
1	F1	96.42± 0.40
2	F2	95.65± 0.33
3	F3	98.75± 0.27
4	F4	97.65± 0.42
5	F5	96.62± 0.27
6	F6	98.99± 0.34

All values are expressed as mean ± SD, n=3

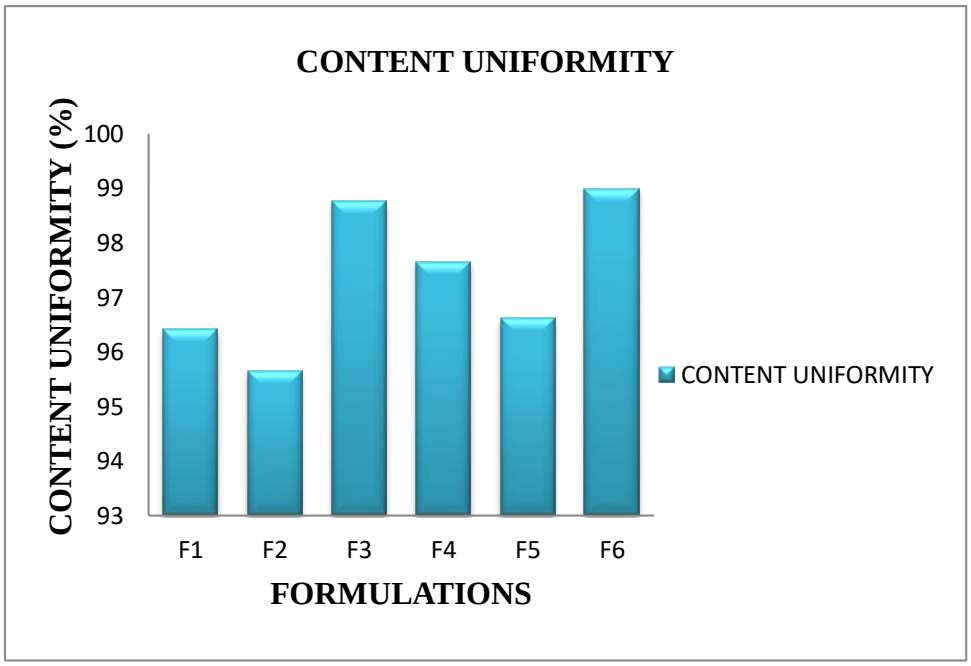


FIG 32: COMPARATIVE CONTENT UNIFORMITY OF FORMULATIONS

DRYING TIME

TABLE 22: COMPARATIVE DRYING TIME OF FORMULATIONS

SNO	FORMULATION	DRYING TIME (min)
1	F1	7min± 44sec
2	F2	6min± 56sec
3	F3	5min± 12sec
4	F4	7min± 20sec
5	F5	6min± 46sec
6	F6	5min± 20sec

All values are expressed as mean ± SD, n=3

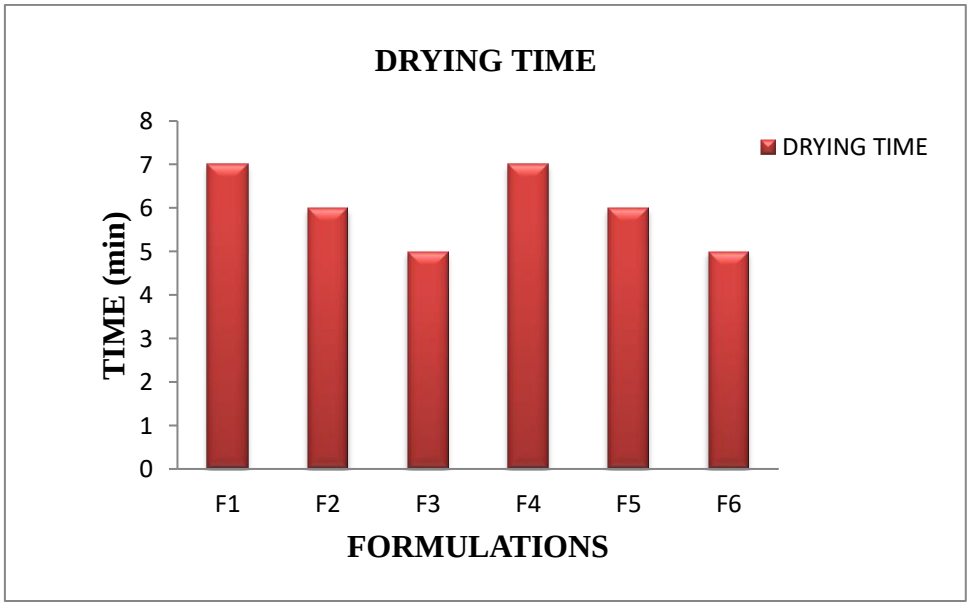


FIG 33: COMPARATIVE DRYING TIME OF FORMULATIONS

FIG 34: FIGURE SHOWING FILM FORMATION OF F6 FORMULATION

IN VITRO DIFFUSION STUDIES

TABLE 23: COMPARATIVE *IN VITRO* DIFFUSION STUDIES OF FORMULATIONS

TIME (hrs)	CUMULATIVE PERCENTAGE OF DRUG RELEASE (%)					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	38.03±0.13	24.79±0.56	10.23±0.34	20.12±0.19	25.79±0.22	13.42±0.46
2	44.88±0.56	30.47±0.23	15.34±0.46	34.16±0.21	29.87±0.26	18.27±0.35
3	53.26±0.11	39.55±0.14	19.67±0.23	44.12±0.25	33.45±0.19	23.27±0.24
4	58.05±0.23	46.29±0.43	27.57±0.56	50.69±0.54	38.98±0.34	30.81±0.31
5	66.83±0.54	50.33±0.10	34.09±0.26	62.82±0.56	45.65±0.54	47.39±0.36
6	80.75±0.67	56.41±0.22	40.23±0.35	75.56±0.66	55.34±0.56	58.53±0.27
7	96.89±0.12	61.54±0.56	66.56±0.34	83.89±0.45	59.86±0.19	66.59±0.19
8	-	64.44±0.19	86.45±0.31	95.60±0.11	64.34±0.43	79.98±0.18
10	-	69.56±0.21	93.45±0.18	-	69.57±0.16	96.56±0.34
12	-	77.45±0.30	95.45±0.24	-	73.98±0.32	98.19±0.25

All values are expressed as mean ± SD, n=3

FIG 35: *IN VITRO* DIFFUSION STUDY

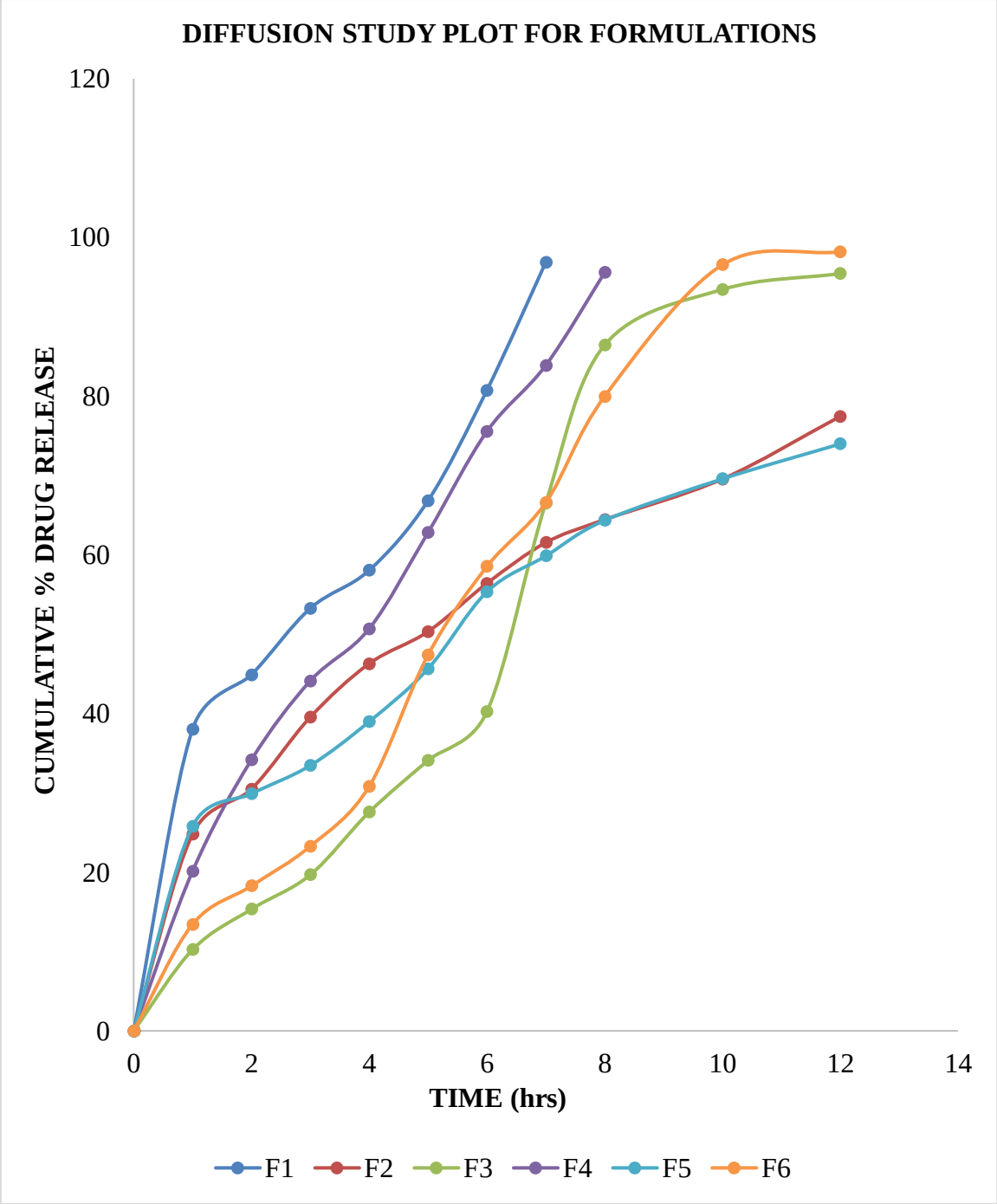
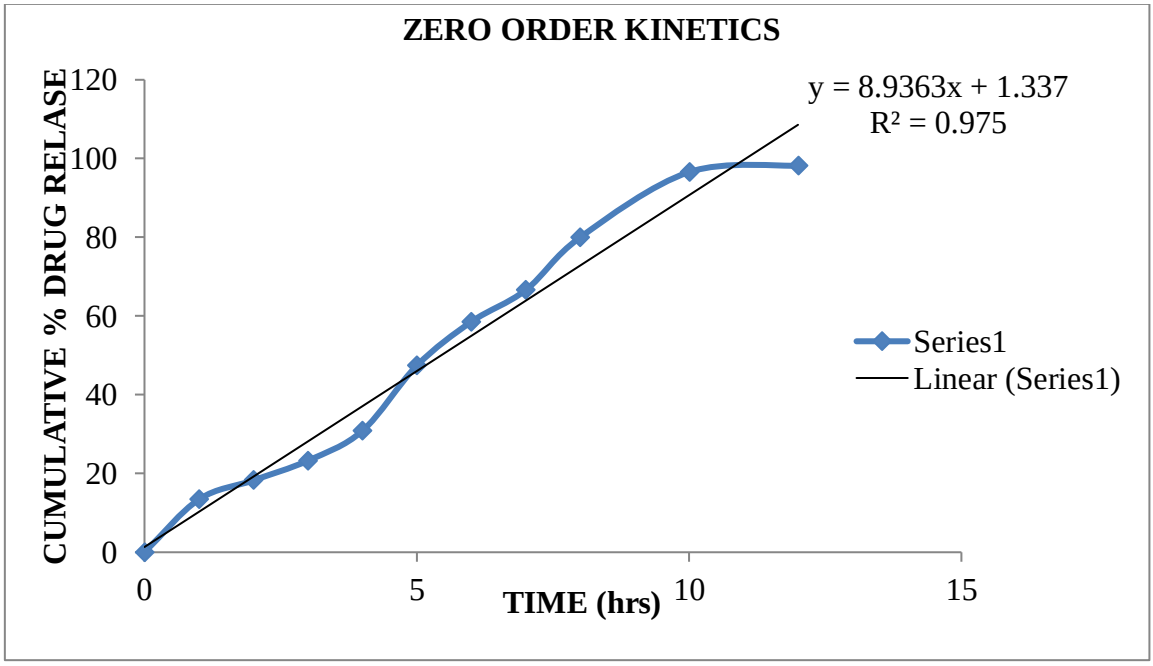


FIG 36: COMPARATIVE *IN VITRO* DIFFUSION STUDIESOF FORMULATIONS

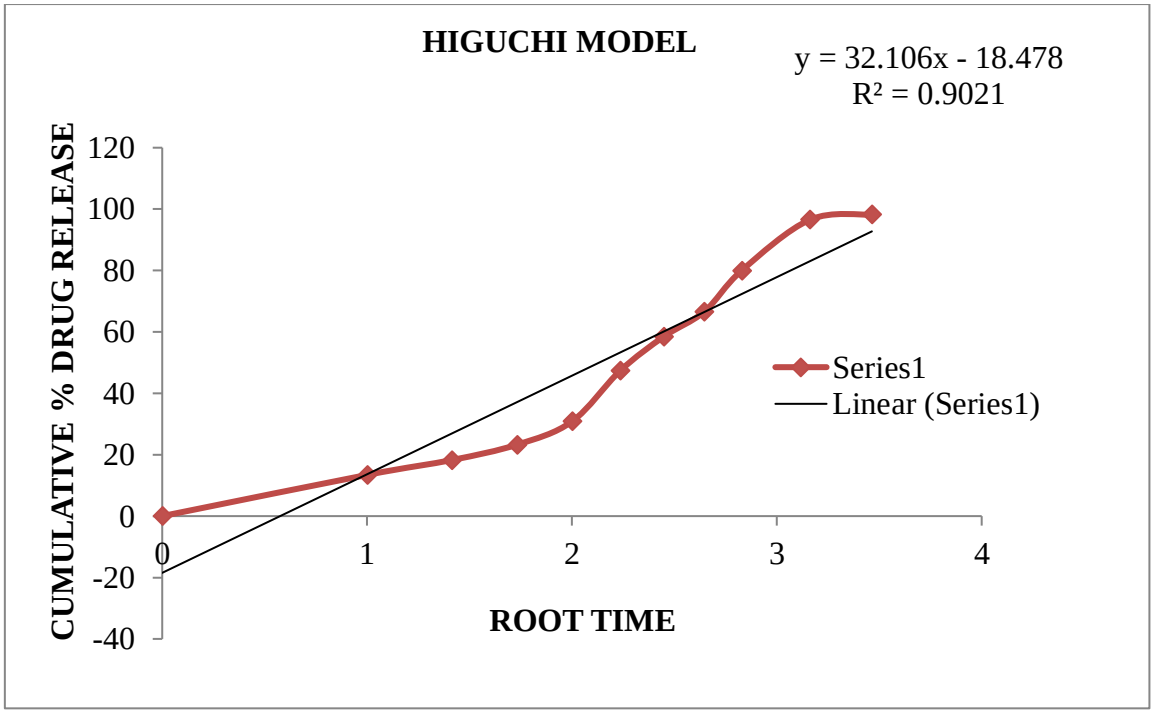
RELEASE KINETIC STUDIES

TABLE 24: COMPARATIVE RELEASE KINETIC STUDIES OF FORMULATIONS

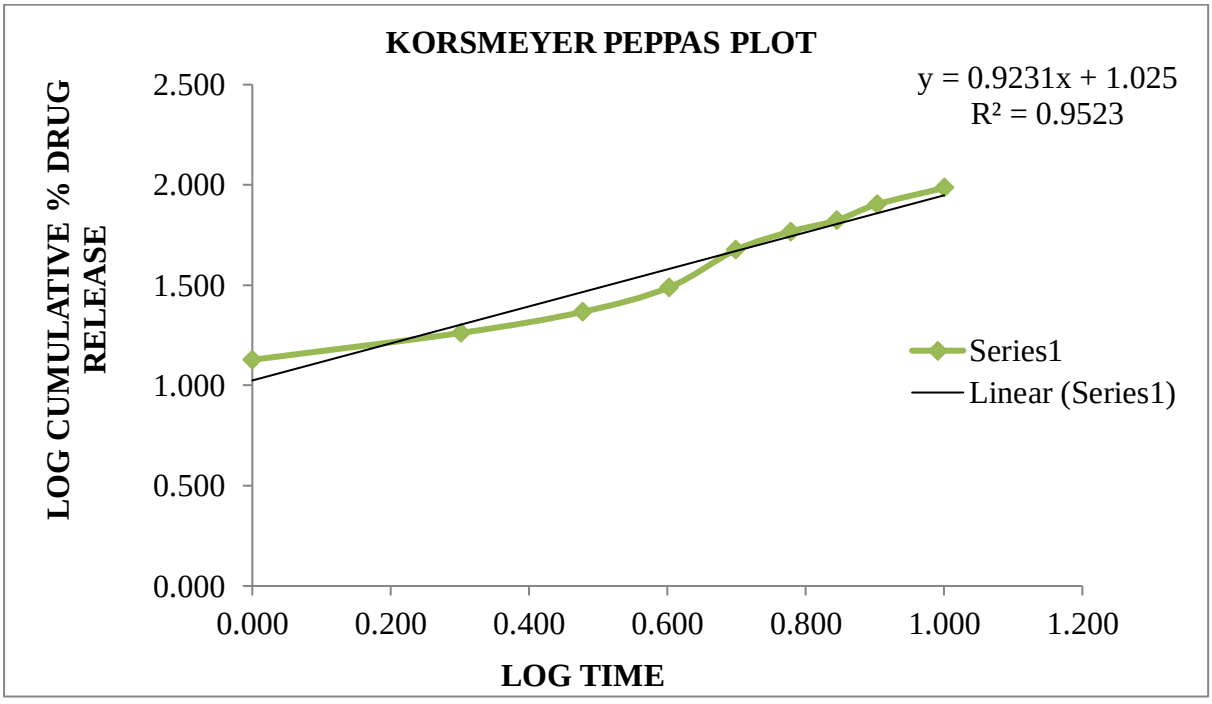
FORMULATION CODE	ZERO	HIGUCHI	PEPPAS		FIRST
	r^2	r^2	r^2	n	r^2
F3	0.9402	0.8949	0.9321	0.887	0.8734
F6	0.9750	0.9021	0.9327	0.859	0.8610



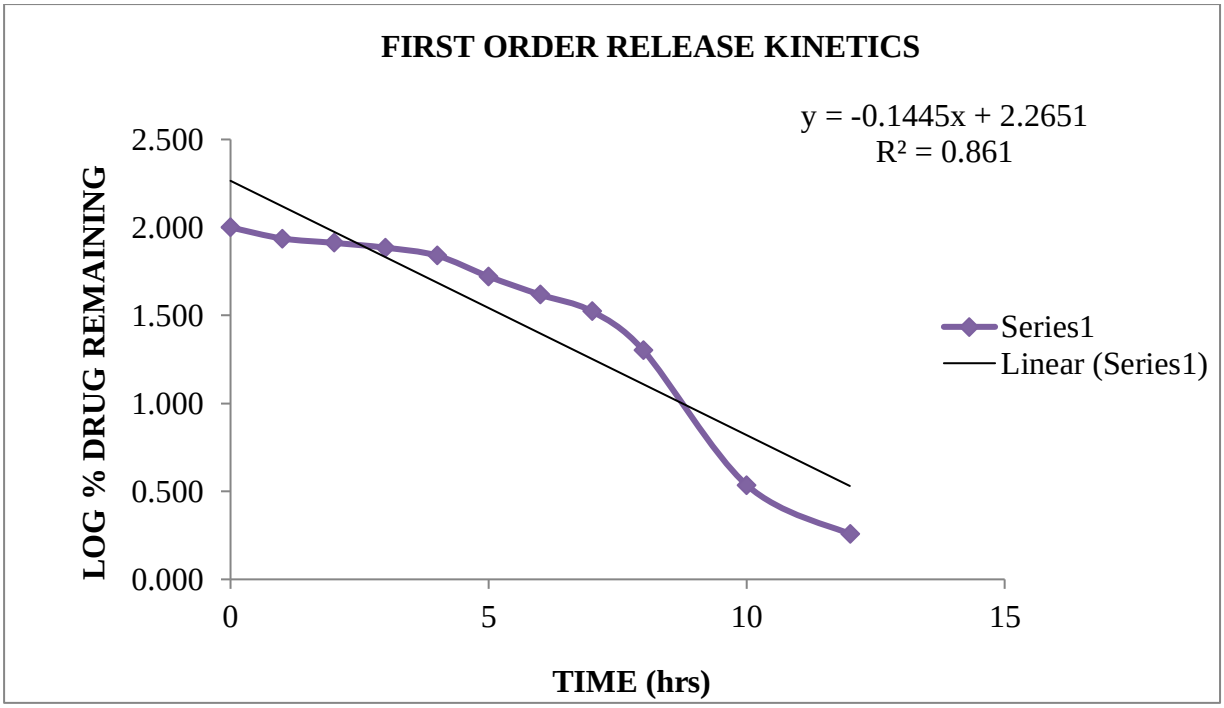
A. GRAPH SHOWING ZERO ORDER RELEASE KINETICS FOR F3 FORMULATION



B. GRAPH SHOWING HIGUCHI MODEL FOR F3 FORMULATION

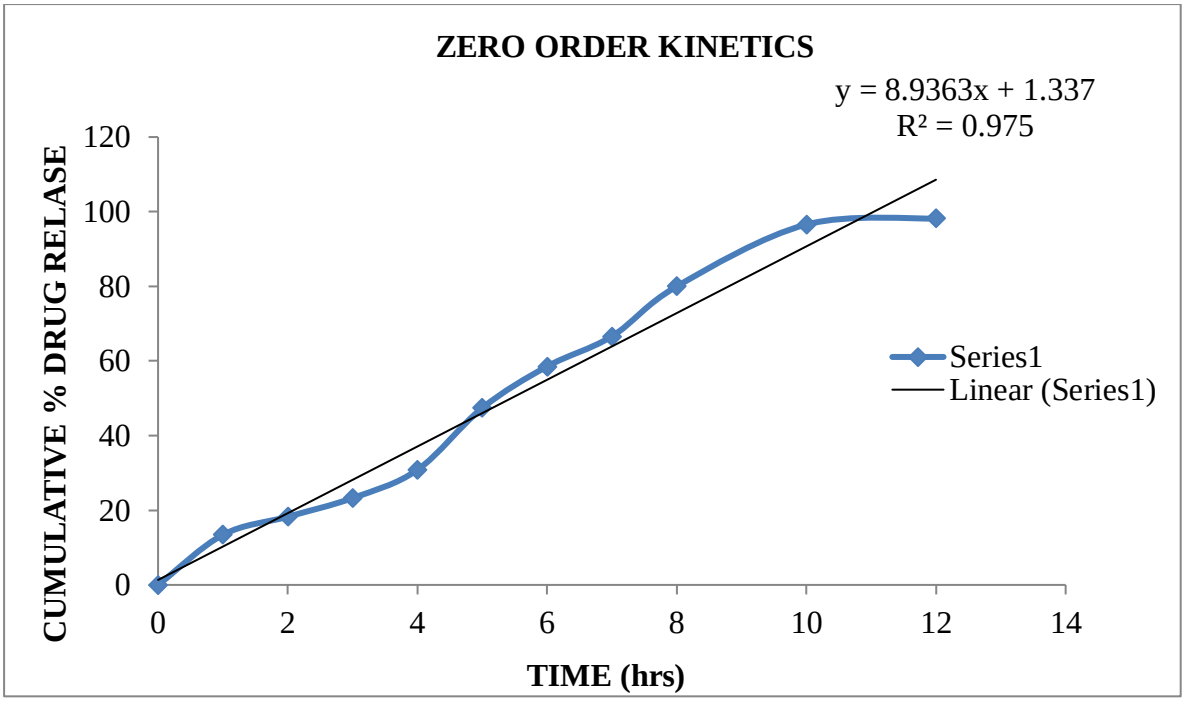


C. GRAPH SHOWING KORSMEYER PEPPAS MODEL FOR F3 FORMULATION

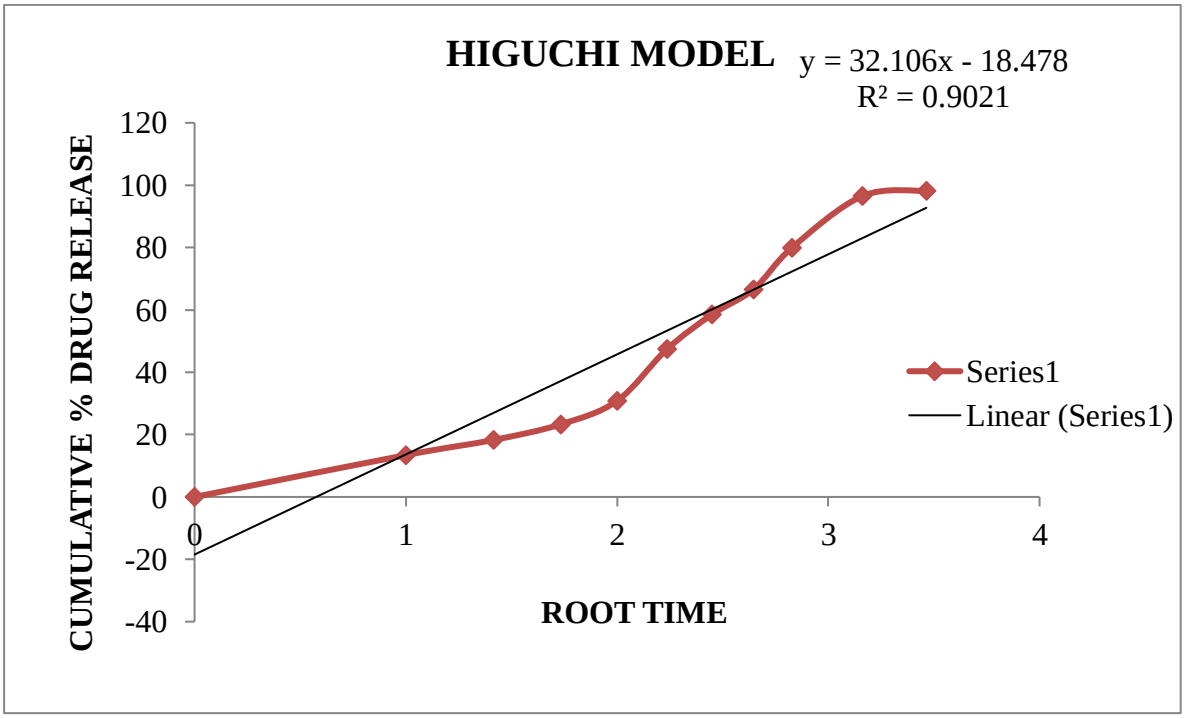


D. GRAPH SHOWING FIRST ORDER RELEASE KINETICS FOR F3 FORMULATION

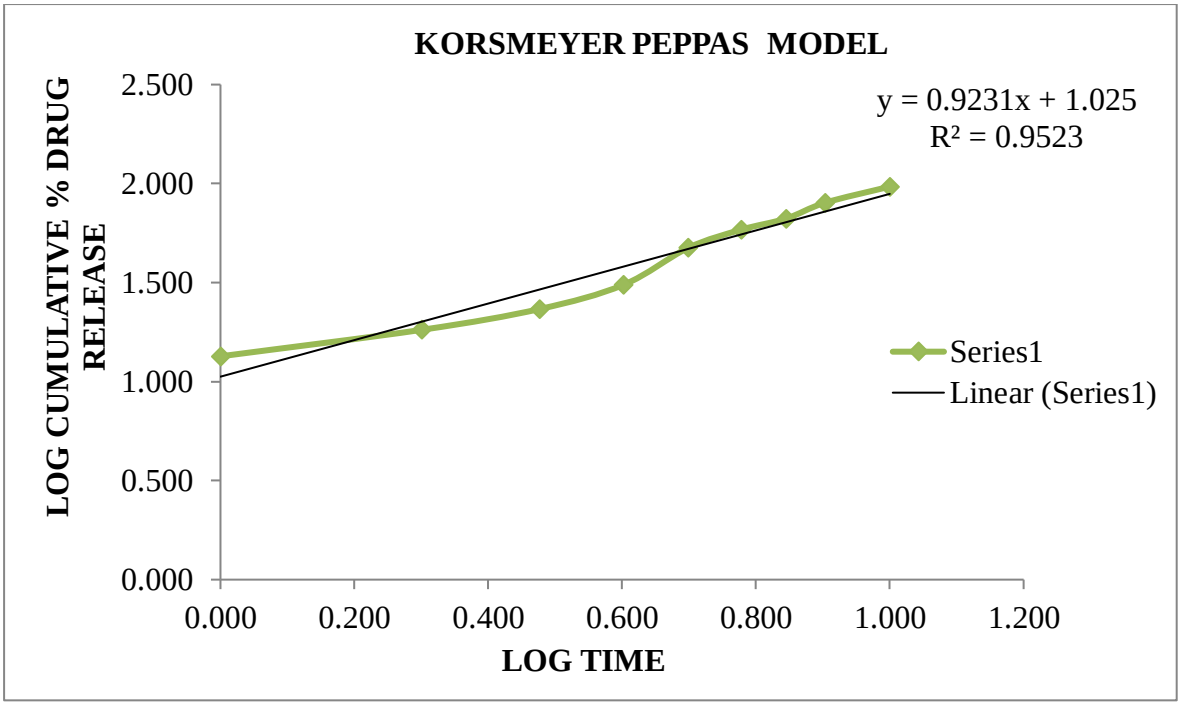
FIG 37: RELEASE KINETICS FOR F3 FORMULATION



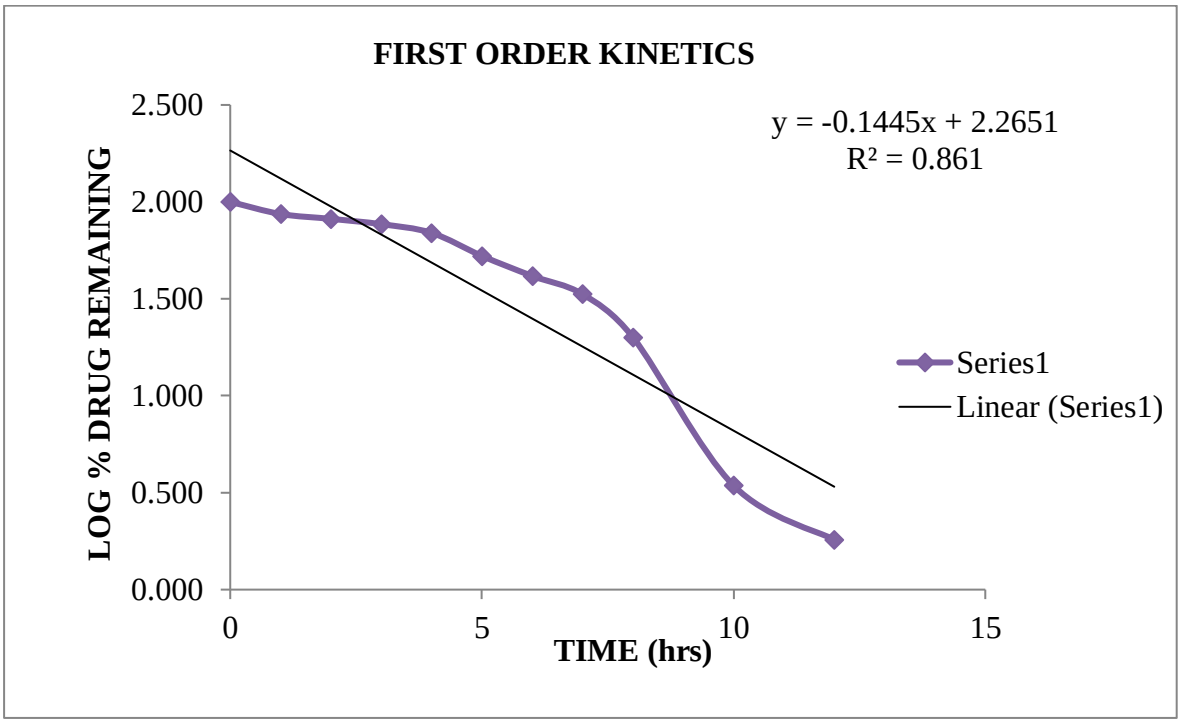
A. GRAPH SHOWING ZERO ORDER RELEASE KINETICS FOR F6 FORMULATION



B. GRAPH SHOWING HIGUCHI MODEL FOR F6 FORMULATION



C. GRAPH SHOWING KORSMEYER PEPPAS MODEL FOR F6 FORMULATION



D. GRAPH SHOWING FIRST ORDER RELEASE KINETICS FOR F6 FORMULATION

FIG 38: RELEASE KINETICS FOR F6 FORMULATION

INVITRO WOUND HEALING ACTIVITY

MTT assay Cell Cytotoxicity

TABLE 25: OD VALUE AT 570 nm

S. NO	TESTED SAMPLE CONCENTRATION (µg/ml)	OD VALUE AT 570 nm (IN TRIPLICATES)		
1.	Control	0.498	0.439	0.507
2.	10µg/ml	0.485	0.454	0.472
3.	20 µg/ml	0.463	0.446	0.455
4.	40µg/ml	0.436	0.439	0.427
5.	60µg/ml	0.408	0.421	0.410
6.	80 µg/ml	0.399	0.379	0.404
7.	100 µg/ml	0.353	0.360	0.376
8.	200 µg/ml	0.331	0.333	0.349
9.	300 µg/ml	0.315	0.325	0.300
10.	400 µg/ml	0.308	0.288	0.279
11.	500 µg/ml	0.259	0.257	0.216

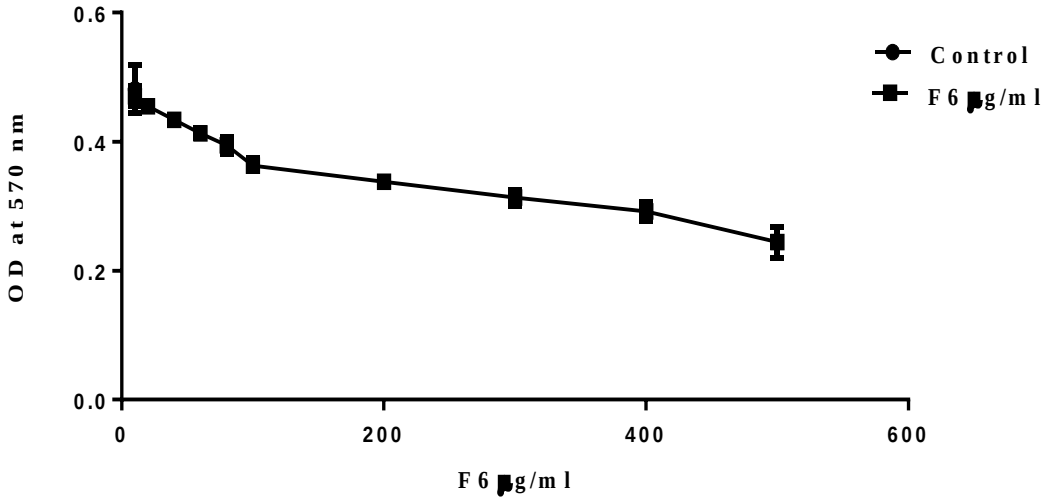


FIG 39: OD VALUE OFCONTROL VS F6 AT 570 nm

TABLE 26: CELL VIABILITY % OF F6

S.NO	TESTED SAMPLE CONCENTRATION (µg/ml)	CELL VIABILITY (%) (IN TRIPLICATES)			MEAN VALUE (%)
1	Control	100	100	100	100
2	10µg/ml	97.3896	103.417	93.0966	97.9676
3	20 µg/ml	92.9719	101.595	89.7436	94.7700
4	40µg/ml	87.5502	100	84.2209	90.5903
5	60µg/ml	81.9277	95.8998	80.8679	86.2317
6	80 µg/ml	80.1205	86.3326	79.6844	82.0458
7	100 µg/ml	70.8835	82.0046	74.1617	75.6832
8	200 µg/ml	66.4659	75.8542	68.8363	70.3854
9	300 µg/ml	63.253	74.0319	59.1716	65.4855
10	400 µg/ml	61.8474	65.6036	55.0296	60.8268
11	500 µg/ml	52.008	58.5421	42.6036	51.0512

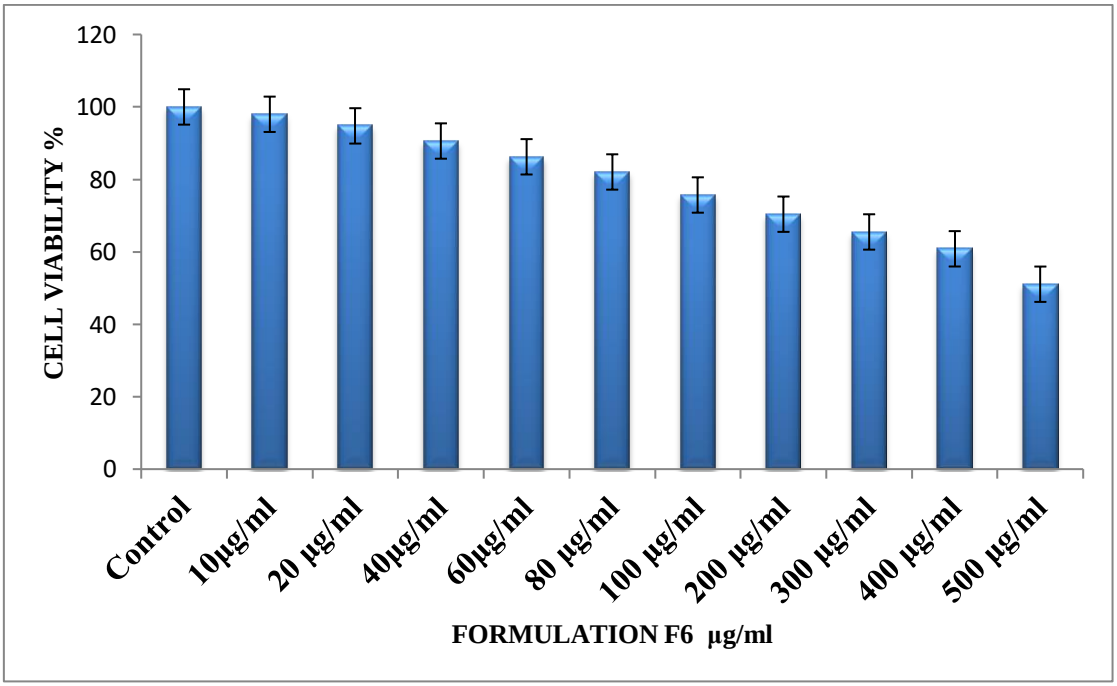


FIG 40: THE EFFECT OF F6 ON VERO CELL VIABILITY AT DIFFERENT CONCENTRATION

TABLE 27: IC50 VALUE OF F6: 130.5µg/ml

log(inhibitor) vs. Normalized response -- Variable slope		
Best-fit values		
LogIC50		2.116
HillSlope		-1.373
IC50		130.5
Std. Error		
LogIC50		0.02611
HillSlope		0.1081
95% Confidence Intervals		
LogIC50		2.062 to 2.169
HillSlope		-1.594 to -1.152
IC50		115.4 to 147.6
Goodness of Fit		
Degrees of Freedom		28
R square		0.9447
Absolute Sum of Squares		1660
Sy.x		7.699
Number of points		
Analyzed	3	30

Images of control cells and F6 treated cells

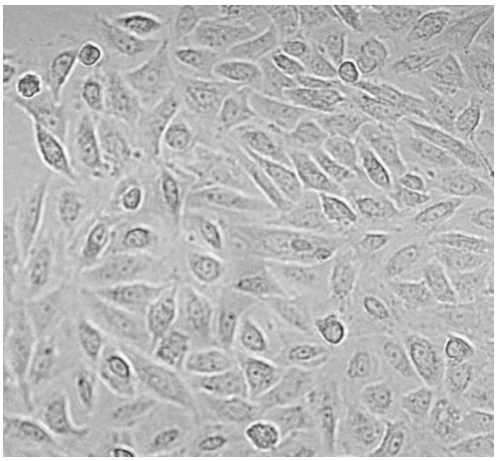


FIG 41: CONTROL

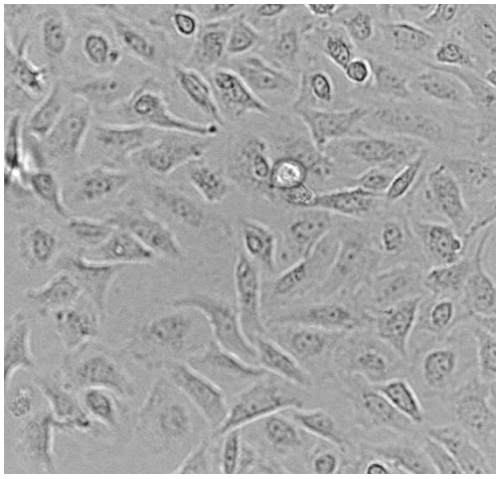


FIG 43: F6 10 µg/ml

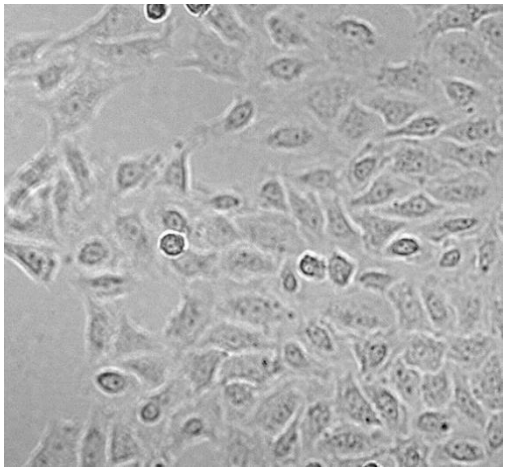


FIG 43: F6 40 µg/ml

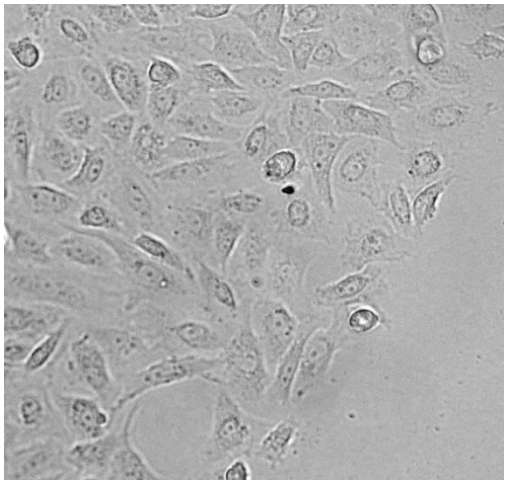


FIG 45: F6 80 µg/ml

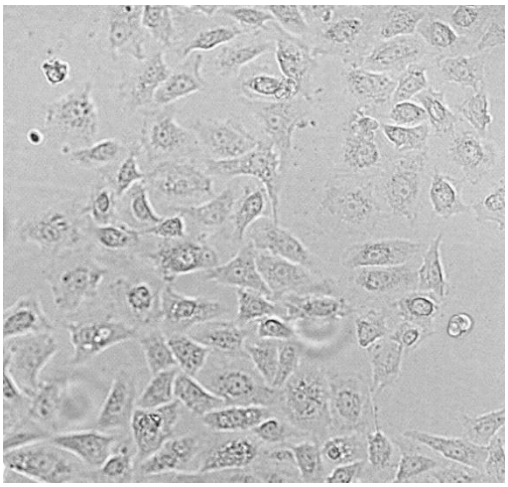


FIG 45: F6 100 µg/ml

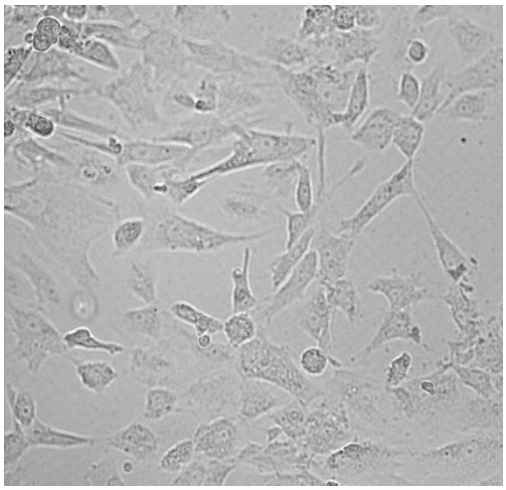


FIG 47: F6 300 µg/ml

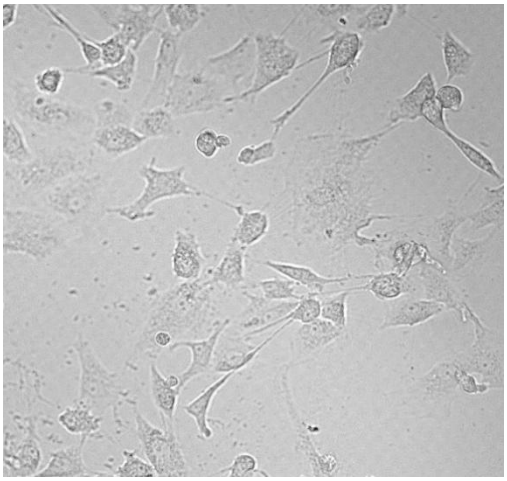


FIG 47: F6 500 µg/ml

Wound healing assay

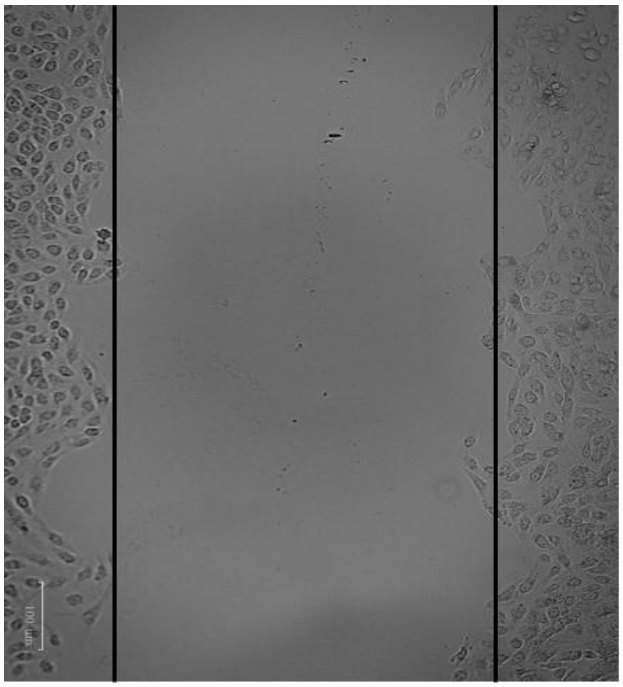


FIG 48: CONTROL SHOWING WOUND AT 0 HOURS

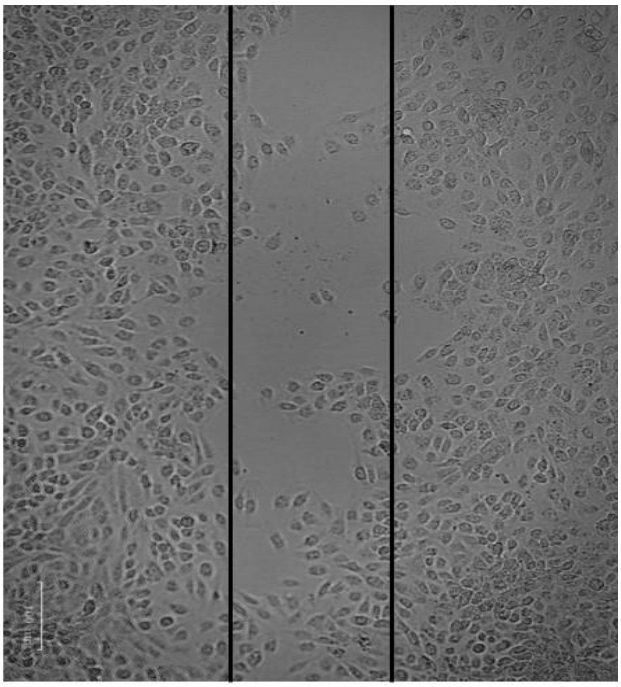


FIG 49: CONTROL SHOWING WOUND AT 24 HOURS

FIG 50: F6 SHOWING WOUND AT 0 HOURS

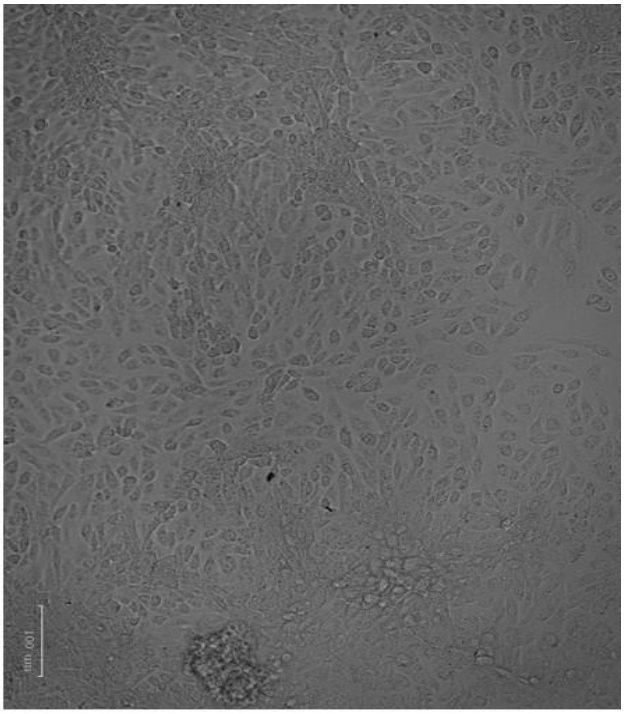


FIG 51: F6 SHOWING WOUND CLOSURE AFTER 24 HOURS

TABLE 28: DENSITOMETRY ANALYSIS OF WOUND HEALING ASSAY FOR CONTROL BY IMAGE J SOFTWARE

TIME	AREA	PERCENT	AREA	PERCENT
0 hr	10396.893	11.1	10470.509	9.80
24hr	84337.143	90.56	89203.679	83.54

TABLE 29: DENSITOMETRY ANALYSIS OF WOUND HEALING ASSAY FOR TREATED BY IMAGE J SOFTWARE

TIME	AREA	PERCENT	AREA	PERCENT
0 hr	10652.345	6.65	10676.496	6.45
24 hr	159377.928	99.50	161653.473	97.68

TABLE 30: DENSITOMETRY ANALYSIS OF WOUND HEALING ASSAY BY IMAGE J SOFTWARE (24 hrs)

SAMPLE DETAILS	PERCENTAGES OF WOUND HEALING (IN DUPLICATES)		MEAN VALUE (%)
Control	90.56	83.54	87.05
Treated with 130µg/ml of F6 sample	99.50	97.68	98.59

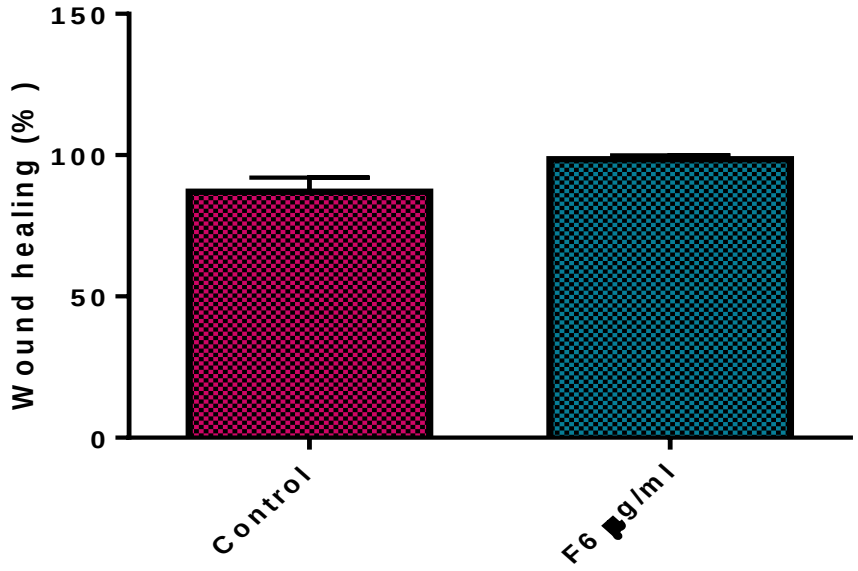


FIG 52: % WOUND HEALING OF CONTROL AND F6

STABILITY STUDIES

TABLE 31: VARIOUS CHARACTERISTICS OF F6 FORMULATION AFTER STABILITY STUDY

STABILITY DURATION	pH	VISCOSITY (cps)	SPREADABILITY (cm)	DRUG CONTENT (%)	DRYING TIME (min)
Before storage	6.7±0.04	4900	5.50± 0.05	98.99± 0.34	5min±20sec
After 1 month	6.7± 0.19	4900	5.50± 0.07	98.99± 0.10	5min±18sec
After 3 month	6.6± 0.05	4900	5.60± 0.12	98.99± 0.21	5min±12sec

TABLE 32: % DRUG RELEASE OF F6 FORMULATION AFTER STABILITY STUDY

TIME (hrs)	BEFORE STORAGE	AFTER 1 MONTH	AFTER 3 MONTH
0	0	0	0
1	13.42± 0.46	11.34± 0.12	10.01± 0.49
2	18.27± 0.35	16.45± 0.43	15.76± 0.33
3	23.27± 0.24	20.12± 0.47	19.45± 0.21
4	30.81± 0.31	29.68± 0.23	28.63± 0.47
5	47.39± 0.36	45.12± 0.37	43.67± 0.19
6	58.53± 0.27	57.68± 0.19	56.37± 0.25
7	66.59± 0.19	69.78± 0.32	65.20± 0.29
8	79.98± 0.18	76.89± 0.54	75.67± 0.38
10	96.56± 0.34	95.78± 0.39	94.23± 0.20
12	98.19± 0.25	97.01± 0.27	96.97± 0.16

All values are expressed as mean ± SD, n=3

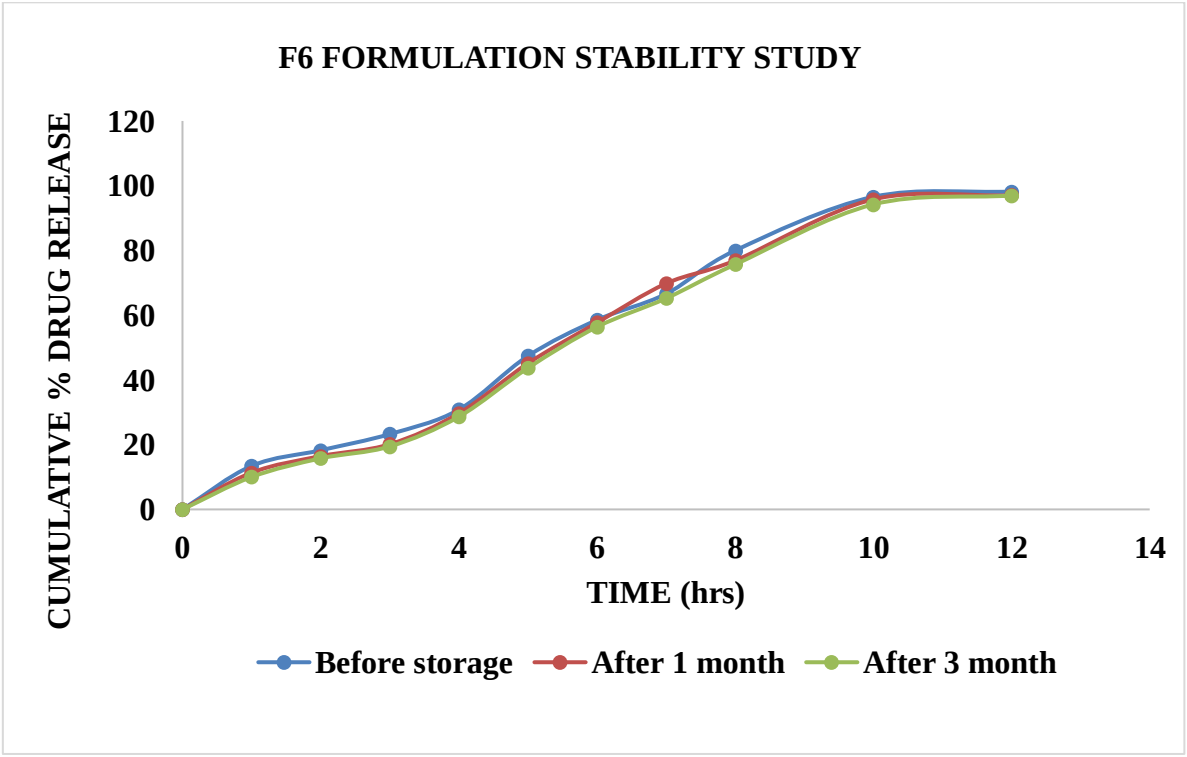


FIG 53: % DRUG RELEASE OF F6 FORMULATION AFTER STABILITY STUDY

TABLE 33: KINETIC STUDY OF F6 FORMULATION AFTER STABILITY STUDY

STABILITY DURATION	ZERO	HIGUCHI	PEPPAS	FIRST
	r^2	r^2	r^2	r^2
Before storage	0.9750	0.9021	0.9327	0.8610
After 1 month	0.9751	0.8925	0.9312	0.8773
After 3 month	0.9749	0.8879	0.9481	0.8856

COMPARISON OF *IN VITRO* RELEASE STUDIES OF OPTIMIZED BATCH WITH MARKETED FORMULATION

TABLE 34: COMPARISON OF IN VITRO RELEASE STUDIES OF OPTIMIZED BATCH WITH MARKETED FORMULATION

TIME (hrs)	% DRUG RELEASE	
	MARKETED PRODUCT	OPTIMIZED FORMULATION
0	0	0
1	26.1± 0.23	13.42± 0.46
2	49.3± 0.34	18.27± 0.35
3	72.4± 0.31	23.27± 0.24
4	95.8± 0.54	30.81± 0.31
5	-	47.39± 0.36
6	-	58.53± 0.27
7	-	66.59± 0.19
8	-	79.98± 0.18
10	-	96.56± 0.34
12	-	98.19± 0.25

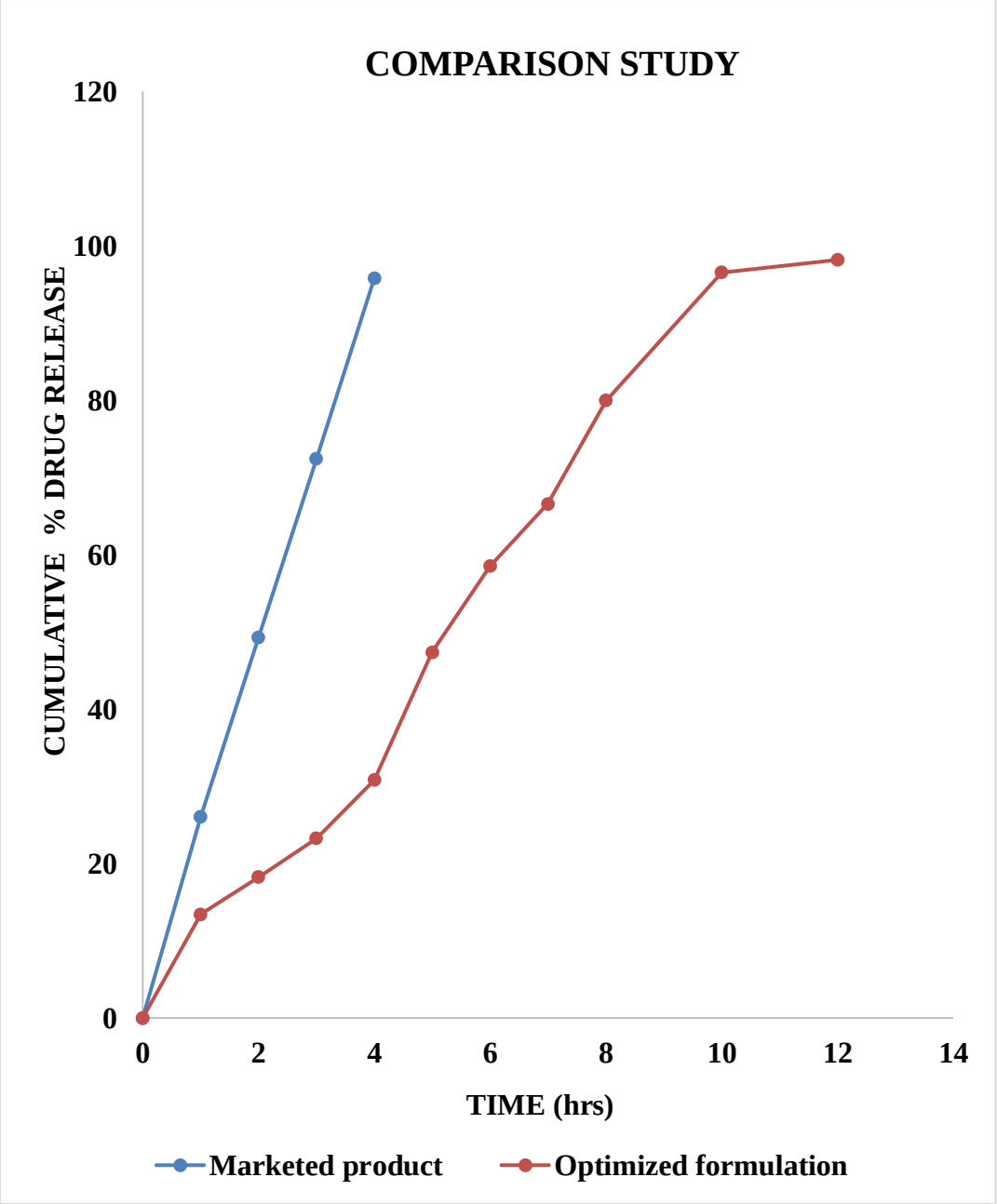


FIG 54: COMPARISON OF IN VITRO RELEASE STUDIES OF OPTIMIZED BATCH WITH MARKETED FORMULATION

9.2. DISCUSSION

The dissolution medium was prepared by using phosphate buffer pH 7.4. **The absorption maxima (λ max) of Papain** were estimated by scanning the drug solution using double beam UV spectrophotometer. The obtained spectrum showed that the λ max was reported as 277.4nm in phosphate buffer pH 7.4 was shown in Fig. 18.

The calibration curve for Papain in phosphate buffer pH 7.4 was measured in λ max of 277.4nm. The correlation coefficient was found to be r^2 – 0.9999 which indicated linearity and obeys Beers law within the concentration range of 10- 60µg/ml (Fig.19).

The **FTIR** graph of drug, polymer and physical mixture of drug with polymer was taken. Physical mixture showed that there was no shifting of functional peaks and all the major peaks present in the spectrum of pure drug were clearly observed in the spectrum of physical mixture. It clearly indicated that there was no interaction between the drug and excipients (Table 14)

The **Trial batches** were prepared by employing varied concentration of polymer medium molecular weight and high molecular weight chitosan 1% - 2% (Table 6).

The **Visual appearance** of the formulation is an important parameter as it has impact on the patient compliance. All the formulations were subjected to visual appearance. The results are given in Table.15 and all the prepared formulation was transparent and clear. F6 formulation was clear and elegant in appearance when compared with other formulations (Fig.28).

The **pH** of all the formulations was found to be satisfactory in the range of 6.5 – 6.7 as depicted in Table. 16. This is considered to be close to the pH of the skin and is considered satisfactory for application with minimal risk of tissue irritation.

The **Homogeneity** of all the formulations was shown in (Table 17). Film-forming gels were formulated using polymers which were completely soluble in the solvent system; thus, the prepared formulations showed good homogeneity with the absence of lumps and were transparent.

The **Extrudability** of all the formulations was shown in (Table 18). The results of formulation F1 and F4 were satisfactory and formulations F2, F3, F5, and F6 were excellent.

The diameters of gels spreading following the **Spreadability test** are found to be between 5 and 7cm (Table 19). This indicates that the spreadability increased with decrease in polymer concentration.

The **Rheological properties** of the formulation are important in topical dosage form. The formulation showed marked increase in **viscosity** with increasing in concentration of

Chitosan in the formulation. The average viscosities of all the formulation were found to be in the range of 2500- 5000 cps (Table 20).

The **drug content** of film-forming gel formulations as shown in (Table21) was found in the range of 96 – 98% indicating uniform distribution of drug in formulation. The formulation F6 was found to have maximum drug content of 98.99% indicating homogeneous drug distribution and insignificant loss of drug during the formulation.

The **Drying time** of film forming gels formulation ranged between 5min – 7min (Table 22). The drying time with the highest values was being observed with formulations of lower polymer concentrations.

The ***In vitro* release study** of Papain from all the formulation in phosphate buffer pH 7.4 was conducted for a period of 12 hrs and result was shown in (Table 23). It can be deduced from *in vitro* diffusion study that formulations F1 & F4 released the drug too quickly revealing their inability to sustain the drug release over 12 hours. This may be attributed to low concentration of drug release modulating polymers and low viscosity of the formulations. On the other hand, formulations F2 & F5 did not release the drug to a considerable extent which could result in a sub therapeutic effect of the drug. Formulations F3 & F6 sustained drug release over 12 hours.

Kinetic analyses of *in vitro* drug release data for the formulation were studied and shown in Table 24. The examination of the regression coefficient values r^2 for F3 and F6 indicated that the drug release followed diffusion-controlled mechanism, as the values are found to be in the range of 0.9402 to 0.9750 for zero order, 0.8949 to 0.9021for higuchi, 0.9321 to 0.9327 for peppas, and 0.8734 to 0.8610 for first order. The results of the release kinetics elucidated from the Korsmeyer Peppas equation show that the release exponent values (n) for the formulations were within the range of 0.859 to 0.887($0.5 < n < 1.0$); this indicates non-fickian (anomalous) release mechanism in these formulations. The results indicated that the drug release follow zero order release, higuchi model with diffusion mechanism.

From the release kinetic results **F6** was selected as the optimized formulation based on the r^2 value which was found to be higher in zero order kinetics, korsmeyer - peppas model release exponent 'n' value was 0.859 ($n<1$), thus following non- fickian type diffusion and the values of higuchi (0.9021) was higher than first order (0.8610). From six formulations F6 showed maximum drug release (98.19%) at the end of 12 hours. Hence this formulation is selected for further evaluations.

Cell cytotoxicity assay for the F6 sample was performed on Vero Cell lines. After 24 hours the **cell viability** effect of F6 against Vero cell lines at different concentrations were determined using the **MTT assay** and the results have been summarized in Tables 25 and 26 and the control mean OD value is obtained as 0.481 from the Table 25. All concentrations of the sample does not induced cell cytotoxicity as illustrated from fig. 41 to 47 and the 50 percentage inhibitory concentration (IC50) for the sample also determined as 130.5µg/ml and given in Table 27.

***In vitro* wound healing activity,** Vero cells were treated with 130.5µg/ml of *F6* sample for 24 hrs. Cell migration at 0, 24 h were captured and wound closure distance was calculated by Image J software and illustrated in Tables 28-30 and Fig 48-51. Percentage wound closure at different time intervals in untreated and F6 treated cells is 87.05% and 98.59% respectively, which have been represented from Fig. 52

From the results of Cell Cytotoxicity (MTT) assay, ***in vitro* skin irritation** data can also be obtained. According to OECD guidelines, irritant chemicals are identified by their ability to decrease cell viability below threshold levels (<50%), test chemicals that produce cell viabilities above the defined threshold, may be considered non irritants (>50%) [110]. From Table 26, it is shown that the F6 concentration ranging from 10µg/ml - 500µg/ml has cell viability >50%, thus indicating F6 does not create skin irritation.

The optimized formulation F6 sealed in vial with rubber cap and kept in humidity chamber maintained 40 ± 2^{o} C75 ± 5% RH for 3months. After 30, 90 days samples are retrieved and analyzed for the pH, viscosity, Spreadability, drug content, drying time, *in vitro* drug release (Table. 31-33). There was no significant change before and after **stability study**.

The ***in vitro* drug release** of the formulation F6 was **compared with that of the marketed formulation**. The release of the optimized formulation was sustained for 12 hours, while the marketed formulation released the drug within 4 hours.

SUMMARY AND CONCLUSION

10. SUMMARY AND CONCLUSION:

10.1. SUMMARY:

The film-forming hydrogel (FFH) is a hydrogel dosage form which transform from the hydrogel to film-type by solvent evaporation after application to the wound site. Over time, the film erodes and is spontaneously removed. Such a film-forming gel shares many protective advantages with the skin barrier itself (e.g., confers protection to the treatment site from environmental threats and is resistant to washout), but also possesses distinct advantages of a gel (e.g., easy application and good adhesiveness to treatment sites). Compared with wound dressing forms, it offers easier use and application, and simpler manufacture. The FFH system can be freely applied to any wound site, even though the wound is curved and shaped. Furthermore, the FFH system was hardly attempted to be developed as a pharmaceutical product for wound healing.

- The λ max of Papain was found to be 277.4nm in phosphate buffer pH 7.4.
- Papain obeys Beer's law within concentration 10- 60µg/ml
- FTIR studies showed that there was no interaction between drugs and excipients
- The film forming hydrogel was prepared by simple agitation method using different concentration of polymer.
- The Pre formulation parameters were within the required limit, indicating suitable to formulate film forming hydrogel
- The Post formulation parameters such pH, viscosity, spreadability, homogeneity, extrudability, drug content, drying time of all the formulation was within the acceptable limits.
- F6 formulation was selected as the best formulation based on *in vitro* release studies. The extent of drug release was found to be 98.19% in 12 hours.
- The drug release follows zero order, non fickian diffusion mechanism.
- Cell cytotoxicity study was done in optimized formulation. This assay was helped in dose fixation for *in vitro* wound healing activity. It shows cell viability >50% in concentration 10- 500µg/ml, indicating presence of non skin irritants.
- *In vitro* wound healing activity was evaluated in Vero cell lines and the formulation enhanced the wound closure in Vero cells.
- Optimized formulation showed controlled release profile than the marketed conventional formulation.
- There was no significant change before and after stability study.

10.2. CONCLUSION

For controlled drug delivery, a topical route is the most popular and successful route. The goal of this work is to develop Papain urea containing film-forming gel for prolonged release based on topical delivery and to treat wound healing in a short duration of time. Film forming gels also proves to be an efficient dosage method for the topical delivery of wound healing. It also stays adhered to the affected portion for a longer time without being rubbed off. It offers prolonged effect than conventional dosage forms, and also there is no need for regular re- application. Since the formulation is prepared with a drug and also the polymer having wound healing effects, the wound healing efficiency will be in higher rate. The prepared topical drug delivery system of Papain urea using chitosan as a polymer was a gel before administration, and forms film after application. The idea will change the therapy of different diseases and provide young researchers and scientists with a broad platform in the field of film forming gel.

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