DEVELOPMENT AND EVALUATION OF BIOACTIVE WOUND DRESSING MATERIAL

A PROJECT REPORT

Submitted by

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BONAFIDE CERTIFICATE

Certified that this project report **"DEVELOPMENT AND EVALUATION OF BIOACTIVE WOUND DRESSING MATERIAL"**, is the bonafide work of **"CHARANNYA SOZHEESVARI, S. ROHIT, R.** and **SHANMUGAM, K."** who carried out the project work under my supervision.

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The report of the project work submitted by the above students in partial fulfillment for the award of Bachelor of Technology degree in Biotechnology of Anna University was evaluated and confirmed to be the report of the work done by the above students and then evaluated.

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LIST OF ABBREVIATIONS

FDA	Food and Drug Administration
PG	Propylene glycol
°C	Degree Celsius
CONC	Concentration
PBS	Phosphate buffer saline
OD	Optical density
DMSO	Dimethyl sulphoxide
LB	Luria broth
rpm	revolutions per minute
nm	nanometer
AG	Agar-Glycerol
AGG	Agar-Glycerol-Gelatin
APG	Agar-Propylene glycol-Gelatin
AP	Agar-Propylene glycol
APgl	Agar-Propylene glycol-glycerol
TAE	Turmeric Acetone Extract
TME	Turmeric Methanol Extract
M	Molarity
S.D	Standard deviation
AMP	Ampicillin

mg/ml	**milligram per millilitre**
µg/µl	**microgram per microlitre**
µm	**micrometer**
mg/kg	**milligram per kilogram**
g	**gram**
hr	**hour**
RT	**Room temperature**

ABSTRACT

ABSTRACT

An attempt was made to prepare agar based film impregnated with turmeric extracts for developing bioactive wound dressing material.4 different concentrations of agar (0.5%, 1.0%, 1.5%, 2.0%) were tested for film forming capacity. Films with agar concentration of 1.5 % were found to be transparent and could be peeled off from the glass mould compared to other concentration. 14 different combinations of fixed agar concentration (1.5 %) and varying composition and concentration of gelatin, glycerol and propylene glycol films were prepared and studied for their mechanical properties like thickness, elongation and tensile strength. Thickness of the films was tested using thickness meter. Two formulations of AGG and $AGGP_2$ were selected as they showed good elongation (%).Water absorption capacity of AGG and $AGGP_2$ film were found to be 2.440g/g and 0.960g/g respectively. Water vapour permeability for AGG film and $AGGP_2$ film were 2.230g/cm^2 and 2.291g/cm^2 respectively.

4% acetone and methanol turmeric extract was added to the selected formulations of agar films. Addition of acetone turmeric extract increased the elongation of the AGG film about 75.71%. The addition of acetone turmeric extract and methanol turmeric extract to $AGGP_2$ film increased the elongation by 84.75% and 47.44% respectively. The water absorption capacity of the films increased on addition of turmeric extract to the films. Percent release of turmeric compound from film was calculated using Franz Diffusion Cell and found to be 0.614% and 0.561% for AGG and $AGGP_2$ film respectively after 4 hrs. Four different microorganisms namely *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Klebsiella sp.,* were tested for

their kill rate. When compared to the control (without film) significant reduction in cell density was noted.

INTRODUCTION

1. INTRODUCTION

The skin is the outer covering of the body. It is the largest organ of the integumentary system made up of multiple layers of epithelial tissues, and guards the underlying muscles, bones, ligaments and internal organs. Because it interfaces with the environment, skin plays a very important role in protecting (the body) against pathogens. Severely damaged skin will try to heal by forming scar tissue. This is often discolored and depigmented. Skin is composed of three primary layers, Epidermis provides waterproofing and serves as a barrier to infection, Dermis serves as a location for the appendages of skin and Hypodermis (subcutaneous adipose layer).

A wound is a type of injury in which the skin is torn, cut or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). Wounds are generally classified as, wound s without tissue loss (e.g. in surgery), and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments example: venous stasis, and iatrogenic wounds such as skin graft donor sites and dermabrasions.

Wounds are also classified by the layers involved, superficial wounds involve only the epidermis, partial thickness wounds involve only epidermis and dermis, and full thickness wounds involve the subcutaneous fat or deeper tissue. Although restoration of tissue continuity after injury is a natural phenomenon, infection, quality of healing, speed of healing, fluid loss and other complications that enhance the healing time represents a major clinical challenge. Majority of wounds heal without any complication. However, chronic non-healing wounds involving progressively more tissue

loss give rise to the biggest challenge to wound-care product researchers. Second major challenge is the prevention of scarring, keloid formation or contractures and a cosmetically acceptable healing

Wound healing, or wound repair, is the body's natural process of regenerating dermal and epidermal tissue. A set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage. These events overlap in time and may be artificially categorized into separate steps: the inflammatory, proliferative, and remodeling phases. In the inflammatory phase, bacteria and debris are phagocytized and removed, and factors are released that cause the migration and division of cells involved in the proliferative phase.

Dermal substitution and wound healing are research areas of medicine in which there have been many recent advances, but neither the commercially available products nor the materials currently described in experimental studies are able to fully substitute for natural living skin. On the other hand, healing of dermal wounds with macromolecular agents such as natural polymers is preferred to skin substitutes owing to many advantages such as biocompatibility, nonirritant and nontoxic properties, and ease and safety of the application on dermis. Modern dressings prevent wounds from drying and inherit properties like do not stick, easy to remove absorb effusions, suitably gas permeable, prevent infections, and generally do not cause irritation and allergies. The increase in the resistance of bacteria to antibiotics is an essential problem.

Wound management is an important area of medical practice. Chronic wounds are painful to the patients and difficult to cure using the normal treatments and are characterized by impairment of wound healing process by

of wound bed formation due to excessive proteolytic cleavage of fibronectin, bacterial infections, degradation of growth factors etc., (Yager and Wnorneh, 1999). Traditionally, we are using herbal products for treating many of our ailments and specifically for wound treatment, there are many Indian herbs described and practiced by people. Presence of growth factor stimulants, anti-microbial compounds are attributed for wound healing properties of these herbs (Mackey and Miller, 2003).

Bandages and dressings are both used in wound management. A bandage is a piece of cloth or other material used to bind or wrap a diseased or injured part of the body. Usually shaped as a strip or pad, bandages are either placed directly against the wound or used to bind a dressing to the wound. A dressing can consist of a wide range of materials, sometimes containing medication, placed directly against the wound. Wound dressings are generally classified as Passive products, Interactive products and Bioactive products, based on its nature of action.

Traditional dressings like gauze and tulle dressings that account for the largest market segment are passive products. Interactive products comprise of polymeric films and forms, which are mostly transparent, permeable to water vapor and oxygen but impermeable to bacteria. These films are recommended for low exuding wounds. Bioactive dressing is which delivers substances active in wound healing; either by delivery of bioactive compounds or dressings is constructed from material having endogenous activity. These materials include proteoglycans, collagen, non-collagenous proteins, alginates or chitosan.

Agar or agar agar is a gelatinous substance derived from seaweed. The gelling agent is an unbranched polysaccharide obtained from the cell walls of some species of red algae, primarily from the genera *Gelidium* and

Gracilaria, or seaweed (*Sphaerococcus euchema*). Chemically, agar is the sulfuric acid ester of a linear galactan, a polysaccharide. Hydrolysis yields the hexose sugars D-galactose and L-galactose, and sulfuric acid in a constant 9:1:1 ratio. In addition, there are several cations associated with the composition, with the principal cation being calcium. These cations are believed to be the cross-linking agents in thick solutions of agar and their subsequent gels. The average chain length of this polysaccharide is anywhere from 200 to 250 sugar residues in length, depending upon the species from which it was originally extracted. Agar is insoluble in cold water, but is slowly soluble in hot water to give a viscous, straw colored solution. A 1% agar solution melts at 80° to 100° C. and sets at 35° to 50° C. to a rigid gel.

Turmeric has been attributed, a number of medicinal properties in the traditional system of medicine. It has got many therapeutic properties including promotion of wound healing. *Curcuma longa* commonly known as turmeric, is a spice used extensively in Eastern cuisine. The root of the plant is normally ground to yield a yellow colored powder, which is then used for various applications. Some of the culinary uses of turmeric are as a coloring and flavoring agent and as a food preservative. Turmeric is also known to have medicinal applications dating back several millennia and has been an important component in the ancient Indian Ayurvedic medicines. However, recently, several properties of turmeric have begun to be vigorously researched and are opening new venues for its medicinal use. Among these properties are its antioxidant, anti-inflammatory, anti-carcinogenic, antimutagenic, anti-thrombotic, hepatoprotective, and antimicrobial, antiviral and anti-parasitic actions ([illegible]). Turmeric contains phenolic compounds called curcuminoids that are chemically related to

curcumin, the main ingredient of turmeric. These curcuminoids are responsible for the yellow color of the root of the *Curcuma longa* plant

OBJECTIVES:

The present study is formulated to design a wound dressing material, agar based film impregnated with turmeric extracts.

1. To prepare turmeric extracts.
2. To prepare agar films impregnated with turmeric extracts.
3. To test the physio-chemical properties of the film.
4. To test the films for their primary skin irritation and wound healing properties in animal models.

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Introduction

The skin is considered the largest organ of the body and has many different functions. The epidermis or outer layer is made of mostly dead cells with a protein called keratin. This makes the layer waterproof and is responsible for protection against the environment. The dermis or middle layer is made up of living cells. It also has blood vessels and nerves that run through it and is primarily responsible for structure and support. The subcutaneous fat layer is primarily responsible for insulation and shock absorbency. Cells on the surface of the skin are constantly being replaced by regeneration from below with the top layers sloughing off. The repair of an epithelial wound is merely a scaling up of this normal process.

In medicine, a wound is a type of injury in which the skin is torn, cut or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin. Wounds that fail to heal in the anticipated time frame and often reoccur are considered chronic wounds. These wounds are visible evidence of an underlying condition such as extended pressure on the tissues, poor circulation, or even poor nutrition.

2.2 History of Wound Treatment

Throughout history man has had to contend with dermal wounds. In primitive societies substances derived from animals, plants and minerals formed the basis of crude remedies (Mollering, 1995) needed to staunch bleeding, reduce swelling, minimise pain, remove damaged tissue, treat infections, mask foul smells and promote healing. Although topical antimicrobial agents were utilised in wound care for thousands of years, during the 19th century the discovery of chemical preservatives and

disinfectants, as well as a better understanding of the nature of infection and inflammation, allowed increased control of wound infection. Paul Ehrlich began the search for chemicals with selective toxicity for infectious agents, rather than non-specific inhibitors, such as antiseptics and disinfectants. The discovery and development of antibiotics during the 20th century provided potent antimicrobial agents with high specificity, which revolutionised clinical therapy and marked the decline of many former remedies.

Honey is an ancient remedy which has been re-discovered for the treatment of wounds (Zumla and Lulat, 1989). Many therapeutic properties have been attributed to honey including antibacterial activity and the ability to promote healing. Turmeric is extensively used as a spice, food preservative and colouring material in Asia. It has been used in traditional medicine as a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Extensive work has been done to establish the biological activities and pharmacological actions of turmeric and its extracts. Curcumin the bioactive component of turmeric has shown to have a wide spectrum of biological actions. Clinically, curcumin has already been used to reduce post-operative inflammation. Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases (Chattopadhyay, 2004).

2.3 Biochemical Process in Wound Healing

Wound healing, or wound repair, is the body's natural process of regenerating dermal and epidermal tissue. When an individual is wounded, a set of complex biochemical events takes place in a closely orchestrated

cascade to repair the damage. These events overlap in time (Stadelmann et al., 1998) and may be artificially categorized into separate steps: the haemostasis, inflammatory, proliferative, and remodeling phases (Figure 2.1).

In the inflammatory phase (lag phase/resting phase), clotting takes place in order to obtain hemostasis, or stop blood loss, and various factors are released to attract cells that phagocytise debris, bacteria, and damaged tissue and release factors that initiate the proliferative phase of wound healing. About two or three days after the wound occurs, fibroblasts begin to enter the wound site, marking the onset of the proliferative phase even before the inflammatory phase has ended.

The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction (Midwood et al., 2004). In angiogenesis, new blood vessels grow from endothelial cells (Chang et al., 2004). As in the other phases of wound healing, steps in the proliferative phase do not occur in a series but rather partially overlap in time. The proliferative phase is also called the reconstruction phase. In epithelialization, epithelial cells crawl across the wound bed to cover it (Garg, 2000).In contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis (Midwood et al., 2004).In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis.

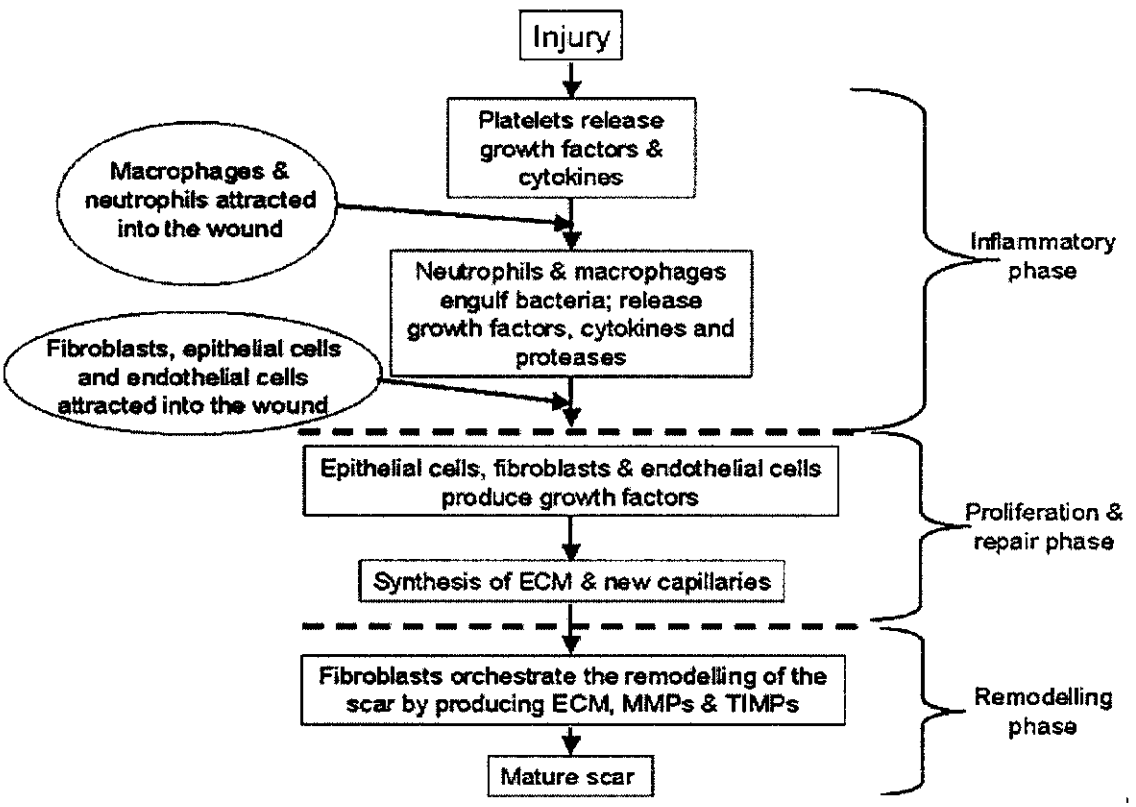

Figure 2.1: Different phases of wound healing

However, this process is not only complex but fragile, and susceptible to interruption or failure leading to the formation of chronic non-healing wounds. An open wound, which is directly exposed to air, will dehydrate and a scab or scar is formed. This forms a mechanical barrier to migrating epidermal cells and are then forced to move in a deeper level of tissue, which prolongs the healing process. Moist healing prevents the formation of scab as the dressing absorbs wound exudate secreted from the ulcer.

2.4 Global Wound Care Market

In the year 2001, the global wound care market was estimated at US$ 13156 million with an annual growth rate of 15%. This projection was for 40-45 million surgical procedures, and other chronic wounds like 8-10 million leg ulcers, 7-8 million pressure sores and an equal number of burn wounds. The US$4.2 billion global advanced wound care segment is the fastest growing area with double-digit growth of 10% per year. The global wound care market is estimated to be worth US$7.2 billion in 2006 and comprises two sectors, traditional and advanced (www.piribo.com). Clinicians are unable to recommend the use of advanced dressing although they are more conducive to healing due to its high cost. However, it requires fewer dressing changes with less care, and because of its faster healing it reduces the resources required. The analysis of wound care market indicates the gradual shift toward the advanced wound dressing.

2.5 Wound Care Materials

The basic function of the wound care materials is providing protection against an infection, blood and exudates absorption, to promote healing and possibly apply a medication to the wound (Horrocks and Anand, 2004). The available materials range from simple cotton gauzes and lint to sophisticated multifunctional systems made from natural or synthetic materials (Miraftab, 2006). A growing number of wound-healing devices such as negative pressure wound therapy (NPWT), oxygen therapy, electrical stimulation, low level laser therapy (LLLT), therapeutic ultrasound and maggot therapy are becoming more prominent.

2.51 Negative pressure wound therapy

Negative pressure wound therapy (NPWT), also known as vacuum assisted closure is a technique of wound closure used to promote healing in large or chronic wounds, fight infection and enhance healing of burns. A vacuum is used to reduce pressure around the wound, drawing out excess fluids and cellular wastes. NPWT seals the wound with gauze or foam dressing and applies negative pressure to the wound bed with a tube threaded through the dressing. Intermittent removal of used instillation fluid supports the cleaning and drainage of the wound bed and the removal of infectious material (Moch et al., 1998).

2.5.2 Topical Hyperbaric Oxygen (THBO) therapy

Oxygen applied directly to the base of an open wound at pressure slightly above atmospheric. Oxygen is required for all new cell growth. Tissue at the base of chronic or non healing wounds tends to be ischemic. Application of topical hyperbaric oxygen induces the growth of new blood vessels at the wound base. The new blood vessels allow an increased flow of oxygenated blood to the wound which begins the healing process. It is bactericidal for anaerobic bacteria e.g. *Staphylococcus aureus* and *E.coli.* However, blood oxygen levels are normally adequate for wound healing. Topical hyperbaric oxygen delivers oxygen directly to the wound (Phillips, 1996).

2.5.3 Maggot therapy

Maggot therapy (also known as maggot debridement therapy (MDT), larvae therapy, biosurgery) is a type of biotherapy involving the intentional introduction by a health care practitioner of live, disinfected maggots (fly larvae) raised in special facilities into the non-healing skin and soft tissue wound(s) of a human or other animal for the purposes of selectively cleaning out only the necrotic tissue within a wound (debridement), disinfection, and promotion of wound healing. The maggots have three principal actions reported in the medical literature:

debride wounds by dissolving only necrotic, infected tissue;
disinfect the wound by killing bacteria; and
stimulate wound healing.

Maggot therapy has been shown to accelerate debridement of necrotic wounds and reduce the bacterial load of the wound, leading to earlier healing, reduced wound odor, and less pain. The combination and interactions of these actions make maggots an extremely potent tool in wound care (Whitaker et al., 2007).

2.6 Features of an ideal wound dressing:

The features of an ideal wound dressing are:

- Sustained antimicrobial activity
- Provides a moist wound healing environment
- Allows consistent delivery over the entire surface area of the wound
- Allows monitoring of the wound with minimum interference
- Manages exudates if problematic
- Comfortable & Conformable
- Provides an effective microbial barrier
- Absorbs and retains bacteria
- Avoids wound trauma on removal.

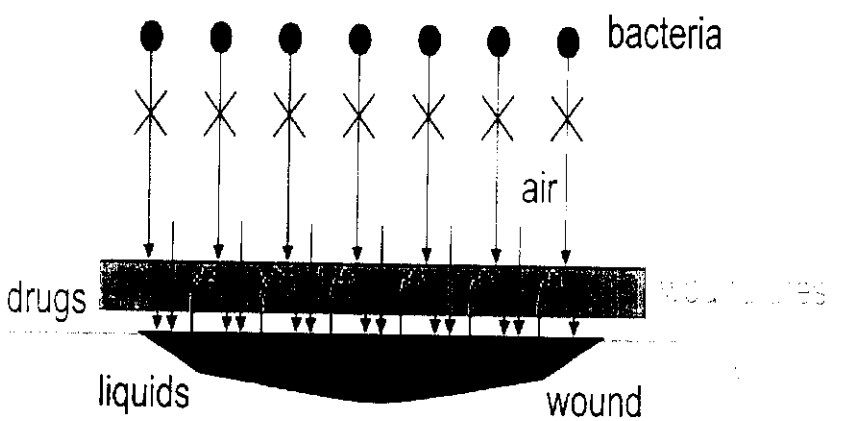

Figure 2.2: A wound dressing functionality.

2.7 Classification of wound dressing products

In the history, the development of wound dressings was managed in accordance to personal experience or historical knowledge. Recently, the development is guided by clinical studies and evidence based medicine. There have been recognized two wound care systems, these being traditional and advanced dressing. Their classification includes 10 dressing classes (www.tycohealth-ece.com)

Gauzes	Bio-dressing
Antiseptics	Skin derivates
Proteolytic Enzymes	Semipermeable Dressings
Absorbents	Occlusive Dressing
Granulation Promoters	Hydrogels

The materials and designs of wound dressings are derived from the application condition. These for the traditional and advanced dressing are summarized in Table 2.1.The wound dressing materials belonging to the traditional and advanced wound dressings are designed according to their functionality. They are presented in Table 2.2 (www.tycohealth-ece.com).

2.7.1 Comparison between Traditional & Advanced Dressings

It's commonly believed that a moist wound is frequently infected. It has been demonstrated that advanced dressings can reduce the incidence of infection by more than 50% in comparison with traditional dressings. The advanced dressings do not adhere to the lesion and so avoid further damage to the wound or its surrounding tissues. The comfort during dressing changes is immediately noticeable and the creation of a moist, warm environment can reduce pain. Everyone knows that dressings represent 5% of the management costs of a chronic wound, whilst 70% of the cost is

absorbed by nursing time. It is evident that if dressings worn over a longer period are used, nursing time can be reduced. This represents a gain, both economically and in clinical resources (www.tycohealth-ece.com).

Table 2.1: Comparison of Traditional and Advanced dressing

Traditional dressing	**Advanced dressing**
Exudate absorption and drying of the wound	Keep a moist environment
Haemostatis	Remove exudates and necrotic tissue
Antisepsis	Keep temperature constant
Protection from infection	Oxygen permeable
Wound covering	Protection from exogeous infection
Easy to handle	Non-traumatic at the dressing change

Table 2.2: Types of Traditional and Advanced wound dressings

Traditional dressing	Advanced dressing
Gauze	Alginates
Lint	Hydrogel
Wadding	Hydrocolloids
Plasters	Foam dressing
	Film dressing

2.7.2 Traditional Wound Dressings

More wound dressings are composite materials consisting of an absorbent layer placed between a wound contact layer and a flexible base material. The wound contact layer should prevent the adherence of the dressing to a wound and be easily removed without disturbing the tissue growth. The function of absorbent layer is to absorb blood or other exudate liquids and provide cushioning effect to protect a wound (Horrocks and Anand, 2004).

Some of these articles can be coated by pressure sensitive adhesives which contribute to wound dressing performance by being stick to the skin around the wound and preventing the abrasion of wound by the material, therefore damaging of new grown tissue (Miraftab, 2006).

A gauze serves as an absorbent material in a pad form (swab) in surgical applications. If yarns are barium sulphate coated and incorporated into a swab, the material is X-ray detectable. Lint is a plain weave cotton fabric that is used as a protective dressing for first aid and mild burn

applications. Wadding materials are also used for wound care applications. They are a high absorbent materials sometimes covered with a nonwoven fabric to prevent wound adhesion or fibre loss as shown in Figure 2.3. The absorbent layer can be made of cellulose pulp, wood pulp, wet laid cotton or viscose fabrics, etc.

Table 2.3: Properties of Traditional wound dressings

Types	**Examples**	**Function**	**Form of Application**
Traditional Dressings	Cotton Wool Gauze & lint	Allow strike through, shed fibres and adhere to the wound + dehydrate the wound.	Used on clean, dry wound or as secondary dressing
Low adherent dressing	Cutilin Melolin Release Setoprime	Suitable for dry wounds or lightly exuding wounds.	Need to be secured with bandage or adhesive tape.

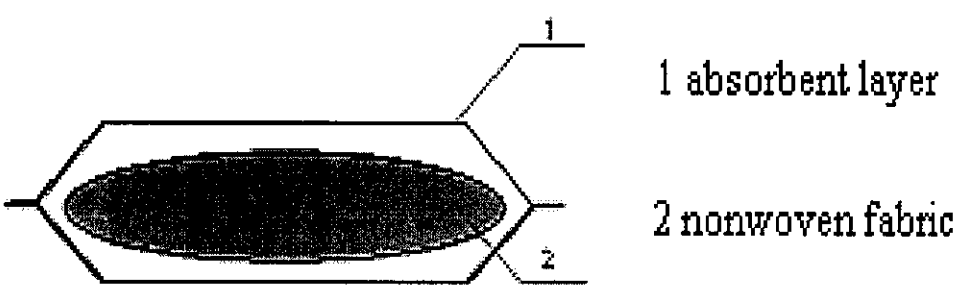

Figure 2.3: Structure of a wadding material.

2.7.3 Modern Wound Dressings

There is a large variability of the materials classified as advanced wound dressing summarized in Table 2.4 (Anand, 1999) according to their origin, functional ability and form of applications. The descriptions of the functionalities of most common dressings are presented in Table 2.5.

2.7.3.1 Film Dressings

Film dressings are non-absorbent, permeable to moisture vapour and oxygen and impermeable to bacteria and viruses. They are typically made of a thin, transparent polymer membrane, which is coated with a layer of acrylic adhesive (Figure 2.4). Film dressings can be also combined with other dressing materials.

2.7.3.2 Hydrogel Dressings

Hydrogels maintain balanced hydration through controlled evaporation. Exudates are absorbed into the gel, moisture evaporates through semi-permeable film backing or a secondary dressing (Figure 2.5). Hydrogel dressings have proven their usefulness in the treatment of chronic wounds. A hydrogel sheet for use as a wound dressing with capillaries permitting wound exudate to pass through the sheet while not permitting bacteria to infect the wound is disclosed. The sheets do not stick to the wound surface and allow large quantities of wound exudate to be removed from the wound. The high water content of the gel is particularly compatible with the exposed surface of the wound and healing is significantly enhanced.

Figure 2.4: Film dressings

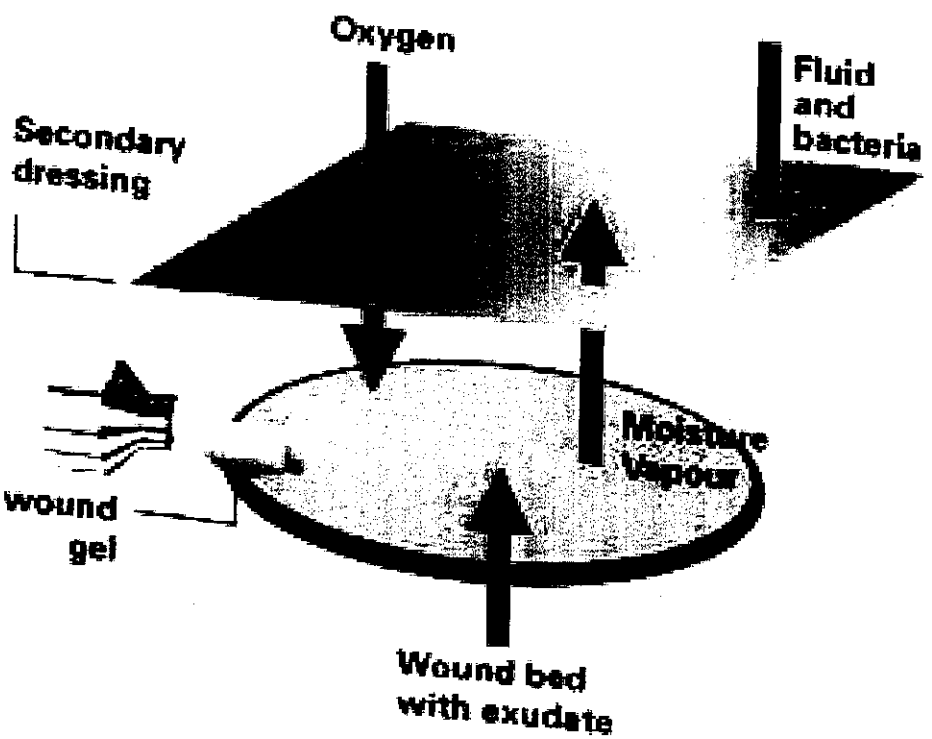

Figure 2.5: Hydrogel Dressings

2.7.3.3 Hydrocolloid Dressings

Hydrocolloid dressings are backed by either waterproof polyurethane foam or a thin film. In some cases, the backing extends beyond the margin of the hydrocolloid to form a border. The totally sealed hydrocolloid at the wound area keeps the moisture in while keeping the bacteria and fluids out (Figure 2.6). This limits any gas exchange between a wound and the environment. Fluid absorption is slow primary trough swelling of particles in the dressing. This swelling allows a soft, non-adherent gel formation over the wound.

2.7.3.4 Calcium Alginate Dressings

Calcium alginate being a natural haemostat, alginate based dressings are indicated for bleeding wounds. The gel forming property of alginate helps in removing the dressing without much trauma, and reduces the pain experienced by the patient during dressing changes. It provides a moist environment that leads to rapid granulation and reepithelialization. In a controlled clinical trial, significant number of patients dressed with calcium alginate was completely healed at day 10 compared with the members of the paraffin gauze group. Calcium alginate dressings provide a significant improvement in healing split skin graft donor sites. In another study with burn patients, calcium alginate significantly reduced the pain severity and was favored by the nursing personnel because of its ease of care. The combined use of calcium sodium alginate and a bio-occlusive membrane dressing in the management of split-thickness skin graft donor sites eliminated the pain and the problem of seroma formation and leakage seen routinely with the use of a bio-occlusive dressing alone (Figure 2.7).

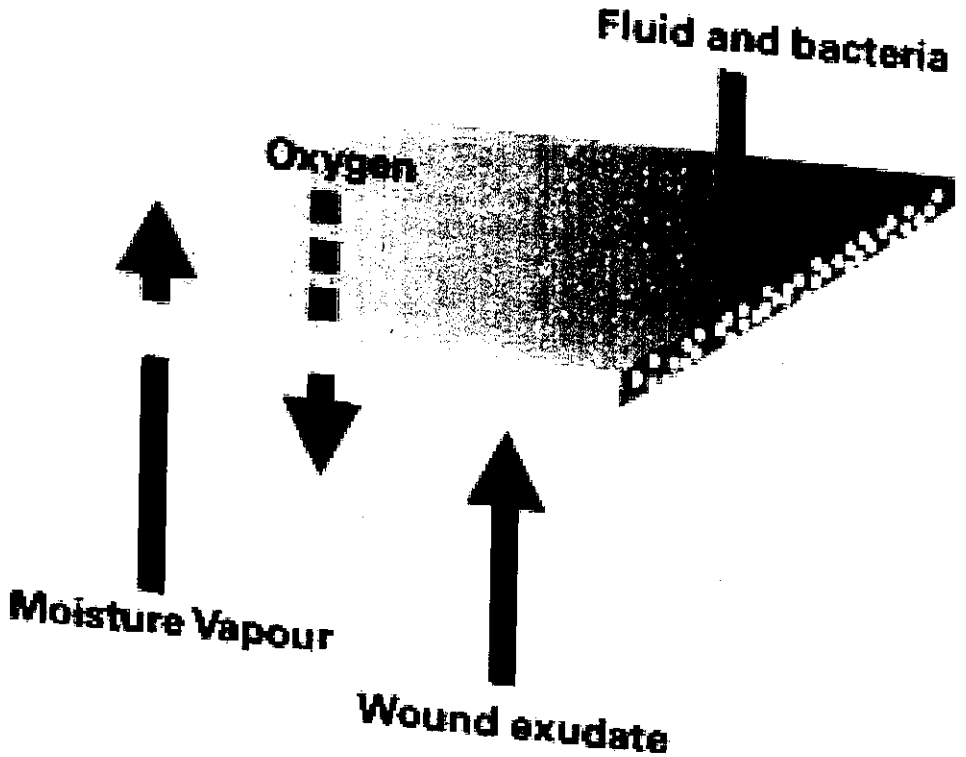

Figure 2.6: Hydrocolloid Dressings

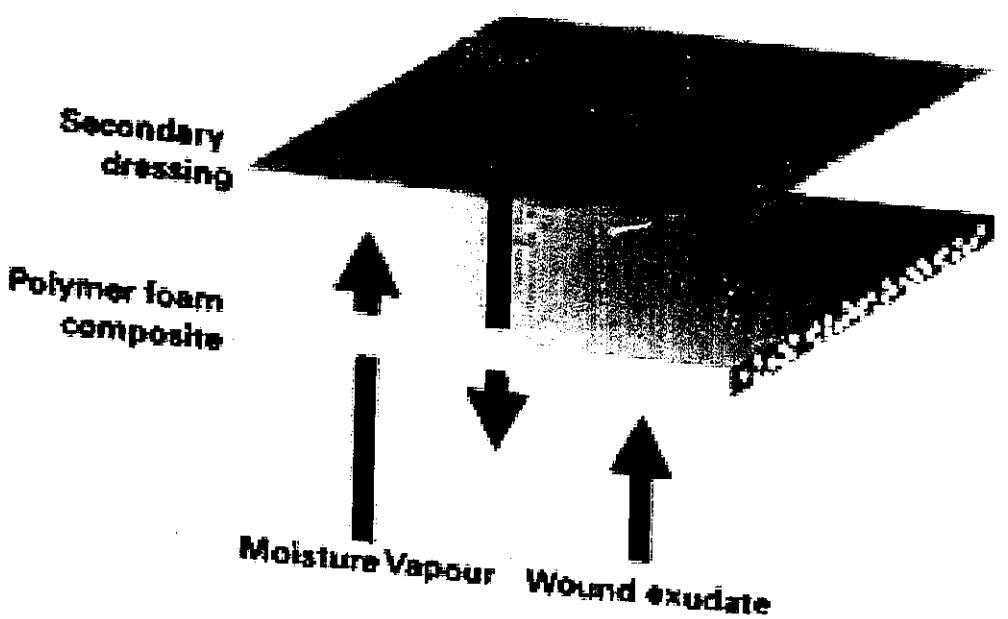

Figure 2.7: Calcium Alginate Dressings

The calcium alginates provide a moist healing environment by converting the exudates into a gel. Reaction between the calcium in the dressing and the sodium in a wound exudates results in a chemical ion exchange, which forms a gel-like substance. The gel conforms to the wound, providing a soft, moist healing environment. Due to its dehydration effect, this dressing should not be used with dry wounds.

2.7.3.5 Foam Dressings

Foam dressings are indicated for a partial thickness or superficial wounds. The open pore structure of a foam dressing gives it relatively high moisture vapour transmission rate (Figure 2.8). Absorptive ability varies by a manufacturer. The pore size, texture and absorbency are controlled by the agents impregnated in the dressing. The degree of occlusion depends on a presence of a film backing.

2.7.3.6 Silicon Dressings

A soft silicone dressing is a dressing coated with a soft silicone as an adhesive or a wound contact layer. The intrinsic properties of soft silicone are such that these dressings may be removed without causing trauma to the wound or to the surrounding skin (www.worldwidewounds.com). There are different types of soft silicone dressings including a traumatic wound contact layers, absorbent dressings for exuding wounds and also a dressing for the treatment of hypertrophic scars and keloids. The soft silicone cannot enter the circulatory system. It is insoluble in wound exudate and the silicone molecules are too big to penetrate through cell membranes or 15 pass through the skin into blood vessels. They therefore cannot be transported around the body to produce any systemic effects.

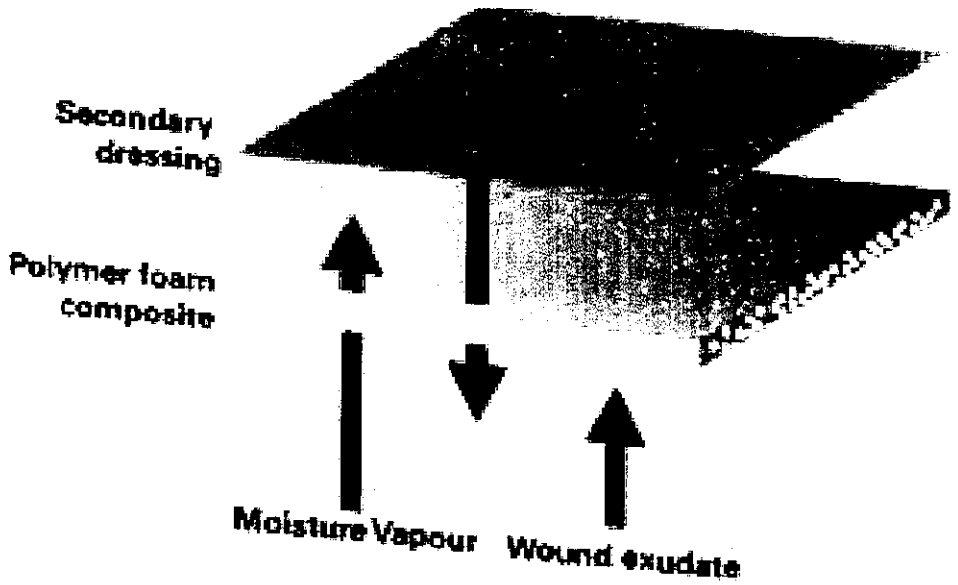

Figure 2.8: Foam Dressings

2.7.3.7 Collagen Dressings

Collagen is the fibre forming protein of mammalian connective tissues. It is the major component of the extracellular matrix forming an organized structure bridging the basal cells to epidermis. At least 10 different types of collagen have been identified. In the wound dressing applications, the collagen has haemostatic and absorbent functions (www.pjonline.com).

2.8 Agar

Agar or agar agar is a gelatinous substance derived from seaweed. The gelling agent is an unbranched polysaccharide obtained from the cell walls of some species of red algae, primarily from the genera *Gelidium* and *Gracilaria*, or seaweed (*Sphaerococcus euchema*). Commercially it is derived primarily from *Gelidium amansii*. Chemically, agar is a polymer

made up of subunits of the sugar galactose. Agar polysaccharides serve as the primary structural support for the algae's cell walls.

2.8.1 Structural Unit and Properties

Agar consists of a mixture of agarose and agaropectin. Agarose is a linear polymer, of molecular weight about 120,000, based on the -(1-3)-β-D-galactopyranose-(1-4)-3,6-anhydro-α-L-galactopyranose unit, the major differences from carrageenans being the presence of L-3,6-anhydro-α-galactopyranose rather than D-3,6-anhydro-α-galactopyranose units and the lack of sulfate groups. Agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts. They gel poorly and may be simply removed from the excellent gelling agarose molecules by using their charge.

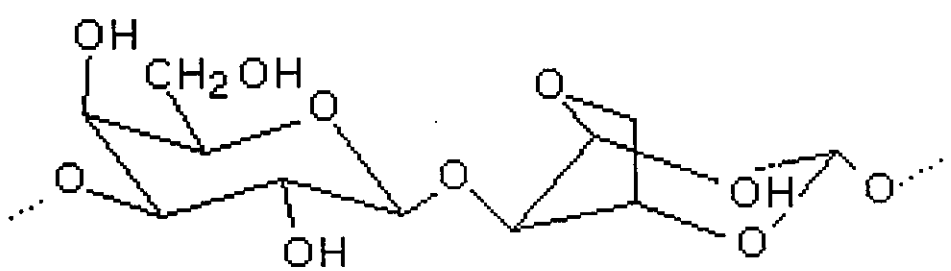

Figure 2.9: Structure of Agar

The quality of agar is improved by alkaline treatment that converts of any L-galactose-6-sulfate to 3,6-anhydro-L-galactose. Agar exhibits hysteresis, melting at 85 °C (358 K, 185 °F) and solidifying from 32-40 °C. (305 - 313 K, 90-104 °F).

2.8.2 Molecular structure

Agarose molecules have molecular weights of about 120,000, The gel network of agarose contains double helices formed from left-handed threefold helices. These double helices are stabilized by the presence of water molecules bound inside the double helical cavity. Exterior hydroxyl

groups allow aggregation of up to 10,000 of these helices to form suprafibers (en.wikipedia.org/wiki/Agar).

2.9 Turmeric

Turmeric (Curcuma longa) is extensively used as a spice, food preservative and colouring material in India, China and South East Asia. It has been used in traditional medicine as a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. For the last few decades, extensive work has been done to establish the biological activities and pharmacological actions of turmeric and its extracts. Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. These include its antiinflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. Its antiinflammatory, anticancer and antioxidant roles may be clinically exploited to control rheumatism, carcinogenesis and oxidative stress-related pathogenesis. Clinically, curcumin has already been used to reduce post-operative inflammation.

Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases.

2.9.1 Chemical composition of turmeric

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%)5. Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%) (Ruby et al., 1995). It has a melting point at 176–177°C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform.

2.9.2 Biological activity of turmeric and its compounds

Turmeric powder, curcumin and its derivatives and many other extracts from the rhizomes were found to be bioactive. Curcumin is also reported to have antibacterial (Shanker and Murthy., 1979), antiamoebic and antiHIV activities. Curcumin also shows antioxidant activity. The volatile oil of C. longa shows antiinflammatory, antibacterial and antifungal activities. The petroleum ether extract of C. longa is reported to have antiinflammatory activity (Arora et al., 1971). Alcoholic extract and sodium curcuminate can also offer antibacterial activity(Ramprasad and Sirsi, 1956). The crude ether and chloroform extracts of C. longa stem are also reported to have antifungal effects.

2.9.3 Anti-inflammatory activity

The colouring principle of turmeric is the main component of this plant and is responsible for the anti-inflammatory property.Curcumin is effective against carrageenin-induced oedema in rats and mice. The natural analogues of curcumin, viz. FHM and BHM, are also potent anti-inflammatory agents. The volatile oil and also the petroleum ether, alcohol

and water extracts of C. longa show anti-inflammatory effects. The antirheumatic activity of curcumin has also been established in patients who showed significant improvement of symptoms after administration of curcumin. to indomethacin and salicylate, has recently been reported. Curcumin offers antiinflammatory effect through inhibition of NFkB activation (Singh and Aggarwal, 1995). Curcumin has also been shown to reduce the TNF-a-induced expression of the tissue factor gene in bovine aortic-endothelial cells by repressing activation of both AP-1 and NFkB. The anti-inflammatory role of curcumin is also mediated through downregulation of cyclooxygenase-2 and inducible nitric oxide synthetase through suppression of NFkB activation. Curcumin also enhances wound-healing in diabetic rats and mice, and in H2O2-induced damage in human keratinocytes and fibroblasts (Phan et al., 2001).

2.9.4 Antioxidant effect

It acts as a scavenger of oxygen free radicals (Subramanian et al., 1994). It can protect haemoglobin from oxidation. In vitro, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H2O2 and nitrite radical generation by activated macrophages, which play an important role in inflammation (Joe and Lokesh, 1994). Curcumin also lowers the production of ROS in vivo. Its derivatives, demethoxycurcumin and bis-demethoxycurcumin also have antioxidant effect (Song, 2001). Curcumin exerts powerful inhibitory effect against H2O2-induced damage in human keratinocytes and fibroblasts (Phan et al., 2001) and in NG cells. This is brought about by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase. Since ROS have been implicated in the development

of various pathological conditions, curcumin has the potential to control these diseases through its potent antioxidant activity.

Contradictory to the above-mentioned antioxidant effect, curcumin has pro-oxidant activity (Kelly et al., 2001). It is reported that curcumin not only failed to prevent single-strand DNA breaks by H2O2, but also caused DNA damage. As this damage was prevented by antioxidant a-tocopherol, the pro-oxidant role of curcumin has been proved. The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of b-diketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant (Figure 2.10) (Rao, 1994).

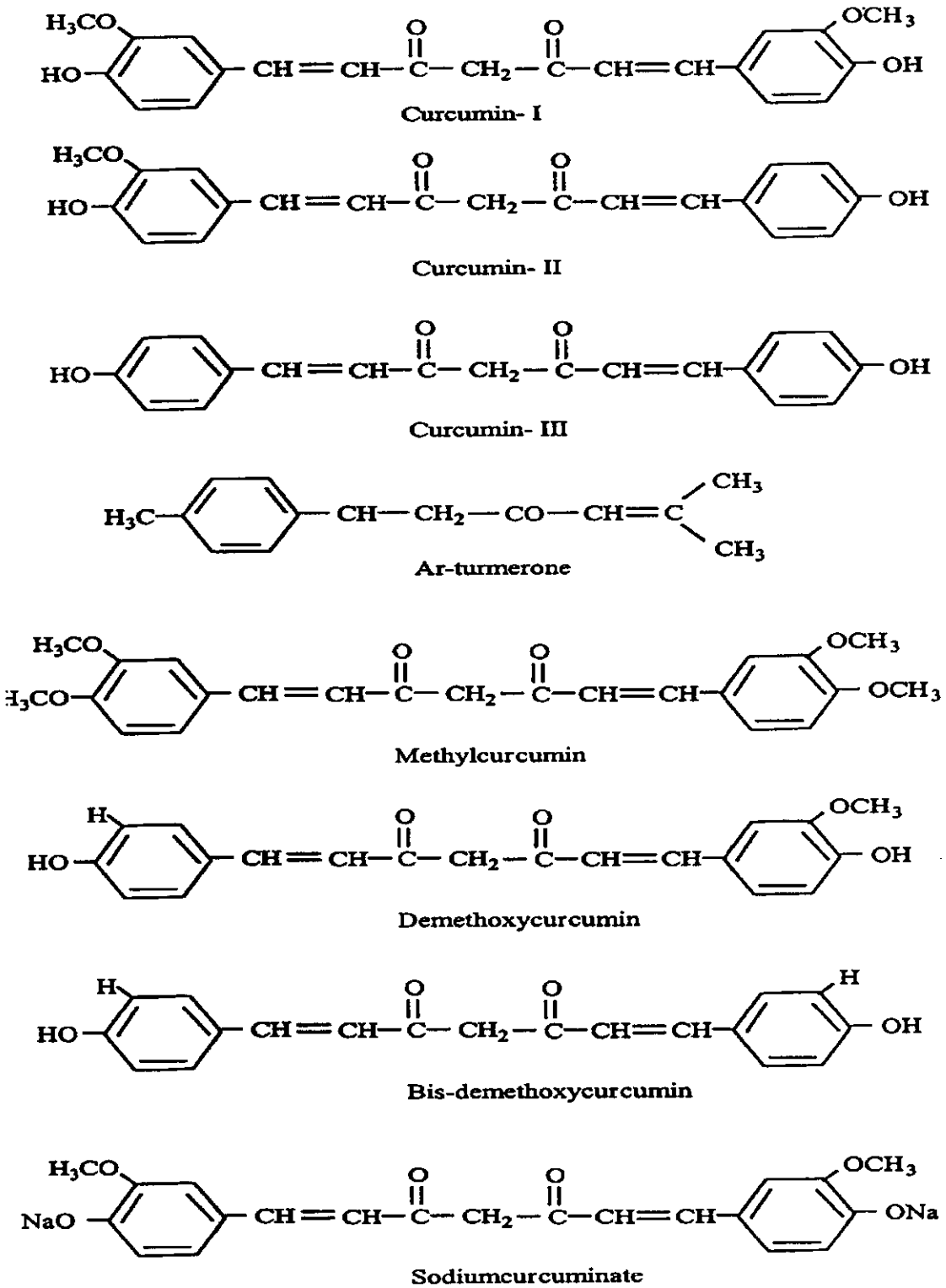

Figure 2.10: Structure of natural curcuminoids

2.9.5 Antimicrobial activity

Both curcumin and the oil fraction suppress growth of several bacteria like Streptococcus, Staphylococcus, Lactobacillus, etc. The aqueous extract of turmeric rhizomes has antibacterial effects (Kumar et al., 2001). Curcumin also prevents growth of Helicobacter pylori CagA+ strains in vitro (Mahady et al., 2002). Crude ethanol extract also possesses antifungal activity (Wuthi-Udomler et al., 2000). Turmeric oil is also active against Aspergillus flavus, A. parasiticus, Fusarium moniliforme and Penicillium digitatum. The ethanol extract of the rhizomes has anti-Entamoeba histolytica activity. Curcumin has anti-Leishmania activity in vitro (Koide et al., 2002). Curcumin has been shown to have antiviral activity. curcumin also shows anti-HIV (human immunodeficiency virus) activity by Inhibiting the HIV-1 integrase needed for viral replication (De Clercq, 2000). It also inhibits UV light-induced HIV gene expression (Taher et al., 2003). Thus curcumin and its analogues may have the potential for novel drug development against HIV.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Chemicals

All chemicals used in the study are of analytical grade. Agar, Glycerol and Acetone were purchased from Qualigens, Mumbai. Propylene Glycol was purchased from Merck, Mumbai. Gelatin was purchased from Loba Chemie.

3.2 Collection of Turmeric rhizome samples

The turmeric samples were collected from the local market as dried rhizome (Figure 3.1) and ground to a fine powder (Figure 3.2) using mesh sieve of size 150μm. Uniform size powder was obtained and used for extract preparation.

Figure 3.1: Turmeric rhizome

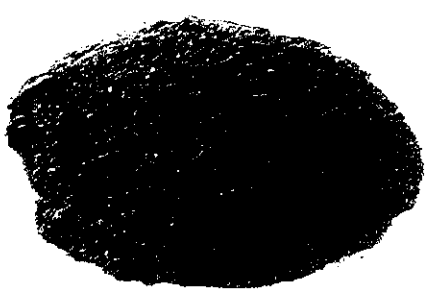

Figure 3.2: Turmeric powder

3.3 Preparation of Turmeric extract

3.3.1 Acetone extract

About 10 g of turmeric powder was extracted with 100 ml of acetone, using a sterile conical flask with intermittent shaking for 72 hours. The extract was filtered using Whatman (No. 1) filter paper and the filtrate was collected in a sterile petridish (initial weight of empty petridish is noted). The solvent was completely evaporated at RT, weighed to obtain final weight of the petridish with the residue and dissolved in DMSO to obtain the acetone extract. This was used for further preparation of Agar-based films.

3.3.2 Methanol extract

About 10 gm of turmeric powder was extracted with 100 ml of ice cold methanol by using a sterile conical flask with intermittent shaking for 72 hours. The extract was filtered using Whatman (No. 1) filter paper and the filtrate was collected in a sterile petridish (initial weight of empty petridish is noted). The solvent was completely evaporated at RT, weighed to obtain final weight of the petridish with the residue and dissolved in DMSO to obtain the methanol extract. This was used for further preparation of Agar-based films.

3.3.3 Concentration of turmeric extract

From the difference of the initial weight and final weight of the petri-plate noted, the weight of the filtrate residue can be obtained. The concentration of the turmeric extract can be obtained from the formula:

$$\text{Conc. of extract } (\mu g / ml) = \frac{\text{Weight of the filtrate residue } (\mu g)}{\text{Amount of DMSO used to dissolve filtrate residue (ml)}}$$

3.4 Preparation of Agar based films

Varying compositions and concentrations of Agar-Agar, Glycerol, Propylene Glycol and Gelatin as shown in Table 3.1 were used for film preparation. A completely dissolved solution is obtained on boiling in a microwave oven for 2-4 minutes. The heated solution is then cast into moulds which on cooling form gels. The cast is then air dried at RT for 48 hours, the film was peeled off from the mould and stored in air tight plastic covers.

3.5 Mechanical properties of Agar based films

The thickness of the films at 10 different locations (center and various corners) was measured using a thickness meter (Figure 3.3). The values are the average of three independent measurements.

The mechanical properties of the films were measured using a texture analyzer- Instron (Figure 3.4) equipped with a 5 Kg load cell. A film strip (dimensions 8 x 2.5 cm) was held between 2 clamps and pulled by the top clamp at the rate of 30 mm/ min (Kalapathy et al., 2000).

The Load at break (N), extension at break (mm) and time at break (sec) were measured when the film broke off. The values are the average of three measurements. The tensile strength and elongation at break were calculated as shown in Equation 1 and 2.

Tensile strength (N/ mm^2) = Breaking force (N)

/ Cross-sectional Area of sample (mm^2) → (1)

Elongation at break (%) = [Increase in length at Breaking point (mm)

/ Initial Length (mm)] x 100 → (2)

Table 3.1: Film composition and concentration

Agar conc (%)	Gelatin conc (%)	Glycerol conc(%)	PG Conc (%)
O.5	-	-	-
1.0	-	-	-
1.5	-	-	-
2.0	-	-	-
1.5	-	5.0	-
1.5	3.0	5.0	-
1.5	3.0	1.0	-
1.5	3.0	2.0	-
1.5	2.5	2.0	-
1.5	2.0	2.0	-
1.5	1.5	2.0	-
1.5	1.0	2.0	-
1.5	0.5	2.0	-
1.5	-	2.0	-
1.5	-	-	2.0
1.5	1.5	-	2.0
1.5	1.5	-	0.5
1.5	1.5	2.0	2.0
1.5	0.5	1.5	0.5

Figure 3.3: Thickness meter

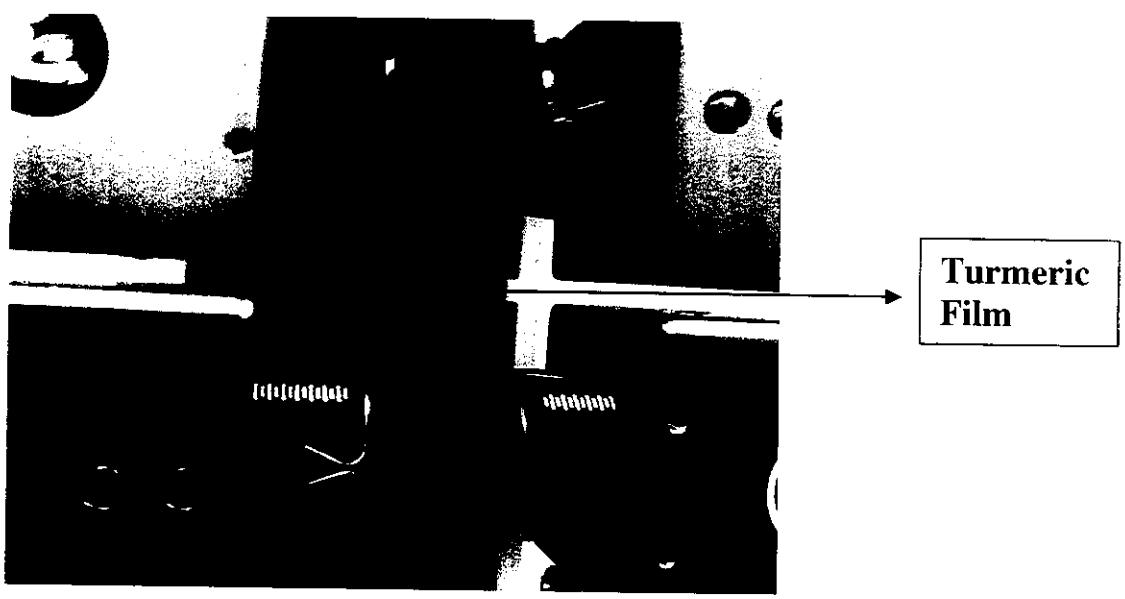

Figure 3.4: Texture analyzer-Instron

3.6 Water Absorption Capacity of the Agar based films

The films are cut to similar weights (initial weights are noted) and suspended in petri plates containing 50 ml of phosphate-buffered saline (PBS) (pH 7.4) and incubated at room temperature (Figure 3.5). Every half an hour intervals, the films were taken out, and the excess water was removed carefully with filter paper and weighed immediately. Measurements were performed in triplicates and are continued until the weight of the films is constant (Shu et al., 2001).

3.7 Water vapour permeability of the Agar based films

The borosilicate glass bottles (capacity, 15 ml; diameter of the top hole, 16 mm) were filled with anhydrous calcium chloride; then the films were tied onto the mouth of the bottles. The average area available for vapour permeation is 8.042 $cm^{2.}$ All bottles were placed in a desiccator (Figure 3.8) containing saturated sodium chloride solution (35.9 g / 100 ml) maintained at room temperature. After 24 days, the containers were weighed using electronic weighing balance (Remunan-lopez and Bodmeier., 1997). Water vapour permeability of the films were calculated using weight gain.

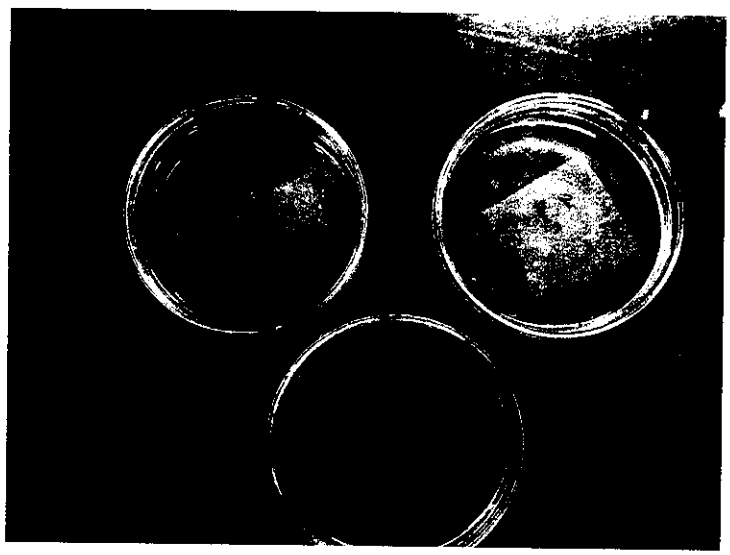

Figure 3.5: water absorption capacity of films

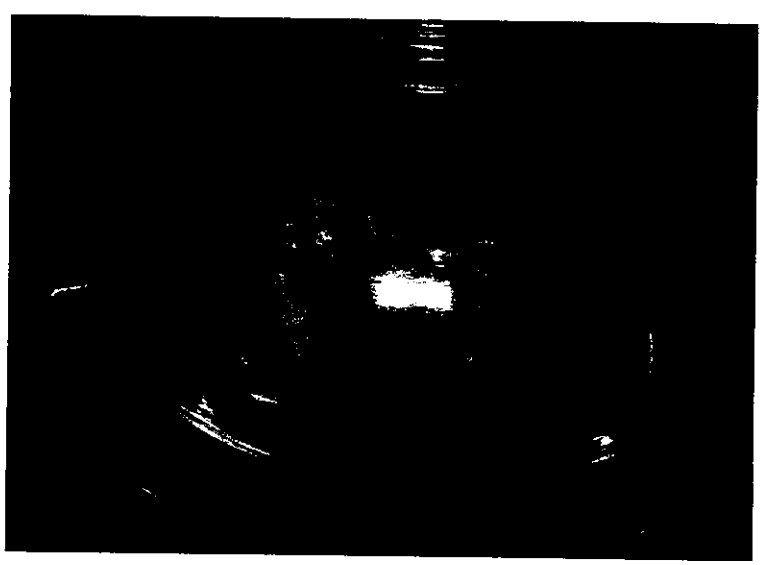

Figure 3.6: Desiccator with agar films tied to the mouth of the bottles

3.8 *In vitro* diffusion study

Each film measuring 9.6 cm^2 were subjected to *in vitro* diffusion testing using Franz diffusion cell (Fig 3.9). A dialysis membrane was clamped between the donor and receptor compartments and the film was placed over the dialysis membrane. The receptor compartment contains phosphate buffer (pH – 7.8) at room temperature. The medium was magnetically stirred and the amount of drug diffusing into the receptor compartment across the membrane were determined by withdrawing 2ml samples over the duration of the experiment and an equivalent amount of diffusion medium was added to the receptor compartment to maintain the constant volume. The samples collected are colorimetrically measured at 422 nm (λ_{max}) using a UV-spectrophotometer. The amount of drug permeating through the skin over 5 hours was then calculated from the total concentration of drug in the patch.

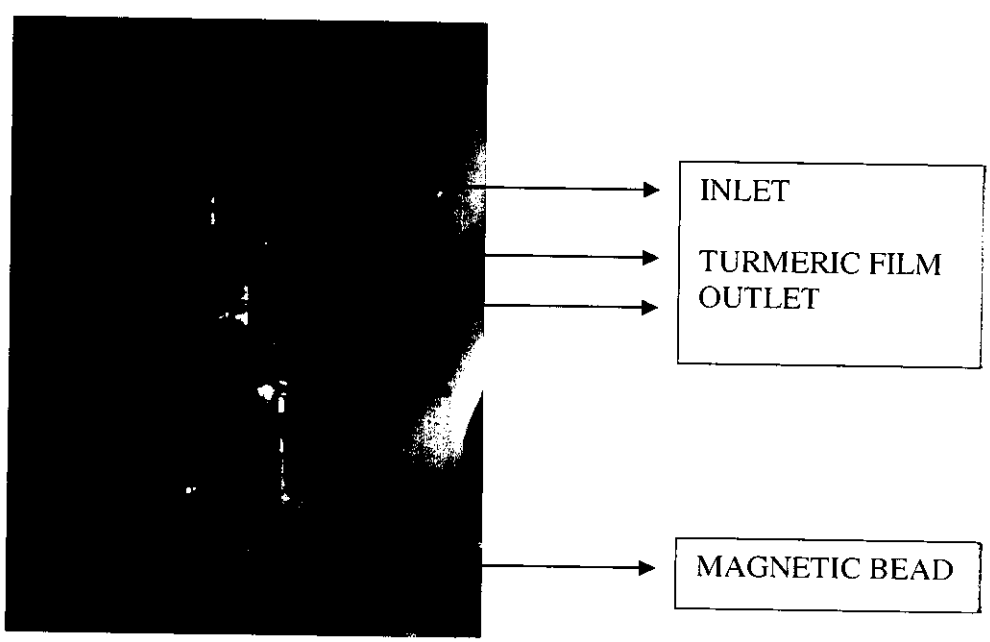

Figure 3.7: Franz diffusion cell

3.9 Anti-microbial testing of Agar based films

3.9.1 Microorganisms tested

Agar based turmeric impregnated films were used for the study of anti-bacterial activity against microorganisms such as *Escherichia coli, Bacillus subtilis, Proteus vulgaris, Klebsiella sp.*

3.9.2 Preparation of culture medium and inoculation

The Luria Bertani agar and broth were used for growing the bacterial cultures. Bacterial culture from slant was inoculated in 50 ml of LB broth and grown at 37°C at 150 rpm overnight.

3.9.3 Liquid culture test

For the liquid culture test, agar based turmeric impregnated films was cut into squares (1.3 cm x 1.3 cm). Three sample squares were immersed in 20 ml nutrient broth (Himedia, Mumbai) in a 25 ml glass bottle. The medium was inoculated with 1 % of over-night grown, *Escherichia coli, Bacillus subtilis, Protease vulgaris, Klebsiella sp* and then transferred to an orbital shaker and rotated at 37 °C at 200 rpm. The culture was sampled periodically (0, 2, 4, 8, 24 hours) during the incubation to obtain microbial growth profiles (Sauch et al., 2007).

The same procedure was repeated for the control films, DMSO was used as negative control and ampicillin and silver nitrate were used as positive control. The optical density (OD) was measured at λ = 600 nm using an ELICO SL 159 UV-VIS SPECTROMETR.

3.10 Animal study of films

3.10.1 Animal

Twelve adult male Wistar Albino rats between two and three months of age and weighing about 200-250g are used in the study. The rats are housed individually in cages, maintained under standard conditions (12 hr light and 12 hr dark cycle at 25 ± 30^0C) (Figure 3.8). The animals were fed with standard rat pellet diet and water. They are into 4 groups of 3 animals in each. The study will be conducted in accordance with the internationally accepted guidelines for laboratory animal use and care.

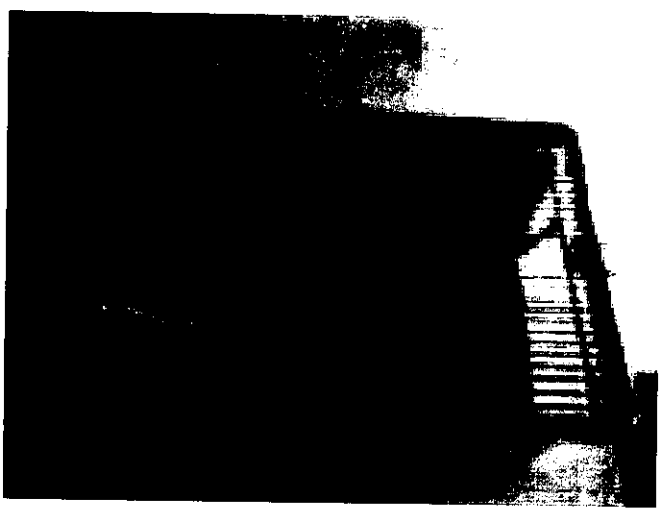

Figure 3.8: Young Wistar Albino male rats

3.10.2 Creation of punch biopsy wounds

The rats are used for the evaluation of superficial burn wounds. The rats are anesthetized with ketamine (25mg/kg of body weight) administered intraperitoneally and the back of the rats will be depilated. Then a heated aluminium stamp maintained at a temperature of 80^0C will be applied for 14 seconds for forming superficial burn wound. Each rat will have 2-3 wounds

of surface area 0.5cm^2. All freshly created wounds will be washed with normal saline before the application of the films.

3.10.3 Application of films and dressing procedures

After wound creation, different films were applied onto the wounds created. The control comprised of gauze soaked with normal saline. The films and the control were placed in such a way that the wounds could be completely covered. All the wounds were then covered with non-adherent occlusive gauzes to hold the films in place and further occluded with hypoallergenic adhesive tape. Finally, a bandage was wrapped around the trunk of the animals to protect the dressing. The bandage and the films were changed every three days until the wound had completely healed.

3.10.4 Examination of punch biopsy wounds

Conditions of the wounds were examined (and photographed) at 0(before film application), 3, 6, days after the wound creation until complete healing. Prior to wound examination, the bandage and the films were removed.

The films were examined for transparency, flexibility, adherence, ease of removal from the wounds without damaging underlying tissues and fluid accumulation using scores of 0-3. A score with a maximum of 3 was given to the desired attributes of the film as wound dressing.

For evaluation of the wounds, the wounds were rinsed with 5ml normal saline from a syringe, gently wiped and graded using scores of 1-3 for dryness of wounds, exudation and scar formation. A score with maximum of 3 was given to conditions preferable in wound healing.

In addition, the outer margin of the wound was traced using a tracing paper and the area determined planimetrically. The degree of wound

contraction (expressed as a percentage) was calculated using the following equation:

$[(A_{day\ 0} - A_{day\ X})/ A_{day\ 0}] * 100$

Where X = 3,6 days after wound creation, A = wound surface area

The period of epithelialization of the wound was also estimated and expressed as the number of days taken for complete epithelialization with no raw wound left.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSIONS

4.1 Preparation of Turmeric extract

Acetone and Methanol extracts of turmeric were prepared as given in materials and methods and the concentration of that particular extract is calculated as below

(Initial weight of the petridish – final weight of the dried extract)/ ml of DMSO used to dissolve the sample and stored at 4°C for further use.

4.1.1 Concentration of Methanol Extract

Initial weight of the Petridish = 180.81g

Final weight of the dried Extract =178.35g

Difference =2.46 g

Volume of DMSO used to dissolve the sample =10 ml

Therefore, Concentration = 2.46g/10 ml

= 0.246 g/ml

=246µg/µl

4.1.2 Concentration of Acetone Extract

Initial weight of the Petridish = 242.42 g

Final weight of the dried Extract = 240.17 g

Difference =2.25 g

Volume of DMSO used to dissolve the sample = 10 ml

Therefore, Concentration = 2.25g/10 ml

=0.225 g/ml

= 225µg/µl

4.2 Preparation of films

The Films of various Compositions and varying Concentrations were prepared as shown in Table 4.1

Table 4.1: Composition and Nature of the Films

AGAR CONC(%)	GELATIN CONC (%)	GLYCEROL CONC (%)	PG CONC (%)	DRYING CONDITION	NATURE OF THE FILM
0.5	-	-	-	Oven (50^0 C)	Sticky, transparent
1.0	-	-	-	Oven (50^0 C)	Sticky, transparent
1.5	-	-	-	Oven (50^0 C)	Sticky, transparent
1.5	-	-	-	RT	Sticky, transparent
1.5	3.0	-	-	RT	Very Stiff
1.5	-	5.0	-	RT	Transparent, non sticky
1.5	3.0	5.0	-	RT	Translucent, non sticky
1.5	3.0	1.0	-	RT	Translucent, non sticky
1.5	3.0	2.0	-	RT	Translucent, non sticky
1.5	2.5	2.0	-	RT	Translucent, non sticky
1.5	2.0	2.0	-	RT	Translucent, non sticky
1.5	1.5	2.0	-	RT	Translucent, non sticky
1.5	1.0	2.0	-	RT	Translucent, non sticky
1.5	0.5	2.0	-	RT	Translucent, non sticky
1.5	-	2.0	-	RT	Transparent, non sticky
1.5	-	-	2.0	RT	Transparent, non sticky
1.5	1.5	-	2.0	RT	Translucent, non sticky
1.5	1.5	-	0.5	RT	Transparent, non sticky
1.5	1.5	2.0	2.0	RT	Translucent, non sticky
1.5	0.5	1.5	0.5	RT	Transparent, non sticky

Among the films discussed on the Table 4.1, seven films were chosen for further testing of Mechanical Properties.

4.3 Mechanical Properties

As shown in Table 4.2, the thickness of the films was in the range of 113 μm to 349.9 μm. The thinnest films were the films with propylene glycol and agar. Addition of Gelatin increased the thickness of the film. Film thickness increasing with increasing gelatin concentration in the formulation. The thickness of the film was highly affected by the addition of Turmeric, as it increased the thickness as shown in Table 4.3 (Sezer et al., 2004).

Mechanical strength values of the film were in the range of 2.810 N to 47.817 N and elasticity values ranged from 1.156% to 16.415%. Strength of the film increased with the addition of propylene glycol along with Gelatin as shown in the table 4.4. In addition, tensile strength of the films changed in inverse proportion to its elasticity (especially for AG, AP, AGG, APG). This is in agreement with the report available (Sezer et al., 2004).

Glycerol provides good elongation property and the addition of gelatin enhances the elongation ability. Among the films chosen, AGG has the maximum elongation capacity. Protection of the wound surface from external factors and elasticity of the dressing are the primary properties of ideal dressing material (Burn, 2001), the main focus on the elongation property, the two films with maximum elongation capacity, AGG and $AGGP_2$ were chosen and the turmeric films were prepared by the addition of turmeric extract into it.

From the Table 4.5, it is shown that the addition of turmeric increases the strength of the film and % increase in elongation is 17.780% to 28.8%.

Table 4.2: Thickness of the Film

Thickness (μm)						
AG	**AP**	**AGG**	**APG**	**APgl**	**$AGGP_1$**	**$AGGP_2$**
150	100	160	140	130	130	180
140	110	150	150	140	120	180
150	110	170	120	140	110	170
120	120	160	160	150	120	170
120	120	170	130	160	110	190
150	110	150	150	150	110	190
160	120	170	140	140	110	190
140	110	180	160	160	120	180
130	120	160	130	150	130	180
130	110	150	140	140	120	170
139	**113**	**162**	**142**	**146**	**118**	**180**

Table 4.3: Thickness of Turmeric Added Films

Thickness (μm)			
AGG (TAE)	**AGG (TME)**	**$AGGP_2$ (TAE)**	**$AGGP_2$ (TME)**
180	150	410	310
170	170	390	310
170	170	400	300
160	150	370	280
160	170	400	270
180	130	390	270
180	140	400	310
200	130	410	290
190	140	410	290
170	140	370	270
175.9	**149**	**394.9**	**289.9**

Table 4.4: Tensile Strength and Elongation Characteristics of the Film

CODE OF THE FILM	BREAKING FORCE (N±S.D)	TENSILE STRENGTH (N/mm^2±S.D)	EXTENSION AT BREAK (mm± S.D)	FILM ELONGATION (%±S.D)	TIME AT BREAK (Sec± S.D)
AG	9.463±3.04	0.047±0.01	9.649±0.00009	12.060±0.00	19.300±0.00
AP	33.859±1.26	0.168±0.006	0.925±0.31	1.156±0.39	1.850±0.63
AGG	5.345±3.55	0.026±0.01	13.133±1.75	16.415±2.192	26.266±3.50
APG	47.817±4.34	0.238±0.02	4.049±0.91	5.061±1.15	8.100±1.83
APgl	2.810±1.88	0.013±0.009	4.216±1.51	5.270+1.89	8.433±3.03
$AGGP_1$	5.402±0.26	0026±1.73	10.038±1.4	12.547±1.74	20.076±2.8
$AGGP_2$	10.433±0.39	0.051±0.002	11.014±0.03	13.836±0.05	20.640±1.60

Table 4.5: Tensile Strength and Elongation Characteristics of the Turmeric Films

CODE	BREAKING FORCE (N±S.D)	TENSILE STRENGTH (N/mm^2±S.D)	EXTENSION AT BREAK (mm± S.D)	FILM ELONGATION (%±S.D)	TIME AT BREAK (Sec± S.D)
AGG (TAE)	22.140±2.45	0.110±0.01	23.076±2.58	28.844±3.23	45.150±3.74
AGG (TME)	32.623± 3.60	0.162±0.01	12.624±1.66	15.780±2.07	25.250±3.32
$AGGP_2$ (TAE)	2.097±1.27	0.036±0.03	20.450±3.67	25.562±4.59	43.400±3.81
$AGGP_2$ (TME)	1.478±0.22	0.007±0.001	16.399±3.59	20.400±0.009	32.800±1.55

4.4 Water Absorption Capacity of the Films

The Water Absorption Capacity of the films ranged from 0.720 g (Table 4.9) to 3.980 g (Table 4.10) /g of the film. It was greater than the water absorption Capacity of the Chitosan Films reported (Shu et al., 2001). As the time interval increase the water absorption capacity of the film also increases as shown in Figure 4.6 to 4.16. It was noted that the thinner films have greater water absorption capacity than the thicker ones. Addition of gelatin seems to increase the water absorption capacity of the films as shown in Figure 4.12. It is stated in the literature that wound fluid should be absorbed in a balanced manner and moisture should be kept under control during wound healing (Lloyd et al., 1998). These findings demonstrated that AGG film have good water asorption capacity.

Addition of Turmeric Extracts to the films seems to have greater influence in increasing the Water absorption capacity as shown in the Table (Table 4.13 to 4.16). The % increase in water absorption was calculated.

Table 4.6: Water Absorption of AG Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.277	-
½ hr	0.475 0.456 0.491	0.474±0.01
1 hr	0.511 0.491 0.480	0.494±0.01
1 ½ hr	0.525 0.492 0.508	0.508±0.01
2 hr	0.539 0.519 0.508	0.522±0.01
2 ½ hr	0.560 0.545 0.492	0.532±0.03
3 hr	0.526 0.525 0.545	0.532±0.01
3 ½ hr	0.569 0.517 0.517	0.532±0.03
4 hr	0.549 0.537 0.539	0.542±0.006
4 ½ hr	0.546 0.540 0.539	0.542±0.003
WEIGHT GAINED (g)/ g of film	0.965	-

Table 4.7: Water Absorption of AP Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.172	-
½ hr	0.504 0.543 0.537	0.528±0.02
1 hr	0.560 0.576 0.554	0.561±0.01
1 ½ hr	0.560 0.603 0.585	0.582±0.02
2 hr	0.618 0.621 0.630	0.623±0.006
2 ½ hr	0.650 0.647 0.660	0.652±0.006
3 hr	0.667 0.659 0.670	0.665±0.005
3 ½ hr	0.655 0.680 0.678	0.671±0.01
4 hr	0.646 0.680 0.684	0.670±0.02
4 ½ hr	0.674 0.678 0.670	0.674±0.004
WEIGHT GAINED (g)/ g of film	2.920	-

Table 4.8: Water Absorption of AGG Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.437	-
½ hr	1.170 1.301 1.181	1.217±0.07
1 hr	1.337 1.382 1.428	1.382±0.04
1 ½ hr	1.368 1.407 1.501	1.425±0.06
2 hr	1.421 1.417 1.536	1.458±0.06
2 ½ hr	1.442 1.420 1.522	1.461±0.05
3 hr	1.461 1.493 1.540	1.498±0.03
3 ½ hr	1.484 1.466 1.570	1.506±0.05
4 hr	1.483 1.465 1.569	1.505±0.05
4 ½ hr	1.480 1.467 1.567	1.504±0.05
WEIGHT GAINED (g)/ g of film	2.440	-

Table 4.9: Water Absorption of APG Film

TIME NTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.282	-
½ hr	1.356 1.402 1.402	1.386±0.02
1 hr	1.389 1.404 1.504	1.432±0.06
1 ½ hr	1.360 1.440 1.524	1.441±0.08
2 hr	1.381 1.401 1.363	1.381±0.02
2 ½ hr	1.332 1.360 1.418	1.370±0.04
3 hr	1.362 1.415 1.360	1.379±0.03
3 ½ hr	1.362 1.412 1.374	1.382±0.02
4 hr	1.379 1.413 1.437	1.409±0.03
4 ½ hr	1.362 1.409 1.443	1.405±0.04
WEIGHT GAINED (g)/ g of film	3.980	-

Table 4.10: Water Absorption of APgl Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.161	-
½ hr	0.239 0.248 0.238	0.242±0.005
1 hr	0.242 0.261 0.238	0.247±0.01
1 ½ hr	0.254 0.274 0.249	0.259±0.01
2 hr	0.250 0.266 0.240	0.252±0.01
2 ½ hr	0.259 0.272 0.258	0.263±0.007
3 hr	0.252 0.292 0.243	0.262±0.02
3 ½ hr	0.265 0.290 0.267	0.274±0.01
4 hr	0.272 0.277 0.283	0.277±0.005
4 ½ hr	0.275 0.288 0.270	0.277±0.009s
WEIGHT GAINED (g)/ g of film	0.720	-

Table 4.11: Water Absorption of $AGGP_1$ Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.079	-
½ hr	0.183 0.202 0.184	0.189±0.01
1 hr	0.181 0.195 0.192	0.189±0.007
1 ½ hr	0.206 0.213 0.202	0.207±0.005
2 hr	0.208 0.209 0.198	0.205±0.006
2 ½ hr	0.209 0.209 0.207	0.208±0.001
3 hr	0.213 0.212 0.199	0.208±0.007
3 ½ hr	-	-
4 hr	-	-
4 ½ hr	-	-
WEIGHT GAINED (g)/ g of film	1.633	-

Table 4.12: Water Absorption of $AGGP_2$ Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.102	-
½ hr	0.200 0.208 0.203	0.204±0.004
1 hr	0.202 0.217 0.216	0.211±0.008
1 ½ hr	0.192 0.218 0.209	0.206±0.01
2 hr	0.191 0.216 0.201	0.202±0.01
2 ½ hr	0.182 0.213 0.207	0.200±0.01
3 hr	0.196 0.203 0.201	0.200±0.003
3 ½ hr	0.185 0.207 0.210	0.200±0.01
4 hr	-	-
4 ½ hr	-	-
WEIGHT GAINED (g)/ g of film	0.960	-

Table 4.13: Water Absorption of AGGT (TAE) Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.110	-
½ hr	0.299 0.308 0.314	0.307±0.006
1 hr	0.345 0.343 0.314	0.343±0.001
1 ½ hr	0.369 0.398 0.410	0.392±0.01
2 hr	0.402 0.412 0.398	0.404±0.006
2 ½ hr	0.433 0.435 0.429	0.432±0.002
3 hr	0.420 0.423 0.419	0.420±0.002
3 ½ hr	0.446 0.449 0.440	0.445±0.003
4 hr	0.452 0.450 0.445	0.449±0.003
4 ½ hr	0.455 0.449 0.443	0.449±0.005
WEIGHT GAINED (g)/ g of film	3.080	-

Table 4.14: Water Absorption of AGGT (TME) Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.109	-
½ hr	0.315 0.305 0.316	0.312±0.005
1 hr	0.364 0.363 0.354	0.360±0.004
1 ½ hr	0.395 0.389 0.390	0.391±0.003
2 hr	0.393 0.405 0.407	0.402±0.006
2 ½ hr	0.409 0.415 0.420	0.414±0.004
3 hr	0.434 0.420 0.410	0.421±0.009
3 ½ hr	0.434 0.429 0.430	0.431±0.002
4 hr	0.450 0.434 0.427	0.437±0.009
4 ½ hr	0.450 0.444 0.427	0.442±0.006
WEIGHT GAINED (g)/ g of film	3.055	-

Table 4.15: Water Absorption of $AGGP_2$ (TAE) Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.195	-
½ hr	0.456 0.453 0.455	0.454±0.001
1 hr	0.496 0.485 0.511	0.497±0.01
1 ½ hr	0.547 0.514 0.528	0.529±0.01
2 hr	0.562 0.551 0.549	0.554±0.005
2 ½ hr	0.593 0.585 0.561	0.579±0.01
3 hr	0.580 0.593 0.601	0.591±0.008
3 ½ hr	0.630 0.596 0.582	0.602±0.02
4 hr	0.663 0.615 0.598	0.625±0.03
4 ½ hr	0.695 0.678 0.605	0.659±0.04
WEIGHT GAINED (g)/ g of film	2.379	-

Table 4.16: Water Absorption of $AGGP_2$ (TME) Film

TIME INTERVAL	WEIGHT GAINED (g)	(g ±S.D)
INITIAL	0.268	-
½ hr	0.584 0.595 0.602	0.594±0.007
1 hr	0.621 0.640 0.636	0.632±0.008
1 ½ hr	0.640 0.652 0.650	0.647±0.005
2 hr	0.653 0.683 0.682	0.672±0.01
2 ½ hr	0.679 0.699 0.707	0.695±0.01
3 hr	0.703 0.734 0.740	0.725±0.01
3 ½ hr	0.716 0.749 0.754	0.739±0.01
4 hr	0.745 0.759 0.787	0.763±0.01
4 ½ hr	0.770 0.773 0.778	0.773±0.003
WEIGHT GAINED (g)/ g of film	1.847	-

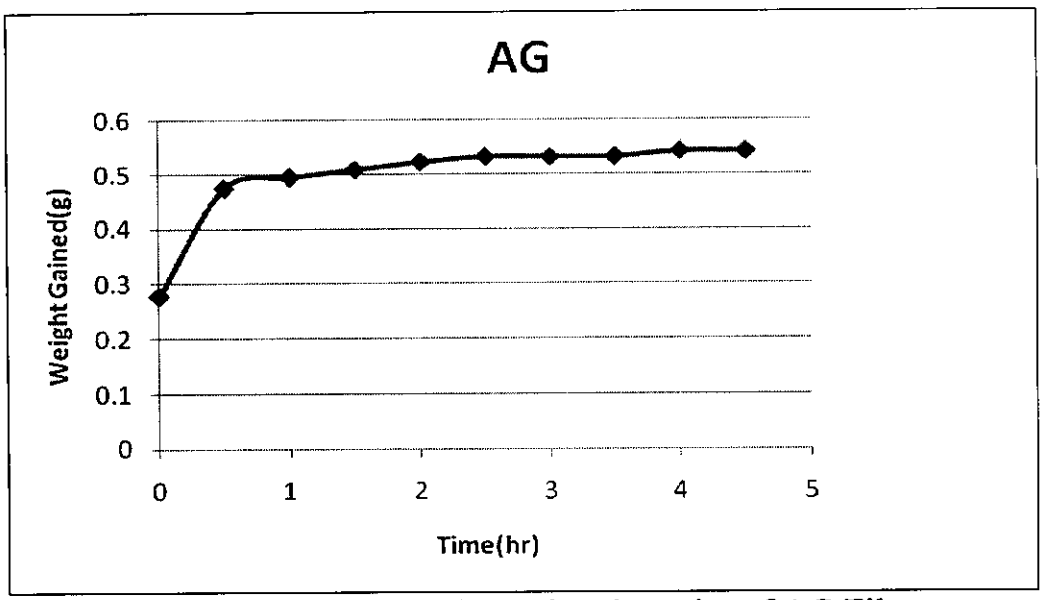

Figure 4.1: Water Absorption Capacity of AG Film

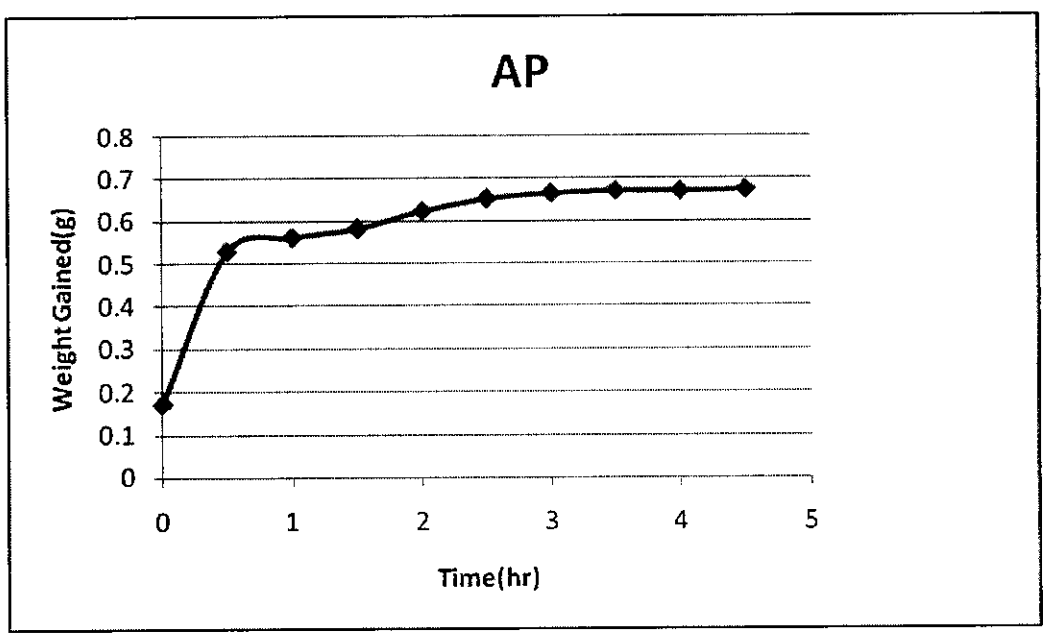

Figure 4.2 : Water Absorption Capacity of AP Film

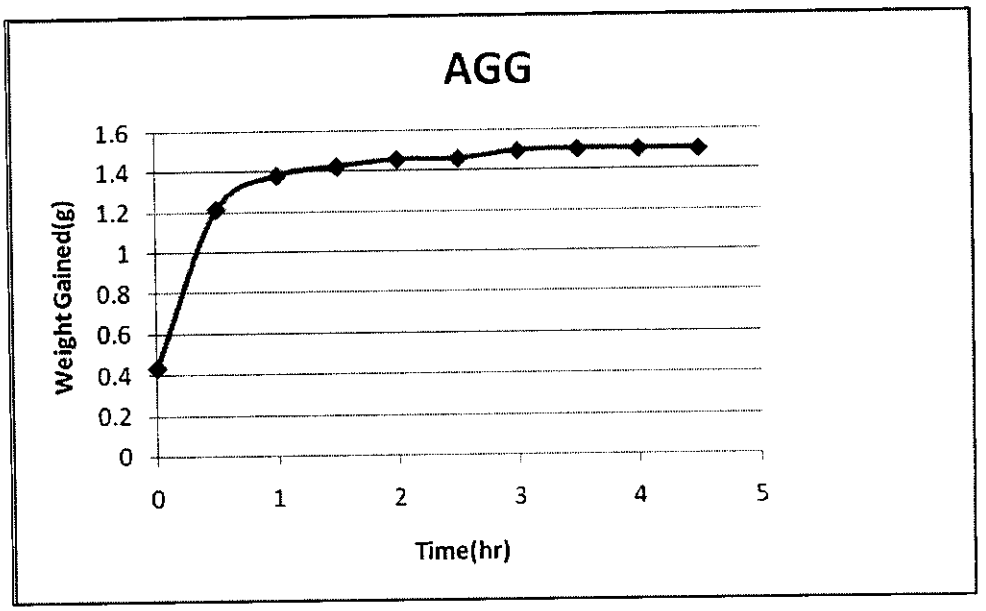

Figure 4.3 : Water Absorption Capacity of AGG Film

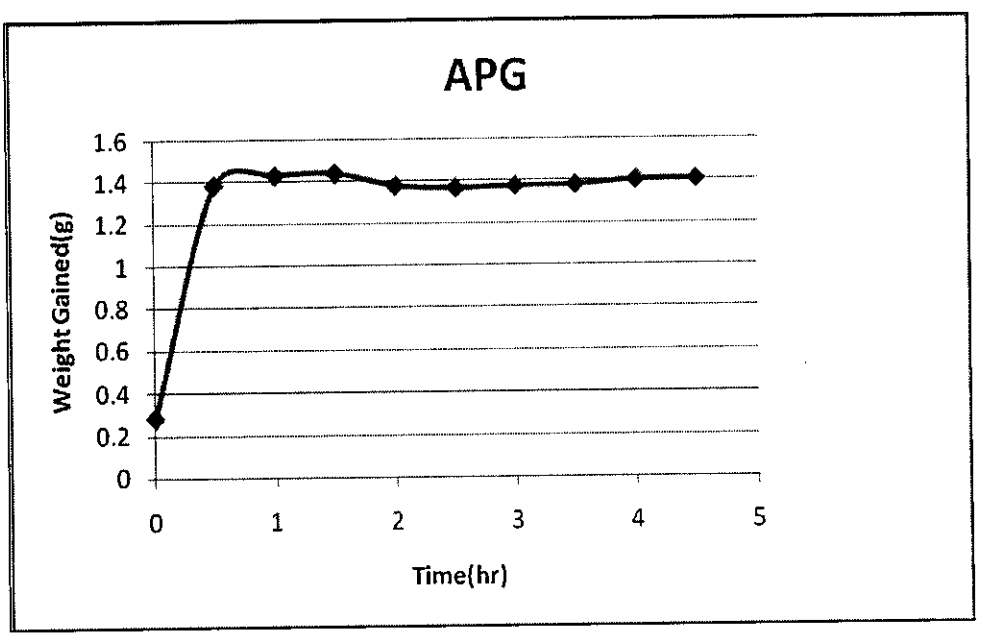

Figure 4.4 : Water Absorption Capacity of APG Film

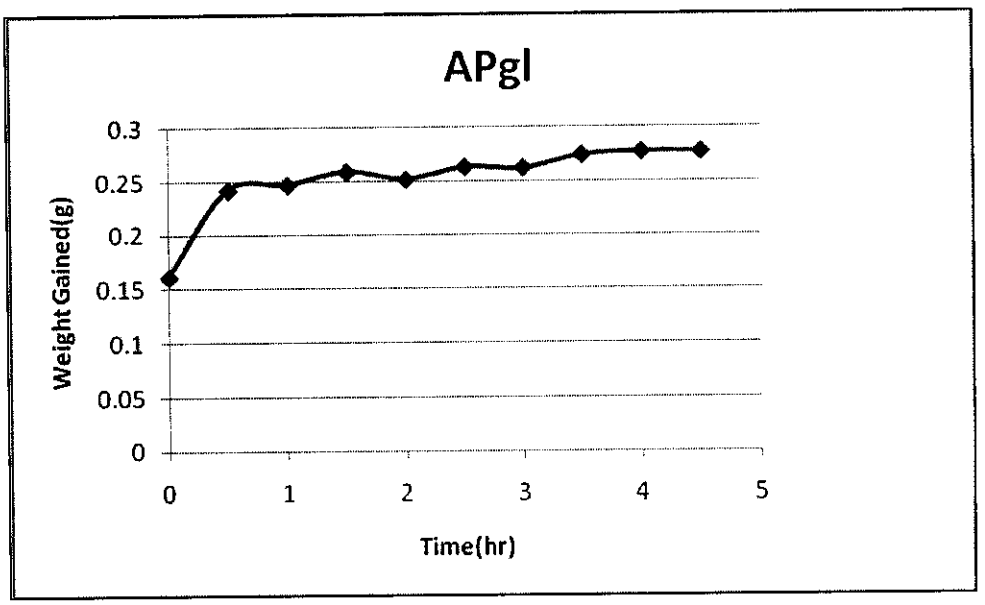

Figure 4.5: Water Absorption Capacity of APgl Film

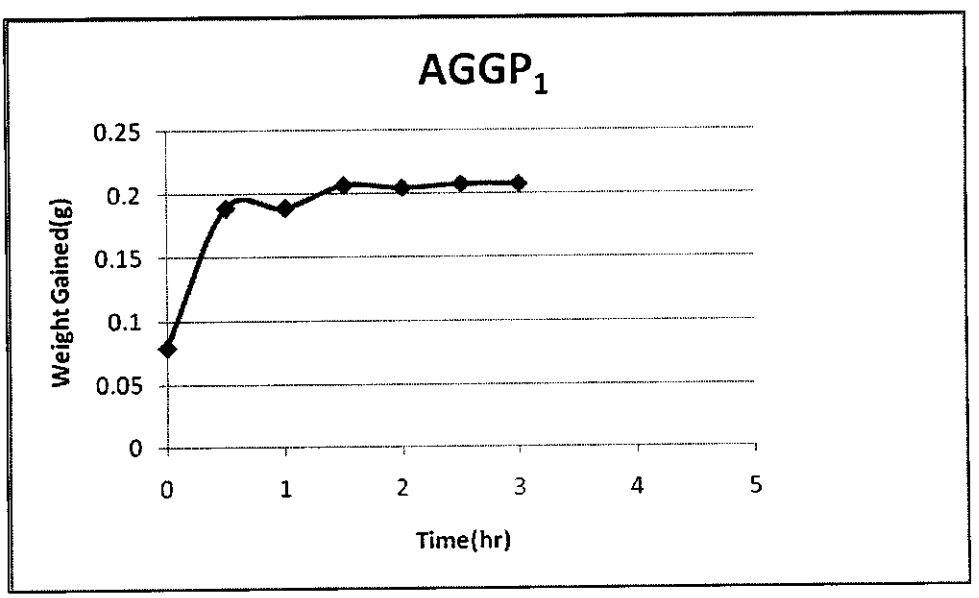

Figure 4.6: Water Absorption Capacity of $AGGP_1$ Film

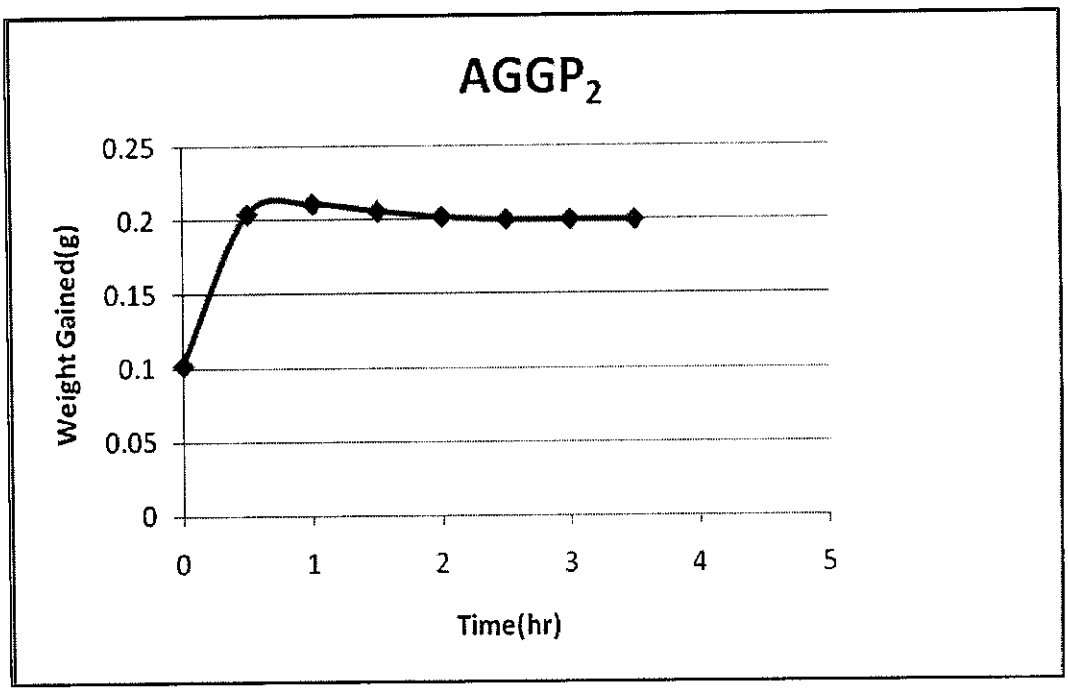

Figure 4.7 : Water Absorption Capacity of $AGGP_2$ Film

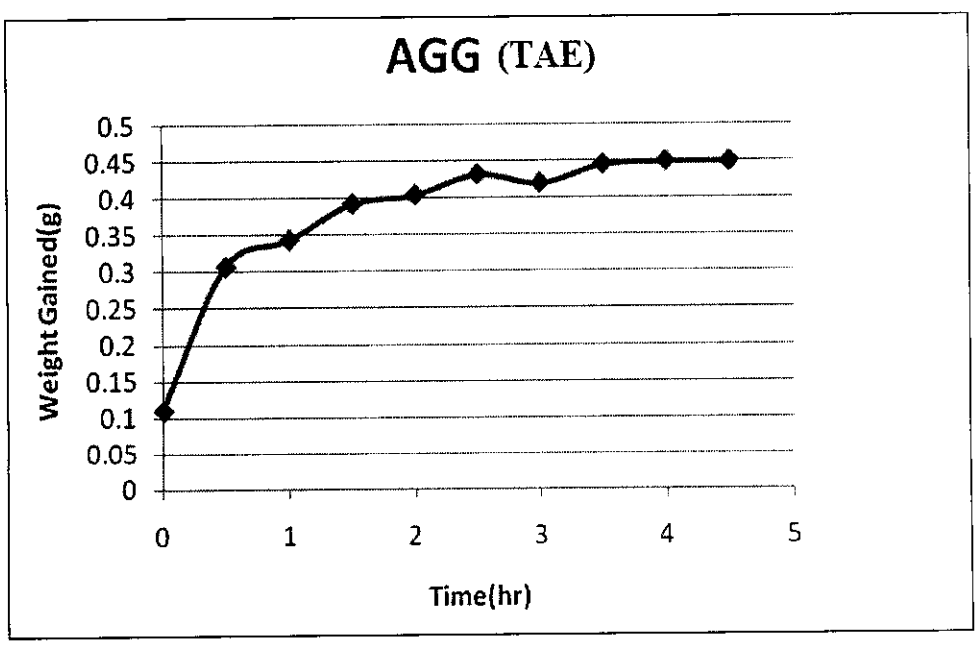

Figure 4.8: Water Absorption Capacity of AGG(TAE) Film

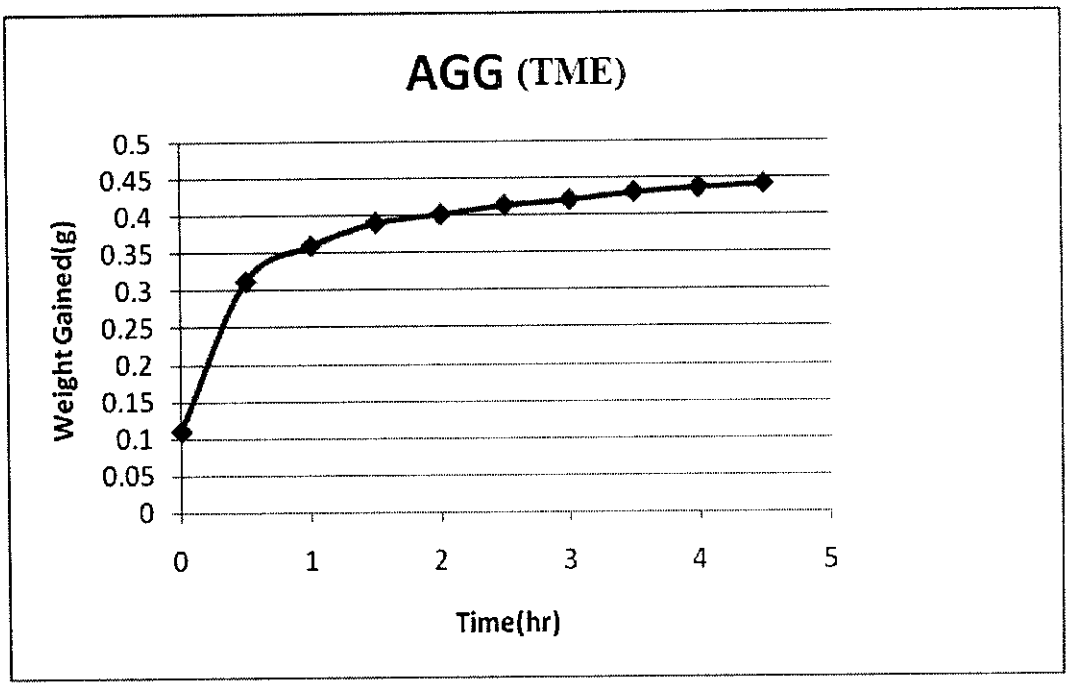

Figure 4.9: Water Absorption Capacity of AGG(TME) Film

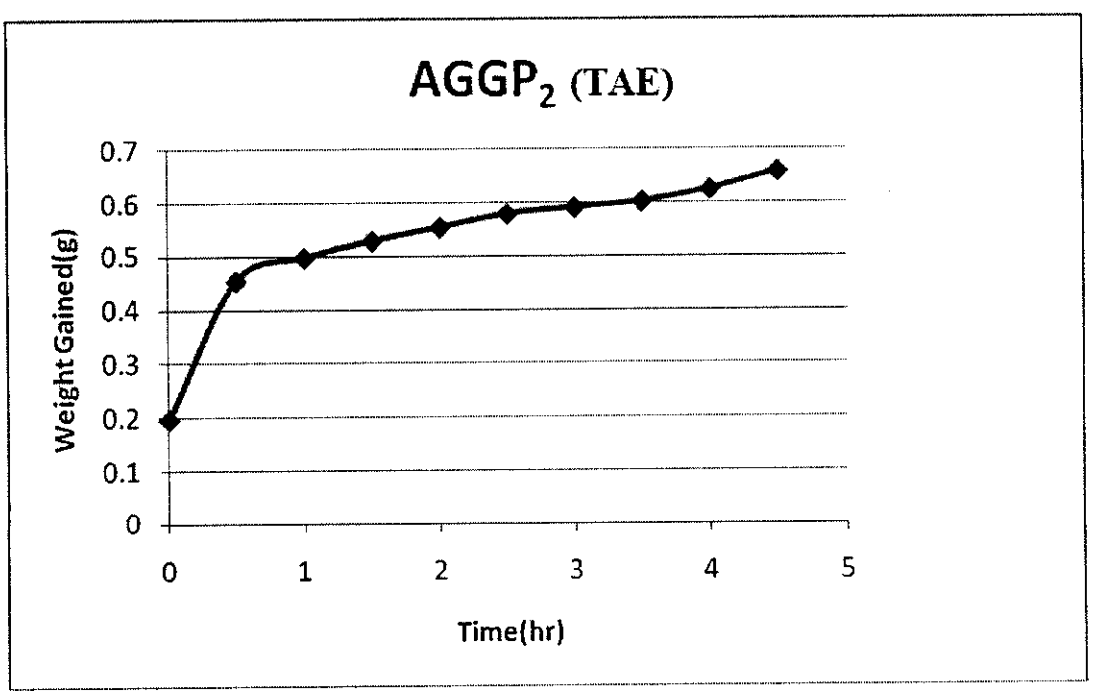

Figure 4.10 : Water Absorption Capacity of $AGGP_2$(TAE) Film

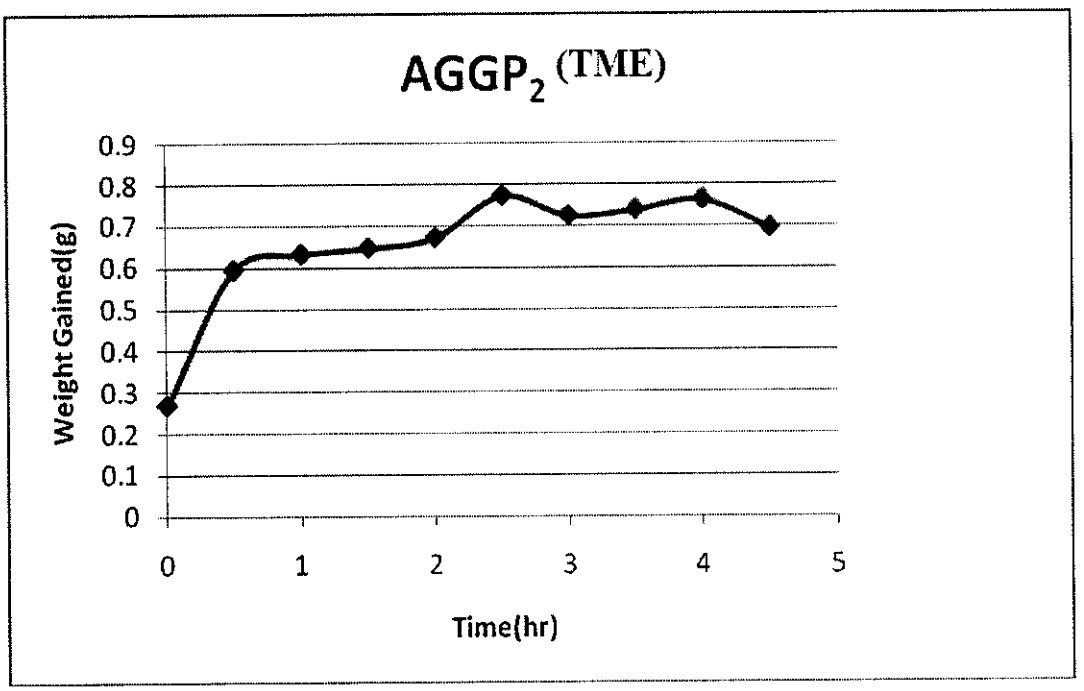

Figure 4.11 : Water Absorption Capacity of $AGGP_2$(TME) Film

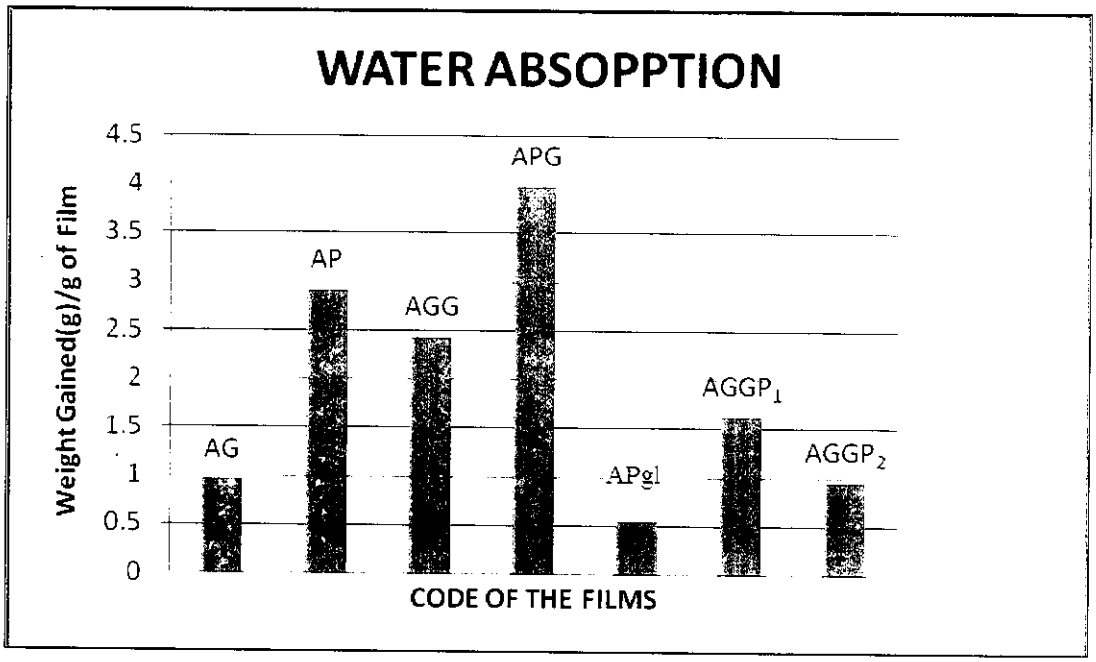

Figure 4.12: Comparison of the Water Absorption Capacity of Different Films

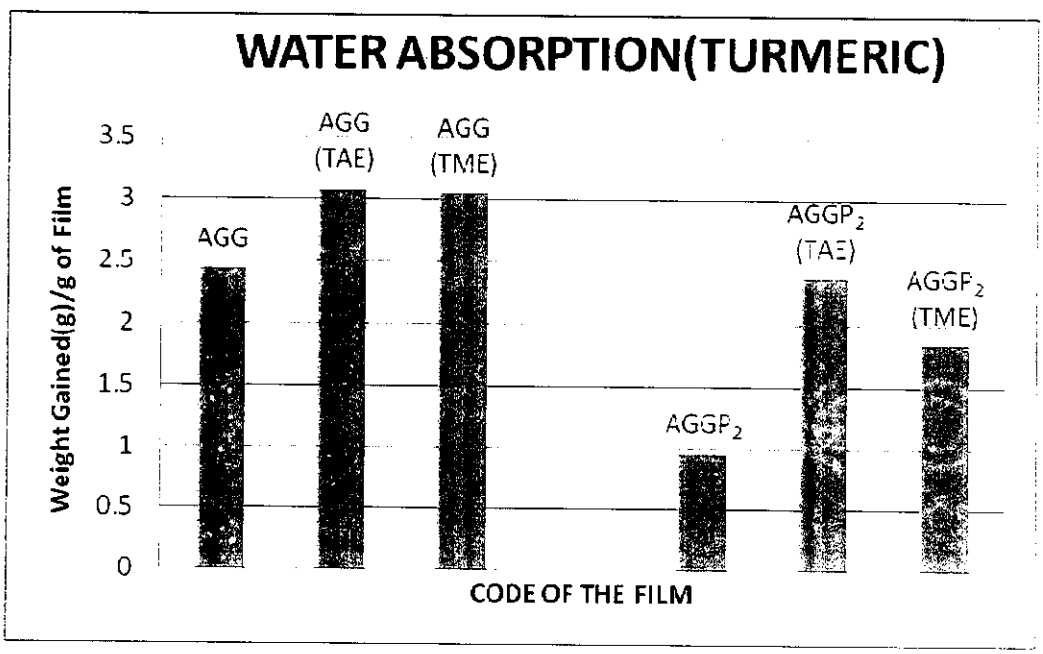

Figure 4.13: Comparison of the water absorption capacity of turmeric films of AGG and $AGGP_2$ formulation with control films without turmeric.

4.5 Water Vapour Permeability of the Films

Water vapour permeability of the films was in the range of 0.913 g to 2.609 g/cm^2 of the films as shown in Table 4.17. Water vapour permeability of the films was significantly decreased with increase in Gelatin concentration in the formulation. These data seems to be similar as reported earlier the permeability of the film decreased with increasing Chitosan concentration (Sezer et al., 2007). Glycerol added films show greater water vapour permeability. According to these findings, using films with optimum water vapour permeability, the treatment is advantageous because moisture and oxygen play an important role during wound healing (Khan et al., 2000). AGG film shows a good water absorption capacity along with good water vapour permeability. Open bottles containing anhydrous calcium chloride placed inside the dessicator is considered as Control.

Table 4.17: Water Vapour Permeability of the Films

FILM CODE	INITIAL WEIGHT(g)	FINAL WEIGHT(g)	WEIGHT GAINED(g)	WEIGHT GAINED(g)/cm^2 OF THE FILM	g ± S.D
AG	17.668	22.981	5.313	2.643	2.609±0.05
	18.483	23.590	5.107	2.541	
	18.448	23.761	5.313	2.643	
AP	17.773	21.753	3.980	1.980	2.010±0.04
	17.776	21.919	4.143	2.061	
	17.301	21.300	3.999	1.989	
AGG	18.008	22.519	4.511	2.244	2.230±0.01
	17.844	22.314	4.470	2.224	
	17.575	22.042	4.467	2.222	
APG	19.190	21.132	1.942	0.966	0.913±0.04
	17.962	19.762	1.800	0.895	
	18.231	20.000	1.769	0.880	
Apgl	20.039	25.223	5.184	2.579	2.426±0.16
	18.648	23.785	4.537	2.257	
	18.752	23.662	4.910	2.443	
$AGGP_1$	18.665	23.455	4.790	2.383	2.529±0.12
	18.615	23.864	5.249	2.611	
	18.743	23.954	5.211	2.593	
$AGGP_2$	18.026	22.367	4.341	2.160	2.291±0.13
	17.545	22.123	4.578	2.278	
	18.390	23.287	4.897	2.436	
CONTROL	21.056	26.659	5.603	2.788	-

Table 4.18 :Water Vapour Permeability of Turmeric Films

FILM CODE	INITIAL WEIGHT(g)	FINAL WEIGHT(g)	WEIGHT GAINED(g)	WEIGHT GAINED(g)/cm^2 OF THE FILM	g ± S.D
AGG (TAE)	18.610	22.799	4.189	2.084	1.982±0.09
	17.933	21.887	3.954	1.967	
	17.707	21.516	3.809	1.895	
AGG (TME)	18.582	22.772	4.190	2.084	2.179±0.09
	19.768	24.148	4.380	2.179	
	18.169	22.742	4.573	2.275	
$AGGP_2$(TAE)	18.151	23.076	4.925	2.450	2.432±0.30
	18.193	22.458	4.265	2.122	
	17.922	23.397	5.475	2.724	
$AGGP_2$(TME)	17.412	21.136	3.724	1.853	1.895±0.18
	17.562	21.772	4.210	2.094	
	17.170	20.665	3.495	1.739	

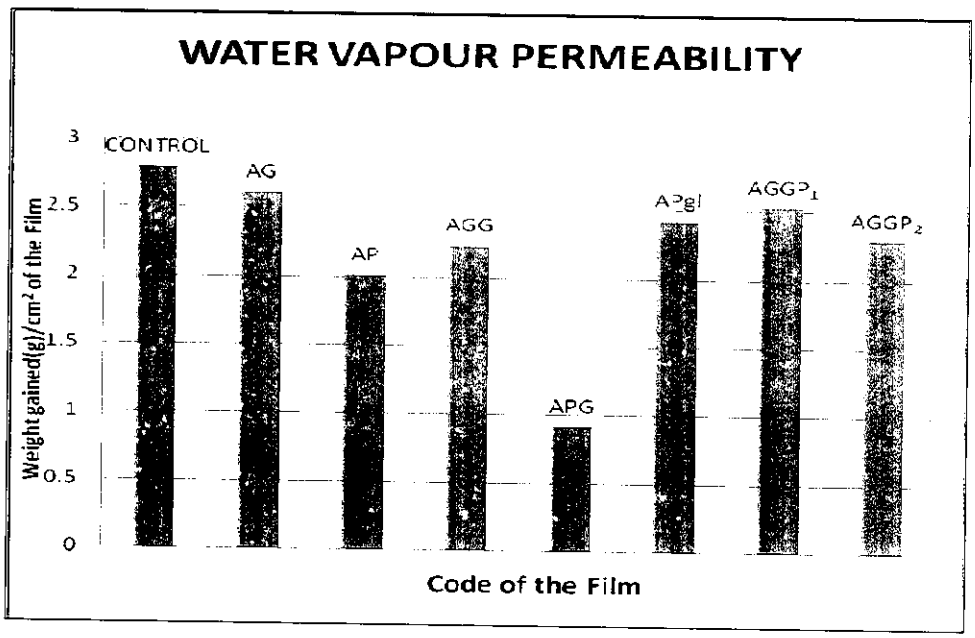

Figure 4.14: Comparison of the Water Vapour Permeability of Different Films

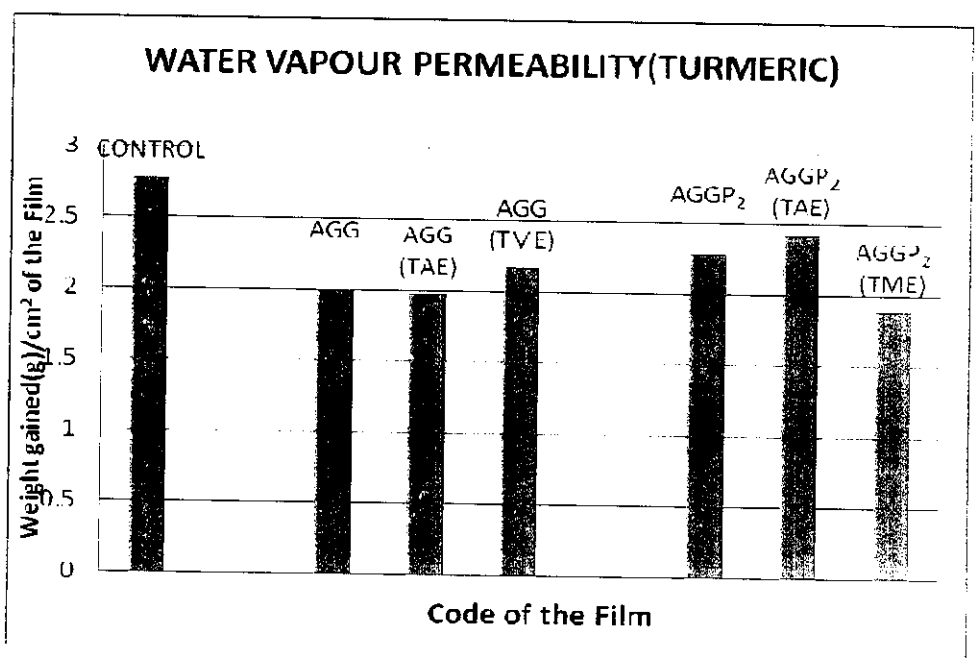

Figure 4.15: Comparison of the Water Vapour Permeability of Turmeric Films of AGG and $AGGP_2$ Formulation.

4.6 *In Vitro* Diffusion Study:

Table 4.19: Concentration of TAE

CONC OF TAE	0.D at 422 nm
10	0.215
20	0.402
30	0.571
40	0.702
50	0.962

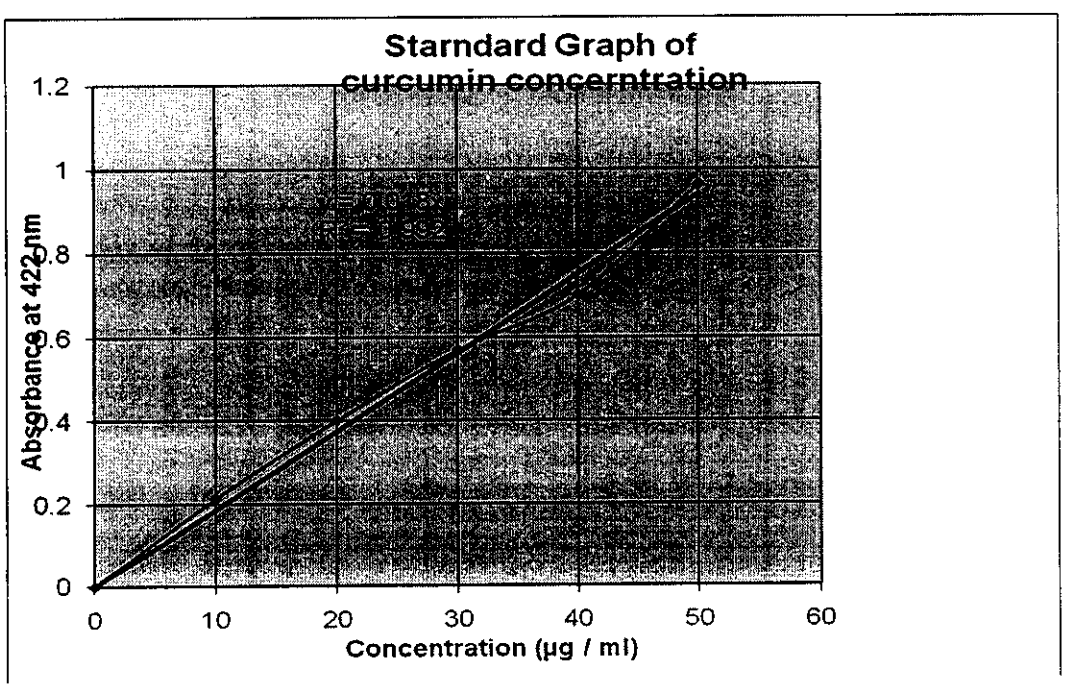

Figure 4.16 Standard Graph of Curcumin Concentration

Table 4.20: Release CONC. From AGG (TAE) Film:

Time intervals (hr)	O.D at 422nm	CONC. (µg / ml)	% Release of Turmeric
0	0.017	0.904	0.020
1	0.086	4.574	0.127
2	0.120	6.383	0.254
3	0.276	14.688	0.489
4	0.221	11.755	0.614
5	0.209	11.112	0.531

Table 4.21: Release Conc. From $AGGP_2$ (TAE) Film:

Time intervals (hr)	O.D at 422nm	CONC (µg / ml)	% Release of Turmeric
0	0.070	3.723	0.086
1	0.092	4.894	0.200
2	0.184	9.789	0.341
3	0.237	12.606	0.520
4	0.217	11.542	0.561
5	0.190	10.106	0.503

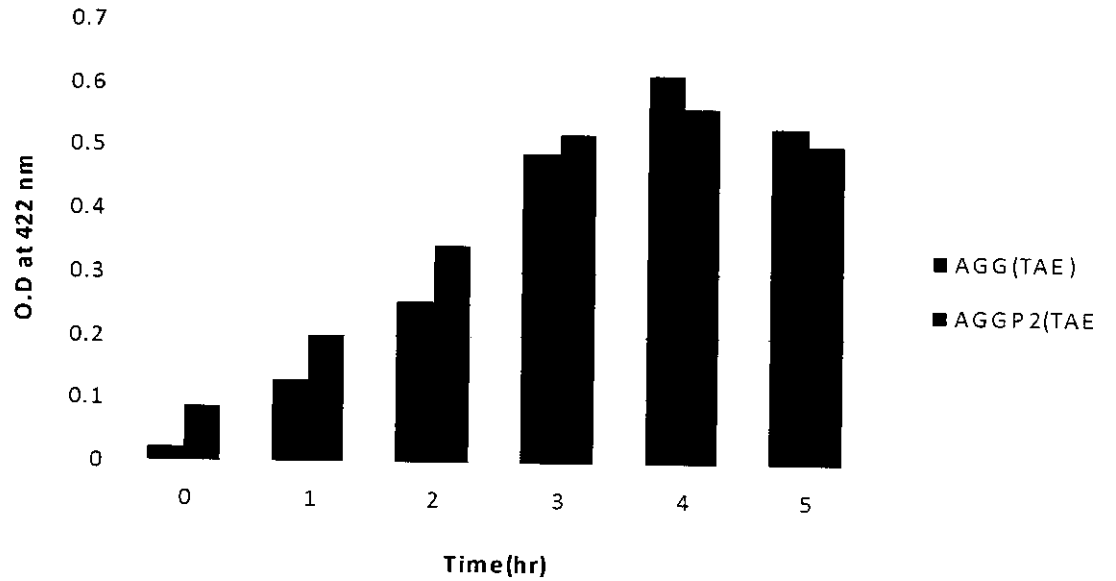

Figure 4.17: Comparison of the release % of Turmeric from AGG and $AGGP_2$ Films

The results were tabulated and studied. It was found that the percent release of AGG (TAE) film was found to be higher.

4.7 Liquid Culture Test

The Liquid Culture test was performed for the Agar based films impregnated with turmeric, against *Bacillus subtilis, Escherichia coli, Klebsiella sp., Proteus vulgaris* and the O.D was read at 600nm. Film impregnated with DMSO was treated as negative control and the film impregnated with ampicillin and silver nitrate was treated as positive control. The results are tabulated and the percent reduction is calculated.

Table 4.22: Liquid Culture Test of AGG Films against *Bacillus subtilis*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGG FILM	0.357 0.304 0.384	0.748 0.569 0.717	1.591 1.790 1.749	1.869 1.976 1.952	-
MEAN±S.D	0.348±0.40	0.678±0.095	1.71±0.105	1.932±0.056	
AGG FILM-WITH TAE	0.386 0.346 0.396	0.463 0.548 0.465	1.274 1.238 1.233	1.739 1.666 1.761	12.72
MEAN±S.D	0.376±0.026	0.492±0.049	1.248±0.022	1.722±0.049	
AGG FILM-WITH TME	0.311 0.312 0.305	0.584 0.596 0.584	1.483 1.421 1.481	1.751 1.751 1.712	11.91
MEAN±S.D	0.309±0.003	0.586±0.007	1.461±0.035	1.738±0.022	
AGG FILM WITH DMSO	0.325 0.296 0.307	0.688 0.605 0.681	1.532 1.664 1.477	1.811 1.940 1.757	6.94
MEAN±S.D	0.309±0.014	0.658±0.046	1.557±0.096	1.836±0.094	
AGG FILM WITH $AgNo_3$	0.318 0.283 0.278	0.420 0.353 0.449	1.407 1.353 1.347	1.747 1.684 1.650	14.19
MEAN±S.D	0.293±0.021	0.407±0.049	1.369±0.033	1.693±0.049	
AGG FILM WITH AMP	0.103 0.163 0.151	0.215 0.197 0.198	0.302 0.291 0.275	0.366 0.371 0.361	81.85
MEAN±S.D	0.139±0.031	0.203±0.010	0.289±0.013	0.358±0.011	
CONTROL BROTH	0.312 0.394 0.368	0.753 0.688 0.724	1.684 1.794 1.780	1.961 1.980 1.979	-
MEAN±S.D	0.358±0.04	0.721±0.03	1.752±0.06	1.973±0.01	

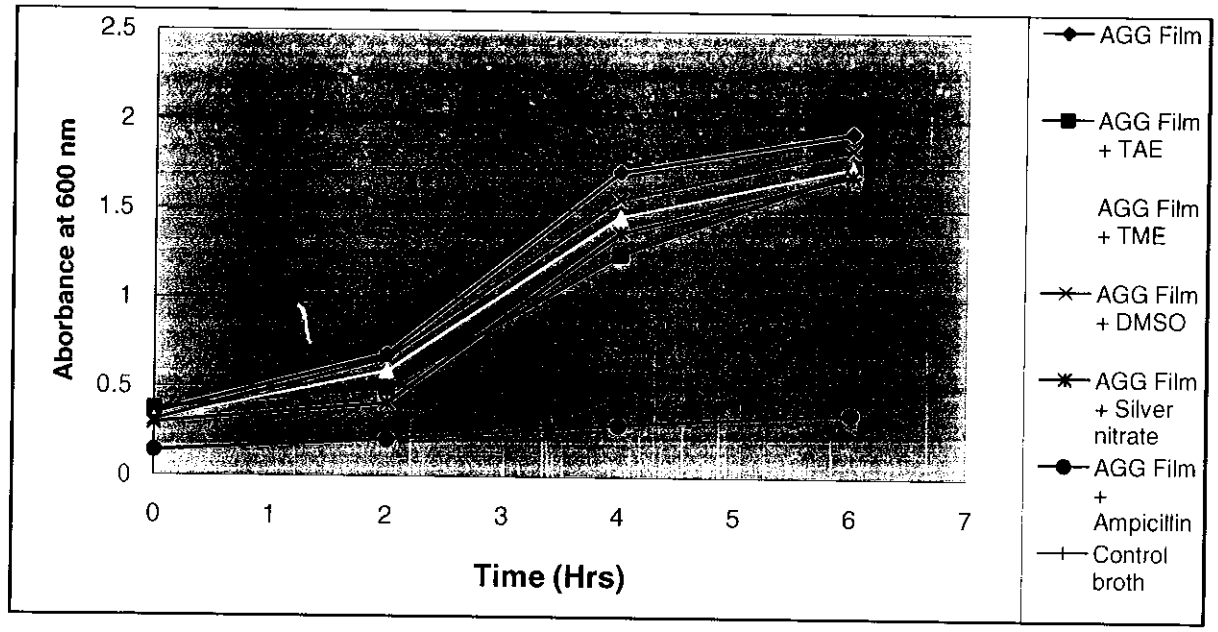

Figure 4.18: Liquid Culture Test of AGG Films against *Bacillus subtilis*

Table 4.23: Liquid Culture Test of AGG Films against *Escherichia coli*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGG FILM	0.377 0.387 0.384	0.757 0.589 0.683	1.391 1.341 1.306	1.658 1.674 1.663	-
MEAN±S.D	0.383±0.005	0.676±0.084	1.346±0.042	1.665±0.008	
AGG FILM-WITH TAE	0.277 0.307 0.323	0.556 0.548 0.621	0.889 0.861 0.868	1.239 1.248 1.295	25.04
MEAN±S.D	0.302±0.023	0.575±0.04	0.873±0.014	1.260±0.030	
AGG FILM-WITH TME	0.353 0.382 0.306	0.614 0.596 0.602	0.804 0.809 0.781	1.168 1.183 1.185	29.86
MEAN±S.D	0.347±0.038	0.604±0.009	0.798±0.014	1.179±0.009	
AGG FILM WITH DMSO	0.365 0.373 0.381	0.668 0.673 0.643	1.253 1.266 1.247	1.517 1.534 1.557	8.62
MEAN±S.D	0.373±0.008	0.661±0.016	1.255±0.009	1.536±0.020	
AGG FILM WITH $AgNo_3$	0.312 0.326 0.378	0.612 0.553 0.572	0.667 0.653 0.647	0.997 1.024 1.065	40.28
MEAN±S.D	0.339±0.034	0.579±0.030	0.656±0.010	1.028±0.034	
AGG FILM WITH AMP	0.333 0.303 0.364	0.430 0.408 0.453	0.502 0.529 0.545	0.596 0.571 0.591	65.13
MEAN±S.D	0.333±0.03	0.430±0.022	0.525±0.021	0.586±0.013	
CONTROL BROTH	0.333 0.303 0.364	0.762 0.701 0.674	1.382 1.361 1.312	1.702 1.684 1.658	-
MEAN±S.D	0.333±0.008	0.712±0.04	1.351±0.03	1.681±0.02	

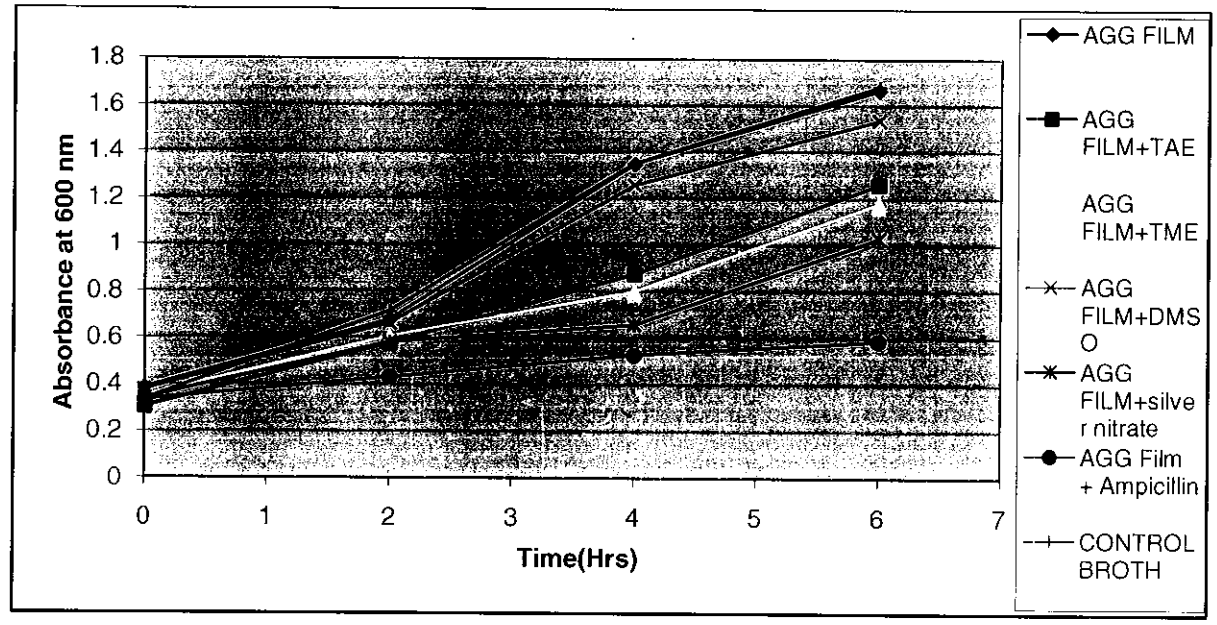

Figure 4.19: Liquid Culture Test of AGG Films against *Escherichia coli*

Table 4.24: Liquid Culture Test of AGG Films against *Proteus vulgaris*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGG FILM	0.087 0.102 0.093	0.537 0.520 0.484	1.376 1.325 1.280	1.689 1.614 1.643	-
MEAN±S.D	0.094±0.007	0.513±0.02	1.327±0.04	1.648±0.03	
AGG FILM-WITH TAE	0.052 0.055 0.057	0.340 0.368 0.339	1.188 1.104 1.155	1.253 1.196 1.204	23.63
MEAN±S.D	0.055±0.003	0.349±0.01	1.149±0.04	1.260±0.03	
AGG FILM-WITH TME	0.050 0.048 0.057	0.314 0.305 0.295	1.159 1.172 1.183	1.152 1.148 1.182	29.69
MEAN±S.D	0.0516±0.004	0.305±0.009	1.171±0.012	1.160±0.018	
AGG FILM WITH DMSO	0.064 0.058 0.067	0.456 0.480 0.431	1.280 1.243 1.202	1.563 1.585 1.589	4.30
MEAN±S.D	0.061±0.034	0.455±0.024	1.275±0.029	1.579±0.014	
AGG FILM WITH $AgNo_3$	0.066 0.056 0.062	0.293 0.280 0.276	0.768 0.735 0.774	1.009 1.071 1.069	36.34
MEAN±S.D	0.061±0.05	0.283±0.008	0.759±0.021	1.050±0.035	
AGG FILM WITH AMP	0.051 0.049 0.050	0.130 0.108 0.153	0.313 0.292 0.335	0.586 0.561 0.571	65.33
MEAN±S.D	0.050±0.001	0.130±0.136	0.313±0.021	0.572±0.012	
CONTROL BROTH	0.112 0.072 0.092	0.540 0.512 0.501	1.401 1.312 1.342	1.692 1.628 1.631	-
MEAN±S.D	0.02±0.02	0.517±0.0.2	1.351±0.04	1.650±0.03	

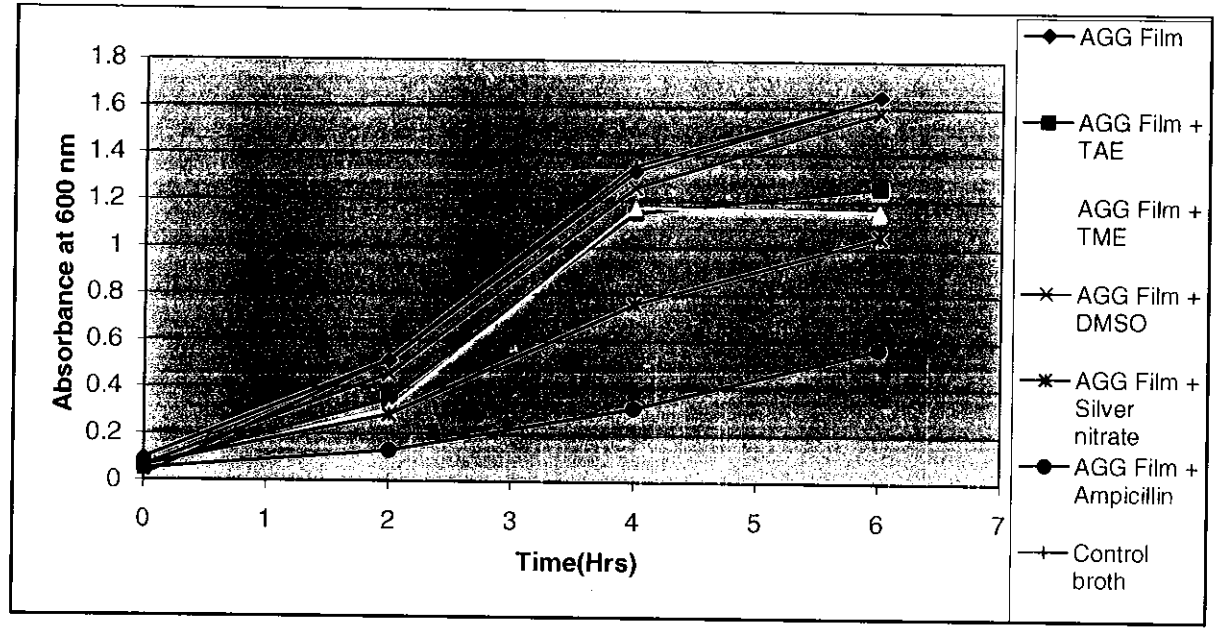

Figure 4.20: Liquid Culture Test of AGG Films against *Proteus vulgaris*

Table 4.25: Liquid Culture Test of AGG Films against *Klebsiella*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGG FILM	0.368 0.373 0.363	0.853 0.839 0.864	1.712 1.734 1.709	1.986 1.998 1.973	-
MEAN±S.D	0.368±0.005	0.852±0.013	1.718±0.013	1.979±0.014	
AGG FILM-WITH TAE	0.363 0.320 0.359	0.687 0.673 0.671	1.559 1.540 1.547	1.784 1.792 1.786	10.03
MEAN±S.D	0.347±0.023	0.677±0.008	1.549±0.275	1.775±0.015	
AGG FILM-WITH TME	0.420 0.401 0.409	0.765 0.773 0.791	1.577 1.583 1.596	1.700 1.798 1.729	11.70
MEAN±S.D	0.410±0.009	0.776±0.011	1.585±0.009	1.742±0.05	
AGG FILM WITH DMSO	0.334 0.328 0.319	0.830 0.845 0.847	1.695 1.683 1.677	1.804 1.838 1.821	7.704
MEAN±S.D	0.327±0.07	0.841±0.009	1.685±0.009	1.821±0.017	
AGG FILM WITH $AgNo_3$	0.341 0.339 0.348	0.579 0.583 0.591	1.412 1.409 1.423	1.625 1.602 1.597	18.49
MEAN±S.D	0.343±0.006	0.584±0.006	1.415±0.007	1.608±0.014	
AGG FILM WITH AMP	0.320 0.319 0.304	0.409 0.402 0.407	0.483 0.491 0.499	0.602 0.610 0.613	69.18
MEAN±S.D	0.314±0.008	0.406±0.003	0.491±0.008	0.608±0.005	
CONTROL BROTH	0.370 0.384 0.393	0.870 0.845 0.890	1.764 1.760 1.743	1.984 1.964 1.972	-
MEAN±S.D	0.382±0.01	0.868±0.01	1.755±0.01	1.973±0.01	

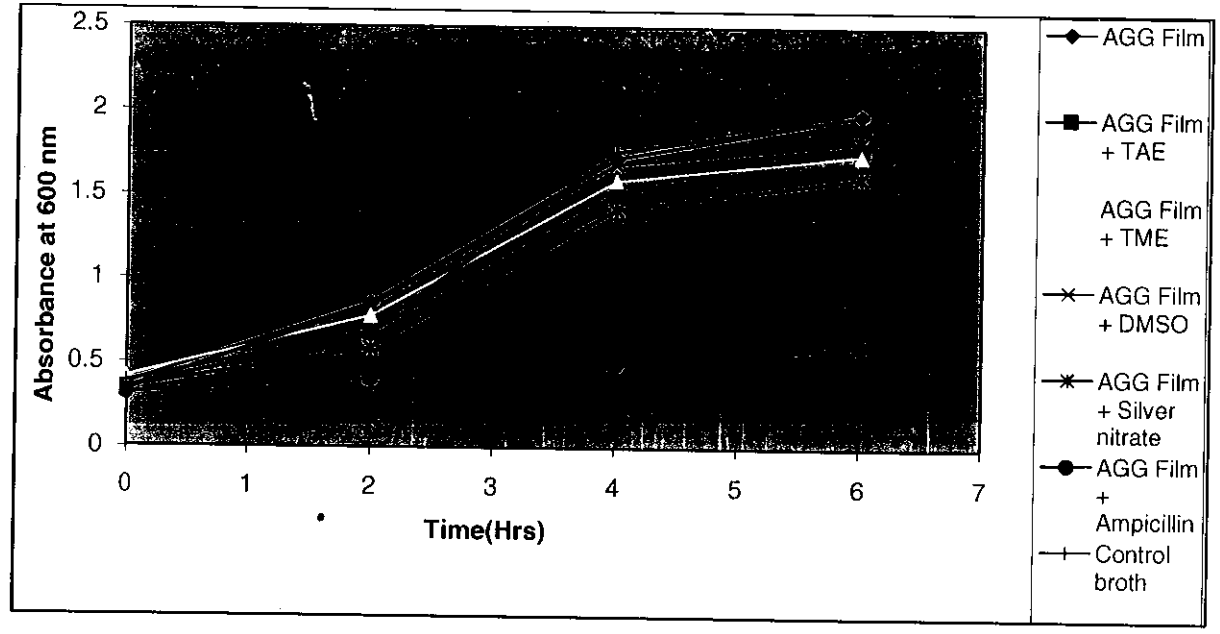

Figure 4.21: Liquid Culture Test of AGG Films against *Klebsiella sp.*

Table 4.26: Liquid Culture Test of AGGP Films against *Bacillus subtilis*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGGP FILM	0.169 0.171 0.179	0.411 0.459 0.436	1.238 1.271 1.203	1.514 1.483 1.490	-
MEAN±S.D	0.173±0.005	0.435±0.02	1.239±0.045	1.496±0.016	
AGGP FILM-WITH TAE	0.173 0.181 0.158	0.287 0.235 0.254	0.789 0.793 0.805	1.137 1.158 1.143	23.65
MEAN±S.D	0.170±0.011	0.259±0.026	0.796±0.008	1.146±0.01	
AGGP FILM-WITH TME	0.155 0.159 0.161	0.231 0.229 0.238	0.765 0.798 0.753	1.126 1.121 1.114	25.38
MEAN±S.D	0.158±0.003	0.233±0.004	0.772±0.023	1.120±0.06	
AGGP FILM WITH DMSO	0.178 0.183 0.185	0.368 0.352 0.347	1.143 1.154 1.148	1.398 1.427 1.419	5.8
MEAN±S.D	0.182±0.003	0.357±0.011	1.148±0.005	1.414±0.015	
AGGP FILM WITH $AgNo_3$	0.145 0.153 0.162	0.212 0.208 0.207	0.715 0.705 0.713	1.089 1.096 1.083	27.44
MEAN±S.D	0.153±0.008	0.209±0.002	0.711±0.005	1.089±0.006	
AGGP FILM WITH AMP	0.125 0.138 0.131	0.198 0.176 0.171	0.376 0.363 0.372	0.484 0.491 0.493	67.42
MEAN±S.D	0.131±0.006	0.182±0.01	0.370±0.006	0.489±0.004	
CONTROL BROTH	0.184 0.173 0.193	0.424 0.448 0.460	1.284 1.268 1.213	1.504 1.480 1.520	-
MEAN±S.D	0.183±0.01	0.440±0.01	1.255±0.03	1.501±0.02	

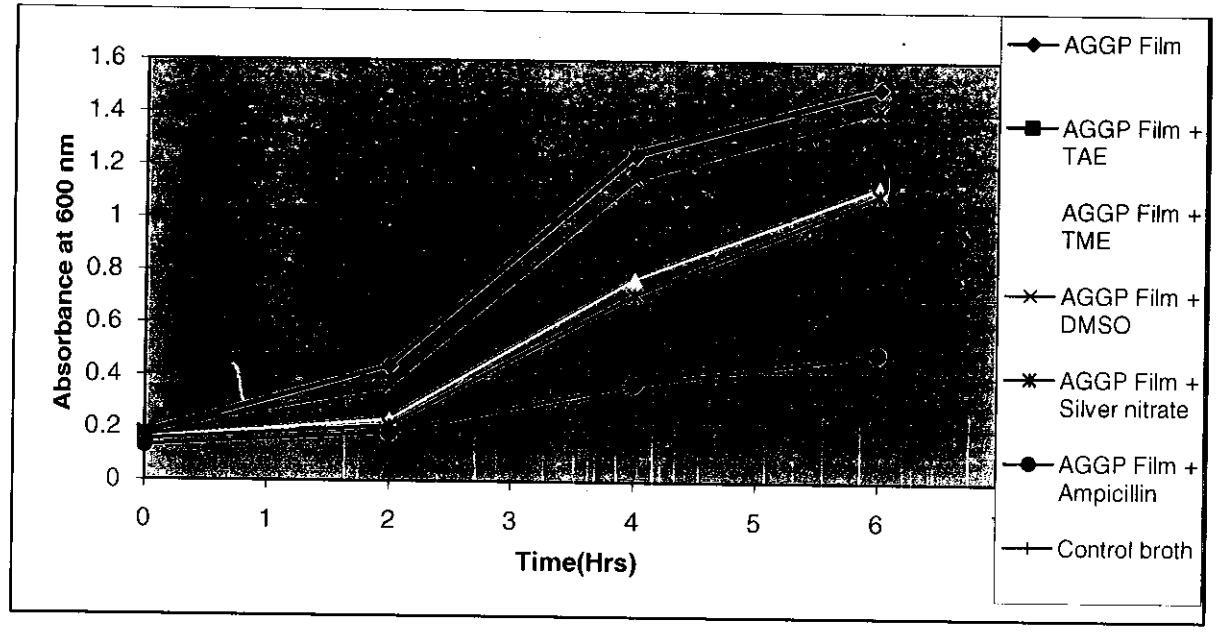

Figure 4.22: Liquid Culture Test of AGGP Films against *Bacillus subtilis*

Table 4.27: Liquid Culture Test of AGGP Films against *Escherichia coli*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGGP FILM	0.274 0.268 0.273	0.684 0.695 0.695	1.271 1.278 1.233	1.667 1.632 1.621	-
MEAN±S.D	0.271±0.003	0.684±0.011	1.261±0.017	1.641±0.017	
AGGP FILM-WITH TAE	0.253 0.249 0.244	0.473 0.489 0.484	0.831 0.847 0.826	1.184 1.192 1.186	28.75
MEAN±S.D	0.248±0.004	0.482±0.008	0.835±0.01	1.187±0.004	
AGGP FILM-WITH TME	0.248 0.256 0.253	0.479 0.503 0.491	0.874 0.912 0.868	1.213 1.278 1.209	25.99
MEAN±S.D	0.252±0.04	0.491±0.012	0.884±0.024	1.233±0.039	
AGGP FILM WITH DMSO	0.258 0.270 0.243	0.622 0.634 0.639	1.167 1.193 1.184	1.587 1.604 1.543	5.28
MEAN±S.D	0.257±0.013	0.631±0.008	1.181±0.013	1.578±0.03	
AGGP FILM WITH $AgNo_3$	0.235 0.241 0.237	0.417 0.420 0.419	0.637 0.688 0.651	0.972 1.008 0.963	41.11
MEAN±S.D	0.238±0.003	0.419±0.001	0.659±0.022	0.981±0.028	
AGGP FILM WITH AMP	0.227 0.213 0.228	0.289 0.290 0.301	0.357 0.352 0.381	0.479 0.453 0.448	72.38
MEAN±S.D	0.223±0.008	0.293±0.066	0.362±0.013	0.460±0.016	
CONTROL BROTH	0.284 0.290 0.263	0.693 0.701 0.710	1.284 1.224 1.293	1.682 1.654 1.663	-
MEAN±S.D	0.279±0.01	0.701±0.008	1.267±0.03	1.666±0.01	

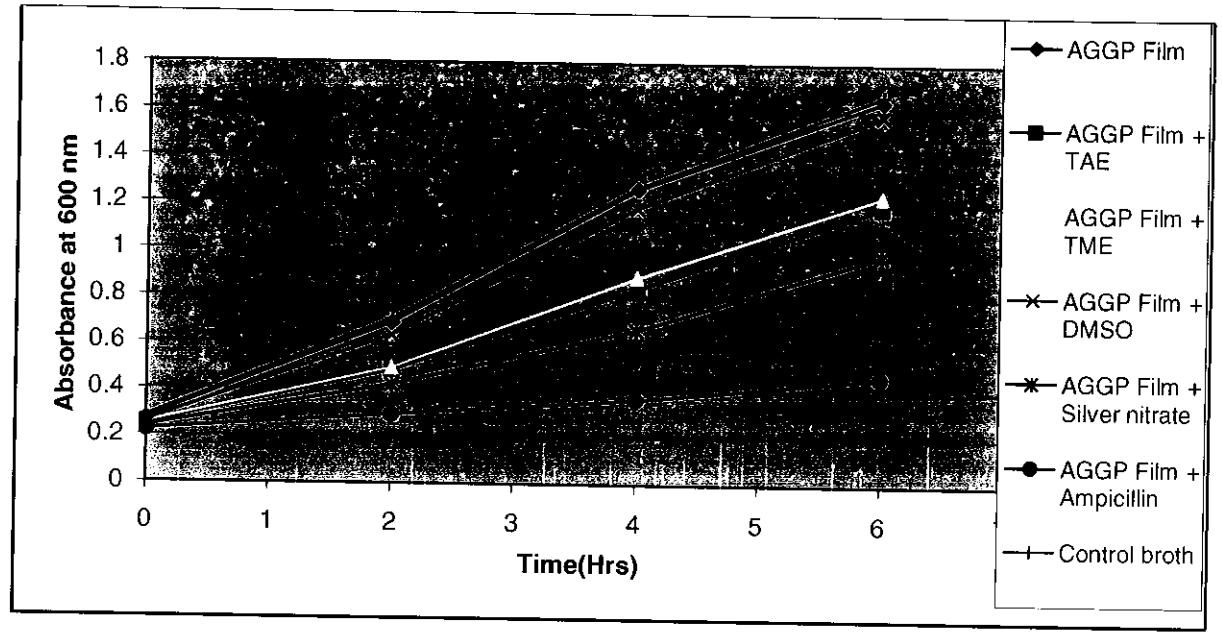

Figure 4.23: Liquid Culture Test of AGGP Films against *Escherichia coli*

Table 4.28: Liquid Culture Test of AGGP Films against *Proteus vulgaris*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGGP FILM	0.161 0.141 0.163	0.851 0.883 0.839	1.450 1.473 1.468	1.703 1.684 1.686	-
MEAN±S.D	0.155±0.012	0.857±0.022	1.464±0.012	1.691±0.010	
AGGP FILM-WITH TAE	0.088 0.079 0.083	0.502 0.531 0.529	1.122 1.026 1.081	1.145 1.130 1.126	33.78
MEAN±S.D	0.083±0.004	0.520±0.016	1.076±0.048	1.133±0.01	
AGGP FILM-WITH TME	0.089 0.084 0.078	0.542 0.539 0.563	1.162 1.062 1.192	1.180 1.165 1.173	31.50
MEAN±S.D	0.084±0.005	0.548±0.013	1.138±0.068	1.172±0.007	
AGGP FILM WITH DMSO	0.093 0.096 0.101	0.780 0.803 0.789	1.266 1.206 1.283	1.349 1.360 1.383	20.28
MEAN±S.D	0.096±0.004	0.790±0.016	1.252±0.04	1.364±0.017	
AGGP FILM WITH $AgNo_3$	0.080 0.074 0.072	0.627 0.637 0.620	1.152 1.086 1.093	1.284 1.281 1.263	25.42
MEAN±S.D	0.075±0.004	0.626±0.008	1.110±0.036	1.276±0.011	
AGGP FILM WITH AMP	0.071 0.097 0.075	0.120 0.178 0.143	0.367 0.352 0.329	0.546 0.531 0.527	68.73
MEAN±S.D	0.081±0.014	0.147±0.029	0.349±0.019	0.535±0.010	
CONTROL BROTH	0.172 0.158 0.184	0.890 0.842 0.856	1.504 1.468 1.490	1.742 1.690 1.701	-
MEAN±S.D	0.171±0.01	0.862±0.02	1.487±0.02	1.711±0.02	

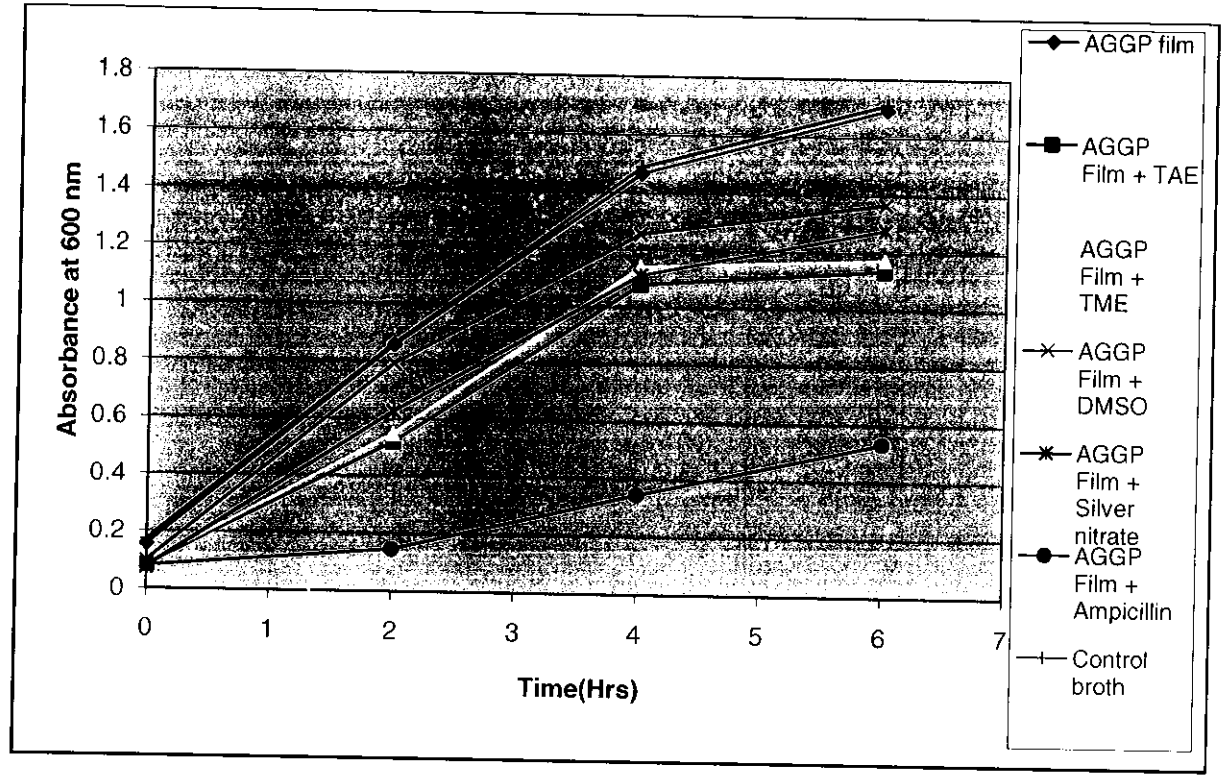

Figure 4.24: Liquid Culture Test of AGGP Films against *Proteus vulgaris*

Table 4.29: Liquid Culture Test of AGGP Films against *Klebseilla*

FILM CODE	0Hrs	2Hrs	4Hrs	6Hrs	% REDUCTION
AGGP FILM	0.347 0.386 0.359	0.748 0.795 0.806	1.575 1.586 1.532	1.807 1.823 1.812	-
MEAN±S.D	0.364±0.019	0.783±0.030	1.564±0.028	1.814±0.008	
AGGP FILM-WITH TAE	0.323 0.339 0.344	0.673 0.649 0.617	1.146 1.129 1.167	1.501 1.492 1.486	18.19
MEAN±S.D	0.335±0.010	0.646±0.028	1.147±0.019	1.493±0.007	
AGGP FILM-WITH TME	0.367 0.355 0.383	0.779 0.703 0.786	1.251 1.247 1.268	1.576 1.578 1.609	12.98
MEAN±S.D	0.368±0.014	0.756±0.004	1.255±0.011	1.588±0.018	
AGGP FILM WITH DMSO	0.358 0.370 0.334	0.712 0.743 0.729	1.320 1.383 1.367	.678 1.694 11.662	8.05
MEAN±S.D	0.354±0.018	0.728±0.015	1.357±0.032	1.678±0.016	
AGGP FILM WITH $AgNo_3$	0.331 0.344 0.337	0.519 0.492 0.503	0.854 0.888 0.862	1.329 1.308 1.343	27.28
MEAN±S.D	0.337±0.006	0.505±0.013	0.868±0.017	1.327±0.017	
AGGP FILM WITH AMP	0.329 0.313 0.328	0.484 0.490 0.501	0.553 0.589 0.581	0.631 0.644 0.647	64.87
MEAN±S.D	0.323±0.008	0.492±0.008	0.574±0.018	0.641±0.008	
CONTROL BROTH	0.384 0.368 0.372	0.809 0.774 0.789	1.574 1.558 1.593	1.843 1.808 1.824	-
MEAN±S.D	0.374±0.008	0.790±0.07	1.575±0.01	1.825±0.01	

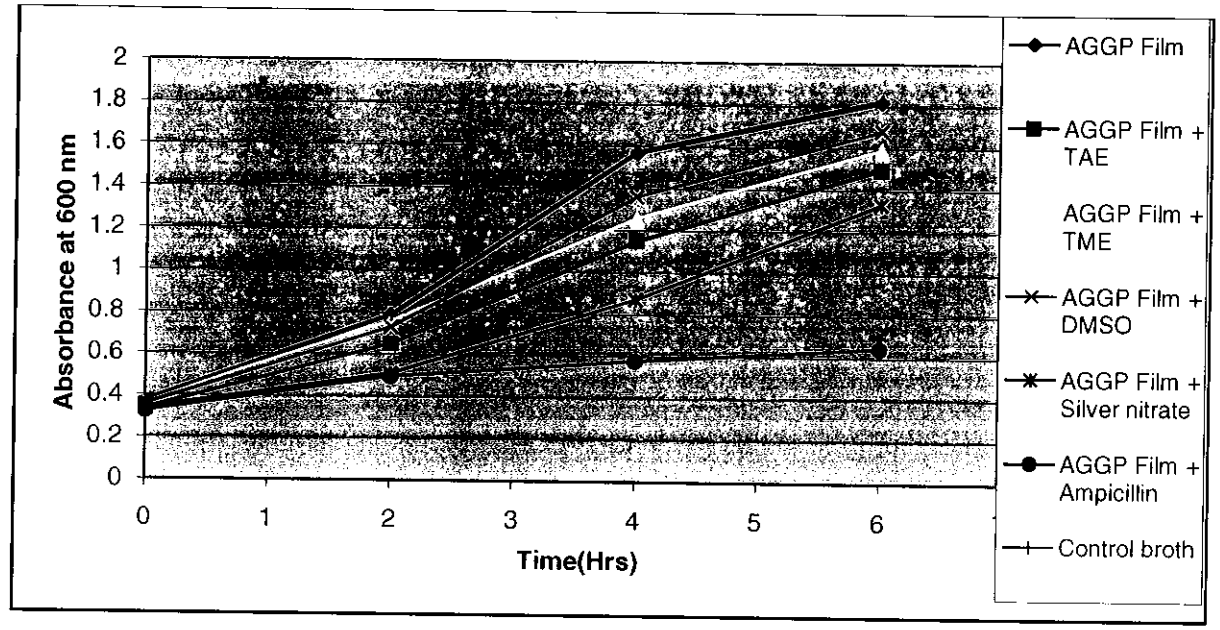

Figure 4.25: Liquid Culture Test of AGGP Films against *Klebseilla sp.*

4.8 Animal study

On obtaining permission from the Institutional Animal Ethics Committee, PSG IMSR, Coimbatore, we purchased twelve male Wistar Albino rats from the Animal House, PSG IMSR. Since the rats weigh around 80 g, and the experiments are to be carried out on Adult rats weighing around 200-300 g, the animals are housed in the animal house until the required growth. Rats are individually housed in polypropylene cages, maintained under standard conditions (12 hr light and 12 hr dark cycle at 25 – 30 ˚C). The animals will be fed with standard rat pellet diet and water. Research will be carried out in accordance with the internationally accepted guidelines for laboratory animal use and care. The experiment report will be approved by Institutional Ethics Committee.

CONCLUSION

CONCLUSION:

- Four different concentrations of agar (0.5%, 1.0%, 1.5%, 2.0%) were tested for film forming capacity. Films with agar concentration of 1.5 % were found to be transparent and could be peeled off from the glass mould compared to other concentrations.

- 14 different combinations of fixed agar concentration (1.5 %) and varying composition and concentration of gelatin, glycerol and propylene glycol were studied for their mechanical properties like thickness, elongation and tensile strength.

- Thickness of the films was measured using thickness tester.

- Agar (1.5 %) + gelatin (1.5%) + glycerol (2 %) (AGG) formulation and Agar (1.5%) + gelatin (1.5%) + glycerol (2%) + propylene glycol (2%) ($AGGP_2$) formulation were selected as they have good elongation of about 16.416% and 13.836% respectively.

- Water absorption capacity of AGG and $AGGP_2$ film were found to be 2.440g/g of the fim and 0.960g/g of the film respectively.

- Water Vapour Permeability for AGG film and $AGGP_2$ film were 2.230g/cm^2 of the film and 2.291g/cm^2 of the film.

- 4% acetone and methanol turmeric extract was added to the selected formulations of agar films.

- On the addition of acetone turmeric extract elongation of the AGG film increased about 75.71% and the addition of methanol extract had no much influence on elongation.

- The addition of acetone turmeric extract to $AGGP_2$ film increased the elongation by 84.75% and the turmeric methanol extract addition increased the elongation by 47.44%.
- The water absorption capacity of the AGG films increased by 26.22% on addition of acetone turmeric extract (TAE) and 25.20% for AGG TME (Tumeric Methanol, Extract) film.
- Water absorption capacity of $AGGP_2$ film increased by 147.8% for TAE and 92.39% for $AGGP_2$ TME film.
- The water vapour permeability of the film decreased on comparison to the control (without any film). The water vapour permeability of the AGG film decreased by 11.12% on addition of TAE and decreased by 2.28% for TME. In the case of $AGGP_2$ film, TAE addition increased the water vapour permeability by 6.15% and decreased the permeability by 17.28%on TME addition.
- Percent release of turmeric compound from film was calculated using Franz Diffusion Cell and found to be 0.614% and 0.561% for AGG and $AGGP_2$ film respectively at 4 hrs.
- Four different microorganisms namely *Bacillus subtilis ,Escherichia coli, Proteus vulgaris,Klebsiella sp.,* were tested for their kill rate. When compared to the control (without film) significant reduction in cell density was noted.
- We have obtained permission from Animal Ethics Committee for animal studies and the primary skin irritation study is still to be carried out.

APPENDICES

Appendix – I

Composition of Luria Bertani broth

Ingredients	**Concentration (g/ lt)**
Casein enzymic hydrolysate	10.00
Yeast extract	5.00
Sodium chloride	5.00

Final pH (at 25° C) 7.0 $\pm$ 0.2

Composition of Nutrient broth Medium

Ingredients	**Concentration (g/ lt)**
Peptone	10.00
Beef extract	10.00
Sodium chloride	5.00

pH after sterilization 7.3 $\pm$ 0.1

Composition of PBS buffer:

8 g of NaCl

0.2 g KCl

1.44 g of Na_2HPO_4

1.24 g of KH_2PO_4

800 ml of H_2O

Adjust the pH to 7.4 and make up the volume to 1L.

APPENDIX – II

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
PSG Institute of Medical Sciences and Research
Avinashi road • Peelamedu • Coimbatore-641004 • India
Phone: 91-422-2570170 • 2598822 • FAX: 91-42225944
Email:psganimalethics@gmial.com
Registration No: 158/1999/CPCSEA

DCRB-PSG/065/2009

12.02.2009 Ref: Proposal-72

Dear Mr. Rohit,

Thank you for presenting your proposal entitled ,

"Development and evaluation of bioactive wound dressing materials"

before the IAEC, for its approval.

The committee suggests the following modifications:

- Inclusion of proof for the active principle being released from the topical formulation to be used, by conducting an in vitro testing.
- Substitution of pentobarbitone by Ketamine as anesthetizing agent for the animals.

The modified proposal with required details can be resubmitted to the Chairman of IAEC for approval

Dr.S.Ramalingam
Chairman
Animal Ethics Committee

To
Dr. N. Saraswathi & Mr Rohit,
Kumaraguru College of Technology
Coimbatore

APPENDIX – III

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
PSG Institute of Medical Sciences and Research
Avinashi Road • Peelamedu • Coimbatore-641004 • India
Phone: 91-422-2570170 2598822 • FAX: 91-42225944
Email:psganimalethics@gmail.com
Registration No: 158/1999/CPCSEA

18th April, 2009

Dear Mr Rohit

Thank you for presenting the study entitled :

"Development and evolution of bioactive wound dressing materials"

The Proposal was approved as such by the committee. We have enclosed Form D which has to be filled up by you. A copy of the completed Form D along with the final report of the study should be submitted to the IAEC on / before 15th April 2010. This is only a provisional approval and the final approval will be made available on receipt of form D and report.

With best wishes for successful completion of the project.

Dr S Ramalingam
Chairman, IAEC

To
Dr N Saraswathi & Mr. Rohit,
Kumaraguru College of Technology
Coimbatore.

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