# Preparation and Characterization of Polyvinyl Alcohol-Gelatin Hydrogel Membranes For Biomedical Applications


**By:**

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**International Islamic University Islamabad**

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Hydrogel

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**2016**


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**FINAL APPROVAL**

**It is certificate that we have read the thesis submitted by Naila Imtiaz and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Biotechnology.**

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A thesis submitted to Department of Biotechnology & Bioinformatics, International Islamic University Islamabad, as a partial fulfilment of requirement for the award of the Degree of MS Biotechnology.

## DECICATION

This thesis is dedicate to my beloved parents and sisters whose hands always rise in prayers for my success. Their moral supports always boost up my will power in every critical situations.

***And***

My Professor Dr Bushra Uzair, thank you so much for your expertise and time which you dedicated to me during experimental work, without your sincere concern it would be impossible. You are one of the best teacher of my carrier.

## DECLARATION

I hereby declare the work present in the following thesis is my own effort, Except where otherwise acknowledged and that the thesis is my own composition. No part of thesis has been previously presented for any other degree.

**Date:**_____

**Naila Imtiaz**

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Last but not the least, I would like to acknowledge my family and friends for their prayers, love and moral support.

**Naila Imtiaz**

## List of Abbreviations

| | |
|---|---|
| PVA | Polyvinyl alcohol |
| PVP | Polyvinylpyrrolidone |
| NP | Nano particals |
| *S. aureus* | *Staphylococcus aureus* |
| *E.coli* | *Escherichia coli* |
| TQ | Thymoquinone |
| MIC | Minimum inhibitory concentration |
| MRSA | Methicillin resistance *Staphylococcus aureus* |
| HCL | Hydrochloric acid |
| SEM | Scanning electron microscope |
| FTIR | Fourier Transformed Infrared |
| XRD | X- Rays Diffraction |
| KBr | Potassium bromide |
| GEL | Gelatin |
| *N.sativa* | *Nigella sativa* |
| HG | Hydrogel |
| HGE | Hydrogel with extract |
| HGAA | Hydrogel with acetic acid |
| VAN | Vancomycin |
| UHG | Uncoated hydrogel |
| ml | Millilitre |
| GEN | Gentamicin |
| GNR | Gram Negative Rods |
| GPC | Gram Positive Cocci |
| mm | Millimeter |
| µg | Microgram |

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# ABSTRACT

The expanding diversity of microbial infection specially those related to chronic skin wound morbidity and biomedical implant rejection has urged to improve the materials that have antimicrobial activity. The emergence of drug resistance is major concern of present time specially Methicillin-resistant Staphylococcus *aureus* (MRSA) has become very common.

The motive of this research work was to plan and create hydrogel membrane by PVA-Gelatin esterification. The membrane was characterized by SEM, FTIR, swelling behaviour, XRD and antimicrobial assay.

SEM of blend showed how different the assembly pattern in each of the hydrogel membrane, voids were not present in membranes treated with acetic acid and extract. FTIR results of blend membrane showed highest peak at 1730 $cm^{-1}$ due to c=c ester bond of very strong intensity. Peak at 1443$cm^{-1}$ due to bending of C-H bond of which is characteristic peak of alkanes and peaks at 3615, 3222$cm^{-1}$ are due to stretching vibration of OH bond. Swelling behaviour of extract and acetic acid added membrane was checked by double distilled water for 8 hrs. These results showed its good water holding capacity. XRD results showed crystallinity was due to gelatine PVA and extract ,acetic acid reduce the crystallinity of membrane. Antimicrobial activity against two skin pathogens (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) was screened. Two strategies were adopted to fortified the HG membrane with antimicrobial agents. One was to impregnate and other was to coat on the surface.

Acetic Acid didn't give inhibition zone when impregnated with HG, while through coating it has given susceptibility zone of 18mm and 16mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. *Nigella sativa* (impregnated) has given zone of 18mm, 20mm for *P.aeruginosa and S.aureus* respectively. When coated it has given susceptibility zone of 18 mm and 16 mm against *S. aureus* and *P. aeruginosa* respectively. The results obtained indicated PVA-Gel blend membrane has a potential to be used as important biomedical applications like skin wound dressings. Although further analysis is required before rule out it as potential dressing.

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# *INTRODUCTION*

## INTRODUCTION:

### 1.1 OVERVIEW

At the point when the skin is breached, microorganisms from the surroundings and skin surface can get entrance to basic tissues where the physical conditions are ideal for colonization and propagation. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are among the most widely recognized pathogens disengaged from both intense and constant injuries of different etiologies. Their pervasiveness has been exhibited in surgical site infection also as in the military setting where they have been credited to bringing about diseases of battle related wounds (Champion *et al.*, 2003; Fadeev *et al.*,2009; Gomez *et al.*,2009; Keen *et al.*, 2010; Co *et al.*, 2011). Different studies have also underscored the nearness of microorganisms and the polymicrobial nature of interminable, non-recuperating wounds (Pfaller *et al.*, 1989; Bowler *et al.*, 2001; Frank *et al.*, 2009; Fazli *et al.*,2009; Gontcharova *et al.*, 2010 ) and the recurrence of *S. aureus* and *P. aeruginosa* has been appeared to be exceedingly high (Gjodsbol *et al.*, 2006; Dowd *et al.*, 2008; Malic *et al.*, 2009; Fazli *et al.*,2009; Martin *et al.*, 2010). This polymicrobial bioburden in wounds exists prevalently as a biofilm impervious to antimicrobial medications (Ebright, 2005; Wolcott & Ehrlich, 2008; James *et al.*, 2008; Black & Costerton, 2010; Nusbaum *et al.*, 2012). *S. aureus,* in its methicillin sensitive and methicillin resistance (MRSA), is a typical artful pathogen, in charge of the greater part of all upper skin contaminations, bringing about expanded dismalness, mortality, and gigantic social insurance costs (Talan *et al.*, 2011). Expanded frequency of MRSA-related diseases in both intense what's more,

constant injuries is very much recorded (Roghmann *et al.*, 2001; Co *et al.*, 2011; O'Hara *et al.*, 2012) and poisons as well as destructiveness components delivered by MRSA contribute in a noteworthy route to the pathogenicity (Schiavo & Van der Goot, 2001).The following image shows the superficial skin abscesses caused by MRSA.

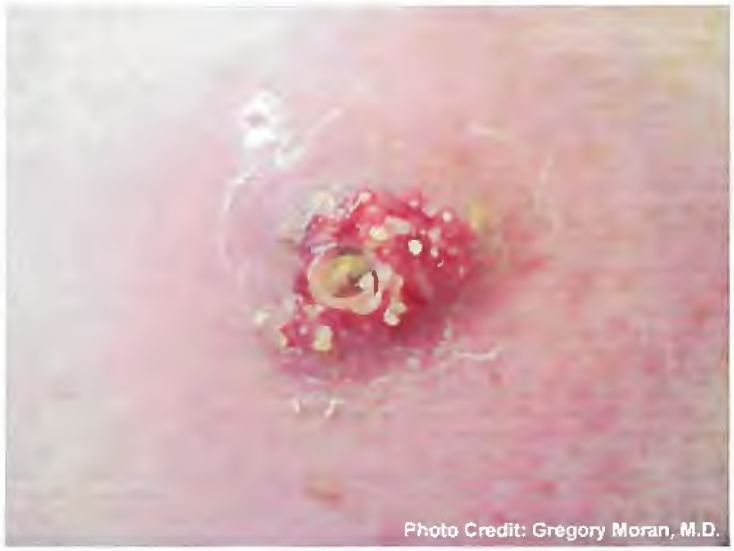



Figure (1.1) Shows the skin abscesses caused by MRSA

The omnipresent of both *S.aureus* and *P. aeruginosa* in intense and incessant injuries ( Gjodsbol *et al.*, 2006). The staggering rate of *staphylococcal* skin and delicate tissue contaminations, as the observing that injuries contaminated with *Pseudomonas* are slower to recover ( Zhao *et al.*, 2010), all evidences lead to think that these two ubiquitous pathogens are likely offenders in bringing on wound contamination furthermore, recovery. Figure (1.2) shows the skin ulcer in diabetic foot caused by *Pseudomonas aeruginosa.* Besides, infection with either *S. aureus* or *P. aeruginosa*

has been observed to cause delay in wound healing in mouse and rabbit wound models (Schierle *et al.*, 2009; Gurjala *et al.*, 2011; Seth *et al.*, 2012).

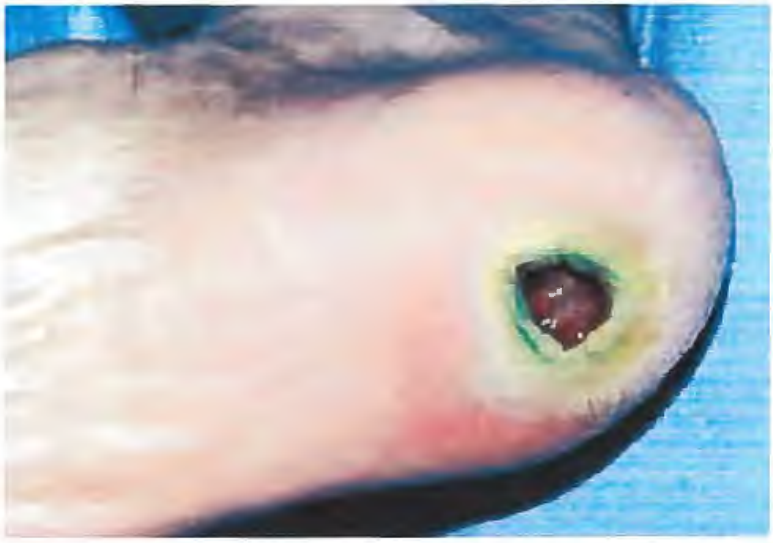


Figure.1.2 Mild diabetic foot infection associated with *Pseudomonas*

The primary negative factor affecting healing of burns and wounds is the loss of skin integrity. It is indicated in the literature that wound and burn healing occur much more quickly with the help of a dressing material (Mutsaers *et al.,* 1997) (Kapoor, 2005). Therefore, in recent years, the use of biopolymers has gained priority in tissue engineering and biotechnology, both as dressing material and in terms of enhancing treatment efficiency (Atiyeh *et al.*, 2005).

Among the wound dressings, special attention has been paid to hydrogels because of their unique interesting properties which can meet the essential requirements of ideal wound dressings including immediate pain control, easy replacement, transparency to allow healing follow up, absorb and prevent loss of body fluids, barrier against hacteria, oxygen permeability, good handling, control of drug dosage and so on (Higa

*et al.,* 1999). The essential negative component influencing mending of blazes and wounds is the loss of skin intactness. It is shown in the previous work that injury and blaze recuperating happen a great deal all the more rapidly with the assistance of a dressing material (Mutsaers et al., 1997) (Kapoor, 2005). Hence, as of late, the utilization of biopolymers has picked up need in tissue building and biotechnology, both as dressing material and as far as upgrading treatment effectiveness (Atiyeh et al., 2005).

Among the injury dressings, uncommon consideration has been paid to hydrogels in view of their novel intriguing properties which can meet the crucial necessities of perfect injury dressings including quick agony control, simple substitution, straightforwardness to permit recuperating postliminary, assimilate and avoid loss of body liquids, obstruction against microorganisms, oxygen porousness, great taking care of, control of medication measurement (Higa et al., 1999).

## 1.2 HYDROGEL:

The term hydrogel portrays three-dimensional system structures acquired from a class of engineered or common polymers which can ingest and hold huge measure of water (Rosiak and Yoshii, 1999). The hydrogel structure is made by the hydrophilic gatherings or areas present in a polymeric system upon the hydration in a watery domain (Gulrez and Al-Assaf, 2011). Hydrogels were initially reported by

Wichterle and Lím in 1960s and their natural applications were progressed.( Wichterle & Lím,1960).

## 1.3 PROPERTIES OF HYDROGEL:

### 1.3.1 HYDROPHILIC:

The water holding capacity and permeability are the most important characteristic features of a hydrogel. The polar hydrophilic groups are the first to be hydrated upon contact with water which leads to the formation of primary bound water. As a result the network swells and exposes the hydrophobic groups which are also capable of interacting with the water molecules. This leads to the formation of hydrophobically-bound water, also called 'secondary bound water'. Primary and secondary bound water are often combined and called 'total bound water'. The network will absorb additional water, due to the osmotic driving force of the network chains towards infinite dilution. This additional swelling is opposed by the covalent or physical cross-links, leading to an elastic network retraction force. Thus, the hydrogel will reach an equilibrium swelling level. The additional absorbed water is called 'free water' or 'bulk water', and assumed to fill the space between the network chains , or the centre of larger pores, macropores, or voids (Hennink & Nostrum, 2002; Hoffman, 2002).

### 1.3.2 BIODEGRADABLE :

Depending on the nature and composition of the hydrogel the next step is the disintegration or dissolution, if the network chain or cross-links are degradable. Biodegradable hydrogels, containing labile bonds, are therefore advantageous in

applications such as tissue engineering, wound healing and drug delivery. These bonds can be present either in the polymer backbone or in the cross-links used to prepare the hydrogel. The labile bonds can be broken under physiological conditions either enzymatically or chemically, in most of the cases by hydrolysis (Hennink & Nostrum, 2002; Hoffman, 2002 ).

### 1.3.3 BIOCOMPATIBILITY :

Biocompatibility is the third most important characteristic property required by the hydrogel. Biocompatibility calls for compatibility of the hydrogel with the immune system and its degradation products formed, which also should not be toxic. Ideally they should be metabolised into harmless products or can be excreted by the renal filtration process. Generally, hydrogels possess a good biocompatibility since their hydrophilic surface has a low interfacial free energy when in contact with body fluids, which results in a low tendency for proteins and cells to adhere to these surfaces. Moreover, the soft and rubbery nature of hydrogels minimises irritation to surrounding tissue (Anderson & Langone, 1999; Smetana, 1993).

### 1.3.4 FLEXIBILITY :

The cross-links between the different polymer chains results in viscoelastic and sometimes pure elastic behaviour and give a gel its structure (hardness), elasticity and contribute to stickiness. Hydrogels, due to their significant water content possess a degree of flexibility similar to natural tissue (Gulrez & Al-Assaf, 2011).

### 1.3.5 DIVERSITY :

It is possible to change the chemistry of the hydrogel by controlling their polarity, surface properties, mechanical properties, and swelling behaviour (Gulrez & Al-Assaf, 2011).

### 1.3.6 STIMULI RESPONSIVE HYDROGELS:

Hydrogels can also be stimuli sensitive and respond to surrounding environment like temperature, pH and presence of electrolyte (Nho *et al.,* 2005). These are similar to conventional hydrogels except these gels may exhibit significant volume changes in response to small changes in pH, temperature, electric field, and light. Temperature sensitive hydrogels are also called as thermogels (Jarry *et al.*, 2002; Schuetz *et al.*, 2008). These stimuli-sensitive hydrogels can display changes in their swelling behaviour of the network structure according to the external environments. They may exhibit positive thermo-sensitivity of swelling, in which polymers with upper critical solution temperature (UCST; temperature at which mixture of two liquids, immiscible at room temperature, ceases to separate into two phases) shrink by cooling below the UCST (Said *et al.,* 2004). Some of the examples of stimuli sensitive hydrogels are poly (vinyl methyl ether) and poly (N-isopropyl acrylamide) gels, kappa-carrageenan-calcium based hydrogels, etc. (Bhardwaj *et al.,* 2005; Sen, 2005). A summary of recent progress in biodegradable temperature sensitive polymers including polyesters, polyphosphazenes, polypeptides, and chitosan, and pH/temperature-sensitive polymers such as sulfamethazine-, poly(b-amino ester)-, poly(amino urethane)-, and

poly(amidoamine)-based polymers is reviewed recently by Nguyen and Lee in 2010 ( Nguyen & Lee, 2010).

## 1.4 USES OF HYDROGELS:

Hydrogel of many synthetic and natural polymers have been produced with their end use mainly in tissue engineering, pharmaceutical, and biomedical fields (Hoare & Kohane, 2008). Due to their high water absorption capacity and biocompatibility they have been used in wound dressing, drug delivery, agriculture, ophthalmic applications, hybrid-type organs (encapsulated living cells) (Benamer *et al.*, 2006; Nho *et al.*, 2005 ; Rosiak *et al.*, 1995; Rosiak & Yoshii, 1999). Wound care, dental Materials, (Al-Assaf *et al.*, 2009), Technical products (cosmetic, pharmaceutical) (Jeremic *et al.*, 1999; Trksak & Ford, 2008; Yoshii & Kume, 2003; Zhao *et al.*, 2003).

## 1.5 GELATIN DEFINITION:

Gelatin or gelatine (from Latin: gelatus meaning "stiff", "frozen") is by-products of collagen obtained from various animals and is a translucent, colourless, brittle (when dry), flavourless foodstuff (Ward, 1977).

### 1.5.1 PRODUCTION OF GELATIN:

Gelatin is produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissues of animals such as domesticated cattle, fish, chicken, pigs, and is a mixture of peptides and proteins. There are many processes whereby

collagen can be converted to gelatin, they all have several factors in common. The intermolecular and intramolecular bonds must be broken which stabilize insoluble collagen, and the hydrogen bonds which stabilize the collagen helix must also be broken (Ward, 1977). The gelatin extraction is done as pretreatment to make the raw materials ready for the main extraction step and to remove impurities which may have negative effects on physiochemical properties of the final gelatin product, the main extraction step, which is usually done with hot water or dilute acid solutions as a multi-stage extraction to hydrolyze collagen into gelatine and recovering treatments including filtration, clarification, evaporation, sterilization, drying, rutting, grinding, and filter to remove the water from the gelatin solution, to blend the gelatin extracted, and to obtain dried, blended and ground final product.

During hydrolysis, the natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Its chemical composition is, in many respects, closely similar to that of its parent collagen. Photographic and pharmaceutical grades of gelatin are generally sourced from beef bones and pig skin (Ward, 1977).

### 1.5.2 PROPERTIES OF GELATIN:

Gelatin promptly breaks down in hot water, and sets to a gel on cooling. Gelatin added specifically to icy water does not mix well. Gelatin is likewise dissolvable in most polar solvents. The solvency of the gelatin is controlled by the strategy for production. Regularly, gelatin can be dissolved in a concentrated acid. Such

scatterings are steady for 10–15 days with almost no synthetic changes and are suitable for covering purposes (Cole, 2000). In spite of the fact that gelatin is 98-99% protein by dry weight, it has less healthful quality than numerous other complete protein sources. Gelatin is surprisingly high in the unnecessary amino acids glycine and proline (i.e., those screated by the human body), while without certain vital amino acids (i.e., those not screated by the human body). It contains no tryptophan and is lacking in isoleucine, threonine, and methionine. The inexact amino corrosive arrangement of gelatin is: glycine 21%, proline 12%, hydroxyproline 12%, glutamic corrosive 10%, alanine 9%, arginine 8%, aspartic corrosive 6%, lysine 4%, serine 4%, leucine 3%, valine 2%, phenylalanine 2%, threonine 2%, isoleucine 1%, hydroxylysine 1%, methionine and histidine <1% and tyrosine <0.5%. These qualities differ, particularly the minor constituents, rely upon the source of the crude material and preparing strategy (Stevens, 1992). Gelatin is additionally a topical haemostatic. A bit of gelatin wipe of proper size is connected on draining injury, squeezed for quite a while and tied in gauze. Haemostatic activity depends on platelets harm at the contact of blood with gelatin, which initiates the coagulation course. Gelatin additionally causes a tamponading impact (blood stream stoppage into a vein by a narrowing of the vessel by an outside power) (Denisenko *et al.*, 2003).

### 1.5.3 USES OF GELATIN:

Gelatin extricated from animal skins and stows away can be utilized for food industry (Choa *et al.*, 2005). In the United States, Latin America, Europe and some Asian nations, pork skin is watered, bubbled, dried and afterward seared to make a nibble nourishment (pork skins) and in U.K they are called "pork scratching". Collagen from skins additionally has a part as an emulsifier in meat items since it can tie vast amounts of fat. This makes it a helpful added substance or filler for meat items. Collagen can likewise be separated from dairy cattle covers up to make the collagen hotdog utilized as a part of the meat business.

Gelatin is added to an extensive variety of nutrients, and additionally shaping a notable fixing in jams and aspic (Jamilah & Harvinder, 2002). Its primary use is the generation of jellied sweets, in view of its "melt in the mouth" properties, but on the other hand is added to a scope of meat items, specifically to meat pies. Gelatin is additionally used for solidified pastries and stabilizer for frozen yogurt. Gelatin is added as a defensive colloid to yogurt, dessert and cream pies. The gelatin is thought to repress the arrangement of ice precious stones and the recrystallization of lactose amid capacity.

Around 6.5% of the aggregate creation of gelatin is utilized as a part of the pharmaceutical business (Hidaka & Liu, 2003). A large portion of it is utilized to make the external covering of cases. Gelatin can likewise be utilized as a coupling and exacerbating specialists in the assembling of cured tablets and pastilles. It is utilized as a vital fixing as a part of defensive balm, for example, zinc gelatin for the treatment of ulcerated varicose veins. Gelatin can be made into a sterile wipe

by whipping it into froth, treating it with formaldehyde and drying it (Estaca *et al.*, 2009). Such wipes are utilized as a part of surgery, furthermore to embed a medication or anti-toxin specifically into a particular region. Since gelatin is a protein, it is utilized as a plasma expander for blood in instances of extremely serious stun and harm. Gelatin is an astounding emulsifier and balancing out specialists for some emulsions and froths. It is utilized as a part of restorative items, and in printing for silk screen printing, photogravure printing and so on (Arvanitoyannis, 2002).

### 1.5.4 BIOMEDICAL USES OF GELATIN:

Yue *et al* prepared blend of gelatin with methacryloyl to form hydogel scaffolds which nearly resemble the extracellular matrix due to the property of metalloproteinase and cell adhesions which enhance cell proliferation within the scaffold ( Yue *et al.*,2015).

Tseng *et al* prepared a porous scaffolds for dermal tissue engineering through freeze drying a mixture of chitosan and gelatin (CG) solution. Their study showed that CG scaffolds crosslinked by glutaraldehyde and 1-(3-dimethylaminopropyl)-3-ethyl-carbodimide hydrochloride (EDC) and seeded with human fibroblasts could function as dermal constructs, while the bioglue coating seeded with keratinocytes could function as an epidermal construct. Such a combination could help regenerate skin with integrated dermal and epidermal layers and a have potential use in tissue-engineered skin ( Tseng *et al* ., 2013).

## 1.6 POLYVINYL ALCOHOL:

Polyvinyl alcohol (PVA), a colourless, water-soluble synthetic resin employed principally in the treating of textiles and paper. PVA is unique among polymers (chemical compounds made up of large, multiple-unit molecules) in that it is not built up in polymerization reactions from single-unit precursor molecules known as monomers. Instead, PVA is made by dissolving another polymer, polyvinyl acetate (PVAc), in an alcohol such as methanol and treating it with an alkaline catalyst such as sodium hydroxide. The resulting hydrolysis, or "alcoholysis," reaction removes the acetate groups from the PVAc molecules without disrupting their long-chain structure. The chemical formula of the resulting vinyl alcohol repeating units is $[CH_2CH(OH)]_n$ ( Manfred, 2000).

### 1.6.1 PROPERTIES OF POLYVINYL ALCOHOL (PVA):

PVA is dissolvable in water and insoluble in all organic solvent. Inadequate evacuation of the acetic acid derivation bunches yields resins less soluble in water and more dissolvable in certain organic solution. PVA is utilized as a part of estimating operators that give more noteworthy quality to material yarns and make paper more impervious to greases and oils . It is additionally utilized as a segment of cements and emulsifiers, as a water-solvent defensive film, and as a beginning material for the readiness of different gums. By response with butyraldehyde (CH3CH2CH2CHO) and formaldehyde (CH2O), PVA can be made into the tars polyvinyl butyral (PVB) and polyvinyl formal (PVF). PVB, an intense, clear,

cement, and water-safe plastic film, is broadly utilized as a part of laminated safty glass, essentially for cars.

Polyvinyl alcohol has fabulous film shaping, emulsifying and glue properties. It is likewise impermeable to oil, grease and solvents. It has high elasticity and adaptability, and additionally high oxygen and fragrance obstruction properties. However these properties are reliant on stickiness, at the end of the day, with higher moister more water is consumed. The water, which goes about as a plasticiser, will then lessen its rigidity, however build its stretching and tear quality (SRI,2008).

### 1.6.2 APPLICATIONS OF POLY VINYL ALCOHOL:

PVA is utilized as thickener, modifier, in polyvinyl acetic acid derivation pastes, material measuring operators, paper coatings, discharge liner, as a water-solvent film helpful for bundling i.e envelope containing clothing cleanser in "liqui-tabs", ladies cleanliness products and grown-up incontinence items as a biodegradable plastic sponsorship sheet, carbon dioxide barrier in polyethylene terephthalate (PET) bottles, as a film utilized as a part of the water exchange printing process, as a structure discharge since materials, for example, epoxy don't stick to it,movie practical effect and youngsters' play putty or ooze when consolidated with borax, utilized as a part of eye drops, (for example, simulated tears to treat dry eyes) and hard contact lens arrangement as an oil, PVA fiber, as fortification in solid, crude material to polyvinyl nitrate (PVN) an ester of nitric acid and polyvinyl alcohol, as

a surfactant for the development of polymer embodied nanobeads, used in defensive compound safe gloves, utilized as a fixative for example accumulation, particularly stool samples, when doped with iodine, PVA can be utilized to energize light, as an embolization specialists in medicinal systems, carotid apparitions for use as manufactured vessels in Doppler stream testing, utilized as a part of 3D printing as bolster structure that can then be disintegrated away.

PVA is broadly utilized as a part of freshwater game angling. Little sacks produced using PVA are loaded with dry or oil based lure and joined to the snare, or the bedeviled snare is set inside the pack and cast into the water. At the point when the sack lands on the lake or waterway base it separates, leaving the snare lure encompassed by ground draw, pellets and so on. This technique pulls in fish to the snare trap (Manfred & Hallensleben, 2000).

### 1.6.3 BIOMEDICAL APPLICATIONS OF POLY VINYL ALCOHOL:

PVA is a standout amongst the most regular and the most established engineered polymer hydrogels that because of its great biocompatibility has been connected in a few progressed biomedical applications e.g. wound dressing (Kenawy *et al.*, 2014), injury administration (Zhao *et al.*, 2003), drug delivery system (Muggli *et al.*, 1998), artificial organs (Yang *et al.*, 2008), and contact lenses (Hyon *et al.*, 1994). Be that as it may, PVA hydrogel has deficient flexible, firm film, and extremely constrained hydrophilicity qualities which limit its utilization alone as an injury dressing polymeric material. Among the different hydrogels depicted in the writing, hydrogels arranged utilizing PVA mixed with polysaccharides and

some other manufactured polymers are appealing due to the plenitude of such polymers, simple for substance derivatization or alteration, and as a rule appropriate biocompatibility (Coviello *et al.*, 2007).

## 1.7 POLVINYL ALCOHOL BLENDS:

Mixed polymers for medicinal applications were beforehand characterized as those focused to interface with natural frameworks to assess, address, and increase the capacity of the body, or supplant any tissues or organs (Lee & Mooney, 2012). At present, biodegradable hydrogel films have been connected seriously in the medicinal business sector, because of their intrinsic biocompatibility (Khor *et al.*, 2011; Lee & Mooney, 2012). In a decades ago, the need of polysaccharides has expanded seriously especially in biomedical applications, since polysaccharides are organic polymers that can be gotten from a few regular sources, for example, microbial sources (e.g. dextran, glucan, and alginate), creature sources (e.g. chitosan and gelatin), and vegetal sources (e.g. starch and cellulose).

PVA has excellent and easy film-forming properties and has been previously blended with synthetic and natural polymers, due to its good water-soluble, biodegradable, noncarcinogenic, and biocompatible characters. Blended materials either natural or synthetic polymers assemble the desirable properties of each material on its own, while blended polymer materials are always mixed with PVA for improving mechanical and physicochemical properties of obtained polymeric materials (Silva *et al.*, 2013).

### 1.7.1. PVA/Sodium alginate (SA):

Alginate is obtained from cocoa green algae, is a characteristic and an anionic direct polysaccharide polymer made out of 1,4-connected b-Dmannuronic acid deposits and 1,4-connected a-L-guluronic acid buildups with changing properties (Ress & Welsh, 1997). The proportion between mannuronic acid and guluronic acid deposits unquestionably changes the versatility of the acquired crosslinked hydrogel (Lee & Mooney, 2012). Alginate polymer has a high hydrophilic, biocompatible and generally practical use, it has been broadly utilized as a part of biomedical applications e.g. wound dressing (Kim *et al.,* 2008), frameworks (Zmora *et al.,* 2002), and dental or surgical impression materials (Nandini *et al.,* 2008). Sodium alginate (SA) is of the most well known regular polymers which has been explored for wound dressing application fusing with PVA polymer as either principle or extra segment to the dressing structure because of its high water swelling capacity which affects the neighborhood wound environment beyond dampness management. In vivo investigates rats displayed obviously new epithelium development with no destructive responses, when the injury is secured with the PVA/SA nanofiber ( Tarun & Gobi, 2012).

### 1.7.2 PVA/Dextran (Dex):

Dextran is a bacterial polysaccharide polymer which comprises of a-1, 6-connected D-glucopyranose deposits with a low rate of a-1,2-, a-1,3-and a-1,4-connected side chains (Sidebotham, 1974). Dextran is obtained from Leuconostoc mesenteroides contains around 5% of a-1, 3-glycopyranosidic linkages, which are generally

maybe a couple glucose deposits long. In the past couple of decades, dextran has been respected a standout amongst the most broadly utilized blood plasma polymer expanders. In view of its great biocompatibility and biodegradability, it is additionally a suitable polymer for arrangement of hydrogels (Kamoun & Menzel, 2010). Gentamicin-stacked PVA/Dex hydrogel movies were produced by Hwang et al, in 2010. The freeze–thawing strategy has been utilized for physically crosslinking of PVA/Dex layers(Hwang *et al.,* 2010).

### 1.7.3 PVA/Starch and Hydroxyethyl starch (HES):

Starch is a standout amongst the most copious and the least expensive polysaccharides. For the most part, starch comprises of around 30% amylose and 70% amylopectin (Zhao *et al.,* 2003). The compound alterations of starch to enhance its properties have pulled in much consideration, not just on the grounds that starch is an extremely shoddy polysaccharide additionally in light of the fact that all starch subsidiaries are biocompatible and biodegradable polymers which make them to be broadly utilized as a part of most pharmaceutical and biomedical applications. In any case, polysaccharide polymers particularly starch has poor hydrophilicity, and it can't frame stable hydrogel alone, in this manner a successful technique has been recommended to shape stable hydrogels made by mixing normal and engineered polymers to meet the benefits of each other (Kaetsu, 1996). Hydroxyethyl starch (HES) is a subsidiary of the characteristic Polymer. HES has important restorative applications as blood plasma expander, Leukapheresis

operators, as cryopreservative (Thomas, 2000), and in blood isotonic electrolyte arrangements, which encourage prove its non poisonous quality, biodegradability, and biocompatibility with the human body (Heins *et al.,* 1998). At present, HES is the most utilized polymer as blood plasma expander (Deitrich, 2001), and hydrogels for medication delivery applications (Kamoun & Menzel, 2012).

### 1.7.4 PVA/Glucan:

(1–3), (1–6)- b-Glucan is a water solvent and biodegradable polymer got from aging of plant brooding (Lee *et al.,* 2003). It indicates antibacterial and antiviral impacts, and is extremely viable as allogeneic, syngeneic, and calming, and displays the injury mending action (Lee *et al.*, 2003). Work done by huang and Brazel 2001 demonstrated that PVA/glucan movies could be utilized as wound dressing that can likewise quicken the injury recuperating as demonstrated (Huang & Brazel, 2001).

### 1.7.5 PVA/Chitosan:

Chitosan, is a copolymer of glucosamine and N-acetyl glucosamine units associated by 1–4 glucosidic bonds , it is surmised by deficient de-acetylation of chitin. Chitosan is a champion amongst the most copious trademark amino polysaccharides. Chitosan has a couple of uses in pharmaceutics, biotechnology, and it is a definitely comprehended material in the damage dressing field (Zhao *et al.,* 2003). It has huge biocompatibility, biodegradability, hemostatic, and splendid antibacterial activity. Chitosan with high nuclear weights is insoluble in water

however can deteriorate in water-acidic destructive course of action (Yang et al., 2008). Yang *et al.* (2004) examined the status of PVA/chitosan hydrogel movies for biomedical applications. This stand-out blend course relied on upon the physical blending between different parts of PVA and water dissolvable chitosan brought after by treatment with formaldehyde to change over –NH2 get-together of chitosan into –N,C cluster in PVA/chitosan layers (Yang *et al.,* 2004). El-Salmawi (2007) displayed the course of action of PVA/chitosan hydrogels using prologue to different estimations of c-radiation influenced crosslinking. Chitosan has been blended to PVA in this study to expect microbiological improvement, for instance, tiny living beings, developments and microorganisms (El-Salmawi, 2007).

### 1.7.6 PVA/Chitosan derivatives:

Among chitosan subsidiaries, N-O-carboxymethyl chitosan (CM-chitosan), (Yang *et al.,* 2008) carboxyethyl chitosan (CE-chitosan), (Xiao & Zhou, 2003) and quaternary chitosan (Q-chitosan) (Tashiro, 2001) have had more intrigue in view of their fantastic antibacterial and antifungal activities. Ignatova *et al.* (2006) proposed photocrosslinked electrospun nano-sinewy PVA/Q-chitosan mats, which displayed high bacterial action against the development of Gram-negative microorganisms E. coli and Gram-positive microscopic organisms Staphylococcus aureus, these nano-fibrous mats demonstrated their high potential for wound dressing applications (Ignatova *et al.*, 2006).

### 1.7.7. PVA/Gelatin (GE):

Gelatin has organic qualities because of its characteristic natural activities, which makes it suitable for use as part of wound dressing materials, drug delivery transporter, and frameworks for tissue engineering (Hago & Li, 2013). Hago and Li (2013) added to another way to deal with get ready interpenetrating polymer system from PVA/GE hydrogels containing trans-glutaminase, the hydrogel segments have been crosslinked by enzymatic and the rehashed freeze–thawing strategy.

### 1.7.8. PVA/Poly(N-vinylpyrrolidone) (PVP):

PVP is a standout amongst the most well known water-solvent, biodegradable, biocompatible, and to a great degree low cytotoxicity manufactured polymers (Razzak *et al.,* 2001). It has been utilized beforehand in light of hydrogels for skin recovery and wound dressing applications by Darwis *et al.* (1993) and Himly *et al.* (1993). They have arranged PVP hydrogel films utilizing radiation crosslinking, PVP hydrogel layers were flexible, fixable, straightforward, and impermeable for microorganisms, the joined cells on the acquired hydrogel layers were suitable just for sound skin yet not for wound dressing or suitable for wound dressing just in a tropical situation (Darwis *et al.,* 1993; Himly *et al.,* 1993).

### 1.7.9 PVA/Polyethylene glycol (PEG):

PEG is a polyether compound, water-dissolvable amphiphilic polymer, clear, terrible, liquid, and thick polymer, in view of its stunning biocompatibility and biodegradability; PEG was used as a piece of mechanical collecting of

pharmaceuticals. Dutta (2012) reported PVA/PEG/CaCl2 hydrogels crosslinked by acquaintance of the hydrogel parts with c-light for wound dressing applications ( Dutta, 2012).

### 1.7.10 WOUND DRESSINGS BASED ON PVA/COMPOSITE POLYMERS OR (BLENDED POLYMERS WITH NANOPARTICLES):

The unbending and delicate nature of the hydrogel polymers might be unfavorable in handling into non-circular polymer frames, for instance layers, movies, or filamentous by means of gel state. Strategies have been recommended to defeat this disadvantage by mixing with high quality good and adaptable composites e.g., certain engineered polymers or nano-fillers (e.g. minerals, muds, or calcium phosphate nanoparticles). Abd El-Mohdy (2013) spoke to Ag nanoparticles bolstered inside of PVA/cellulose acetic acid derivation/gelatin composite hydrogels have been effectively orchestrated utilizing gamma radiation-prompted crosslinking as novel in-situ strategy for wound dressing purposes. The outcomes showed that Ag nanoparticles repressed the crystallization level of PVA-based gel, however Ag nanoparticles in view of PVA/cellulose acetic acid derivation/gelatin hydrogels were found to have antimicrobial action against different organism and microorganisms. In the interim, the antimicrobial movement was fundamentally enhanced by the expanding of AgNO3 nanoparticles content in composite hydrogel. In any case, the perfect hydrogel composite (without Ag-nanoparticles) indicated higher hindrance toward in vitro bacterial grip contrasted with Ag-nanocomposite hydrogel (Abd El-Mohdy,

2013). Correspondingly, PVA/chitosan/Ag nanoparticles stringy mats were readied by electrospinning for wound mending applications (Li *et al.*, 2013).

## 1.8 TECHNIQUES TO PREPARE HYDROGEL:

The different readiness methods received are physical cross-connecting (Hennink & Nostrum, 2002), compound cross-connecting (Barbucci *et al.*, 2004), joining polymerisation (Said *et al.*, 2004), and radiation cross-connecting (Fei *et al.*, 2000; Liu *et al.*, 2002). Such alterations can enhance the mechanical properties and viscoelasticity for applications in biomedical and pharmaceutical fields (Rosiak *et al.*, 1995; Rosiak & Yoshii, 1999; Barbucci *et al.*, 2004; Nho & Lee, 2005). The general strategies to deliver physical and chemical gels are portrayed underneath.

### 1.8.1 PHYSICAL CROSS-LINKING:

There has been an expanded enthusiasm for physical or reversible gels because of relative simplicity of creation and the benefit of not utilizing cross-linking agents. These specialists influence the uprightness of substances to be entangled (e.g. cell, proteins) and also the requirement for their evacuation before application. Watchful determination of hydrocolloid sort, focus and pH can prompt the arrangement of a wide scope of gel compositions and is at present a range getting extensive consideration, especially in the sustenance business. The different strategies reported in previous work to acquire physically cross-connected hydrogels are:

#### 1.8.1.1. HEATING/COOLING A POLYMER SOLUTION:

Physically cross-connected gels are shaped when cooling hot arrangements of gelatine or carrageenan. The gel arrangement is because of helix-development, relationship of the helices, and framing intersection zones (Funami *et al.*, 2007).

**1.8.1.2. IONIC INTERACTION:**

Ionic polymers can be cross-connected by the expansion of di-or tri-valent counterions. This technique underlies the rule of gelling a polyelectrolyte arrangement (e.g. Na+ alginate-) with a multivalent particle of inverse charges (e.g. Ca2+ + 2Cl-). Some different samples are chitosan-polylysine (Bajpai *et al.*, 2008), chitosan-glycerol phosphate salt (Zhao *et al.*, 2009), chitosan-dextran hydrogels (Hennink & Nostrum, 2002).

**1.8.1.3. COMPLEX COACERVATION:**

Complex coacervate gels can be framed by blending of a polyanion with a polycation. The fundamental rule of this technique is that polymers with inverse charges stick together and structure solvent and insoluble complexes relying upon the fixation and pH of the separate arrangements. One such sample is coacervating polyanionic xanthan with polycationic chitosan (Esteban and Severian, 1999; 2000; 2001).

**1.8.1.4 H-BONDING:**

H-fortified hydrogel can be obtained by bringing down the pH of fluid arrangement of polymers conveying carboxyl groups. Samples of such hydrogel is

a hydrogen-bound CMC (carboxymethyl cellulose) system shaped by scattering CMC into 0.1M HCl (Takigami *et al.*, 2007).

#### 1.8.1.5 MATURATION (HEAT INDUCED AGGREGATION):

Conglomeration of the proteinaceous segments, affected by thermal treatment, builds the atomic weight and thusly creates a hydrogel structure with upgraded mechanical properties and water tying capacity (Aoki *et al.*, 2007).

#### 1.8.1.6 FREEZE-THAWING:

Physical cross-connecting of a polymer to shape its hydrogel can likewise be accomplished by utilizing solidify defrost cycles. This system includes the arrangement of microcrystals in the structure because of stop defrosting. Illustrations of this kind of gelation are stop defrosted gels of polyvinyl alcohol and xanthan (Giannouli & Morris, 2003; Hoffman, 2002; 2004).

## 1.9. CHEMICAL CROSS-LINKING:

Chemical cross-connecting secured here includes uniting of monomers on the foundation of the polymers or the utilization of a cross-linkers to connection two polymers chains. The cross-connecting of common and manufactured polymers can be accomplished through the response of their useful functional (for example, OH, COOH, and NH2) with cross-linkers, for example, aldehyde e.g. glutaraldehyde, (Hennink & Nostrum, 2002).

### 1.9.1. CHEMICAL CROSS-LINKERS:

Cross-linkers, for example, glutaraldehyde (2008), epichlorohydrin (2002) have been broadly used to get the cross-connected hydrogel system of different manufactured and regular polymers. The procedure principally includes the presentation of new particles between the polymeric chains to create cross-connected chains. One such case is hydrogel arranged by cross-connecting of corn starch and polyvinyl alcohol utilizing glutaraldehyde as a cross-linker (Pal *et al.*, 2006).

### 1.9.2. GRAFTING:

Grafting includes the polymerisation of a monomer on the foundation of a preformed polymer. The polymer chains are enacted by the activity of chemical reagents, or high vitality radiation treatment.

#### 1.9.2.1. CHEMICAL GRAFTING:

In this sort of grafting, macromolecular spines are initiated by the activity of a concoction reagent i.e. starch united with acrylic acid by utilizing N-vinyl-2-pyrrolidone (Spinelli *et al.,* 2008). Such hydrogels demonstrate a brilliant pH-subordinate swelling conduct and have perfect trademark to be utilized as medication and vitamin delivery gadget in the small digestive tract.

#### 1.9.2.2. RADIATION GRAFTING:

Grafting can also be initiated by the use of high energy radiation such as gamma and electron beam (Said *et al.*,2004).

### 1.10 RADIATION CROSS-LINKING:

Radiation cross-connecting is generally utilized method since it doesn't include the utilization of compound added substances and in this manner holding the biocompatibility of the biopolymer. Likewise, the change and disinfection can be accomplished in single step and thus it is a savvy procedure to adjust biopolymers having their end-utilize particularly in biomedical application (Lugao & Malmonge, 2001).

### 1.10.1 AQUEOUS STATE RADIATION:

Light of polymers in weakened arrangement will prompt concoction changes as an aftereffect of 'circuitous activity' of radiation. The water radiolysis produces responsive free radicals which can cooperate with the polymer solute (Clark, 1963).

### 1.10.2. RADIATION IN PASTE:

The cross-connecting of hydrocolloids in fluid glue like conditions state has gotten significant consideration as of late. Under these conditions the grouping of the polymer is high such that both direct activity of the radiation can shape free radicals furthermore there is additionally adequate water present to be radiolysed to frame •OH and related radicals (Wach *et al.*, 2003; Shen *et al.*, 2006).

### 1.11. BIOMEDICAL IMPORTANCE OF HYDROGEL:

In 1960s, for more than fifty years prior hydrogels have been improved by Wichterle and Lim (1960) and have been connected in various biomedical controls

e.g. contact lenses, absorbable materials (Queiroz *et al.*, 2001). In 1980s, Lim and Sun (1980) have uncovered calcium alginate microcapsules for cell engineering, while Yannas' gathering adjusted engineered hydrogels with some regular polymers e.g. collagen to acquire novel dressing materials, demonstrating ideal conditions for healing blazes and wound dressing (Yannas *et al.*, 1981, 1983; Yannas & Forbes, 1982; Yannas, 1985). Since this date polymer hydrogels proceed to interest researchers.

In this way, much consideration has been given to the utilization or change of various polymeric materials that can be utilized presently for biomedical gadgets to satisfy the over expanded requirement for those materials in medicinal applications. Consistently, a huge number of individuals are presented to blazes by high temp water, flares, mischances, and bubbling oil, and these mishaps result in significant handicaps or even once in a while passing. Particularly in grown-ups, and over matured individuals dermis recovery can't happen suddenly once more (Cigdem & Senel, 2008). Since autologous skin has constrained accessibility and connected with extra scarring, this customary methodology for a considerable loss of dermis can't meet the necessities, and dressing materials got to be inescapable for skin tissue or mending (Cigdem & Senel, 2008). Winter (1962) has declared the original of wound dressing polymeric materials and indicated ideal situations for wound repair ( Winter, 1962).

### 1.12 ANTIMICROBIAL HYDROGELS :

Antimicrobial hydrogels are to a great degree alluring materials for use as wound dressings and fillers. Because of their high water content, gels give a soggy, vigorously hydrated environment to the injury territory, encouraging cell immunological action vital to the injury mending process.

The joining of silver NPs into a given hydrogel permits the arrangement of half and half materials that show antimicrobial properties and are profitable for biomedical applications. Hydrogels of unmistakable structure have been utilized to plan antimicrobial hydrogel-silver NPs frameworks. These incorporate hydrogels got from manufactured polymers, for example, PVA, PVP, and poly(acrylamide-co-acrylic acid), and in addition characteristic polymers, for example, gelatin and alginate ( Yu *et al.*, 2007).

As for union, a few techniques have been utilized to fuse silver NPs into a given hydrogel's system. Thomas *et al.* arranged a poly(acrylamide-co-N-vinyl-2-pyrrolidone) hydrogel stacked with silver NPs using a one of a kind taking in/breathing out (BI-BO) strategy (Thomas *et al.*, 2009) . Resultant gels show antibacterial action against *E. coli* in a way that is reliant on the quantity of BI-BO cycles the gel had been subjected. As the quantity of cycles expand, the movement of the resultant gel increments.

Travan *et al.* portrayed the readiness of silver NPs in the vicinity of a chitosan-inferred arrangement, Chitlac. The Chitlac-NP arrangement was then blended with an alginate arrangement shaping a hydrogel. The creators demonstrated that the

silver NPs were immobilized in the gel and that the gel gave antimicrobial activity against S. *aureus*, S. *epidermidis*, E. *coli*, and P. *aeruginosa*. The antimicrobial activity of the material is probably because of the destablization of the bacterial film when microscopic organisms come into direct contact with the material (Travan., 2009).

Gold NPs are likewise known for their antibacterial properties (Hernandez-Sierra *et al.*, 2008; Cui *et al.*, 2012). Similar to the methodologies brought with silver NPs, antibacterial hydrogels have additionally been created through the joining of gold NPs into their networks. (Marsich *et al.*, 2011). Taking point of preference of the known antimicrobial properties of both silver-and gold-NPs, Reddy *et al.* portrayed a framework where bi-metallic, silver-gold NPs were shaped all through the systems of acrylamide (AM)- 2-acrylamido-2-methyl-1-propanesulfonic acidic (AMPS) hydrogels (Reddy *et al.*, 2012). This bimetallic gel shows higher antibacterial action when contrasted with gels containing either silver or gold NPs alone.

### 1.12.1 HYDROGELS LOADED WITH ANTIBIOTICS:

The hydrophilic ability of numerous gels gives a solubilizing domain to small structure antimicrobials. For instance, ciprofloxacin (Tsou *et al.*, 2005), gentamicin (Li *et al.*, 2011), teicoplanin (Peng *et al.*, 2010) and amoxicillin ( Chang *et al.*, 2010) have each been utilized to plan dynamic gels.

Tsou *et al* in 2005 reported that the small molecule of ciprofloxacin assisted in assembly process, and incorporated in the gel's structure. The same non-covalent collaborations of the molecule into the peptide network allow its eventual controlled discharge. The gel indicated activity against S. *aureus,* E. *coli* and a clinical strain of Klebsiella *pneumoniae.* Vital for its natural application, the hydrogel did not demonstrate critical cytotoxicity towards human red platelets or mouse fibroblast cell cultures. This and other ciprofloxacin-stacked hydrogels have potential use as wound dressings (Tsou *et al.*, 2005).

### 1.12.2 HYDROGELS LOADED WITH ANTIMICROBIALS:

Hydrogels have designed to convey all the more comprehensively acting antimicrobial agent (Halpenny *et al.,* 2009). The utilization of antimicrobial agent, rather than commonly used antibiotics can be favorable concerning the rise of antibiotic resistance(Jiang *et al.,* 2012).

### 1.12.3 NIGELLA SATIVA:

The broad investigates utilizing current logical systems were completed by different analysts on *Nigella sativa* since it is accepted to be a marvelous herb that can cure numerous infirmities. Various pharmacological activities of *Nigella sativa* have been examined in the previous couple of decades i.e, antibacterial activities, antifungal action, hostile to schistosomiasis action, cancer prevention agent action, antidiabetic action, anticancer action, mitigating, pain relieving action, immunomodulatory action, cardiovascular action, gastro-defensive action, hepato-

defensive action, nephroprotective action, aspiratory defensive action and against asthmatic impacts, testicular-defensive action, neuro-pharmacolgical exercises, anticonvulsant action, preventative and against ripeness action, and antioxytocic action (Ahmad *et al.,* 2013).

### 1.12.4 ANTIBACTERIAL ACTIVITY NIGELLA SATIVA:

The antibacterial impact of ground dark seeds have been concentrated on in an adjusted paper plate dissemination technique. A clear resistance against the growth of *Staphylococcus aureus* was seen by concentration of 300 mg/mL with distilled water as negative control, this restraint was affirmed by utilizing the positive control Azithromycin. The resistance got was higher with *Nigella sativa* ground seeds from Hadramout than with *Nigella sativa* ground seeds from Ethiopia. The positive hindrance might be credited to the two essential dynamic elements of *Nigella sativa,* TQ (thymoquinone) and melanin (Bakathir & Abbas, 2011). Diverse crude concentrates of *Nigella sativa* were tried for antimicrobial viability against various bacterial disengages which involved 16 Gram negative and 6 Gram positive pathogens. These confines demonstrated numerous resistances against anti-infection agents, exceptionally the Gram negative ones. Rough concentrates of Nigella *sativa* demonstrated a promising impact against a portion of the test strains. The best concentrates were the unrefined alkaloid and water separates. Gram negative pathogens were influenced more than the gram positive ones (Morsi, 2000). Antibacterial activity of *Nigella sativa* against clinical isolates of

methicillin resistance *Staphylococcus aureus* was researched in 2008 by Hannan *et al.* Every tried strain of methicillin resistance *Staphylococcus aureus* were touchy to ethanolic concentrate of *Nigella sativa* at a centralization of 4 mg/circle with a MIC scope of 0.2-0.5 mg/mL (Hannan *et al.,* 2008). Antibacterial action of *Nigella sativa* against and triple treatment in destruction of *Helicobacter pylori* in patients with non-ulcer dyspepsia was done. It was demonstrated that *Nigella sativa* seeds have clinically helpful hostile to *H. pylori* movement, practically identical to triple treatment (Salem *et al.,* 2010). The antibacterial action of TQ and its biofilm inhibition potencies were explored on 11 human pathogenic microbes. TQ displayed a huge bactericidal movement against different human pathogenic microscopic organisms particularly Gram positive cocci (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* (Chaieb *et al.,* 2011).

### 1.13 ANTIMICROBIAL ACTIVITY OF ACETIC ACID:

Acetic acid has been commonly used in medicine for more than 6000 years for the disinfection of wounds and especially as an antiseptic agent in the treatment and prophylaxis of the plague. Ryssel *et al,* in 2009 checked the antimicrobial activity against the typical bacterial spectrum of a burn unit, including *Escherichia coli*, P. *vulgaris,* P. *aeruginosa,* A. *baumannii, Enterococcus faecalis, Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* (MRSA) and beta-haemolytic *Streptocaccus* group A and B (Ryssel *et al.*, 2009).

**AIMS AND OBJECTIVE:**

1: Preparation and characterization of Hydrogel membranes.

2: Investigation of antimicrobial potential of hydrogel membrane made by mixing crude extract of *Nigella sativa* seeds and Acetic Acid against skin pathogens.

# *MATERIAL AND METHODS*

# CHAPTER 2

# MATERIAL AND METHODS:

## 2. MATERIALS:

PVA (molecular weight 40 g/mol) was obtained from sigma chemicals. Gelatin(for bacteriological purposes) was obtain from Musa & Sons. 37% HCl obtained from sigma chemicals. Double distilled used throughout the work was prepared in laboratory. Acetic acid (10%) was prepared in lab. Extract from *Nigella Sativa* seeds was prepared by grinding in lab and extracted by ethyl acetate in IIUI laboratory.

### 2.1 LIST OF MATERIALS:

| Name | Manufacturer |
|---|---|
| Crystal violet | Sigma-Aldrich,USA |
| Gram Iodine | Sigma-Aldrich,USA |
| Safranin | Sigma-Aldrich,USA |
| Acetone | Sigma-Aldrich,USA |
| Acetic acid | Sigma-Aldrich,USA |
| McConkey Agar | Oxoid Ltd,England |
| Blood Agar | Oxoid Ltd,England |
| Nutrient Agar | Oxoid Ltd,England |
| Hydrogen peroxide | Sigma-Aldrich,USA |
| Vancomycin disc(20mg) | Oxoid Ltd,England |

| | |
|---|---|
| **Gentamycin disc** | **Oxoid Ltd,England** |
| **Ethyl acetate** | **BDH Laboratory Supplies, England** |

## 2.2 LIST OF APPARATUS:

| Name | Manufacturer |
|---|---|
| **Glass petri plates** | **PYREX labware** |
| **Glass slides** | **VWR Scientific** |
| **Glass test tubes** | **PYREX labware** |
| **Drying oven** | **DHG9053A-No.0802449 China** |
| **Hot plate** | |
| **Magnetic stirrer** | |
| **Incubator** | **DHP9052.China** |
| **Water bath** | **Clifton** |
| **Reagents bottles** | **PYREX lab ware** |
| **Light microscope** | **Olympus CK2, Japan** |
| **Safety cabnet level II(NU-540)** | **Lab Grade ES** |
| **SEM** | **Quanta FEG 450** |

| | |
|---|---|
| **FTIR** | **(FT/IR-4100 type A)** |
| **X-RD** | **STOE – Germany, θ-θ** |

## 2.1. PREPARATION OF HYDROGEL MEMBRANE:

This method was derived and modified from Kunal Pal and his co-researchers (Pal *et al.*, 2006).

PVA 10% w/v (100 ml ) were made at 100°C at hot plate while fastly stirring (magnetic stirrer) at 3500 rpm by adding 10g of PVA dry powder in 100ml of double distilled water. A homogeneous transparent solution were obtained after 1 hour. Temperature of solution was maintained constant and checked by hanging thermometer in the container. 0.05ml of HCl (37%) and 2.5g gelatin were added to the PVA solution at 70 °C while constant stirring 200 rpm for 45 minutes to carry out the esterification reaction between PVA and gelatin. Final solution was sonicated to remove air bubbles for 2 hrs and 2ml of glycerol was also added to overcome the brittleness of membrane. Total of 100ml solution were sub divided into three equal parts viz 33.3ml each. In solution (1) no antimicrobial agent was added, in solution (2) 5ml *Nigella Sativa* seeds extract added and in solution (3) 5ml Acetic Acid (10%) was added. All were solutions mixed well and poured in petri dish for air dry at room temperature over night. Next day membranes were peeled off and saved in sterile zip lock packets. As shown in flow chart **(2.1)** and figures **(2.1- 2.3)** (Pal *et al.*, 2006).


**Figure.2.1. Representing the experimental setting for the preparation of hydrogel membrane**

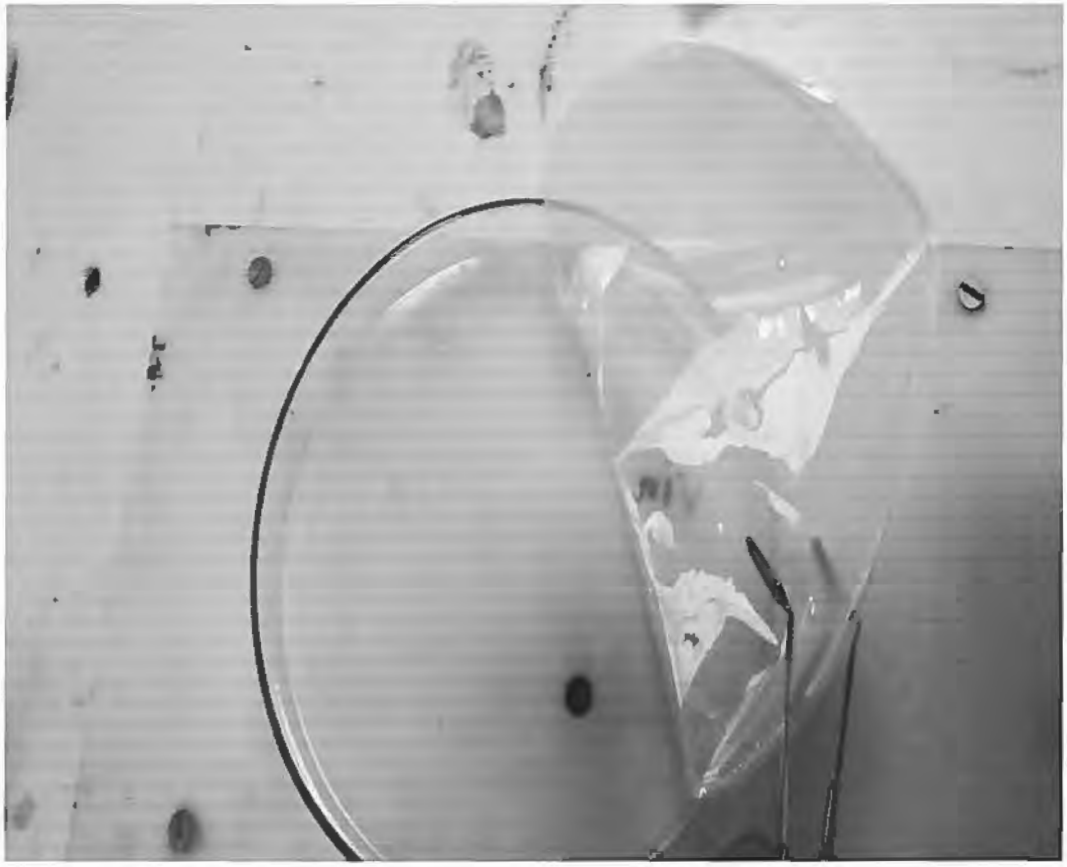


**Figure 2.2. Representing the prepared Hydrogel membrane pealed off from Petri dish**

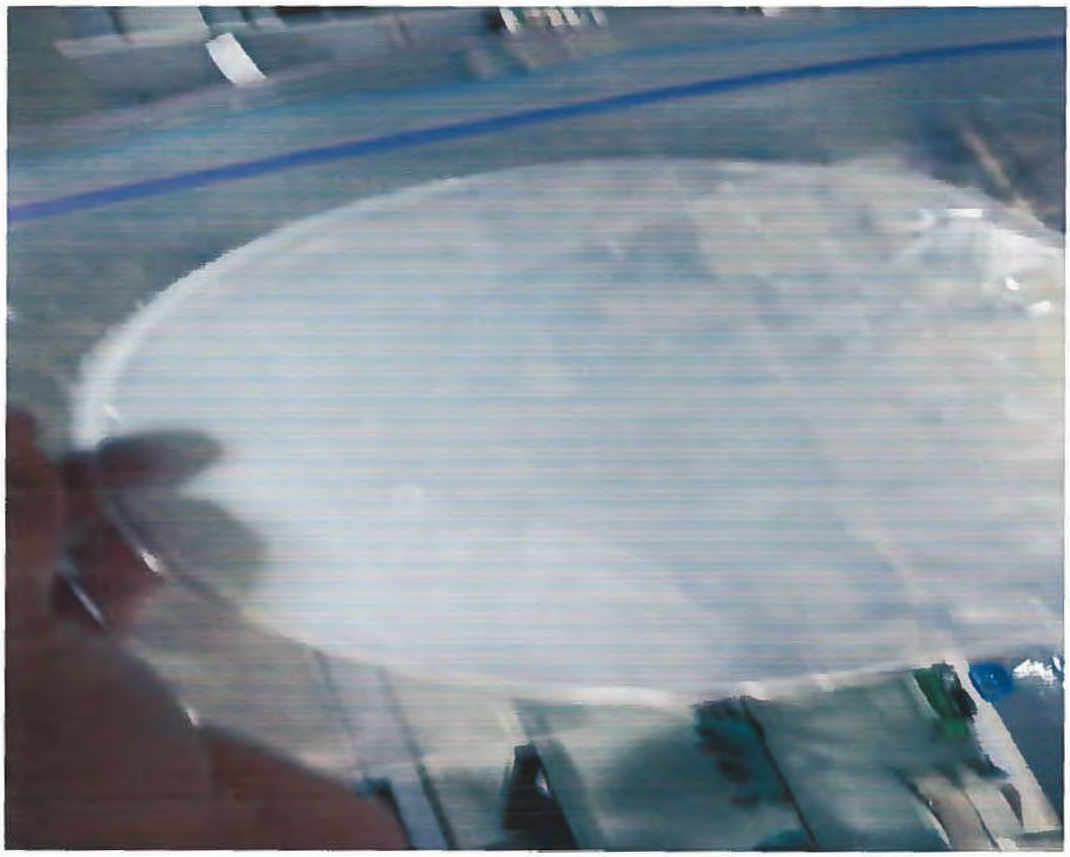
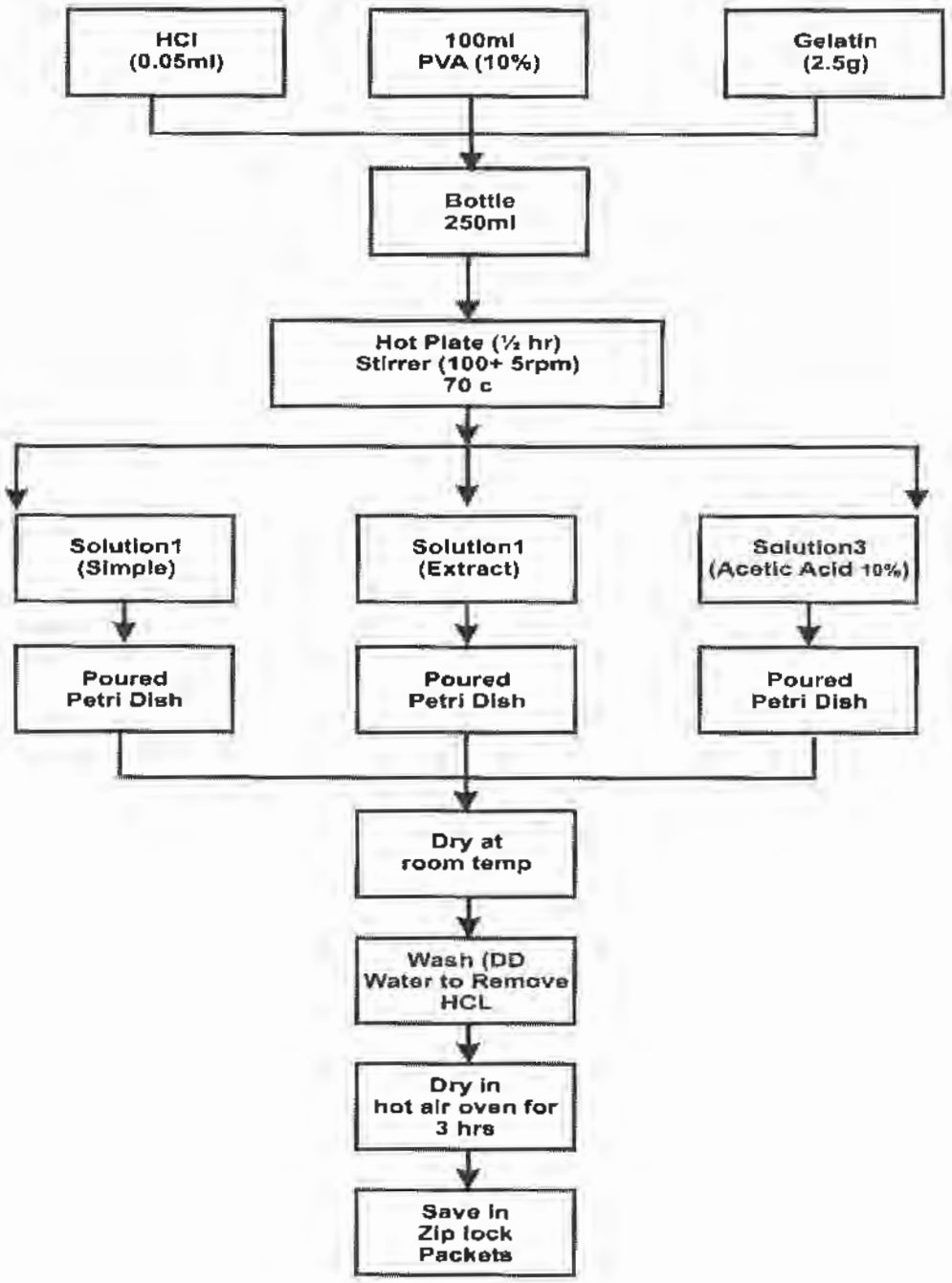


**Figure 2.3. Represents the prepared hydrogel membrane saved in zip lock**

## 2.1 FLOW CHART OF METHODOLOGY FOR PREPARATION OF HYDROGEL MEMBRANE:



## 2. 2. CHARACTERISATION OF HYDROGEL MEMBRANE:

The hydrogel membrane prepared was characterized by the following techniques.

### 2.2.1. SCANNING ELECTRON MICROSCOPY (SEM) OF HYDROGEL MEMBRANE:

For analysis 1/1 cm samples were prepared. It was assured that samples were contamination free.The scanning electron microscopy (SEM) images of the extract and acetic acid impregnated samples were obtained using a "Quanta FEG 450 Scanning Electron Microscope". In these analyses, the samples were coated with gold before SEM measurements. This coating is required to obtain a clear image of an insulating material, but is so thin (200 A°) that it does not hinder the identification of specific minerals (Welton, 2003). At the end of the SEM analysis, SEM micrographs were obtained.

### 2.2.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY OF HYDROGEL MEMBRANE:

Fourier transform infrared spectroscopy (FTIR) was used to characterize the presence of specific chemical groups in Acetic Acid and Extract impregnated hydrogel membranes. PVA hydrogel, PVA crosslinked with gelatin (PVA/Gel) were milled and mixed in a ratio of 1.0% to KBr powder dried for 24 hours at 120 °C. FTIR spectra were obtained in the range of 4000-400 $cm^{-1}$,scanning speed 2mm/sec, with 4$cm^{-1}$ resolution, by using Model (FT/IR-4100 type A). The cross

linking of PVA with gelatin is monitored by FTIR spectroscopy ( Fonseca *et al.*, 2006).

### 2.2.3. SWELLING BEHAVIOUR OF HYDROGEL MEMBRANE:

For swelling studies samples were cut and initial weight was determined (Wi). The samples were then further kept in distilled water at room temperature to study swelling behaviour. The samples were taken out from distilled water at regular intervals of time and weight was determined (Wf). Care was taken to ensure that there was no water present during weighing. The process was continued until saturation of final weight is observed. Swelling percentage was calculated from (Kokabi *et al.*, 2007).

Swelling % = [ Wf-Wi/ Wi ]×10

### 2.2.4. X- RAYS DIFFRACTION OF HYDROGEL MEMBRANE:

The hydrogel membranes with and without antimicrobial were subjected to X-ray diffraction (STOE – Germany, θ-θ) using CuKa radiation generated at 40KV and 40 mA; the range of diffraction angle was 10 - 70° 2□. 0.02° step size of 2□ was maintained at scan speed of 2 s/step (Ricciardi R *et al.*, 2004).

### 2.2.5. SAMPLE COLLECTION AND ISOLATION OF PATHOGENS:

Skin wounds swabs were collected from general OPD patients of Holy Family Hospital Rawalpindi. Samples were immediately processed in the microbiology laboratory of Holy Family Hospital. Swabs were inoculated onto the Blood agar and McConkey agar followed by over night incubation at 37°C.

### 2.2.5.1 IDENTIFICATION OF BACTERIA:

Bacterial specimens were identified by using following identification methods.

I. Gram Staining

II. Biochemical Test

- Catalase test
- Oxidase test

### I. GRAM STAINING

Single colony from fresh bacterial culture was subjected to Gram staining as described earlier by (Garedew *et al.*, 2012). Briefly, bacterial smear was prepared on glass slide. After heat fixing, it was flooded with crystal violet dye for two minutes. Slide was rinsed with tape water. Afterward slide was covered with Gram iodine (mordant) for one minute. Then rinsed with water and decolorized with absolute ethanol for 30 seconds. After this smear was rinsed with water and flooded with Safranin (counter stain) for one minute. At the end rinsed with water, dried and observed under microscope.

### II. BIOCHEMICAL TESTS

- **Catalase Test:**

Catalase test was performed to determine the presence of enzyme catalase in bacteria. Fresh bacterial colonies (18-24hrs old) were placed on glass slides with the help of inoculation loop. Afterwards, a drop of 3% hydrogen peroxide was

introduced on each slide with the help of dropper. Slides were observed for immediate bubble formation(Chester,1979).

- **Oxidase Test:**

An oxidase reagent (tetramethyl-p-phenylenediamine) was mixed with 5ml of distilled water and immediatly poured on to the filter paper placed in a petri dish. The bacterial inoculum was obtained with a cotton-tipped swab instead of a loop or wooden applicator, and the reaction required less than 10 seconds and changed in colour of bacterial colonies was note down (Jeffrey *et al*., 1982).

### 2.2.5.2. EXTRACTION OF BIOACTIVE COMPOUNDS:

2 grams of *Nigella sativa* seeds were crushed to granules using stone rotor and poured into a beaker and 8ml ethyl acetate was added and kept at room temperature for three days, uncovered to evaporate the solvent as maximum as it could and only small amount of concentrated solvent having extract remained in the container. Extract was made by the ratio of 1:4 w/v (Zuo *et al;* 2012). This concentrated extract was stored in sterile container in refrigerator until further use.

### 2.2.6. SCREENING OF ANTIMICROBIAL COMPOUND IMPREGINATED AND COATED HYDROGEL MEMBRANE AGAINST SKIN PATHOGENS

- Samples were collected with cotton swab and carefully transported to microbiology lab in transporting medium (amies). Immediately inoculated on MacConkey, Blood agar (aerobic) and Chocolate medium (anaerobic) and incubated at 37 ❑ C for 24 hours. Biochemical testing was done for further

identification of bacterial species, these includes catalase, oxidase. Two sets of nutrients agar plates were made, one for the impregnated hydrogels and other for coated hydrogels. Identified organisms were inoculated on these plates for susceptibility testing. Wells were made in these plates by plastic tips and in set one 100µl of hydrogels having antimicrobial agents [Extract, Acetic Acid(10%)] impregnated was poured into wells and in set 2 hydrogel membrane pallets coated with antimicrobial agents [extract, acetic acid(10%)] were added (Wallace *et al.*, 1965). Then these plates were placed in incubators for over night incubation at 37 □ C. Next day the zone of inhibitions were observed.

# *RESULTS*

## RESULTS:

### 3.1 SCANNING ELECTRON MICROSCOPY OF HYDROGEL MEMBRANE

For confirming the formation of hydrogel membranes impregnated with antimicrobial compounds i.e Acetic Acid and *Nigella sativa*, scaning electron microscopy of all membranes was done. Although all membranes were looked alike but their microscopic structures were totally different which revealed their uniqueness. The SEM image of simple hydrogel membrane clearly showing the voids as shown in figure **(3.1.1)**. The topographic view of hydrogel membrane with 10%acetic acid showing crystals which revealed its unique structure. Moreover it do not have voids unlike simple membrane as shown in figure **(3.1.2)**. Figure **(3.1.3)** representing the topographic view of hydrogel membrane with *Nigella sativa* seeds extract used as antimicrobial agent. Figure **(3.1.4)** representing the transverse view of hydrogel membrane with *Nigella sativa* seeds extract, which proved the thickness is even with minor differences and are 263.48um, 267.83um and 274.80um at different points, having average thickness of 268.70um. Figure **(3.1.5)** showing the transverse section of membrane with Acetic Acid (10%), with thickness of 137.34um.Where as the thickness of simple hydrogel membrane is 242.11um as shown in figure **(3.1.6)**.

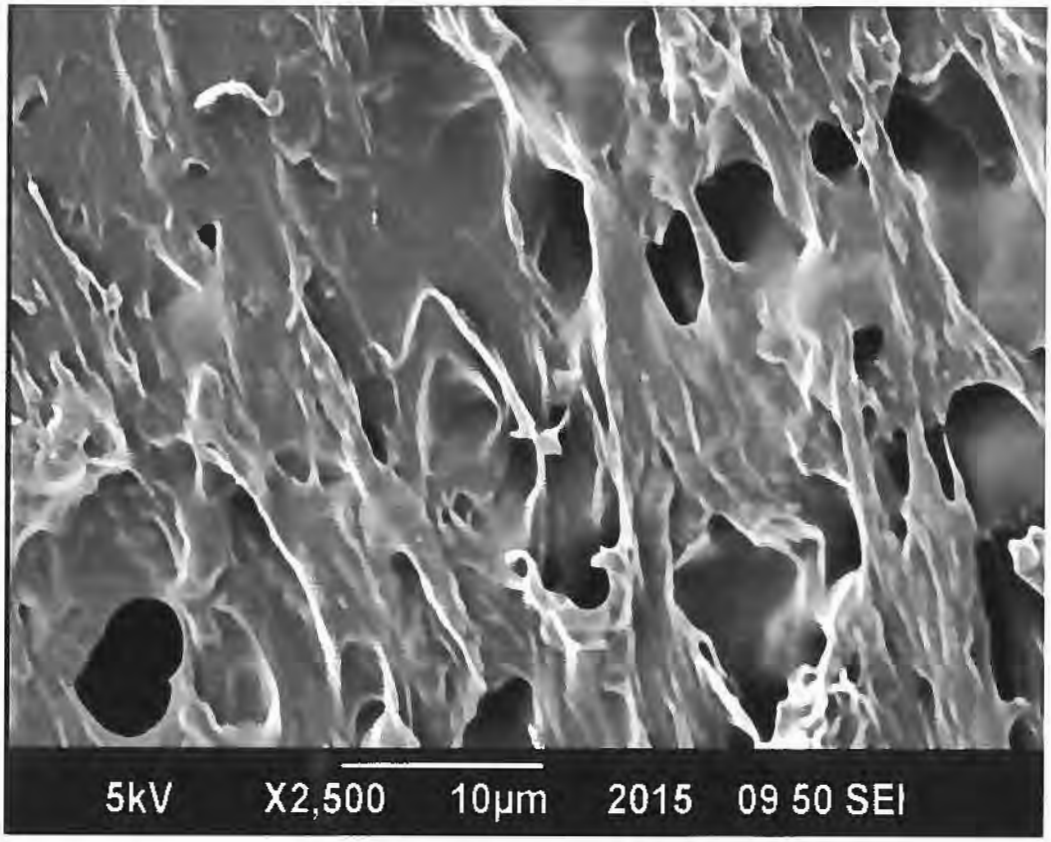



**Figure (3.1.1) SEM image representing the topographic view (surface) of simple HG membrane(without antimicrobial agent )**

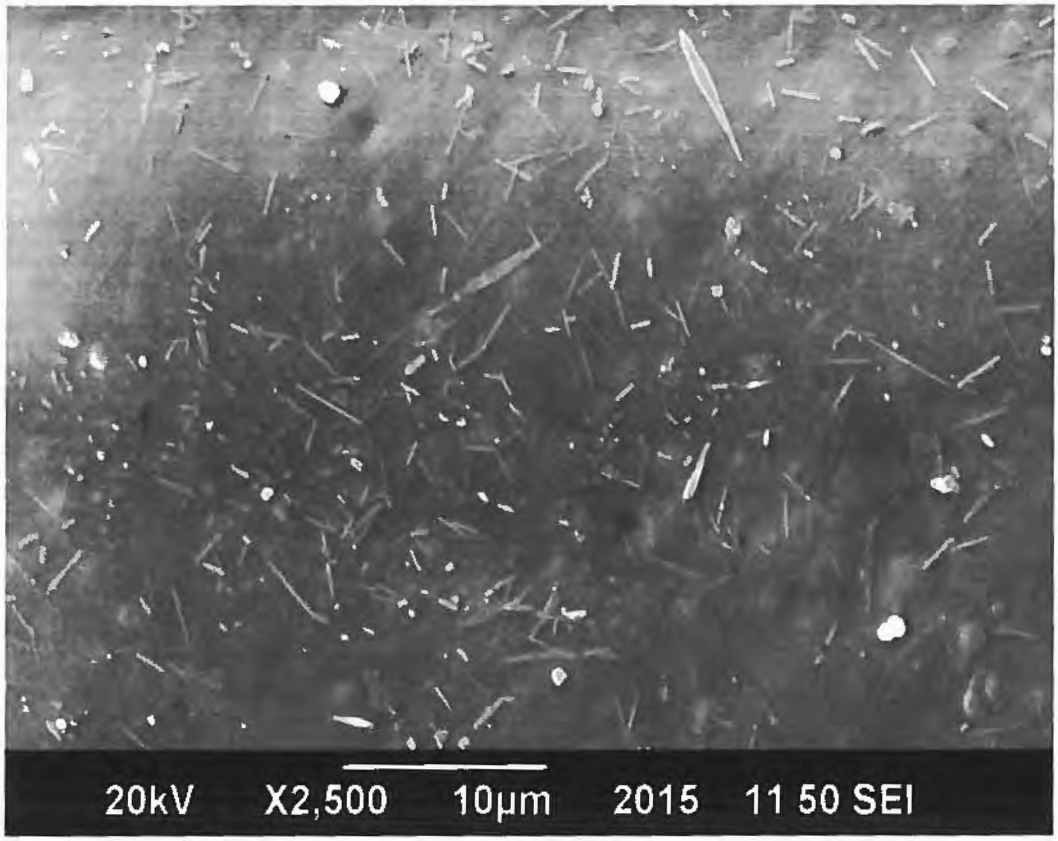



**Figure ( 3.1.2) SEM image representing the topographic view(surface) of HG membrane with Acetic Acid(10%)**

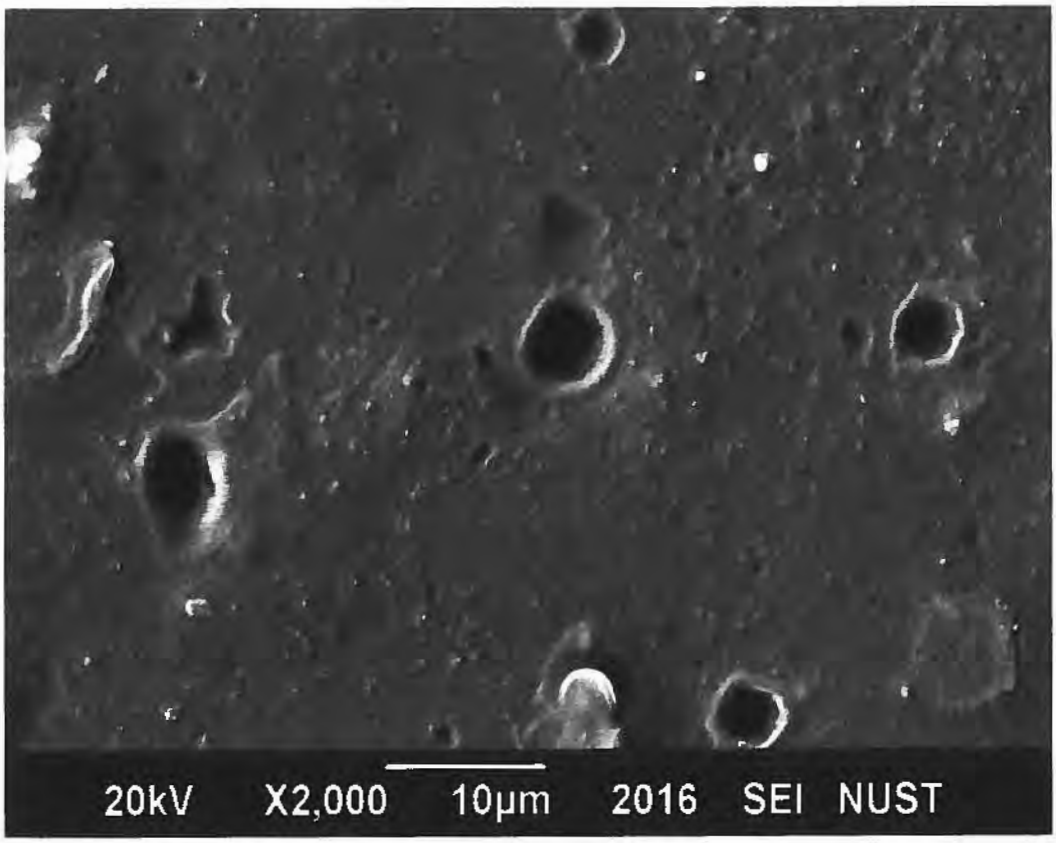



**Figure (3.1.3) SEM image representing the topographical view (surface) of HG membrane with extract (*Nigella Sativa*)**

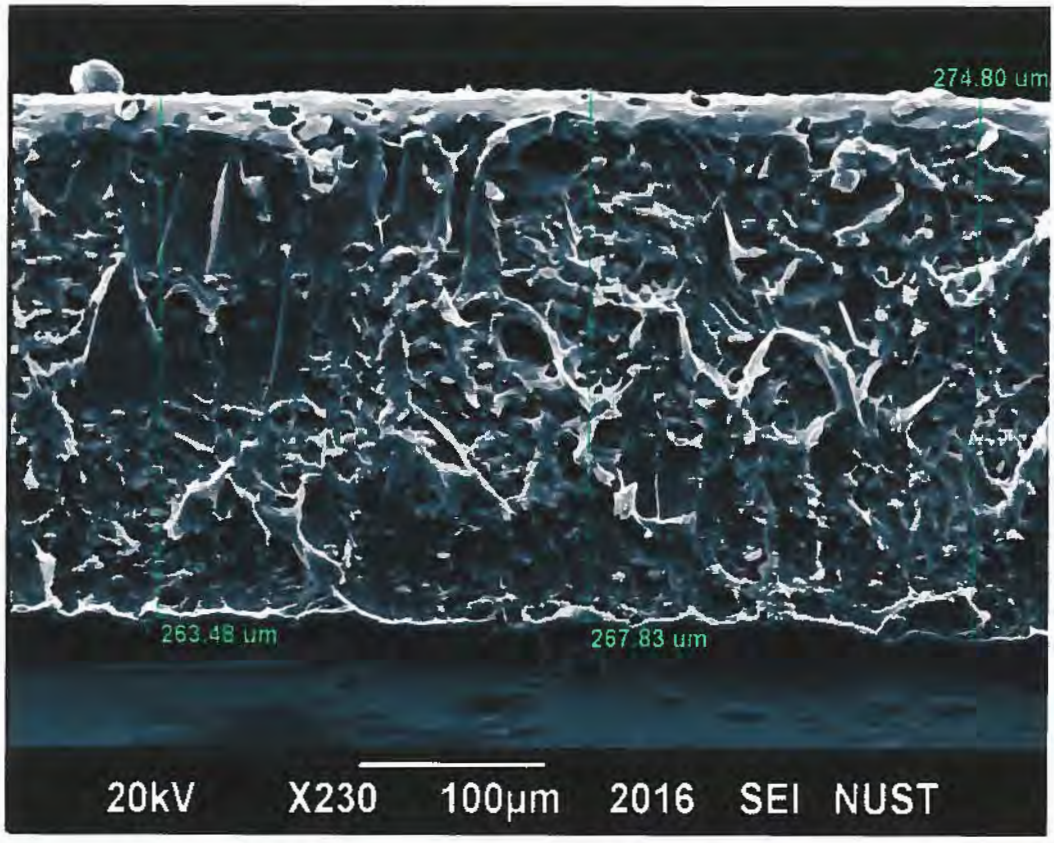
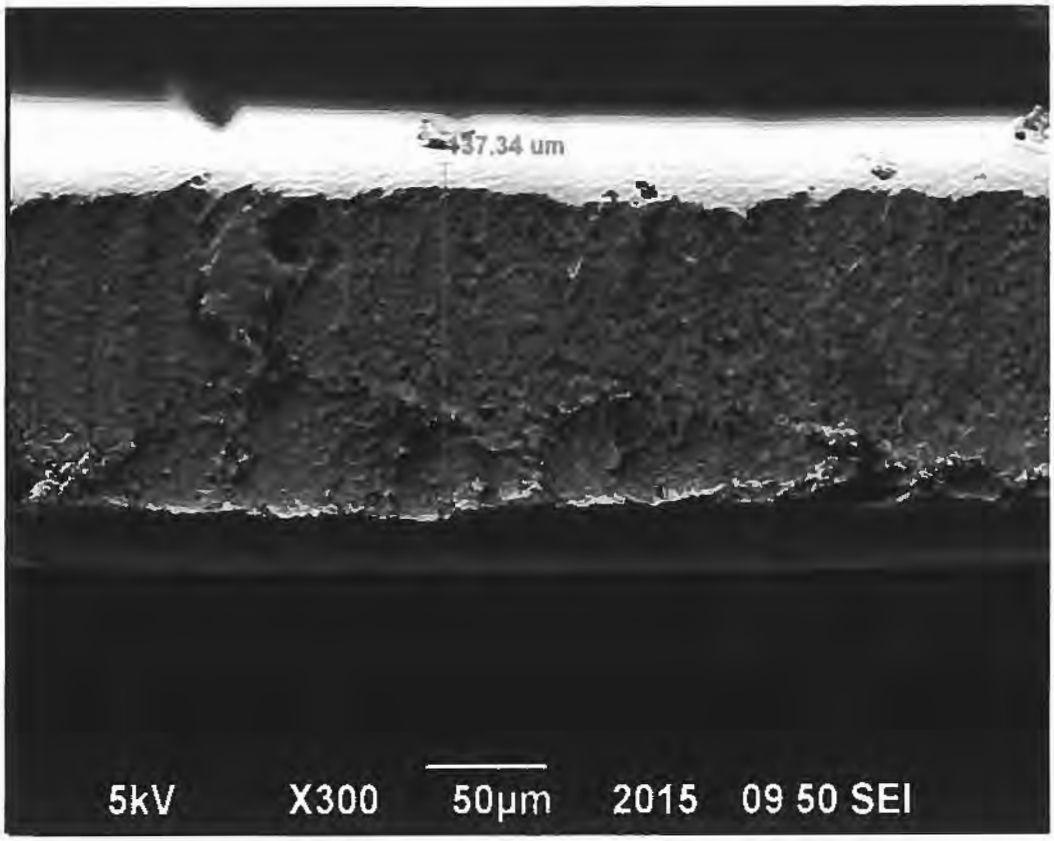



**Figure (3.1.4) SEM image representing the transverse view(thickness) of HG membrane with *Nigella sativa* seeds extracts**




**Figure( 3.1.5). SEM image representing the transverse view(thickness) of HG membrane with Acetic Acid(10%)**

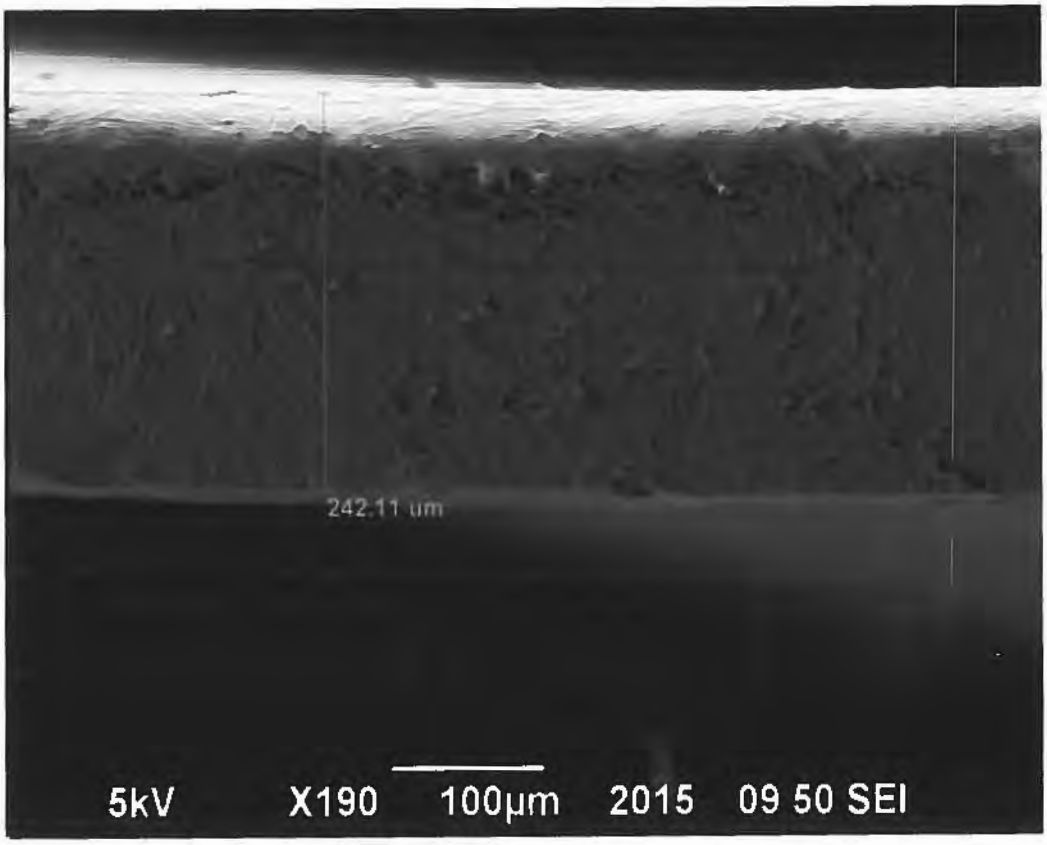



**Figure (3.1.6). SEM image representing the transverse view(thickness) of simple HG membrane(without antimicrobial)**

### 3.2. FOURIOR TRANSFORM INFRARED SPECTROSCOPY (FTIR):

The FTIR spectra of Hydrogel membrane with Acetic Acid has shown strong peaks at 1730cm$^{-1}$ due to C=C ester bond of very strong intensity. Peak at 1443cm$^{-1}$ due to bending vibrations of C—H bond of medium intensity, which are characteristics peaks of the alkanes structure. Peaks at 3615, 3222 cm$^{-1}$ are due to stretching vibration of O—H bonds and H bond repectively. For instance it can be observed intramolecular and intermolecular hydrogen bonding are expected to occur among PVA chains due to high hydrophilic forces (Fonseca *et al.*, 2006). While the spectra of hydrogel membrane with extract(*Nigella sativa*) showed peak at 3291cm$^{-1}$ which is due to asymmetrical stretching of $-NH_3^+$ in amino acids. The peak at 1652cm$^{-1}$ due to two bands stretching C=O and $NH_2$ in primary amides. The peak at 943 cm$^{-1}$ due to $CH_2$ out of plane wagging in $CH=CH_2$ of vinyl compounds. Peaks at 1087 cm$^{-1}$ due to C—O stretching. These results supports that PVA functional groups had crosslinked with the gelatine amine ( Meyers 2000; Mansur *et al.*, 2004 ; Mansur *et al.*, 2002).

As shown in figures **(3.2.1** and **3.2.2).**

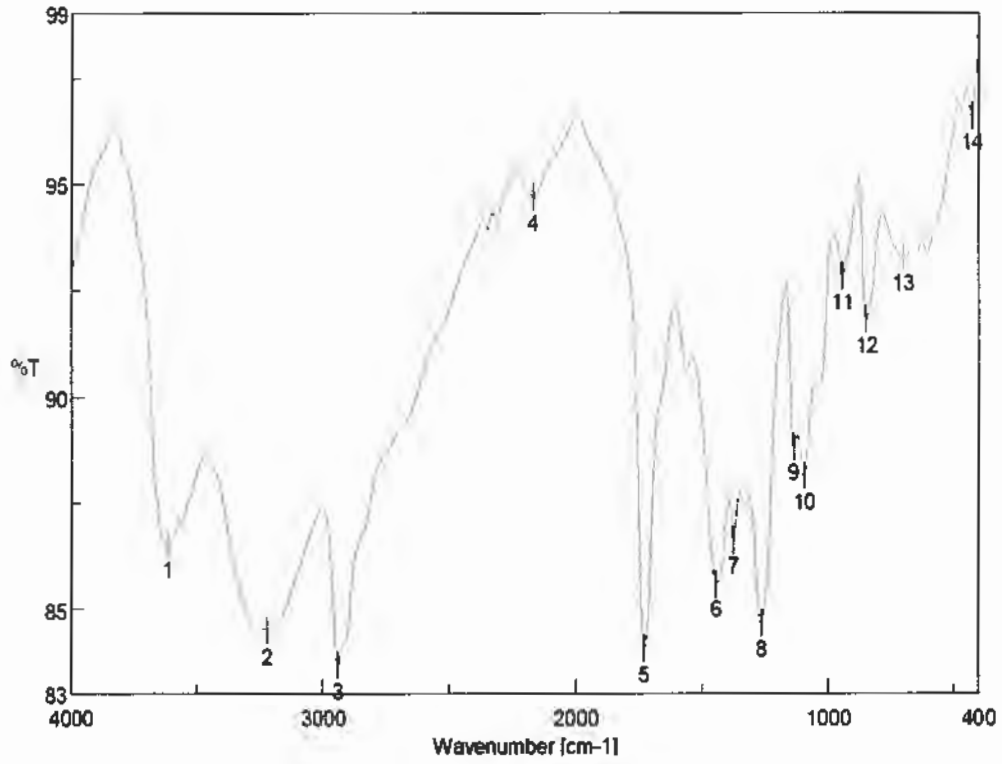



**Figure (3.2.1). Representing the FTIR spectra of hydrogel membrane with Acetic Acid**

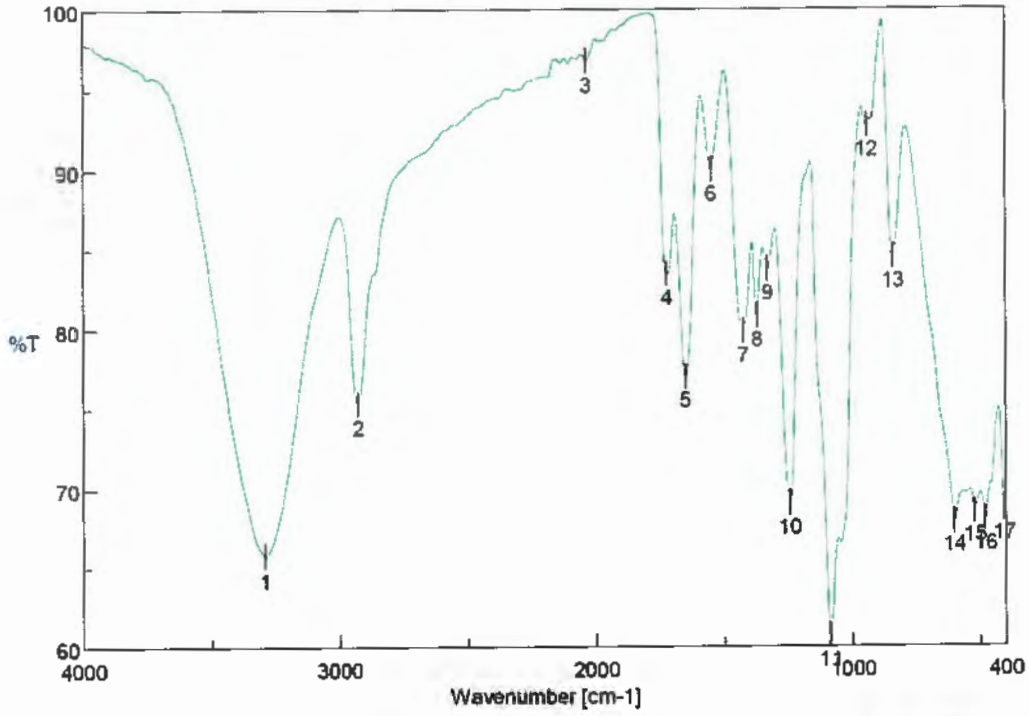



**Figure (3.2.2). Representing the FTIR spectra of hydrogel membrane with Extract( Nigella sativa)**

### 3.3. SWELLING BEHAVIOUR OF HYDROGEL MEMBRANE:

Considering the significance of swelling conduct of mixed hydrogels, swelling rates of two mix hydrogels tests have been evaluated. Double distilled water was used as swelling medium. Sample weights were observed at intervals of 2 hours for 8 hours at room temperature. The influence of acetic acid (10%) on the swelling behaviour of PVA-Gelatin blends and with Extract was evaluated. Swelling percentage curves of membranes are shown in Figure **(3.3.1)**. In case of PVA-Gelatin composites it can be seen there was a steep increase in swelling percentage during the first two hours of the study which further increased gradually and showed saturated state for next six hours. Membrane with extract showed slightly more swelling although not big difference. With addition of acetic acid in blend membrane swelling percentage was found to be decreased as compared to blend with extract. Results have indicated that swelling percentage curves of PVA-gelatin with extract and acetic acid increase quickly with the immersion time. But almost after 2 hours, curves for all the samples got saturated. Figures **(3.3.2, 3.3.3 & 3.3.4)** representing these results. All these results proved that the membrane prepared have a potential to be used as wound dressing.

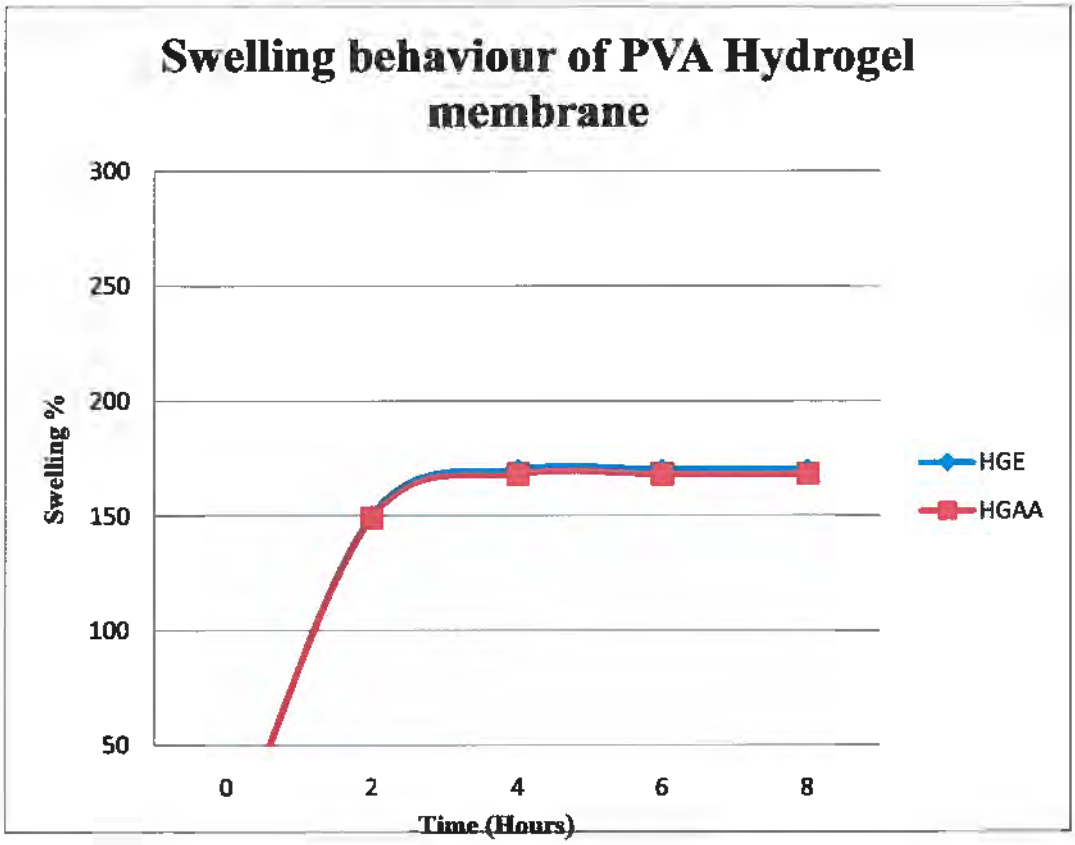



**Figure 3.3.1 Represents the swelling graph of hydrogel membrane with intervals of 2 hrs**

**Key:**

**HGE= Hydrogel with Extract**

**HGAA= Hydrogel with Acetic Acid (10%)**

**Time interval for weighing is 2 hrs**

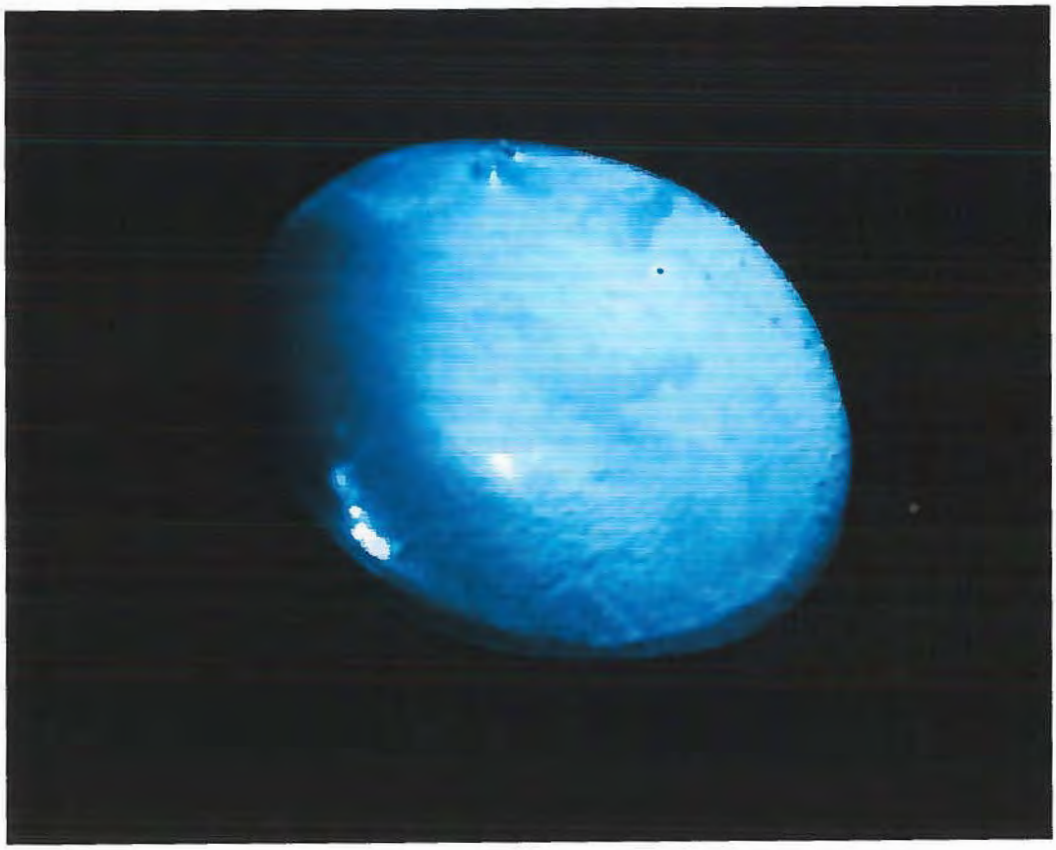


**Figure 3.3.2. Representing the dry form of hydrogel membrane**

**Figure (3.3.3). Representing the wet form of hydrogel membrane with extract**

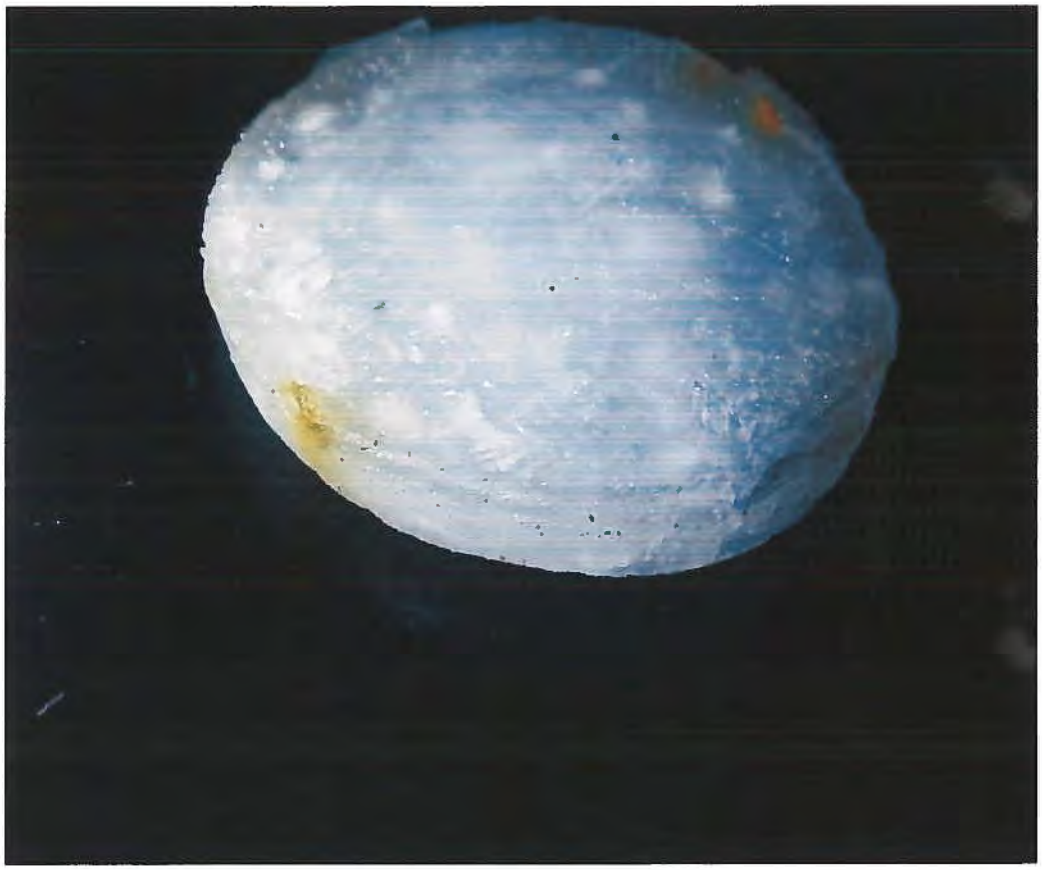


**Figure (3.3.4). Representing the wet form of hydrogel membrane with Acetic acid(10%)**

### 3.4. X-RAY DIFFRACTION 0F HYDROGEL MEMBRANE:

XRD patterns of synthesized hydrogel membranes i.e. can be observed in figures **(3.4.1-3.4.3)**. It can be observed that simple membrane (without antimicrobial) had intense peaks at 20°, 28°, and 44° 2θ. The latter two were associated to presence of gelatin in the hydrogel. Similarly, sharp peak of PVA was present in other two hydrogel membranes at 20°. The intensity of sharp gelatin peaks was decreased in HG membrane with acetic acid. However in HG membrane with extract, all peaks were present, indicated the presence of PVA and gelatin (Ricciardi R *et al.*, 2004).

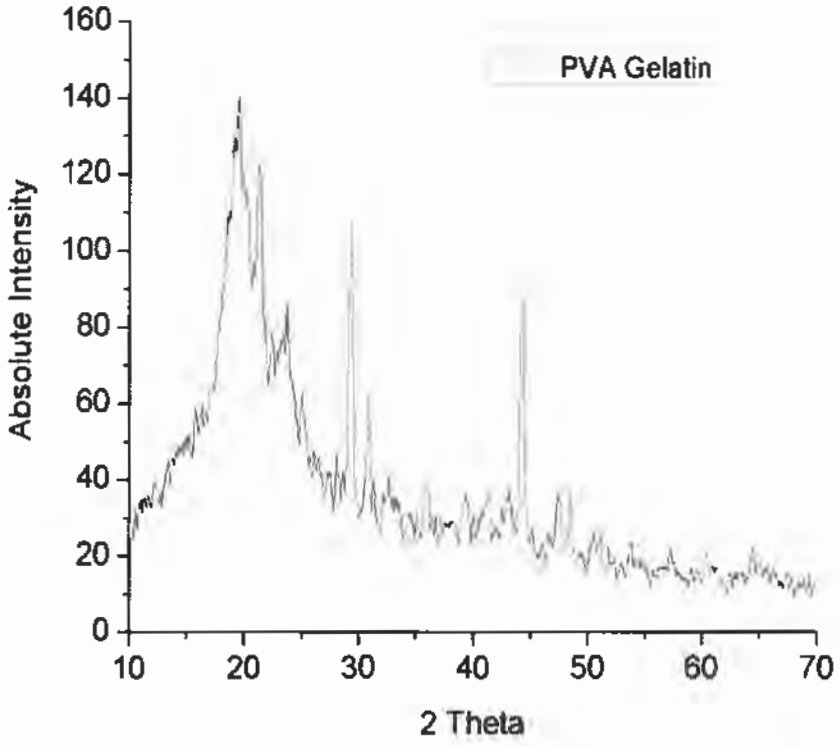



**Fig 3.4.1: Representing the XRD pattern of simple HG membrane (without antimicrobial)**

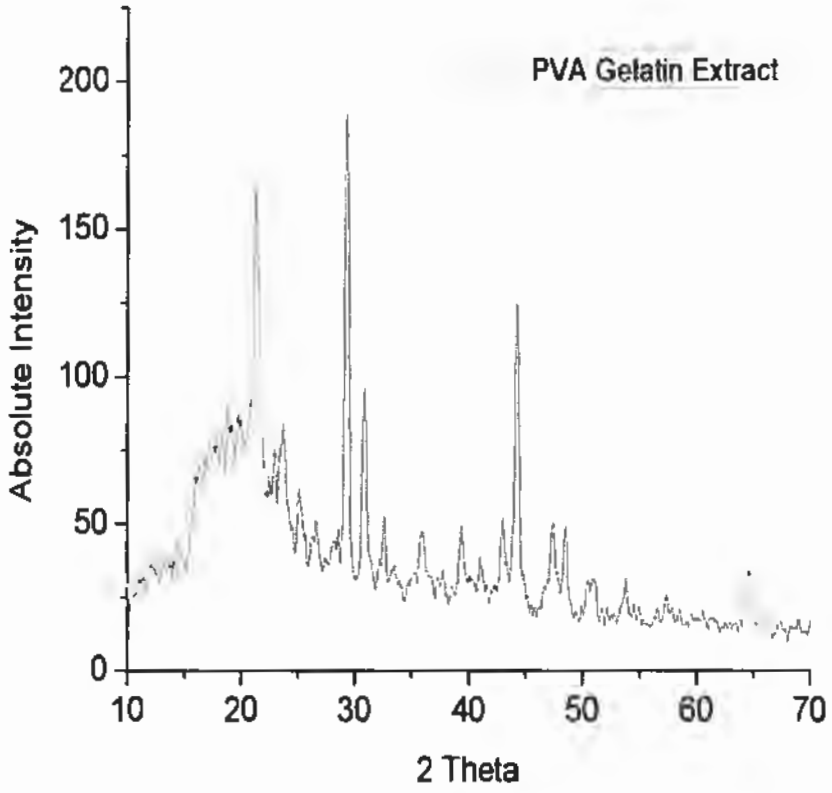



**Fig 3.4.2: Representing the XRD pattern of HG membrane with extract (Nigella *sativa*)**

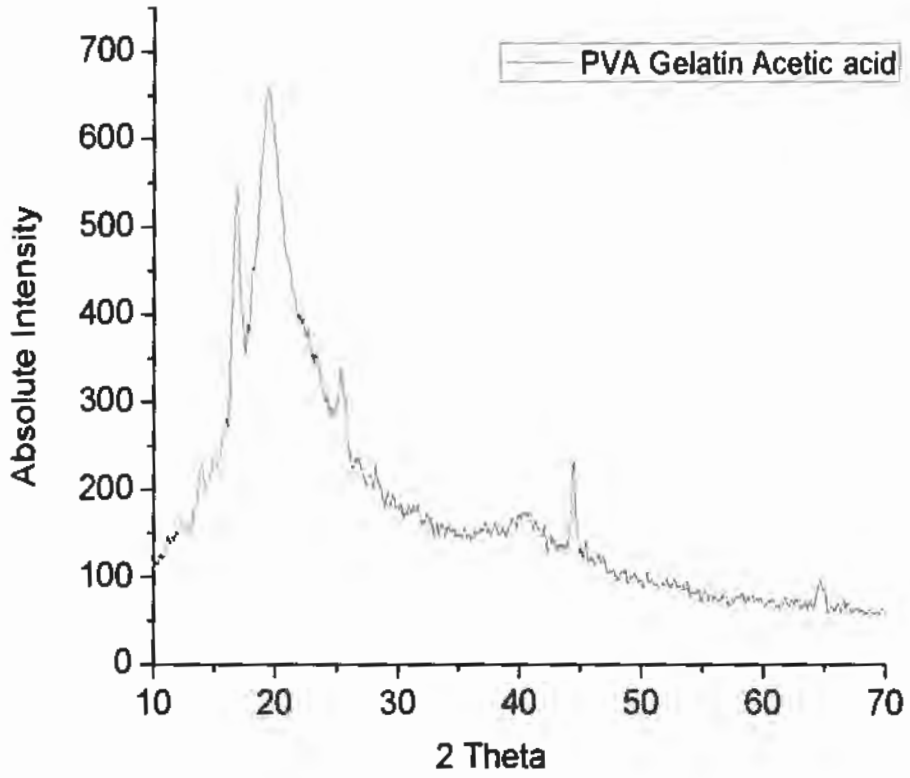



**Figure 3.4.3. Representing the XRD pattern of HG membrane with Acetic Acid**

### 3.5. SAMPLE COLLECTION AND ISOLATION OF PATHOGENS:

Blood agar and nutrients agar plates after 24 hours incubation were observed for the growth of pathogens as shown in figures (**3.5.1 and 3.5.2** ).

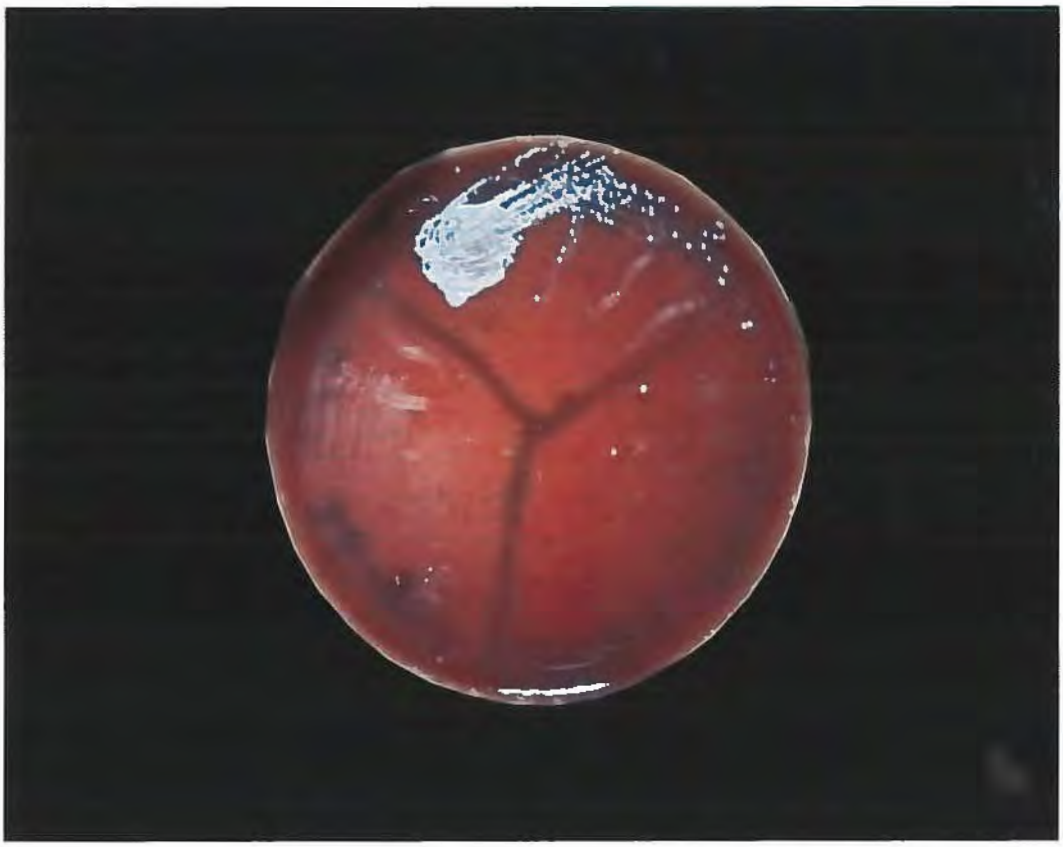


**Figure (3.5.1). Blood agar plates representing the isolated colonies of *Staphylococcus aureus***

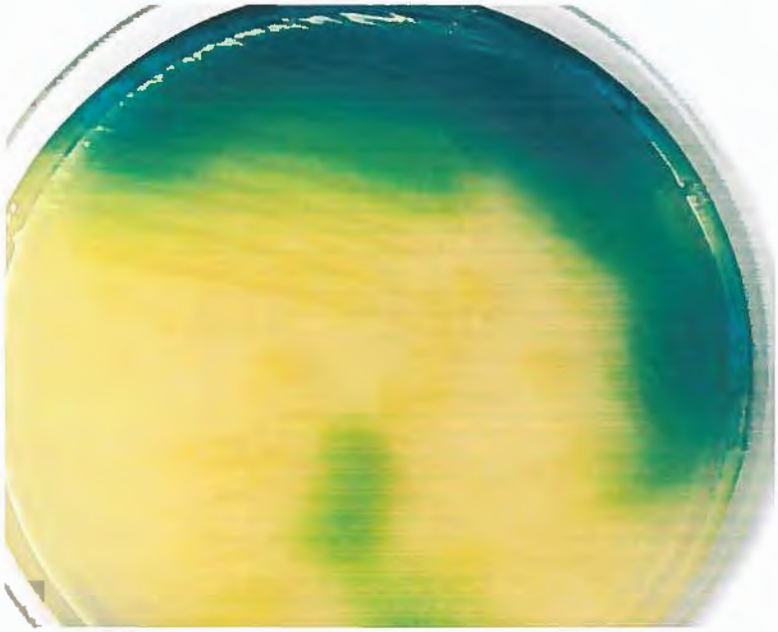


**Figure (3.5.2) Nutrient agar plate clearly representing the pigment producing *Pseudomonas aeruginosa***

## 3.6. IDENTIFICATION OF BACTERIA:

### I GRAM STAIN:

Examination of gram staining slides under microscope revealed that organisms were Gram positive cocci and Gram negative rods.

### II BIOCHEMICAL TESTS:

- **Catalase Test**

Catalase test was done to determine the presence of catalase enzyme which reduces hydrogen peroxide into oxygen and water. Immediate bubble and water formation was observed after emulsifying bacterial colonies with 3% hydrogen peroxide drops. These results indicated that bacterial sample was catalase positive. Figure **(3.6.1 )** represents catalase test results of bacterial sample.

- **Oxidase Test:**

**Filter Paper Method**

Filter paper was placed in petri dish and added 5 ml of freshly prepared oxidase reagent. Through a sterile cotton swab, a small inoculumn of test organisms from a culture plate was inoculated on wet filter paper as shown in figure **(3.6.2)**.

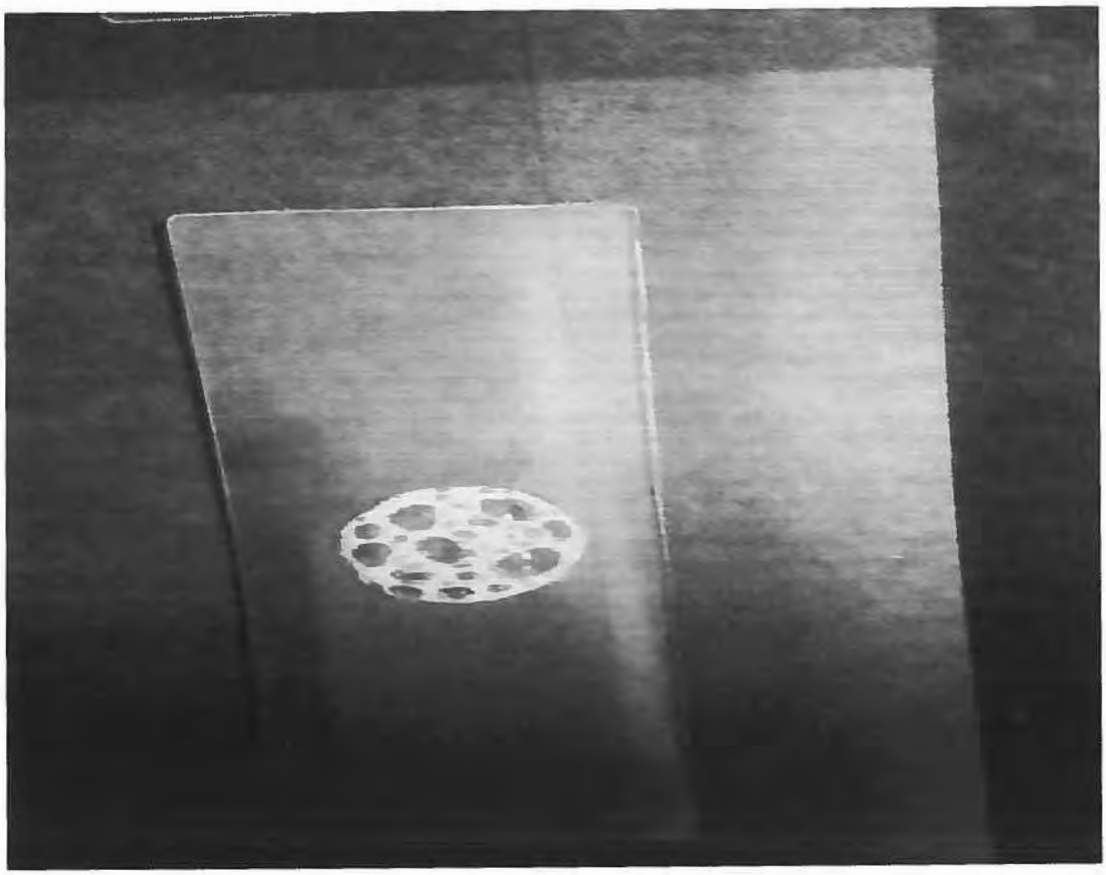


**Figure 3.6.1 Representing the bubbles due to catalase producing**

***Staphylococcus aureus***

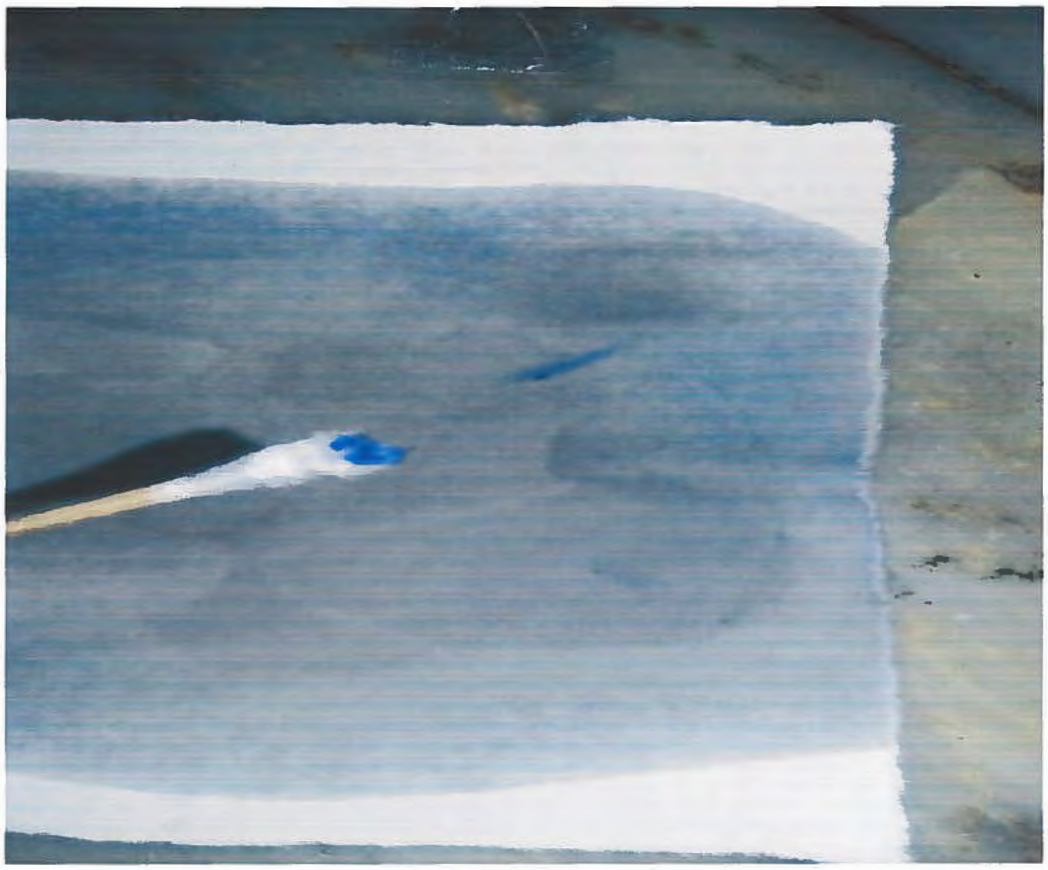


**Figure 3.6.2. Represents purple colour due to oxidase positive colonies of *Pseudomonas aeruginosa***

## 3.7. SCREENING OF ANTIMICROBIAL COMPOUND IMPREGINATED AND COATED HYDROGEL MEMBRANE AGAINST SKIN PATHOGENS

Antimicrobial activities of all hydrogel membranes were evaluated by using agar well diffusion method as mention in materials and methods. Experiments were repeated three times for conformation and the data collected was the average assessment of the antimicrobial activity.

A scale was designed to categorize the antimicrobial activities. Zone of inhibition less than 5mm were considered as resistance. Zone of inhibition more than 5mm were considered as sensitive. Antibiotic disc of Gentamicin (GEN=10μg) and Vancomycin (VAN=30mg) were used as positive control. Simple hydrogel without augmentation with antimicrobial agents was used as negative control (UHG).

Petri plates of figures **(3.7.1 to 3.7.4)** dipicts sensitivity zones of skin pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* with Hydrogel membranes.

The antimicrobial activity of Hydrogel (HG) impregnated with *Nigella sativa* seeds extracts (HGE) showed significant sensitivity zones against *Pseudomonas aeruginosa* and *Staphylococcus aureus* i.e 18mm & 20mm respectively.

Hydrogel membrane impregnated with 10% Acetic acid (HGAA) did not show antimicrobial activity against both pathogens. Here two hypothesis were made either the membrane could not release the acetic acid or cross linker might have changed the chemistry of acetic acid during reaction. As shown in figures **(3.7.1, 3.7.2, 3.7.3, 3.7.4)** and tables **(3.7.1, 3.7.2)**.

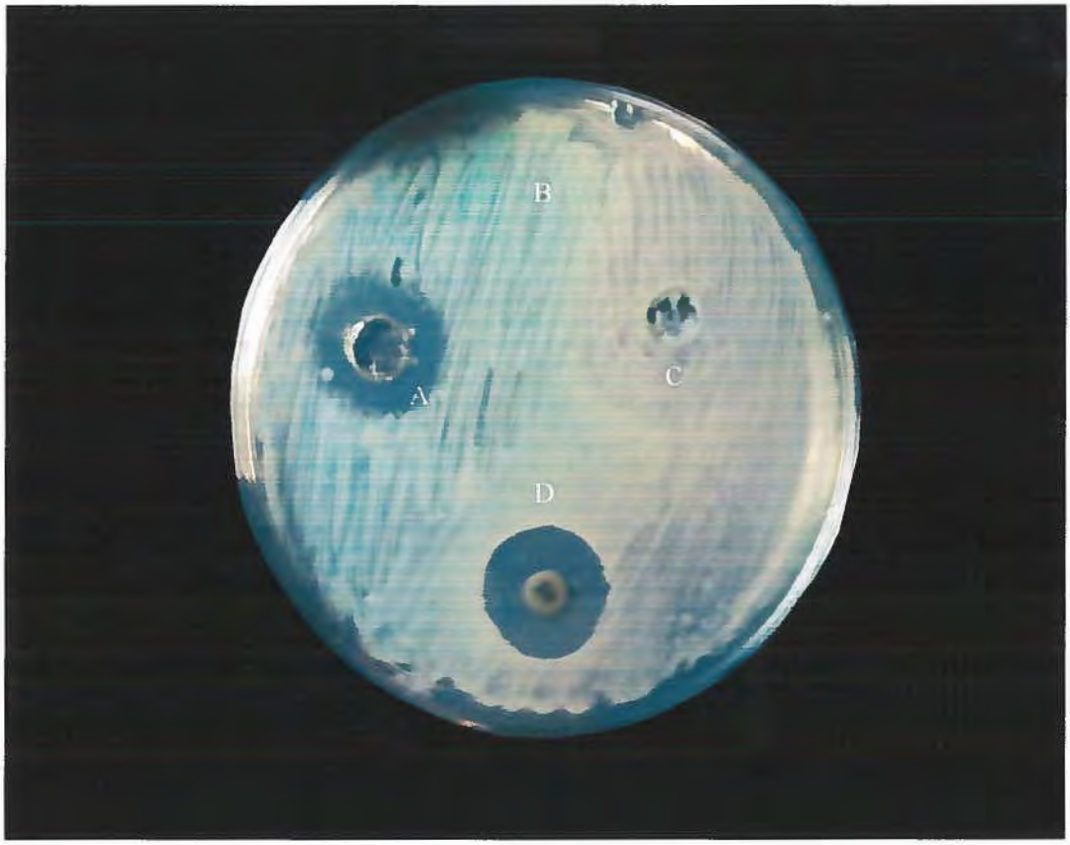



**Figure 3.7.1. Petri plate representing Antimicrobial activity of HG impregnated with *Nigella sativa* extract and acetic acid against *Pseudomonas aeruginosa***

**Key:**

**A= Extract Impregnated Hydrogel**
**B= Simple (negative control)**
**C= Acetic acid impregnated Hydrogel**
**D= Antibiotic (positive control)**

**Table 3.7.1.Screening of antimicrobial activity of HG impregnated with extract and Acetic acid (10%) against GNR:**

| **Gram negative bacteria** | **sample** | | **Positive control** | **Negative control** |
|---|---|---|---|---|
| | HGE | HGAA | | |
| *Pseudomonas aeruginosa* | 18 mm | (---) | Gentamycin | Uncoated HG |
| | | | 20 mm | (---) |

Gentamycin= 10µg

GNR= Gram negative rods

**Table 3.7.2. Screening of antimicrobial activity of HG impregnated with extract and Acetic acid (10%) against GPC:**

| Gram positive bacteria | sample | | Positive control | Negative control |
|---|---|---|---|---|
| | HGE | HGAA | | |
| *Staphylococcus aureus* | 20 mm | (---) | Vancomycin | Uncoated HG |
| | | | 22 mm | (---) |

Inhibition zone diameter were expressed as mean ±SD (mm)

Vancomycin= 30mg

**Table 3.7.3.Screening of antimicrobial activity of HG coated with extract and Acetic acid (10%) against GNR:**

| Gram negative bacteria | sample | | Positive control | Negative control |
|---|---|---|---|---|
| | HGE | HGAA | | |
| *Pseudomonas aeruginosa* | 16 mm | 16mm | Gentamicin | Uncoated HG |
| | | | 20 mm | (---) |

**Table 3.7.4. Screening of antimicrobial activity of HG coated with extract and Acetic acid (10%) against GPC:**

| Gram positive bacteria | sample | | Positive control | Negative control |
|---|---|---|---|---|
| | HGE | HGAA | | |
| *Staphylococcus aureus* | 18 mm | 18 mm | Vancomycin | Uncoated HG |
| | | | 22 mm | (---) |

Inhibition zone diameter were expressed as mean ±SD (mm)

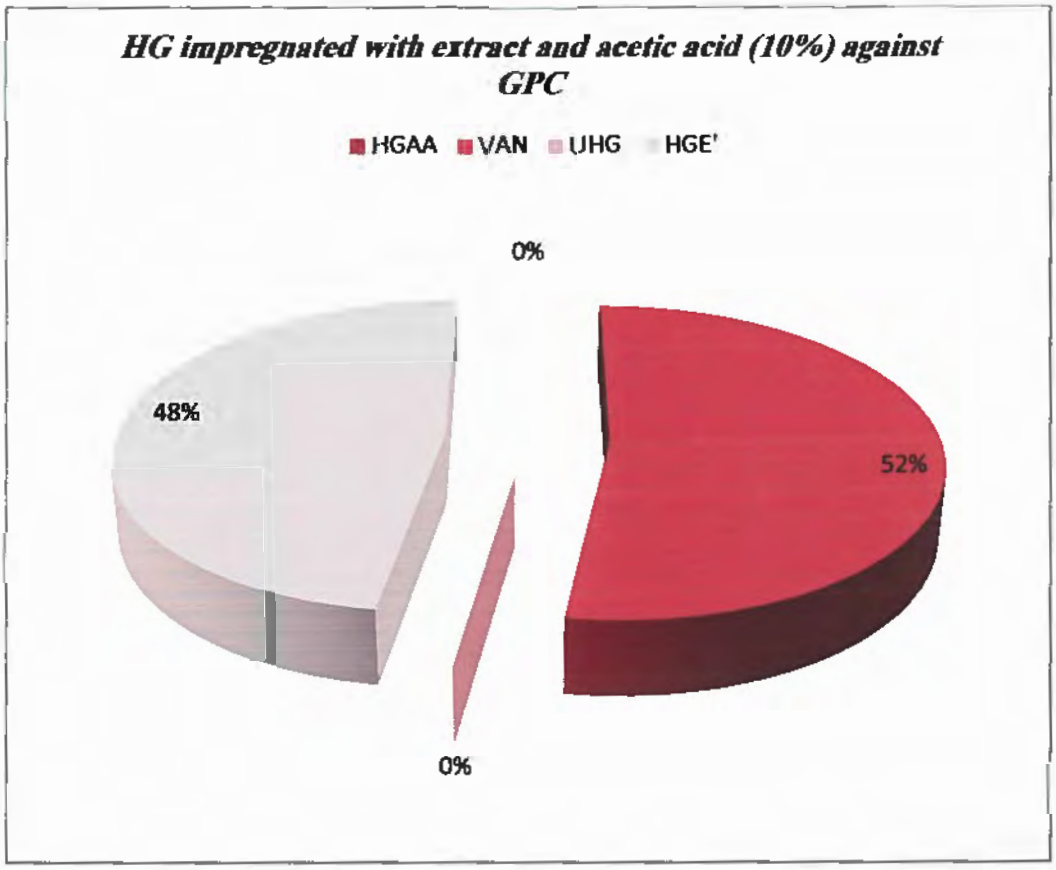



**Figure 3.7.5 Pie chart representing percent inhibition of Gram positive cocci(GPC)**

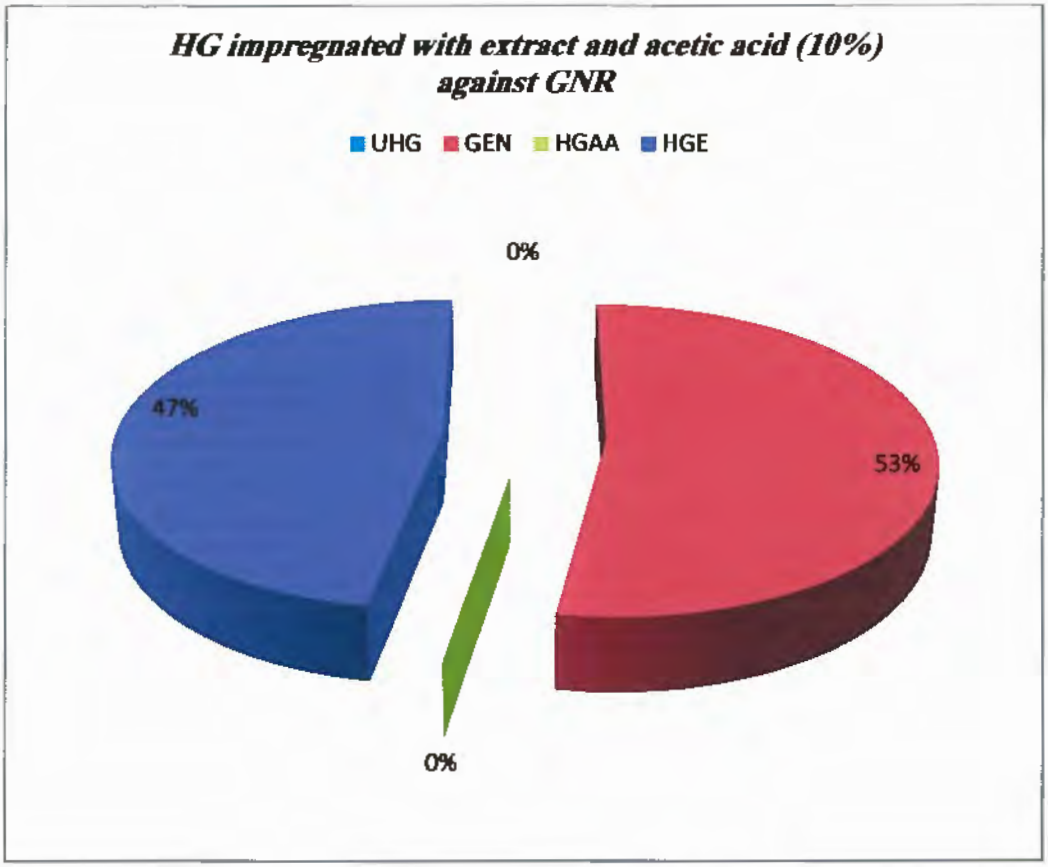



**Figure 3.7.6 Pie chart representing percent inhibition of Gram negative rods(GNR)**

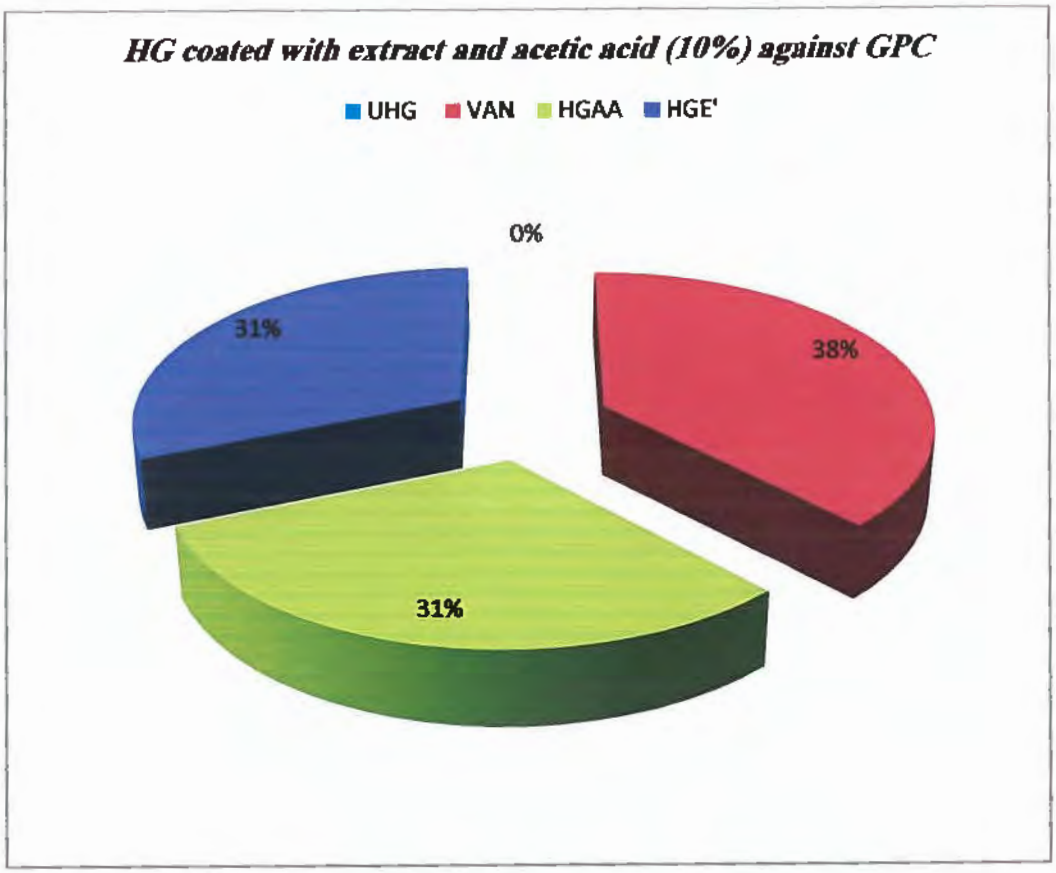



**Figure 3.7.7 Pie chart representing percent inhibition of Gram positive cocci(GPC)**

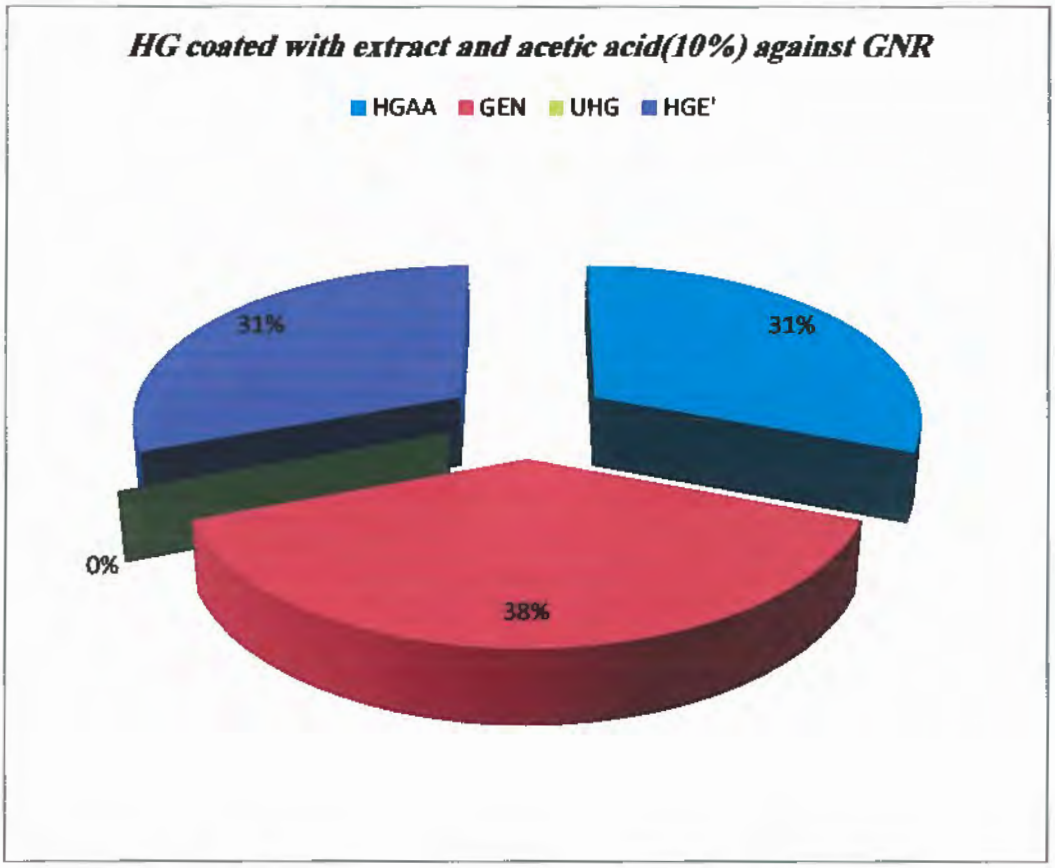



**Figure 3.7.8 Pie chart representing percent inhibition of Gram negative rods(GNR)**

# *DISCUSSIONS*

# *AND*

# *CONCLUSION*

## 4.1 DISCUSSION:

Microbial diseases, created by microscopic organisms and growths, are a genuine health issue, particularly regarding wound recovering and biomedical implant failure. (Siddiqui *et al.*, 2010; Edwards *et al.*, 2004). *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli, Pseudomonas aeruginosa* and *Candida* species are examples of pathogens commonly related to infections (Doyle *et al.*, 2011). Infection can delay in the wound recovery process causes tissue bleakness and relying upon the seriousness of infection, sepsis can happen. Concerning implant, contamination at the implant tissue interface can prompt embed disappointment, which requires implant replacement and substitution. Different medical devices, for example, catheters can go about as vehicles that present contamination from the hospital environment to the patient. To treat wound and implant infection, distinctive techniques have risen to create materials having antimicrobial activity. Materials can be impregnated with antimicrobial compounds that are discharged gradually (Woo *et al.*, 2000; Campbell *et al.*, 2000).

Hydrogels offer a helpful beginning stage to engineer antimicrobial materials. They are a class of exceptionally hydrated biomaterial. Polysaccharides, for example, alginate, dextran, and chitosan, alongside the proteins gelatin and fibrin, are samples of common polymers that frame all around considered hydrogels. Poly(vinyl alcohol) (PVA), polyethylene oxide (PEO), and poly(acrylic acid) (PAA) are samples of

hydrogel-shaping engineered polymers. Numerous hydrogels are biocompatible and can be intended to have mechanical properties like regular tissues, and in this way have been utilized as a part of a variety of uses including drug conveyance, surface coatings for implants, epitome of cells for three-dimensional cell culture, and tissue designing (Peppas *et al.,* 1997; Censi *et al.*, 2012; Seliktar, 2012).

Among various synthetic hydrophilic polymers used in the preparation of hydrogels, PVA has a prime position in biomaterials science because of its inherent nontoxicity, noncarcinogenicity, good biocompatibility, and desirable physical properties such as rubbery or elastic nature and a high degree of swelling in aqueous solutions.

Recently, gelatin has been demonstrated to exhibit activation of macrophage (Anderson *et al.,* 1984), (Tabata *et al.,* 1987) and high-hemostatic effect (Chvapil, 1982) consequently, it has been used in a wide variety of wound dressings (Neumann *et al.,* 1981), (Takahashi *et al.,* 1993) and as a biomaterial in tissue engineering. The presence of higher levels of pyrrolidines in gelatin results in the formation of stronger gels.

In the present study scanning electron microscopy of all the sample was done. Blend membrane showed voids which is one of characteristics of hydrogel membrane and is important for its hydrophilicity (figure 3.1.1). The surface view of the membrane with Acetic Acid (10%) revealed crystals, which may be due to partial dissolution of acetic acid in the final solution (figure 3.1.2). The surface morphology of the membrane

having seeds extracts depicts granular rough surface and a few aerosols (figure 3.1.3).

The microscopy of transverse sections of all three membranes revealed thickness is even, which is important for membrane composition (figure 3.1.4 – 3.1.6).

Fourier Transformed Infrared Spectroscopy is a common technique that is used to find the functional groups present in a material. In the current study FTIR spectra revealed strong peaks at $1730cm^{-1}$ for membrane with Acetic Acid (figure 3.2.1), which was not present after mixing of extract (*Nigella sativa*), these peaks shifted to $3291cm^{-1}$ in blends of PVA–Gelatin which is due to asymmetrical stretching of $-NH_3^+$ in amino acids(figure 3.2.2). Similar work was done by Pal *et al,* in 2007(Pal *et al.*, 2007).

Wound dressing must have an ability to retain wound liquids and exudates. It gets to be important to portray the swelling qualities of hydrogels with a specific end goal to settle on its application. Swelling % decide the time of substitution of wound dressing. In present study, the first hydrogel membrane (HGE) showed swelling % of 150% after 2 hrs of soaking and reached to equilibrium of weight after next 2 hrs that was 170% and remained constant at the $8^{th}$ hrs.

There was not any significant difference observed in the hydrophilicity of both membrane. The second hydrogel membrane (HGA), also gradually gain weight in first 2 hrs time with percentage of 149% and got saturated for next 6 hrs with 168%.

Both membranes showed gradually increase in weight in first two hrs and it reached to equilibrium after 2 hrs time, which was further observed for next 6 hrs but no

increased in swelling % at that interval of time (figure 3.3.1 to 3.3.4). Similar method for swelling study was adopted by Kokabi *et al,* in 2007( kokabi *et al.,* 2007).

Antimicrobial activity of the extract and Acetic Acid coated and impregnated hydrogel membrane was tested by nutrient agar diffusion assay (Gonzalez & Maiolo, 2012).

There was not any zone of inhibition, incase of uncoated hyxdrogel(UHG),which indicates that hydrogel prepared lack antimicrobial potential.

Results of the antimicrobial assay showed when Acetic Acid (10%) impregnated membrane don't give any sensitivity zone, it was assumed that it couldn't released from the hydrogel matrix. But when coated it has given zones of 16mm for *P.aeruginosa* and 18mm for *S. aureus.*

The antimicrobial activity of Hydrogel (HG) with *Nigella sativa* seeds extracts (HGE) have showed significant sensitivity zones in both conditions (coated/impregnated) against *Pseudomonas aeruginosa* and *Staphylococcus aureus* i.e 16mm & 18mm respectively (coated), 18mm & 20mm respectively (impregnated). Apparently there was not a drastic differences in results but slightly increase in sensitivity incase of membrane with *Nigella sativa.*

## 4.2 CONCLUSION:

In current study PVA-Gelatin composite hydrogel membrane with antimicrobial impregnated and coated have been prepared by conventional solution casting method. Polyvinyl alcohol (PVA) at 10%(w/v) has been used for hydrogel formation. The synthesized PVA-Gelatin hydrogel have been characterized using scanning electron microscopy, fourier transform infrared spectroscopy. For checking wound dressing characteristics swelling studies were performed and antimicrobial activity was also accessed. Scanning electron microscopy results showed differences in composition. Fourier transform infrared spectroscopy analysis of the PVA-Gelatin well represented the intercalation of components. The swelling studies showed that membrane have good water holding ability. Antimicrobial activity against two human pathogens was accessed which was significant. The synthesized composite hydrogel was found to satisfy various ideal wound dressing characteristics like moisture maintenance, water absorption and protection from secondary infections proving its wound dressing potential.

**RECOMMENDATION:**

Following recommendation are suggested on the score of the finding of this study.

1. Water vapours transmissibility rate of the hydrogel membrane should be done as it is compulsory for any material to rule out it as wound dressing.
2. As antimicrobial activity was checked against only two pathogens, it should also be checked against other skin pathogens.
3. Before declare it as potential wound dressing it should be assayed on animal models.

# *REFERENCES*

## REFERENCES:

Abd El-Mohdy, H.L., 2013. Radiation synthesis of nanosilver/poly vinyl alcohol/cellulose acetate/gelatin hydrogels for wound dressing. Journal Polymer Res. 20, 177–188.

Anderson, J. M. &Langone, J.J. (1999) Issues and perspectives on the biocompatibility and immunotoxicity evaluation of implanted controlled release systems. Journal of Controlled Release57, 107-113.

Anderson, J. M; Miller, K. N. (1984)Biomaterial biocompatibility and the macrophage.JournalBiomaterials, 5(1), 5-10.

Allison A. Campbell,Lin Song , X. Shari Li, Bradley J. Nelson, Craig Bottoni,Dan E. Brooks &E. Schuyler DeJong(2000), Development, characterization, and anti-microbial efficacy of hydroxyapatite-chlorhexidine coatings produced by surface-induced mineralization"Article

Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK,Siddique NA,Damanhouri ZA, and Anwar F.,( 2013) A review on therapeutic potential of Nigella*sativa*: A miracle herb, Asian Pac Journal Tropical Biomedicine ; 3(5): 337–352.

Atiyeh, B.S.; Hayek, S.N. & Gunn, S.W. (2005) New technologies for burn wound closure and healing-review of the literature, Burns, 31,944-956.

Aoki, H., Al-Assaf, S., Katayama, T. & Phillips, G. O. (2007a) Characterization and properties of Acacia senegal (L.) Willd.var. senegal with enhanced properties (Acacia (sen) SUPER GUM(TM)): Part 2--Mechanism of the maturation process. *Food Hydrocolloids* 21, 329-337.

Aoki, H., Katayama, T., Ogasawara, T., Sasaki, Y., Al-Assaf, S. & Phillips, G. O. (2007b) Characterization and properties of Acacia senegal (L.) Willd.var. Senegal with enhanced properties (Acacia (sen) SUPER GUM(TM)): Part 5. Factors affecting the emulsification of Acacia senegal and Acacia (sen) SUPER GUM(TM).Food Hydrocolloids21, 353-358.

Arvanitoyannis IS., (2002) Formation and properties of collagen and gelatin films and coating. In: Gennadios A, editor. Protein-based films and coatings. Boca Raton: Press: 275-304.

A., Mansouri, B. &Youcef, S. L. (2006) Synthesis and characterization of hydrogels based on poly(vinyl pyrrolidone). Nuclear Instruments and Methods in Physics Research . 248, 284–290.

Bhardwaj, Y. K., Kumar, V., Acharya, A. &Sabharwal, S. (2005) Synthesis, characterization and utilization of radiation synthesized stimuli-responsive hydrogels and membranes. In Radiation synthesis of stimuli-responsive membranes, hydrogels and adsorbents for separation purposes, (ed. I. A. E. Agency), International Atomic Energy Agency, Vienna.coatings. In: Gennadios A, editor. Protein-based films and coatings. Boca Ratón: CRC Press; 275–304.

Black CE, Costerton JW (2010) Current concepts regarding the effect of wound microbial ecology and biofilms on wound healing. Surgical Oncology Clinics of North America 90:1147–1160.

Bajpai, A. K., Shukla, S. K., Bhanu, S. &Kankane, S. (2008) Responsive polymers in controlled drug delivery. Progress in Polymer Science 33, 1088-1118.

Barbucci, R., Leone, G. &Vecchiullo, A. (2004) Novel carboxymethylcellulose-based microporous hydrogels suitable for drug delivery. journal. Biomaterial. Science. Polymer Edn15, 607-619.

Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management.Clinical Microbiology Reviews14: 244–269.

Benamer, S., Mahlous, M., Boukrif,Bakathir HA, Abbas NA.,( 2011) Detection of the antibacterial effect of *Nigella sativa* ground seeds with water. Africian Journal TraditionalCompl Alternative Medicine. ;8(2):159–164.

Choa SM, Gub YS, Kima SB.,( 2005) Extracting optimization and physical properties of yellow fin tuna (Thunnusalbacares) skin gelatin compared to mammalian gelatins. Food Hydrocolloids;19(2):221–229.

Chaieb K, Kouidhi B, Jrah H, Mahdouani K, Bakhrouf A., (2011) Antibacterial activity of Thymoquinone, an active principle of *Nigellasativa* and its potency to prevent bacterial biofilm formation. BMC Compl Alternative Medicine. ;11:29.

Chvapil, M. (1982).Considrations of manufacturing principles of a synthetic burn dressing. Journal Biomaterial Mater Res. 16 (1), 245-263.

Cigdem, A.R.C.A.H., Senel, S., (2008) Chitosan based systems for tissue engineering Part II: soft tissues. FABAD Journal.Pharmaceutical.Science. 33, 211–216.

Cole, CGB (2000), "Gelatin", in Francis, FJ, Encyclopedia of Food Science and Technology, 2nd edition, John Wiley & Sons, 1183–1188.

Coviello, T., Matricardi, P., Marianecci, C. & Alhaique, F. (2007) Polysaccharide hydrogelsfor modified release formulations. Journal of Controlled Release 119, 5-24.

Cui Y, Zhao Y, Tian Y, Zhang W, Lü X, Jiang X.(2012) The molecular mechanism of action of bactericidal gold nanoparticles on *Escherichia coli*.Biomaterials. 33(7):2327-33

Chester B., (1979) Semi-quantitative Catalase Test as an Aid in Identification of Oxidase and Nonsaccharolytic Gram- Negative Bacteria, Journal of Clinical Microbiology,10,525-528.

Champion HR, Bellamy RF, Roberts CP, Leppaniemi A (2003) A profile of combat injury. Journal Trauma 54: S13–19.

Co EM, Keen EF, 3rd, Aldous WK (2011) Prevalence of methicillin-resistant *Staphylococcus aureus* in a combat support hospital in Iraq. Mil Med 176: 89–93.

Darwis, D., Hilmy, N., Hardiningsih, L., Erlinda, T., (1993)Poly(Nvinylpyrrolidone)

hydrogels: 1 Radiation polymerization and crosslinking of N-vinylpyrrolidone. Radiation Physics Chemistery.42, 907– 910.

Deitrich, H.J., (2001)Kolloide in der IntensivmedizinAnaesthesist. Der Anaesthesist 15, 54–68.

Dutta, J., (2012) Synthesis and characterization of c-irradiated PVA/ PEG/CaCl2 hydrogel for wound dressing. American Journal Chemistery. 2:6–11.

Dowd SE, Sun Y, Secor PR, Rhoads DD, Wolcott BM, et al. (2008) Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiology 8: 43.

Edwards R, Harding KG, (2004) Bacteria and wound healing,Current Opinion Infectious Diseases.17(2):91-6.

Doyle JS, Buising KL, Thursky KA, Worth LJ, Richards MJ, ( 2011) Epidemiology of infections acquired in intensive care units. Americian Journal Respiratory and Critical Care Medicine; 32(2):115-38.

Denisenko ,PyotrProkopovich (2003 ) Modern drugs : Pharmacological directory PetrProkopovichDenisenko. ISBN 978-5-7654-2738-5.

Elizabeth Fonseca of Reisa , Fábia S. Cmp Camp osa , AndreyLagea Pereira , Romulo CerqueiraLeitea , LuizGuilhermeHeneineb , Wander Luiz Vasconcelosc , Zelia Ines PortelaLobatoa , Herman Sander Mansurc( 2006 ) Synthesis and Characterization of Poly (Vinyl Alcohol) Hydrogels and Hybrids for rMPB70 Protein Adsorption.Materials Research. 9(2), 185-191.

Ebright JR (2005) Microbiology of chronic leg and pressure ulcers: clinical significance and implications for treatment.Nursing Clinics of North America 40: 207–216.

El-Salmawi, K.M., (2007) Gamma radiation-induced crosslinked PVA/ chitosan blends for wound dressing. Journal Macromolecular Sciences. Part A: Pure AppliedChemistery. 44:541–545.

Estaca JG, Montero PF, Martín F, Gómez-Guillén MC (2009) Physico-chemical and film-forming properties of bovine-hide and tuna-skin gelatin: a comparative study. Journal Eng. 90(3):480–486.

Esteban, C. &Severian, D. (2000) Polyionic hydrogels based on xanthan and chitosan for stabilising and controlled release of vitamins, WO0004086(A1) (ed.U. United States Patent), KemestrieInc [CA], USA.

Fakirov, S. Sarac, Z. Anbar, T. Boz, B. Bahar, I. Evstatiev, M.; Apostolov, A. A. Mark, J. E. Kloczkowski, A. (1996). Mechanical properties and transition temperatures of cross-linked orientedgelatin. Journal Colloid Polymers Science, 274 (4): 334-341.

Fei, B., Wach, R. A., Mitomo, H., Yoshii, F. &Kume, T. (2000) Hydrogel of biodegradable cellulose derivatives. I. Radiation-induced crosslinking of CMC. Journal of AppliedPolymer Science78, 278-283.

Fung, L. K.;Saltzmann, W. M. (1997). Polymeric implants for cancer chemotherapy Journal Advances Drug Delivery Review, 26, 209.

Funami, T., Hiroe, M., Noda, S., Asai, I., Ikeda, S. &Nishimari, K. (2007) Influence ofmolecular tructure imaged with atomic force microscopy on the rheological behavior ofcarrageenan aqueous systems in the presence or absence of cations. Food Hydrocolloids.21: 617-629.

Fadeev, SB &Nemtseva, NV (2009) Formation of biofilms by agents of surgicalsoft tissue infections. Microbiological Epidemiology Immunobiology:114–117.

Fazli M, Bjarnsholt T, Kirketerp-Moller K, Jorgensen B, Andersen AS, *et al.*(2009) Nonrandom distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus*in chronic wounds. Journal of Clinical Microbiology 47: 4084–4089.

Giannouli, P. & Morris, E. R. (2003) Cryogelation of xanthan.Food Hydrocolloids 17, 495-501.

Garedew L., Berhanu A., Mengesha D., and Tsegay G., (2012) Identification of Gram- negative bacteria from critical control points of raw and pasteurized

cow milk consumed at Gondar town and its suburbs, Ethiopia, BMC Public Health ,12: 1-7.

Gao D, Heimann R.B. (1993). Structure and mechanical propertiesof superabsorbent poly(acrylamide)montmorillonite com-positehydroegls. Journal of Polymer Gels Networks;1 (1):225–46.

Gomez R, Murray CK, Hospenthal DR, Cancio LC, Renz EM, et al. (2009) Causes of mortality by autopsy findings of combat casualties and civilian patients admitted to a burn unit.Journal of the American College of Surgeons.208: 348–354.

Gontcharova V, Youn E, Sun Y, Wolcott RD, Dowd SE (2010) A comparison of bacterial composition in diabetic ulcers and contralateral intact skin. The Open Microbiology Journal 4: 8–19.

Gjodsbol K, Christensen JJ, Karlsmark T, Jorgensen B, Klein BM, et al. (2006) Multiple bacterial species reside in chronic wounds: a longitudinal study. International Wound Journal 3: 225–231.

Gurjala AN, Geringer MR, Seth AK, Hong SJ, Smeltzer MS, *et al.* (2011) Development of a novel, highly quantitative in vivo model for the study of biofilm-impaired cutaneous wound healing. Wound Repair Regeneration 19: 400–410.

Hannan A, Saleem S, Chaudhary S, Barka M, Arshad MU., (2008 ) Anti-bacterial activity of *Nigellasativa*against clinical isolates of methicillin resistant *Staphylococcus aureus*. JournalAyub Medical College Abbottabad.;20(3):72–74.

Hago, E.E., Li, X., (2013) Interpenetration polymer network hydrogels based on gelatin andPVA by biocompatible approaches: synthesis and characterization. Advances Material Science Eng., ID 328763, 1–8.

Heins, D., Kulicke, W.M., Kauper, P., Thielking, H., 1998. Characterization of acetyl starch by means of NMR spectroscopy and SEC/MALLS in comparison with hydroxyethylstarch. Starch 50, 431–437.

Hernández-Sierra JF, Ruiz F, Pena DC, Martínez-Gutiérrez F, Martínez AE, Guillén Ade J, Tapia- Pérez H, Castañón GM. (2008)The antimicrobial sensitivity of *Streptococcus*mutans to nanoparticles of silver, zinc oxide, and gold. Nanomedicine. 4(3):237-40

Hidaka S, Liu SY., (2003) Effects of gelatin on calcium phosphate precipitation: a possible application for distinguishing bovine bone gelatin from porcine skin gelatin. Journal of Food Composition Analysis. 16(3):477–483.

Hoare, T. R. &Kohane, D. S. (2008) Hydrogels in drug delivery: Progress and challenges.Polymer 49, 1993-2007.

Hoffman, A. S. (2002) Hydrogels for biomedical applications. Advanced Drug DeliveryReviews 43 3–12.

Huang, X., Brazel, C.S., (2001) Review On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. Journal Controlled Release 73, 121–136.

Hwang, M.R., Kim, J.O., Lee, J.H., Kim, Y., Kim, J.H., Chang, S.W., Jin, S.G., Kim, J.A., Lyoo, W.S., Han, S.S., Ku, S.K., Young, C.S., Choi, H.G., (2010) Gentamicin- loaded wound dressing with polyvinyl alcohol/dextran hydrogel: gelcharacterization and in vivo healing evaluation. American Association of Pharmaceutical ScientistsTechnology,11: 1092–1103.

Hyon, S.H., Cha, W.I., Ikada, Y.,Kita, M., Ogura, Y., Honda, Y., (1994)Poly(vinyl alcohol)hydrogels as soft contact lens material. Journal Biomaterial Science Polymer Ed. 5, 397–406.

HJ Yu , Xu XY , Chen XS , Lu TC , Zhang PB , Jing XB (2007) "Preparation and antibacterial effect of PVA-PVP hydrogels containing silver nanoparticles. Journal of Applied Polymer Science, 103, (1): 125-133.

Ignatova, M., Starbova, K.,Manolova, N., Rashkov, I., (2006)Electrospunnano-fibre matswith antibacterial properties from quaternized chitosan and poly(vinyl alcohol).Carbohydrates Research. 341: 2098–2107.

Jamilah B, Harvinder KG., (2002) Properties of gelatins from skins of fish-black tilapia (Oreochromismossambicus) and red tilapia (Oreochromis nilotica) Food Chemistry ;77(3):81–84.

Jeffrey J. Tarrand and Dieter H. M. Groschel (1982)Rapid, Modified Oxidase Test for Oxidase- Variable Bacterial Isolates. 772-774.

Jarry, C., Leroux, J. C., Haeck, J. &Chaput, C. (2002) Irradiating or autoclaving chitosan/polyol solutions: Effect on thermogelling chitosan-beta-glycerophosphate systems. Chemical & Pharmaceutical Bulletin, 50: 1335-1340.

James GA, Swogger E, Wolcott R, Pulcini E, Secor P, *et al.* (2008) Biofilms in chronic wounds. Wound Repair Regeneration 16: 37–44.

Kaetsu, I., (1996)Stimule-sensitive hydrogels. In: Dumitriu, S. (Ed.), Polysaccharides inMedicinal Application. Marcel Dekker, New York, 243–265.

Kamoun, E.A., Menzel, H., (2010) Crosslinking behavior of dextran modified with hydroxyethyl methacrylate upon irradiation with visible light- effect of concentration, coinitiator type, and solvent. Journal Applied Polymer Science. 117, 3128–3138.

Kenawy, E., Kamoun, E.A., MohyEldin, M.S., El-Meligy, M.A., (2014) Physically Crosslinkedpoly(vinyl alcohol)-hydroxyethylstarch blend hydrogel membranes: synthesis and characterization for biomedical applications. Arabian Journal of Chemistry. 7, 372–380.

Khor, E., Wu, H., Lim, L.Y., Guo, C.M., (2011)Chitin-methacrylate: preparation, characterization, and hydrogel formation. Materials 4, 1728–1746.

Khoylou, F. &Naimian, F. (2009) Radiation synthesis of superabsorbent polyethylene oxide/tragacanth hydrogel. Radiation Physics and Chemistry78, 195-198.

Kim, J.O., Park, J.K., Kim, J.H., Jin, S.G., Yonga, C.S., Li, D.X., Choi, J.Y., Woo, J.S., Yoo,B.K., Lyoo, W.S., Kim, J.A., Choi, H.G., (2008) Development of polyvinyl alcohol–sodium alginate gelmatrix- based wound dressing system containing nitrofurazone.International Journal Pharmaceutical, 359: 79–86.

Kuang, Q. L., Zhao, J. C., Niu, Y. H., Zhang, J. & Wang, Z. G. (2008) Celluloses in an ionic liquid: the rheological properties of the solutions spanning the dilute and semidiluteregimes. Journal of Physical Chemistry B,112: 10234-10240.

Keen EF, 3rd, Robinson BJ, Hospenthal DR, Aldous WK, Wolf SE, *et al.* (2010) Incidence and bacteriology of burn infections at a military burn center. Burns 36: 461–468.

Lee, K.Y., Mooney, D.J., (2012) Alginate: properties and biomedical applications. Progress Polymer Science, 37: 106–126.

Lee, S.B., Jeon, H.W., Lee, Y.M., Lee, Y.W., Song, K.W., Park, M.H., Nam, Y.S., Ahn, H.C.,(2003) Bio-artificial skin composed of gelatin and (1–3), (1–6)-b-Glucan. Biomaterials, 24: 2503–2511.

Li, C., Fu, R., Yu, C., Li, Z.,Guan, H., Hu, D., Zhao, D., Lu, L., (2013)Silver nanoparticle/chitosan oligosaccharide/poly(vinyl alcohol) nanofibers as wounddressing: a preclinical study. International Journal Nano medicines 8, 4131–4145.

Lim, F., Sun, A.M., (1980)Microencapsulated islets as bioartificial endocrine pancreas. Science, 210: 908–910.

Lin SY, Chen K-S, Run-Chu L. (2001) Design and evaluation ofdrug loaded wound dressing havingthermoresponsive,adhesive, absorptive and easy peeling properties. Journal Biomaterials. 22 (1) : 2999–3004.

Liu, P., Zhai, M., Li, J., Peng, J. & Wu, J. (2002b) Radiation preparation and swelling Behaviour ofsodiumcarboxymethyl cellulose hydrogels.Radiation Physics and Chemistry,63: 525– 528.

Manfred L. Hallensleben(2000)Polyvinyl Compounds, Others in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim. doi:10.1002/14356007.a21_743

Nandini, V.V., Venkatesh, K.V., Nair, K.C., (2008) Alginate impressions: a practical perspective. Journal of Conservative Dentistry. 11: 37–41.

Manfred L. Hallensleben., (2000) Polyvinyl Compounds, Others in Ullmann's Encyclopediaof Industrial Chemistry, Wiley-VCH, Weinheim. doi:10.1002/14356007.

Mansur HS, Oréfice RL, Mansur AAP. (2004) Characterization of poly(vinyl alcohol)/poly (ethylene glycol) hydrogels and PVA-derived hybrids by small-angle X-ray scattering and FTIR spectroscopy, Polymer. 45(21):7193-7202.

Mansur HS, Oréfice RL, Pereira MM, Lobato ZIP, Vasconcelos WL, Machado LJC. (2002) FTIR and UV-vis study of chemically engineered biomaterial surfaces for protein immobilization. Spectroscopy-An International Journal; 16 (3-4):351-360.

Marsich E, Bellomo F, Turco G, Travan A, Donati I, Paoletti S.(2013) Nano-composite scaffolds forbone tissue engineering containing silver nanoparticles: preparation, characterization and biological properties.Journal of Material Science and Medicine. 24(7):1799-807.

Martinez, L.R.; Han, G.; Chacko, M.; Mihu, M.R.; Jacobson, M.; Gialanella, P.; Friedman, A.J. Nosanchuk, J.D. & Friedman J.M. (2009) Antimicrobial and healing efficacy ofsustained release nitric oxide nanoparticles against *Staphylococcus aureus*skininfection, Journal of Investigative Dermatology, 129(10), 2463-2469.

Mao, J.; Zhao, L.; De Yao, K.; Shang, Q.; Yang, G.; Cao, Y. (2003)Study of novel chitosan-gelatin artificial skin in vitro. Journal of Biomedicine Material Research 64: 301-308.

Meyers RA (ed.) (2000)Interpretation of Infrared Spectra, A Practical Approach, John Coates in Encyclopedia of Analytical Chemistry. Chichester: John Wiley & Sons Ltd, 10815-10837.

Morsi NM.,(2000) Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. ActaMicrobiolPolonica. 49(1):63–74.

Martin JM, Zenilman JM, Lazarus GS (2010) Molecular microbiology: new dimensions for cutaneous biology and wound healing. Journal of Investigative Dermatology 130:38–48.

Malic S, Hill KE, Hayes A, Percival SL, Thomas DW, *et al.* (2009) Detectionand identification of specific bacteria in wound biofilms using peptide nucleicacid fluorescent in situ hybridization (PNA FISH). Microbiology 155: 2603–2611.

Neumann P. M.;Zur, B.; Ehrenreich, Y. (1981)Gelatin-based sprayable foam as a skin substitute.Journal of BiomedicineMaterial Research. 15(1): 9-18.

Nguyen, M. K. & Lee, D. S. (2010) Injectable Biodegradable Hydrogels. Macromolecules Bioscience.10: 563-579.

Nho, Y.C. & Lee, J.H. (2005) Reduction of postsurgical adhesion formation with hydrogels synthesized by radiation.Nuclear Instruments and Methods in Physics Research B 236: 277–282.

Nho, Y.C., Park, S.E., Kim, H.I. & Hwang, T.S. (2005) Oral delivery of insulin using pHsensitive Hydrogels based on polyvinyl alcohol grafted with acrylic acid/methacrylic acid by radiation.Nuclear Instruments and Methods in Physics Research B 236: 283–288.

Nusbaum AG, Gil J, Rippy MK, Warne B, Valdes J, et al. (2012) Effective method to remove wound bacteria: comparison of various debridement modalities in an in vivo porcine model.Journal of Surgical Research 176: 701–707.

Onuki, Y., Nishikawa, M., Morishita, M. &Takayama, K. (2008) Development of photocrosslinkedpolyacrylic acid hydrogel as an adhesive for dermatological patches: Involvement of formulation factors in physical properties and pharmacological effects. International Journal of Pharmaceutics,349: 47-52.

O'Hara FP, Amrine-Madsen H, Mera RM, Brown ML, Close NM, *et al.* (2012) Molecular Characterization of *Staphylococcus aureus* in the United States 2004–2008 Reveals the Rapid Expansion of USA300 Among Inpatients and Outpatients.Microbiology Drug Resistant.

Palumbo, F. S., Pitarresi, G., Mandracchia, D., Tripodo, G. &Giammona, G. (2006) Newgraft copolymers of hyaluronic acid and polylactic acid: Synthesis and characterization. Carbohydrate Polymers,66: 379-385.

Pal K, Banthia AK, Majumdar DK.(2007)Preparation and Characterization of Polyvinyl Alcohol–Gelatin HydrogelMembranes for Biomedical Applications American Association of Pharmaceutical Scientists,8(1): 21.

Peppas, N. A; Mongia, N. K. (1997) Ultrapure poly(vinyl alcohol) hydrogels with mucoadhesive drug delivery characteristics. European Journal Pharmaceuticals Biopharmacy 43 (1): 51-58.

Pfaller MA, Wey S, Gerarden T, Houston A, Wenzel RP (1989) Susceptibility of nosocomial isolates of Candida species to LY121019 and other antifungal agents.Diagnosis Microbiology Infection Diseases 12: 1–4.

Queiroz, A.C., Santos, J.D., Monteiro, F.J., Gibson, I.R., Knowles, J.C., (2001) Adsorption and release studies of sodium ampicillin from hydroxyapatite and glass-reinforced hydroxyl apatite composites. Biomaterials 22: 1393–1400.

Rauen, K. L; Smith, D. A; Heinemann, W. R; Sehuin, R; Stoughton, P. (1993) Humidity sensor based on conductivity measurements of a poly (dimethyl diallylammonium chloride) polymer film. Sens Actuators B .17 (1): 61-68.

Razzak, M. T., Darwis, D., Zainuddin&Sukirno. (2001) Irradiation of polyvinyl alcohol and polyvinyl pyrrolidone blended hydrogel for wound dressing. Radiation Physics and Chemistry62:107-113.

Ress, D.A., Welsh, E.J., (1997) Secondary and tertiary structure of polysaccharides in solutions and gels. AngewandteChemie International Edition in English 16: 214–224.

Rosiak, J. M. & Yoshii, F. (1999) Hydrogels and their medical applications.Nuclear Instruments and Methods in Physics Research B 151: 56-64.

Rosiak, J. M., Ulanski, P. &Rzeinicki, A. (1995) Hydrogels for biomedical purposes. Nuclear Instruments and Methods in Physics Research B 105: 335-339.

Rosiak JM, Rucinska-Rybus A, Pekala W (1989) Method ofmanufacturing of hydrogel dressings. Patent USA, No.4: 871, 490.

Ruiz, J.;Mantecon, A.; Cadiz, V. (2002) Investigation of Loading and Release in PVA-Based Hydrogels. Journal of Applied Polymer Science, 85: 1644-51.

Ryssel H, Kloeters O, Germann G, Schäfer T, Wiedemann G, Oehlbauer M.,(2009) The antimicrobial effect of acetic acid--an alternative to common local antiseptics, Burns. 35(5):695-700.

Roghmann MC, Siddiqui A, Plaisance K, Standiford H (2001) MRSAcolonization and the risk of MRSA bacteraemia in hospitalized patients withchronic ulcers. Journal Hospital Infection 47: 98–103.

Salem EM, Yar T, Bamosa AO, Al-Quorain A, Yasawy MI, Alsulaiman RM., (2010) Comparativestudy of *Nigellasativa* and triple therapy in eradication of Helicobacter Pylori in patients with non-ulcer dyspepsia. Saudi Journal Gastroenterology: 16(3):207–214.

Said, H. M., Alla, S. G. A. & El-Naggar, A. W. M. (2004) Synthesis and characterization of novel gels based on carboxymethyl cellulose/acrylic acid prepared by electron beam irradiation.Reactive & Functional Polymers 61:397- 404.

Schuetz, Y. B., Gurny, R. & Jordan, O. (2008) A novel thermoresponsive hydrogel based onchitosan. European Journal of Pharmaceutics and Biopharmaceutics 68:19-25.

Seliktar D, (2012)Designing cell-compatible hydrogels for biomedical applications Science. 336(6085):1124-8.

Sen, M. (2005) Radiation synthesis of stimuli responsive hydrogels and their use for the separation and enrichment of water pollutants. In Radiation synthesis of Stimuliresponsivemembranes, hydrogels and adsorbents for separation purposes, (ed. I. A. E. Agency), International Atomic Energy Agency, Vienna.

Shalumon, K.T., Anulekha, K.H., Nair, S.V., Chennazhi, K.P., Jayakumar, R., (2011) Sodiumalginate/poly(vinyl alcohol) nanoZnO composite nanofibers for

antibacterial wound dressing. International Journal Biological Macromolecules 49: 247–254.

Siddiqui, A.R; Bernstein, J.M. (2010) Chronic Wound Infection: Facts and Controversies. Clinics in Dermatologyclindermatol 28: 519-526.

Sidebotham, R.L., (1974)Dextrans. Advance Carbohydrates Chemistry Biochemicals 30, 371–444.

Silva, F.E.F., Di-Medeiros, M.C.B., Batista, K.A., Fernandes, K.F.,(2013) PVA/polysaccharides blended films: mechanical properties. Journal of Material, 1–6.

Singh, A., Hosseini, M. &Hariprasad, S. M.(2010) Polyethylene Glycol Hydrogel Polymer Sealantfor Closure of Sutureless Sclerotomies: A Histologic Study. American Journalof Ophthalmology.150:346-351.

Smetana, K. (1993) Cell biology of hydrogels.Biomaterials 14:1046-1050.

SRI Consulting Chemical Economic Hand book. (2007). Report Polyvinyl Alcohol, published ,abstract. Retrieved July 30, 2008.

Syed K. H. Gulrez, Saphwan Al-Assaf and Glyn O Phillips (2011). Hydrogels: Methods ofPreparation, Characterisation and Applications, Progress in Molecular and Environmental Bioengineering From Analysis and Modeling to TechnologyApplications, Prof. AngeloCarpi (Ed.), ISBN: 978-953-307-268-5.

Seth AK, Geringer MR, Galiano RD, Leung KP, Mustoe TA, *et al.* (2012) Quantitative Comparison and Analysis of Species-Specific Wound Biofilm Virulence Using an In Vivo, Rabbit-Ear Model.Journal of the American College of Surgeon.215(3):388-99.

Stevens, P.V. (1992). Unknown Food Australia 44 (7): 320–324. Retrieved2005-08-11.

Schiavo G, van der Goot FG (2001) The bacterial toxin toolkit. Nature Reviews Molecular Cell Biology 2: 530–537.

Schierle CF, De la Garza M, Mustoe TA, Galiano RD (2009) *Staphylococcal*

biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. Wound Repair Regeneration 17: 354–359.

Tabata, Y.; Ikada, Y. J (1987).Macrophage activation through phagocytosis of muramyl dipeptide encapsulated in gelatin microspheres.JournalPharmacy Pharmacol. 39 (9):698-704.

Talan DA, Krishnadasan A, Gorwitz RJ, Fosheim GE, Limbago B, *et al.* (2011) Comparison of *Staphylococcus aureus* from skin and soft-tissue infections in US emergency department patients, 2004 and 2008. Clinical Infectious Diseases 53: 144–149.

Takahashi, H.; Miyoshi, T.; Boki, K. (1993). Study on hydrophilic properties of gelatin as a clinical wound dressing. II. Water-absorbing property and hemostatic effect of gelatin. Tokushima Journal Exp Medicine, 40(3-4):169-175.

Takigami, M., Amada, H., Nagasawa, N., Yagi, T., Kasahara, T., Takigami, S. &Tamada, M. (2007) Preparation and properties of CMC gel. Transactions of the Materials Research Society of Japan, 32, 3- 32: 713-716.

Tarun, K., Gobi, N., (2012) Calcium alginate/PVA blended nanofiber matrix for wounddressing. Indian J. Fibre Text. Res. 37: 127–132.

Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, Scarpa T, Semeraro S, Turco G, GennaroR, Paoletti S.(2009)Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity. Biomacromolecules. 10(6):1429-35

Tashiro, T., (2001) Antibacterial and bacterium adsorbing macromolecules. Macromolecules Mat Eng. 286, 63–87.

Thomas, S., (2000) Alginate dressings in surgery and wound management: Part 1. Journal of Wound Care 9: 56–60.

Thomas V, Yallapu MM, Sreedhar B, Bajpai SK.(2007) A versatile strategy to fabricate hydrogel-silver nanocomposites and investigation of their antimicrobial activity.Journal Colloid Interface Science. 315(1):389-95.

Tseng HJ, Tsou TL, Wang HJ, Hsu SH.,(2013)Characterization of chitosan-gelatin scaffolds for dermal tissue engineering. J Tissue EngRegen Med. 7(1):20-31.

Tsou TL, Tang ST, Huang YC, Wu JR, Young JJ, Wang HJ. (2005) Poly(2-hydroxyethyl methacrylate) wound dressing containing ciprofloxacin and its drug release studies.Journal Material Science & Material Medicine. 16(2):95-100.

Uslu, I., Keskin, S., Gul, A., Karabulut, T.C., Aksu, M.L., (2010) Preparation and properties ofelectrospunpoly(vinyl alcohol) blended hybrid polymer with Aloe vera and HPMCas wounddressing. Hacet. Journal Biol. Chem: 38, 19--25.

VichayaratRattanaruengsrikul, NuttapornPimpha and Pitt Supaphol (2009) Development of Gelatin Hydrogel Pads as Antibacterial Wound Dressings DOI: 10.1002/mabi.200900131.

Ward, A.G. Courts, A. (1977) The Science and Technology of Gelatin. New York: Academic Press. ISBN 0-12-735050-0.

Wichterle, O., &Lím, D. (1960) Hydrophilic gels for biological use. Nature, 185, 117.

Winter, G.D., (1962) Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig.Nature 193:293–294.

Woo G.L.Y., Mittelman M.W, Santerre , J.P., (2000)Synthesis and characterization of a novel biodegradable antimicrobial polymer, Biomaterials (21) 1235-1246.

Wu, M., Bao, B., Yoshii, F., Makuuchi, K., (2001) Irradiation of crosslinked, poly(vinylalcohol) blended hydrogel for wound dressing.Journal of Radioanalyticaland Nuclear Chemistry. 250: 391–395.

Wolcott RD, Ehrlich GD (2008) Biofilms and chronic infections. JAMA 299:2682–2684.

Wallace L. Guess, S. Alan Rosenbluth,Beverly Schmidt andJohn Autian, (1965) Agar diffusion method for toxicity screening of plastics on cultured cell monolayers, Journal of Pharmaceutical Sciences. 54, 1545–154.

Xiao, C., Zhou, G., (2003) Synthesis and properties of degradable poly(vinyl alcohol) hydrogel. Polymer Degradation and Stability 81: 297–301.

Yang, D., Zhang, J. Z., Fu, S., Xue, Y. & Hu, J. (2010) Evolution process of polymethacrylate hydrogels investigated by rheological and dynamic light scattering techniques. Colloids and Surfaces A: Physicochemical and Engineering Aspects 353, 197-203.

Yang, J.M., Su, W.Y., Leu, T.L., Yang, M.C., (2004) Evaluation of chitosan/PVA blended hydrogel membranes. Journal Membranes Science 236, 39–51.

Yang, X., Liu, Q., Chen, X., Yu, F., Zhu, Z., (2008) Investigation of PVA/ws-chitosan hydrogels prepared by combined gama-irradiation and freeze–thawing. Carbohydrates Polymers 73, 401–408.

Yannas, (1985) Process for Forming Multilayer Bioreplaceable Blood Vessel Prosthesis. U.S.Patient. 4:787-900.

Yannas, Burke, J.F., Orgill, D.P., Skrabut, E.M., (1983) Method of Promoting the Regeneration of Tissue at aWound.U.S. Pat. 4,418,691.

Yannas, Forbes, M.J., (1982) Procedures for Preparing Composite Materials from Collagen and Glycosaminoglycan. U.S. Patient 4,350,629.

Yannas, Gordon, P.L., Huang, C., Silver, F.H., Burke, J.F., (1981)Crosslinked Collagen-Mucopolysaccharide Composite Materials.U.S. Patient. 4,280,954.

Yoshii F, Zhanshan Y, Isobe K,Shiozaki K, Makunchi K. (1999)Electron beam crosslinked PEO and PEO/PVA hydrogelsfor wound dressing. Journal of Radiation Physics Chemistry. 55(5): 133–8.

Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N, Khademhosseini A.,( 2015) Synthesis, properties, and biomedical applications of gelatine methacryloyl (GelMA) hydrogels. Biomaterials. 73:254-71.

Zhao, L., Mitomo, H., Zhai, M., Yoshii, F., Nagasawa, N., Kume, T., (2003) Synthesis of antibacterial PVA/CM-chitosan blend hydrogels with electron beam irradiationCarbohydrates. Polymers. 53: 439–446.

Zhao, Q. S., Ji, Q. X., Xing, K., Li, X. Y., Liu, C. S. & Chen, X. G. (2009) Preparation and characteristics of novel porous hydrogel films based on chitosan andglycerophosphate. Carbohydrate Polymers 76: 410-416.

Zmora, S., Glicklis, R., Cohen, S., (2002) Tailoring the pore architecture in 3-D alginate scaffolds by controlling the freezing regime during fabrication. Biomaterials 23: 4087–4094.

Zuo, GY., Zhang J.X., Yang X.C., Han J., Wang C.G., and Qi-Bian Z., (2012) Evaluation of traditional Chinese medicial plants for anti-MRSA activity with reference to the treatment record of infectious diseases, Molecules,17: 2955-2967.

Zhao G, Hochwalt PC, Usui ML, Underwood RA, Singh PK, *et al.* (2010)Delayed wound healing in diabetic (db/db) mice with *Pseudomonas aeruginosa* biofilm challenge: a model for the study of chronic wounds. Wound Repair Regeneration 18: 467–477.