# Development of SA/PEG with Ag/ZnO Nanoparticles Hydrogel Membrane for Wound Dressing


**By**

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**2023**

# Development of SA/PEG with Ag/ZnO Nanoparticles Hydrogel Membrane for Wound Dressing

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**MS in Chemical Engineering**

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**H-12, Islamabad, Pakistan**

**August, 2023**

## THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS thesis written by Mr **Sohail Zafar** (Registration No 00000362996), of School of Chemical & Materials Engineering (SCME) has been vetted by undersigned, found complete in all respects as per NUST Statues/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

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## MASTER'S THESIS WORK

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| S/No: | Code: | Title: | Core/Elective: | CH: | Grade: |
|---|---|---|---|---|---|
| 1. | CHE-847 | Chemical Kinetics& Reactor Design | Compulsory | 3.0 | B |
| 2. | CHE-843 | Separation Processes In Che | Compulsory | 3.0 | B+ |
| 3. | RM-898 | Research Methodology | Additional | 2.0 | Q |
| 4. | ENE-809 | Waste Water Treatment & Design | Elective | 3.0 | A |
| 5. | EME-921 | Momentum Heat & Mass Transfer In CHE | Compulsory | 3.0 | A |
| 6. | CHE-873 | Membrane Technology | Elective | 3.0 | A |
| 7. | CHE-814 | Product Technology | Elective | 3.0 | B+ |
| 8. | ENS-830 | Cleaner Production Technologies | Elective | 3.0 | A |
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FORM TH-4

## MASTER'S THESIS WORK

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Title: Development of SA/PEG with Ag/ZnO Nanoparticles Hydrogel Membranes for Wound Dressing.

Presented on: 28 Sep 2023 at: 1430 hrs in SCME Seminar Hall

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# Dedication

By the grace of Almighty Allah, who is the most Beneficent and the most merciful

This research is dedicated to my parents, who have always been my source of guidance and support.

To my supervisor who shared his knowledge, gave advice, and encouraged me to fulfill my tasks.

And to all my fellows, with whom I worked with and shared good memories.

# Acknowledgements

All praises to Almighty Allah, without His will nothing can happen, who favored us with the capacity to think and made us anxious to investigate this entire universe. Incalculable greetings upon the Holy Prophet Hazrat Muhammad (PBUH), the reason for the creation of the universe and wellspring of information and blessing for whole humankind.

From the core of my heart, I am thankful to my research supervisor, Dr. Bilal Khan Niazi for her unwavering technical and moral support and enlightening me with a research vision and pushing me for excellence. Her quest for perfection and excellence had been a source of inspiration and driving force. It is her consistent and encouragement that empowered me to achieve this onerous milestone.

I extend my sincere gratitude towards my guidance and committee members: Dr. Usman Liaqat, Dr. Zaib Jahan, and Dr. Tahir Baig for guiding and supporting me in my research course. It would not have been possible without them. I express my gratitude to all committee members for sharing his knowledge and experience regarding research work.

I am thankful of My Seniors who shared their knowledge regarding experimental techniques, and they motivated me in this entire research work. Without any doubt, SCME's supporting staff coordinated with me while I was working on different equipments.

I am highly obligated to my Parents and siblings for their never-ending love. Thanks for believing in me, wanting the best for me, and inspiring me to follow my passion.

**Sohail Zafar**

# Abstract

The present study was focused on the fabrication and characterization of polymeric wound dressings, composed of sodium alginate and Polyethylene glycol. Calcium chloride was added to the polymeric solution for cross-linking. Silver and zinc oxide nanoparticles were incorporated into the formulated hydrogel as an antibacterial agent. The hydrogels were synthesized via the solution casting and tested against different characterization techniques i.e. XRD, SEM, FTIR, and TGA. The XRD pattern of Sodium alginate and polyethylene glycol hydrogel denoted by SAPEG observed peaks 9.8°, 19.4°,23.1°, and 26.5° were related to the face-centered structure of sodium alginate and PEG hydrogel. The SEM results showed the phase separation between SA and PEG membranes. Furthermore, nanoparticles were also homogeneously dispersed in the hydrogel. The mass loss of pristine SAPEG hydrogel membrane is 100% at 31°C. However, the mass loss is 15.5% at 743°C. The maximum tensile strength was 30.8 MPa for SAPEG/0.01Ag/0.02ZnO hydrogel membrane. The effective results of cytotoxicity were of hydrogel membrane SAPEG/0.01Ag/0.02ZnO. The contact angle was reduced after the incorporation of nanoparticles from 27.5° to 15.6°. The antibacterial activity was investigated against Escherichia coli and Staphylococcus aureus. The maximum zone of inhibition was 15 ± 0.67 achieved against E.coli for SA/PEG/0.03ZnO. The maximum zone of inhibition was 27.25 mm achieved against S.Aureus for SA/PEG/0.01Ag/0.02ZnO. The formulated hydrogel membranes have demonstrated the capability to be used as a wound dressing.

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# Acronym

SA Sodium Alginate

PEG Polyethylene Glycol

SEM Scanning Electron Microscope

FTIR Fourier Transmission Infrared Spectroscopy

TGA Thermogravimetric Analysis

AFM Atomic Force Spectroscopy

UV Ultraviolet Spectroscopy

# Chapter 1

# Introduction

## 1.1 Background

Wound healing is a complex biological process that aims to restore the structure and function of damaged tissues. Chronic wounds can be classified as sores or ulcers present a significant challenge in the medical field due to their slow healing and high risk of infection. Traditional wound dressings have limitations in promoting efficient healing, necessitating the development of innovative and effective wound-healing strategies. Hydrogels holds certain important properties such as biocompatibility, moisture retention and rapid wound healing. The main purpose of this study is to prepare wound healing hydrogels by using the polymers named as sodium alginate and polyethylene glycol with incorporated hydrogels [1].

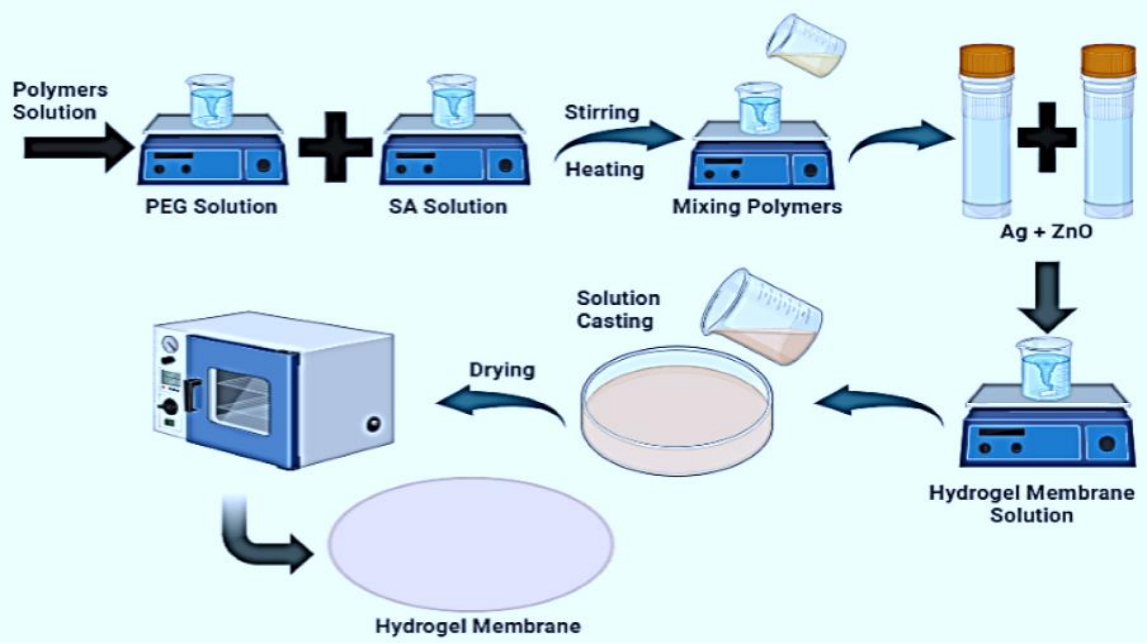


**Figure 1:** Hydrogel membrane solution casting process [1]

Hydrogels are made up of polymers that show properties of biocompatibility, biodegradability, moisture retention, and the ability to absorb water and exudates for efficient wound healing. Mainly, the three-dimensional hydrogel is composed of both physical and chemical crosslinking which allows it to show moisture retention properties for wound healing without dissolving itself into water [2]. The composed hydrogel is hydrophilic in nature. Moreover, wound healing has been seen as a critical process that contains several phases in which matrix mechanisms perform together for the growth and redevelopment of tissues [3]. The wound-healing process is classified into four major stages such as homeostasis, inflammation, proliferation, and remodeling [4]. It should be essential to ensure effective care of the wounds as they could be worse if do not treat them properly within time. This composed hydrogel is effective for wound healing because of its accelerated wound healing property, biocompatibility, moisture retention, mechanical strength, and antibacterial properties. According to a report, the United States invests 20 billion dollars every year in chronic wound management [5].

Furthermore, hydrogel membrane act as a moisture retention and remove damaged tissue to heal wound [6]. The cross-linked polymer structure in hydrogel holds the potential to swell in the form of gel mass when it absorbs the exudates and retains them while isolating the bacteria, odorous molecules, and detritus from the exudate [7]. Their aqueous contents support to diffuse the oxygen and vapor into the wound thus providing a soothing effect [8].

The novelty of the present research can be described as thermo-responsive hydrogels synthesized for wound healing by using an effective polymers such as sodium alginate and polyethylene glycol [9]. The sodium alginate shows an important function in healing mechanism and PEG makes good film which is water soluble and hydrophilic polymer [10]. In addition, sodium alginate shows efficient characteristics such as the safety of wounds from the bacterial burden, giving a moisture retention property to wounds, supports re-modeling and it express significant properties which are useful for wound healing such as rapid healing of wound, biodegradability and biocompatibility [11]. On

the other hand, polyethylene glycol is the hydrophilic and water-soluble polymer and these products can be called as polyethylene oxides [12].

Additionally, the change of structure and characteristics of Sodium alginate/ Polyethylene glycol hydrogel was recorded by (TGA) and (SEM). This hydrogel was made by using a solution-casting process [8]. The silver and zinc oxide nanoparticles were incorporated into the hydrogel membranes at different compositions to increase the tensile strength and antibacterial properties of hydrogel membranes for wound healing applications.

Moreover, the synthesized hydrogels have potential to show effective wound dressing by using conjugated polymers with incorporated nanoparticles [13]. Different characterization was done such as SEM, AFM, XRD, FTIR, UV spectroscopy, ultimate tensile strength, gel fraction, thermogravimetric analysis, antibacterial activity testing, and cytotoxicity test done to investigate hydrogel membrane properties. The wound healing effectiveness of hydrogels was assessed with the help of vitro study [14].

## 1.2 Problem Statement

- Hydrogels possess unique advantages such as lightweight, stretch-ability, biocompatibility, and biodegradability
- In previous hydrogels still, there is need to achieve higher mechanical properties in wound dressing hydrogel
- Previous hydrogels are not giving faster wound healing, mechanical properties, and antimicrobial property collectively.

## 1.3 Research Objectives

To overcome the existing challenges in hydrogel wound dressing, the following objectives were identified. The objectives of this research are given below:

- Synthesis of PEG/Sodium Alginate with Ag/ZnO hydrogel.
- Investigation of Physical, Morphological and Mechanical Properties of formulated hydrogel.
- Antimicrobial and cytotoxicity investigation of formulated hydrogel.

## 1.4 Scope of Study

The following scope was established to ensure that the research would be carried out in the time available

- The synthesized hydrogels made of sodium alginate and polyethylene glycol hydrogel membrane for wound healing application have valuable place in regenerative medicine.
- This research will play a significant role in medical field by introducing a novel biomaterial that has a potential to serve as an advanced wound dressing with antibacterial and mechanical characteristics.
- This hydrogel will also offer a cost-effective and clinically feasible solution for the treatment of wounds and an increased quality of life for affected individuals.

## 1.5 Rationale

- Sodium alginate can be defined as a natural biopolymer which shows effective properties such as biocompatibility, biodegradability, and hydrophilic nature. These properties makes sodium alginate effective for wound healing applications [15]. While, Polyethylene glycol can be defined as a water-soluble synthetic polymer that is used for different biomedical purposes because of its potential to increase material properties. Moreover, mixing polyethylene glycol with sodium alginate leads to the hydrogel with biodegradability, and antibacterial properties [16].

## 1.6 Chapter Summary

The thesis has been mainly designed in the following way

- **Chapter 1**: In the first chapter discusses the theoretical aspects of polymer synthesis-based studies.
- **Chapter 2**: It provides a comprehensive review of the literature on hydrogels, wound healing, and the properties of sodium alginate and polyethylene glycol.
- **Chapter 3:** It details the methodology and experimental procedures used in

preparation hydrogels.

- **Chapter 4**: In Chapter 4, all the outcomes and results of the characterization of hydrogel membranes synthesis and characterizations. At the end the conclusion of this research and future guidelines of research are presented.
- **Chapter 5**: Conclusions and future recommendations of the research are presented in the last part of the thesis.

# Chapter 2

# Literature Review

## 2.1 Hydrogel

### 2.1.1 Background

Hydrogels can be defined as the 3-D network of polymers which have ability to absorb and sustain huge amount of water [18]. Hydrogels have been remained in demand because of their versatile properties including biodegradability and biocompatibility [19]. They are cost effective and gives better wound healing rates. Hydrogels have been showing good characteristics in releasing drug. Many studies have shown that hydrogels with effective composition and polymers are good for wound dressing rather than basic wound dressing. Those hydrogels are useful in tissue regeneration on wound site and provides good healing rate to the wounds [20].

This chapter provides a comprehensive review of the existing literature related to hydrogels, wound healing, and the properties of sodium alginate and polyethylene glycol. The aim is to establish a strong theoretical foundation for the development and application of a sodium alginate and polyethylene glycol hydrogel for advanced wound healing.

### 2.1.2 Wound Healing Mechanisms

It has been remained crucial to develop effective hydrogels for wound healing as the process of wound healing is complex. There are different stages with different requirements are involved in wound healing mechanism. The first stage in wound healing is hemostasis where the bleeding starts from the wound. The second stage in wound healing is inflammation where the wound starts to swell and the third stage in wound healing mechanism is proliferation where the tissues gets together on the surface of wound site. Moreover, the last stage in wound healing is migration or remodeling in which tissues or cells regenerate for wound healing [21]. Blood clotting occurs to prevent excessive bleeding. In the inflammation phase, immune cells, growth factors, and cytokines are

released to remove debris and initiate the repair process. The proliferation phase involves the formation of new tissue through cell proliferation and angiogenesis. Finally, during remodeling, the wound undergoes structural reorganization to improve its strength and function. Hydrogels can influence various stages of wound healing by providing a conducive microenvironment for these processes [22].

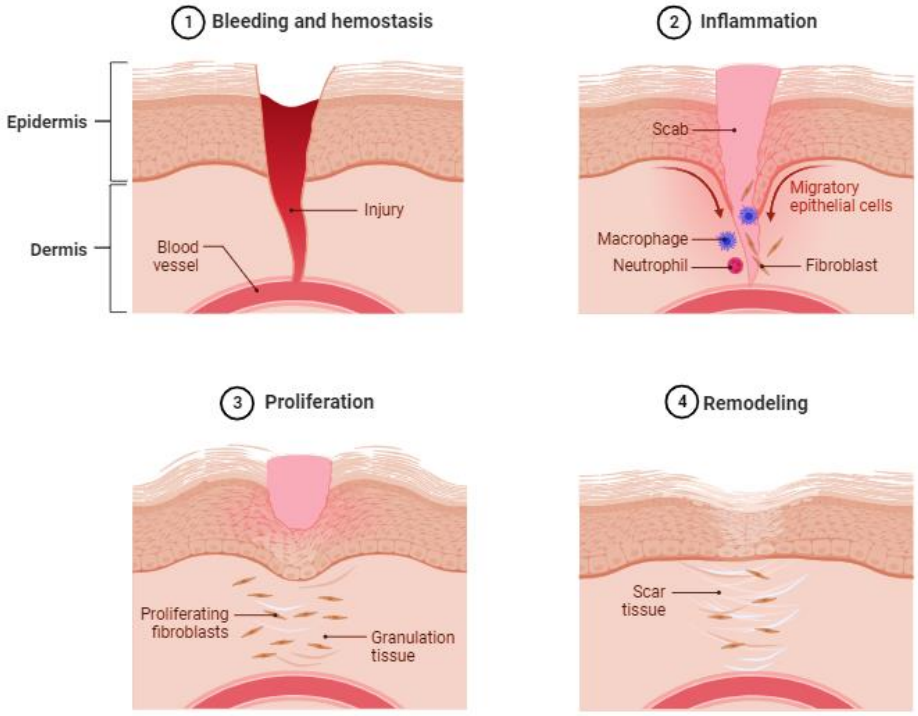


**Figure 2:** Wound healing Mechanism [22]

### 2.1.3 Sodium Alginate in Wound Dressings

Sodium alginate can be defined as the polymer that is taken from brown algae. Sodium alginate hold many properties related to wound healing. It is an effective polymer in terms of wound healing. The major properties of sodium alginate are biocompatibility and strength. Moreover. Calcium chloride ion crosslinking is essential with sodium alginate in order to make a beneficial wound dressing hydrogel.

These properties make sodium alginate an ideal material for wound dressings. When applied to wounds, sodium alginate hydrogels form a protective barrier that absorbs

exudates, maintains a moist environment, and aids in wound debridement. Additionally, its biodegradable nature allows for the gradual release of encapsulated therapeutic agents, contributing to enhanced wound healing [23].

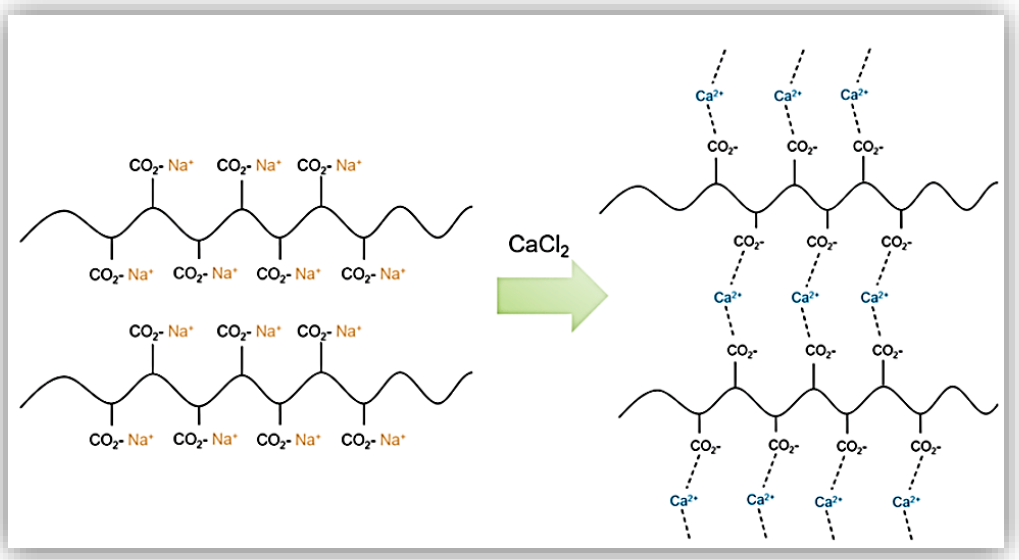


**Figure 3:** Sodium alginate polymeric structure [23]

### 2.1.4 Polyethylene Glycol in Biomedical Applications

Polyethylene glycol can be defined as the most important synthetic polymer which has been used for different purposes such as in membranes preparation for water filtration and in hydrogels preparation for biomedical purposes. There are many uses of PEG polymer are available. It has significant properties as it shows characteristic of good film, strength and it is also not poisonous which is good for hydrogel applications. The polyethylene glycol have number of various grades and every grade have different properties for certain application. Moreover, polyethylene glycol have been showing efficient characteristics regarding wound healing purposes. The PEG can be used to make blend with different natural or synthetic polymers. The PEG has ability to show strength, biocompatibility, cost effective and effective for wound healing use [24].

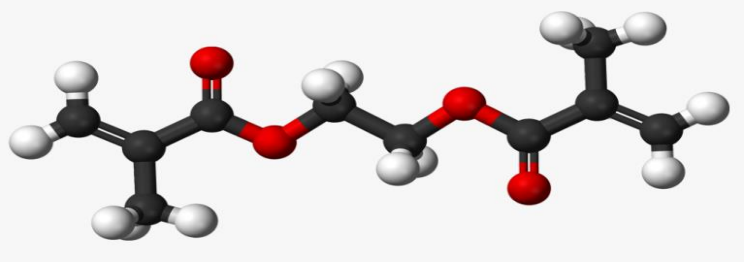

**Figure 4:** Polyethylene glycol polymeric structure [24]

### 2.1.5 Composite Hydrogels for Wound Healing

It has been seen that the composite hydrogels has been playing an critical role in wound healing applications. As these composite hydrogels increases strength of hydrogels with their antibacterial property as well which is good for wound healing purposes. The composite hydrogel can be developed by making blend of two or more than two different polymers. Furthermore, it also has been seen that the composite hydrogels releases impact that strength the individual components capacity. In addition, it is good to make a blend of PEG with sodium alginate because it enhances hydrogel antibacterial, mechanical and biocompatibility properties. Many reported researches has shown that the composite hydrogels are good for wound healing application because of their extensive useful properties [25].

### 2.1.6 Properties of Hydrogel

Hydrogels have been gaining importance because of their versatile properties related to wound dressing and their vast applications such as environmental and pharmaceutical applications. [26]. The key properties of hydrogels include:

**Hydrophilicity**

Hydrogels have a most important characteristic named as moisture retention which is useful to absorb water molecules and to sustain a huge quantity of water. This property creates a moist environment when applied to wounds, promoting cell migration, proliferation, and tissue regeneration [27].

**Biocompatibility**

Most hydrogels are biocompatible, meaning they are well-tolerated by living tissues and do not elicit significant immune responses or toxicity. This property is essential for their use in wound healing [26].

**Swelling Behavior**

Hydrogels have one more unique characteristic that they used to become swelled in the existence of water. But one can control the swelling b behavior of hydrogel by controlling composition of hydrogel. It has been counted as an benefit in wound healing hydrogels because it gives moisture retention to the wound and supports wound to heal rapidly.

**Mechanical Properties**

The mechanical strength and elasticity of hydrogels can be tailored to suit applications. Crosslinking density and the choice of polymers influence the hydrogels. Some hydrogels possess excellent mechanical properties comparable to natural tissues bearing uses in tissue engineering [27].

**Biodegradability**

Many hydrogels are biodegradable, meaning they can be broken down and absorbed by the body over time. Biodegradability is advantageous for wound dressings and scaffolds, as it permits the hydrogel to gradually degrade as tissue heals or regenerates.

**Porosity**

Hydrogels can be designed with a controlled porous structure, permitting diffusion of oxygen, and waste products in tissue engineering applications. The porous nature of hydrogels also facilitates cell infiltration and tissue integration.

**Thermoresponsiveness**

Many hydrogels have been showing thermo responsive characteristics and this characteristic had opened the door of development of advanced hydrogels in purpose of

temperature variations. Moreover, the thermo responsive hydrogels also offers good properties. As they are effective in slow drug release applications.

**Adhesive Properties**

Many particular hydrogels have been remained in use for regenerating biological tissues by giving efficient properties of tissues regeneration and biocompatibility with cells. In addition, this characteristic is most beneficial property for wound healing hydrogels as it supports hydrogel dressing in protection to get harm from any kind of cracking and it also increase the satisfaction of patients.

**Controlled Drug Release**

Control drug release is another kind of application for this purpose hydrogels are also used with certain materials and composition. Moreover, the hydrogels has been giving efficient pathways for drug releasing. Furthermore, control drug release applications have importance in many serious injured patients who need treatment with slow drug release rate. In the end, the hydrogels developed for control drug release are also good for therapeutic agents in an effective manner based on certain requirements of applications.

**Versatility**

Hydrogels have been remained effective because of their synthesized ability. This versatility allows researchers to tailor hydrogels for specific applications, making them adaptable for diverse uses in medicine, biotechnology, and environmental sciences.

Overall, the unique combination of hydrophilicity, biocompatibility, swelling behavior, mechanical properties, and tunable drug release capabilities makes hydrogels a promising and valuable class of materials with vast potential in various fields.

## 2.2 Classifications of wound healing hydrogel

Wound healing hydrogels can be classified based on various criteria, including composition, and intended application. Here are some common classifications of wound healing hydrogels [28]:

### 2.2.1 Composition-based classification

**a) Synthetic hydrogels:**

The synthetic hydrogel can be seen an a type of hydrogels and it is basically developed from different polymers named as PEG and PVA etc. These polymers are good in properties and gives a unique characteristic for wound dressings.

**b) Natural hydrogels:**

Natural hydrogels are derived from biopolymers found in nature. Examples include hydrogels based on alginate, chitosan, collagen, hyaluronic acid, and gelatin. These hydrogels are biocompatible, biodegradable, and may exhibit inherent characteristics for wound healing [29].

### 2.2.2 Origin-based classification

**a) Plant-based hydrogels:**

Hydrogels derived from plant-based sources, such as alginate extracted from brown algae, are commonly used in wound dressings.

**b) Animal-derived hydrogels:**

Hydrogels derived from animal sources, such as collagen extracted from animal tissues, can provide a scaffold that mimics the natural extracellular matrix, supporting cell migration and tissue regeneration in wound healing.

**c) Synthetic hydrogels:**

As mentioned earlier, these hydrogels are entirely synthetic and are produced through chemical processes, providing precise control over their properties.

### 2.2.3 Application-based classification

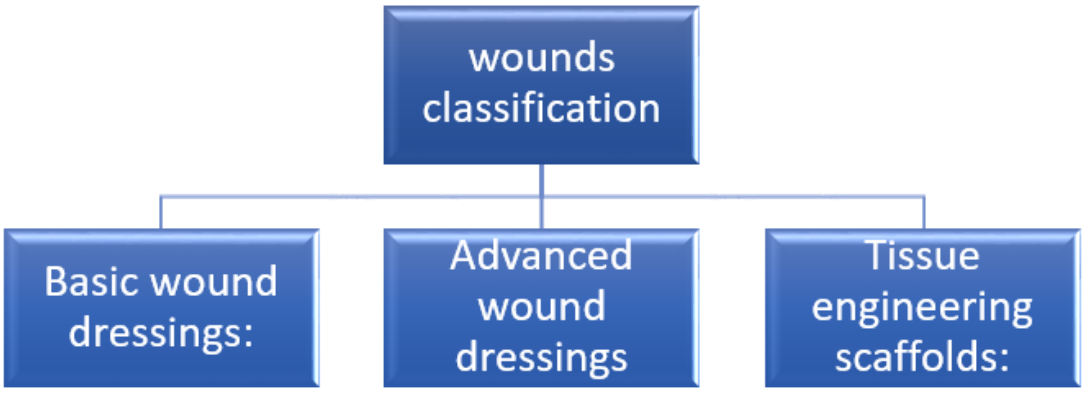


**Figure 5:** Wound classification hierarchy [28]

**a) Basic wound dressings:**

The hydrogels used for basic wound healing are different and they are developed in a different way with different certain properties of polymers. These types of hydrogels gives an faster healing rate and moisture retention and protect the wound from surrounding environment.

**b) Advanced wound dressings:**

Advanced hydrogel dressings may incorporate additional features such as antimicrobial agents, growth factors, or nanoparticles for controlled drug delivery to enhance wound healing outcomes.

**c) Tissue engineering scaffolds**

It is very important to understand that different hydrogels have been prepared for certain application as no one single hydrogel can fulfill all requirements. So hydrogels have been developed on the basis of applications. Thus, hydrogels for tissue regeneration are designed in this way that they will be useful in the second stage of wound healing which is proliferation in which the tissue regenerate on the surface of wound for better wound healing.

### 2.2.4 Crosslinking-based classification

**a) Physical hydrogels:**

There are two different major methods to cross-linked hydrogels. The first one method is called as physical crosslinking. In this method, the hydrogel are cross-linked and prepared with the help of physical crosslinking between different bonds of polymers and it is also a good way to cross link polymers for better efficiency in terms of gel fraction and contact angle of hydrogel membranes. The main forces used in the physical crosslinking are Vander Waals forces. These prepared hydrogels are effected by pH because of their responsive nature.

**b) Chemical hydrogels:**

The second method to prepare or cross-linked hydrogel is known as chemical cross-linked method. In this method, the polymers of hydrogel are chemically cross-linked. The bonding in chemical cross-linked hydrogels is covalent bonding and they used to form efficient smooth network between bonds. According to the reported research studies, these hydrogels are more effective and can be used for long term use. These hydrogels can be preserved. Furthermore, it should be important to understand that the hydrogels are specific for different wound healing applications. For example, hydrogels for third degree wounds are different while hydrogels for simple wounds are different. It all depends on the application and their composition. It means that every hydrogel have some kind of limitation. In addition, the selection of hydrogels have an important effect on wound healing application and it's have a serious effect in the healing treatment. A right hydrogel with right composition can heal the wound rapidly as compared to unbalanced composition hydrogel [29].

## 2.3 Preparation methods of hydrogel membrane

**Preparation methods of hydrogel membrane**

Hydrogel membranes have been developed with the help of different useful method and every method have some advantages as well as limitations. Some important methods for the development of hydrogel have been shown below [30].

**Solution Casting**

The solution casting method has been remained well known among all other methods. This method is easy to handle and to prepare hydrogel membranes. This method contains on the principle of mixing polymers solutions such polyethylene glycol and sodium alginate. After mixing properly and heating at the desired temperature conditions the solutions has to be placed or casted on the petri dishes in a specifics amount. After that the petri dished places in the oven for drying at required temperature. In the end, as all the moisture will evaporated and the hydrogel is cross-linked properly then the final hydrogel membranes will be obtained [31].

**Electrospinning**

The electro spinning can be described as a tool that have been used to develop Nano fiber hydrogel. It is a method in which the prepared solution of polymer injects into the syringe in a specific amount and pass through the electric field. After that the syringe solution will be putted on the collector plate. After some time the hydrogel liquid present in it will be successfully evaporated and left behind a dry hydrogel. Moreover, the method named as electro spinning permits for the membranes fabrication as well but it required more surface. In other words, it is good for wound healing applications [31].

**Freeze-Drying**

The other effective method for the preparation of hydrogel membranes which are porous is freeze drying method. It has been sued to prepare hydrogel membranes from many years. In this method, the solution of polymers freezes to make it solid and the solid hydrogel solution will be passed through the specific process named as sublimation to remove the liquid or moisture e contents. The sublimation will be done under low pressure for efficient process. The obtained hydrogel will be porous in nature and good for water retaining and cell infiltration purposes.

**Photopolymerization**

Photopolymerization can be defined as a toll which is used for membrane preparation. In this method, the crosslinking of polymers of hydrogel will be done with the help of light initiator. Mainly, the solution of hydrogel membranes will be dissolved with the photointiator and placed in open area where the wavelength of light will approached it and the crosslinking of the hydrogel solution will be started. This method have several advantages as one of the major advantage of this method is that it controls the hydrogel content and can make patterned hydrogels.

**Dip Coating**

Dip coating method is an effective method for hydrogels preparation. This method can be defined as the hydrogel membranes fabrication done on solid substrates. Basically, the substrates places into the solution of polymers and then after some time the substrates will be removed from the hydrogel solution at slow rate. Furthermore, the hydrogel will developed a thin layer on the surface of placed substrate and with the passage of time moisture will be evaporated. The solution will become gel type. Thus the method dip coating have been permitting to develop smooth and uniform hydrogels.

**Template Synthesis**

Another efficient method for hydrogel development is template synthesis. In this method, a sacrificial template is used to develop membrane but afterwards this template will be withdrawn. It gives membrane a certain shape. Moreover, the templates are available in different natures such as solid or scaffold etc. After that the precursor will be placed near to the template and permitted it to form gelation. As soon as the hydrogel develops the template will be withdrawn from it. And the final product hydrogel membranes will be available. Furthermore, these methods have been remained cost effective and efficient in forming hydrogels with different shapes and sizes. No doubt the selection of method of hydrogel preparation is depends on the application [32].

## 2.4 Natural and synthetic hydrogels

Natural and synthetic hydrogels has been classified as two different categories of hydrogels. Both have their own limitations and applications. The comparison between natural and synthetic hydrogels are given below:

### 2.4.1 Natural Hydrogels

**Composition**

Natural hydrogels are good for wound dressings. These types of hydrogel have been drawn from those polymers that are available in nature. The examples of these types of polymers are SA, PEG, Chitosan, gelatin [33].

**Biocompatibility**

The natural hydrogels have been gaining important in pharmaceutical industries because of their properties. Natural hydrogels have been counted as biocompatible and moisture retention hydrogels and they do not damage cells or tissues. These properties of natural hydrogels made them effective for wound healing applications. These hydrogels have been playing their role in tissue remigration applications as well [33].

**Bioactive Properties**

Many natural hydrogels contains efficient properties including proliferation of cells, tissues recreation. The good example of bioactive material hydrogel is collagen-based hydrogels integration [19].

**Swelling Behavior**

The swelling behavior is an important aspect of every hydrogel. The natural hydrogels have been showing swelling behavior in the existence of fluid. It shows that how much the hydrogel can absorb water or swell due to retained water [34].

**Ease of Modification**

The ease of modification is the most important thing when we prepare hydrogel membranes. Its process should be easy to handle and cost effective. On the other side. It

has been seen that these hydrogels have some limitation rather than to synthetic hydrogels [19].

### 2.4.2 Synthetic Hydrogels

**Composition:** Synthetic hydrogels are entirely man-made and are created from synthetic polymers, such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyacrylamide, and poly(N-isopropylacrylamide) [36].

**Customizable Properties:** Synthetic hydrogels offer more control over their composition, crosslinking density, and mechanical properties. This enables precise tuning of the hydrogel's characteristics to suit specific applications.

**Mechanical Strength:** Hydrogels for wound healing should have ultimate tensile strength so that those hydrogels will be able to tolerate the stress of during applying on the human skin and also should have biocompatibility [36].

**Consistency and Purity:** Synthetic hydrogels can be produced with consistent purity, minimizing the risk of impurities or allergens compared to natural hydrogels [20].

**Biocompatibility:** While many synthetic hydrogels are biocompatible, their biocompatibility may vary depending on the specific polymer used and the presence of any residual monomers or crosslinking agents [37].

In summary, natural hydrogels offer inherent bioactivity and biocompatibility, while synthetic hydrogels provide greater strength. The choice between natural and synthetic hydrogels depends on the specific application and the desired characteristics of the hydrogel for the intended use. In some cases, a synthetic components may be employed to achieve synergistic effects and create hybrid hydrogel materials.

## 2.5 Physical and chemical cross linking hydrogels

Hydrogels can be cross-linked using physical or chemical methods, each imparting distinct properties to the resulting hydrogel. Here's a comparison of physical and chemical crosslinking methods [38]:

### 2.5.1 Physical Crosslinking

**Definition:** Physical crosslinking involves creating temporary links or associations between polymer chains without forming covalent bonds. These interactions rely on physical forces.

**Reversibility:** Physical crosslinks are generally reversible, meaning the hydrogel can swell and deswell reversibly in environmental conditions like temperature, pH, or ionic strength.

### 2.5.2 Advantages

**Mild Conditions:** Physical crosslinking is often conducted under mild conditions, preserving the biological activity of sensitive bioactive molecules or cells encapsulated within the hydrogel.

**Biocompatibility:** Physical crosslinked hydrogels are often biocompatible, making them suitable for biomedical applications.

**Injectable Hydrogels:** Some physically crosslinked hydrogels can be prepared as injectable formulations, allowing for minimally invasive delivery.

### 2.5.3 Disadvantages

**Lower Mechanical Strength:** Physical crosslinked hydrogels tend to have lower mechanical strength and stability compared to chemically crosslinked hydrogels.

**Limited Long-term Stability:** The reversibility of physical crosslinks can lead to reduced long-term stability, which may be a concern for some applications.

### 2.5.4 Chemical Crosslinking

**Definition:** Chemical crosslinking can be defined as the process in which the interaction between different polymer chain occurs and they formed covalent bonds for stable network [39].

**Permanence:** Chemical crosslinks are generally permanent and do not undergo significant reversibility under normal physiological conditions.

### 2.5.5 Advantages

**Enhanced Mechanical Strength:** Chemically crosslinked hydrogels tend to exhibit higher mechanical strength and stability compared to physically crosslinked hydrogels.

**Long-term Stability:** The permanent nature of chemical crosslinks contributes to the long-term stability and durability of the hydrogel.

**Tunable Properties:** The choice of crosslinking chemistry allows for precise control over hydrogel properties, degradation rate, and drug release kinetics.

### 2.5.6 Disadvantages

**Harsher Conditions:** Chemical crosslinking often requires the use of chemical initiators or catalysts and may involve harsh reaction conditions that could affect the encapsulated bioactive molecules or cells.

**Limited Biocompatibility**

It has been observed that some crosslinking agents are poisonous which causes swelling issues in hydrogels or at the wound site after implementation. It makes them limited for use. In other words, the cross-linked hydrogels has been giving reversibility and proper developing indications which makes then good for the wound healing applications or drug release applications. While, the chemical cross-linked hydrogel has been providing better tensile strength, biocompatibility and moisture retention properties. This makes them effective for certain applications. Moreover, the selection of cross linker is totally based on the certain conditions of hydrogel.

## 2.6 Structural components of hydrogels

Hydrogels can be descried as 3-D networks made up of different parts of structure that participates in characteristics of hydrogel. There are many components of structure of hydrogel are available. Some of the main hydrogel components are given below.

**Polymer Chains**

Basically, the polymer chains is also known as the main backbone of hydrogel membranes. As these polymeric chains has been remained responsible for tensile strength, stability of hydrogel and effectiveness.

**Solvent or Swelling Medium**

The swelling mechanism is another important property of hydrogel in this property the hydrogels gain ability to absorb and retain water or exudes while applying on the surface of skill to treat wounds. As the hydrogel implements on the skin the exudates will take place in the polymeric chains of hydrogel and the swelling occurs it all depends on the interaction of polymeric chains with the type of solvent.

**Crosslinking Density**

Crosslinking density can be defined as the total number of crosslinks occurred in the polymeric chain network of hydrogel. If the hydrogel have more crosslinking ability in chains then it will be more effective for wound healing applications as it will treat the wound effectively.

**Pores and Void Spaces**

Hydrogels have been recognizing in different forms and in nature. Some hydrogels are porous so it contains pores and void spaces in themselves. Mainly, the effect if pores or void spaces in hydrogels shows that the permeability, swelling behavior and water retaining stability of hydrogel will be effected by increasing or decreasing the pores in the hydrogel.

**Biologically Active Molecules (Optional)**

It has been considered that in many cases the hydrogels can be joined with active molecules includes enzymes, growth factor etc. moreover, these active molecules have ability to get deceived physically and joined with the chains of polymers of hydrogel. It effects the hydrogels controlled release properties.

### Nanoparticles or Nanofibers (Optional)

Many hydrogels get the incorporation of nanoparticles to increase their antibacterial, mechanical and cytotoxicity properties for wound healing purposes. It has been observed that the incorporated nanoparticles have ability to support the structure of hydrogel membranes and to prevent them from any sudden damage. These incorporated nanoparticles increases hydrogel ability to treat wound effectively by raising their antimicrobial properties. The crosslinking density, shape of hydrogel, stability and many other factors are also influenced by the incorporation of nanoparticles.

### Current Challenges in Wound Dressing Technology

Many latest things have been done in wound dressing hydrogels or polymers. Moreover, it is also true that many challenges has been persisting the wound healing techniques. The sever wound forms an important burden on clinical side because of their confrontation to conventional treatments. Thus, these critical challenges has made it essential to create well effective wound dressing by using efficient polymers with nanoparticles for wound dressing purposes.

### Conclusion

This literature review establishes the scientific basis for the development and application of a sodium alginate and polyethylene glycol hydrogel for wound healing. It highlights the potential benefits of hydrogel-based dressings, the properties of sodium alginate and polyethylene glycol, and the importance of composite hydrogels in wound healing research. The reviewed literature forms the foundation for the subsequent chapters, where the proposed hydrogel will be synthesized, characterized, and evaluated for its wound-healing efficacy.

# Chapter 3

# Materials and Methods

## 3.1 Materials

Polyethylene glycol (mol. Wt. = 6000 g/mol), sodium alginate, calcium chloride, and glycerin were purchased from Sigma Aldrich pvt limited, Islamabad. Deionized water was used in the whole experiment. The zinc acetate, sodium hydroxide was purchased from scientific hub pvt limited, Islamabad. While the silver nitrate and sodium borohydride were also purchased from Sigma Aldrich pvt limited, Islamabad. However, the zinc oxide and silver nanoparticles were prepared in the laboratory, chemical engineering department, Nust University, Islamabad. All the chemicals were of analytical grade and used as received without any further purification treatment. All the chemicals were of analytical grade and used as received without any further purification treatment.

## 3.2 Methods

### 3.2.1 Preparation of Hydrogel

The SAPEG membranes were synthesized via the solution casting method by using a constant amount of polymers. The separate solutions were prepared by mixing 1g of sodium alginate in 30ml deionized water and 1g PEG in 15ml in deionized water. Afterwards both solutions were mixed to make SAPEG blend. The required composition of polymers were achieved by mixing both polymers such as Sodium alginate and polyethylene glycol. The polymeric solution was heated at 25 ± 2°C for 30 min. The silver and zinc oxide nanoparticles were also incorporated into the hydrogel membranes. The pure and mixture solutions were cast on Petri dishes by water evaporation. The petri dishes of hydrogel membranes were placed in the oven and dried at 60 ± 2°C. After complete drying the hydrogel membranes were detached from the Petri dishes with different formulations and used for further study. SAPEG with incorporated nanoparticles hydrogels were prepared for wound healing application.

### 3.2.2 Table 1: Composition of Hydrogels

| Hydrogels | Polyethylene Glycol (g) | SODIUM ALGINATE (g) | ZINC OXIDE NPs (g) | SILVER NPs (g) | Deionized Water (ml) |
|---|---|---|---|---|---|
| SAPEG | 1 | 1 | - | - | 45 |
| SAPEG/ZnO | 1 | 1 | 0.030 | 0 | 45 |
| SAPEG/Ag/ZnO | 1 | 1 | 0.020 | 0.010 | 45 |
| SAPEG/Ag/ZnO | 1 | 1 | 0.010 | 0.020 | 45 |
| SAPEG/Ag | 1 | 1 | 0 | 0.030 | 45 |

### 3.2.3 Preparation of ZnO Nanoparticles

The zinc oxide nanoparticles had been prepared in the laboratory by using the effective method named as precipitation method. There were different temperature and stirring conditions while using this method. Moreover, zinc acetate and sodium hydroxide were used as major chemicals for zinc oxide nanoparticles preparation with solvent-deionized water. The 10g of zinc acetate dehydrate ($Zn(CH_3(COO))_2 \cdot 2H_2O$) in 50 mL of deionized (DI) water. Similarly, the solution of sodium hydroxide (NaOH) was prepared by dissolving 2.0 g of NaOH pellets in 50 mL DI water. Both solutions were prepared separately and after their preparation, they were mixed with continuous stirring. The stirring rate was 280 rev $min^{-1}$, while the reaction temperature was varied at 30 and 70°C. Moreover, after the completion of process, the solid white precipitated zinc oxide nanoparticles were separated from the solution and passed through the process of centrifugation. After centrifugation, the nanoparticles were dried and prepared for further use [40].

### 3.2.4 Preparation of Ag Nanoparticles

The chemical reduction method was used to synthesize silver nanoparticles. The sodium borohydride was used in the preparation of silver nanoparticles as a reducing agent. The sodium borohydride solution was prepared in deionized water. A specific amount of solution of sodium borohydride $NaBH_4$ was taken and added drop wise in the silver nitrate ionic solution to prepare the silver nanoparticle. The total sodium borohydride dissolved in silver ionic solution solution was 30mL The reaction mixture was agitated on a magnetic stirrer. The solution turned to light yellow after the addition of 2 mL of silver nitrate and to brighter yellow when all of the silver nitrate had been added.

$$\mathbf{AgNO_3 + NaBH_4 \rightarrow Ag + H_2 + B_2H_6 + NaNO_3} \quad (1)$$

The total time for the addition process was 4 minutes. After that the stirring had stooped and the stir bar had taken out. Furthermore, the parameters of reaction such as time of stirring and amount of reagents should be controlled to obtain stable yellow colloidal silver. The initial color of silver nanoparticles solution was darkish yellow and after some time it turns into grayish color. The silver nanoparticles were settled down in the bottom of solution [41].

## 3.3 Characterization Techniques

### 3.3 .1 X-ray Diffraction

The XRD analysis of hydrogel membranes was conducted to investigate crystal structure and phase identification of hydrogel membranes. The samples were simply cut and placed in an XRD machine to analyze the crystal structure. The membranes had been used directly for the XRD analysis. The voltage and current of the X-ray remained at 40kVmA and 4omA [42]. The step time for scanning the samples was 0.5 s/step and the angle was 2θ ranges from 0∘∘ to 90∘∘. The speed of scanning was 0.02°/min [43].

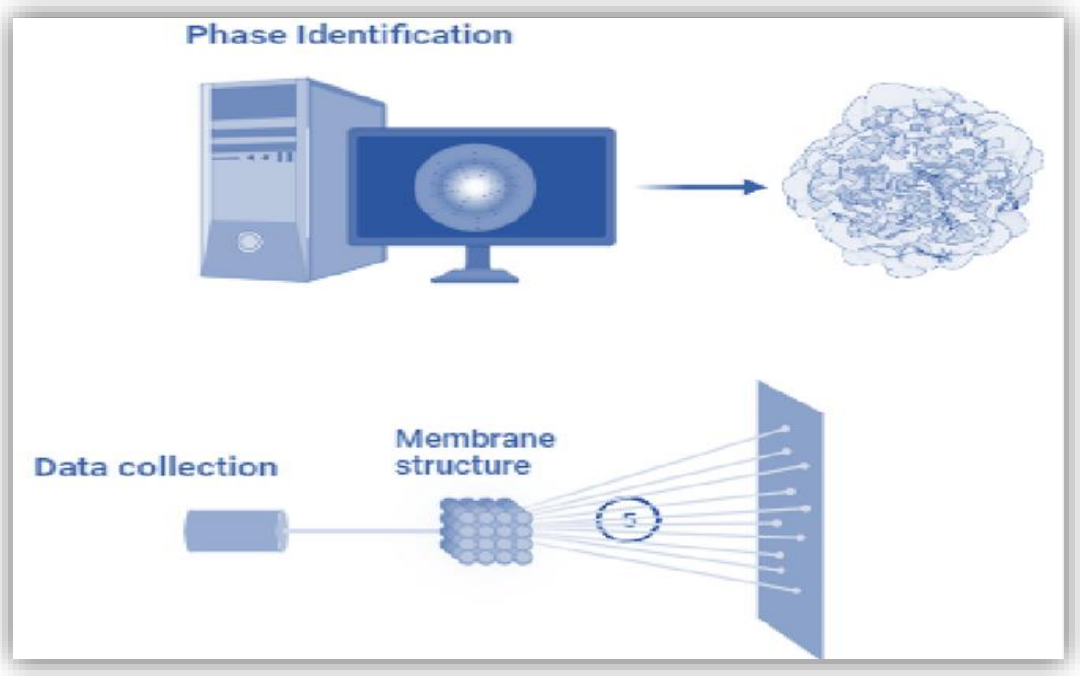


**Figure 6:** X-ray Diffraction Technique [43]

### 3.3 .2 Scanning Electron Microscopy

Scanning electron microscopy (SEM) has been used for the characterization of hydrogel membranes due to its ability to provide detailed surface information and high-resolution imaging [44]. The morphology and structure analysis of the sample, pore size, surface analysis, and cross-sectional analysis of the sample was analyzed by using SEM (JSM-64900). A thin layer of platinum was used to conceal the sample before investigation. The voltage provided to samples was 10 kV and 20 kV.

### 3.3 .3 Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy has been used to investigate functional groups, content of material and to analyze structural characteristics of membranes. The FTIR test gives important information related to properties and composition of hydrogel membranes. The FTIR analysis was done from **450-4000 $cm^{-1}$**. However, the frequency during analysis was **32**Hz and resolution was 4 $cm^{-1}$. The pellets of hydrogel membranes were formed to place in the FTIR machine [45].

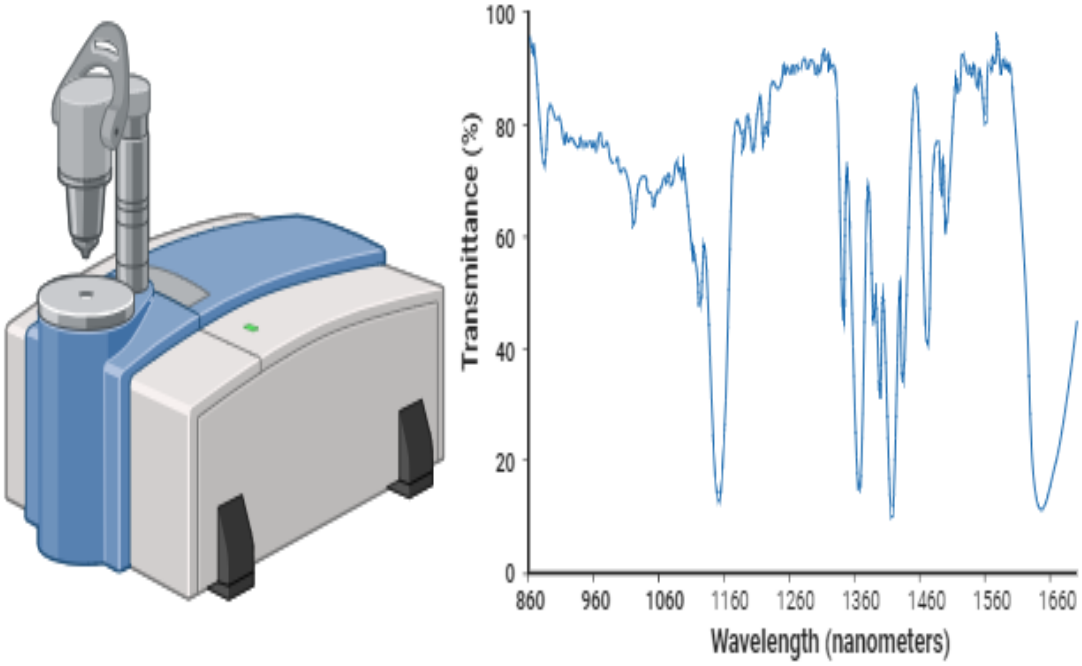


**Figure 7:** Fourier Transform Infrared Spectroscopy [45]

### 3.3 .4 Gel fraction

The gel fraction test had been conducted for hydrogel membranes to analyze crosslinking in the material and it helps to analyze mechanical strength as well as stability of membranes. The synthesized hydrogel membranes were also characterized via gel fraction test. The samples were cut into equal size such as 1x1 cm2. The initial weights (Wi) of all samples were calculated in weight balance and after that constant weight was achieved of the membrane by putting them in oven. Afterward, the samples were putted into deionized water for 96 hrs [48]. Then the samples were withdrawn from water and putted in oven until their constant weight was not achieved. The final weight of samples was Wt. The samples were dried at 37°C temperature. The percentage of gel fraction had recorded with the help of below formula[49].

**Gel fraction % = Wi/Wt x 100**

### 3.3 .5 Thermogravinetric Analysis

The thermogravimetric analysis can be defined as a technique which is used to investigate the thermal stability, decomposition behavior and kinetics of hydrogel membranes. It measures the weight loss of membranes against different temperatures. It also measures heat flow through membranes with respect to time [50]. The thermogravimetric analysis was performed by using a (Q2000 Series) thermal analysis unit [7]. Moreover, the sample weight was between 4 to 5mg. The samples had been removed with dry nitrogen but the temperature was raised from 30 °C to 800 °C with step time (10 °C/min) [13].

### 3.3.6 Ultraviolet Spectroscopy

The ultraviolet spectroscopy technique has been used for analyzing the polymer structure, monitoring cross-linking reactions, assessing polymerization and curing processes, quantifying analyses, and studying stability and degradation. It also gives a transmission analysis of hydrogel membranes against absorbance peaks [51].

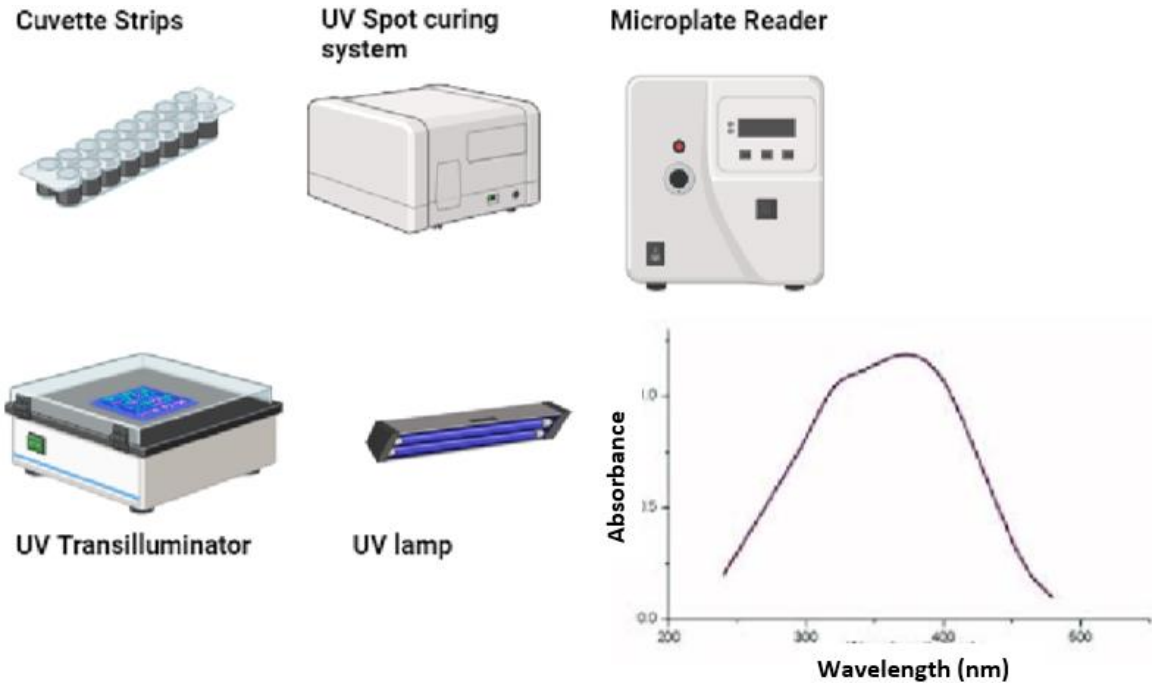


**Figure 8:** Ultraviolet Spectroscopy [51]

### 3.3.7 Tensile Strength

The ultimate tensile strength of hydrogel membranes has been remained an important mechanical property that analyses its potential to withstand pulling forces without any breakage or showing any significant deformation. The prepared hydrogel membranes were cut in standard dimensions with no surface defects and joined with holding clamps of the universal testing machine to place in the machine for investigating tensile strength [52]. The strain rate had remained fixed at 10 $N/mm^2$. However, the supported clamps could move forward direction and rate of stress of formulated hydrogels was noted. The main aim of measuring the tensile strength of a membrane is to investigate the tensile strength of a membrane which is important for its wound healing application.

### 3.3.8 Antibacterial

The antibacterial activity of hydrogels can be investigated by using method named disc diffusion. In this method, two different strains were used. One is positive strain which was Staphylococcus aureus and other is negative which was Escherichia Coli [53]. The main aim of strains was to distinguish between positive and negative bacteria to analyze antibacterial activity. Furthermore, bacterial had grown in test tube holding broth. After that, it was placed into shaking water bath at 37 °C. On the other hand, the solution of agar poured in the petri dishes homogeneously and it had become solidified. The spreading of bacterial was done on plates. The samples of formulated hydrogels was cut into equal dimensions such as 6 mm disks and were located over the agar plates. The petri dishes contained bacterial and hydrogel membrane samples were putted into an incubator for 1 day at 37 °C. Afterwards, the plates had taken out from the incubator and one of inhibition was noted with the help of vernier calipers. One membranes sample was controlled and that was sodium alginate and polyethylene glycol,SAPEG without any incorporated particles. All the instruments used was autoclaved before use to avoid any contamination.

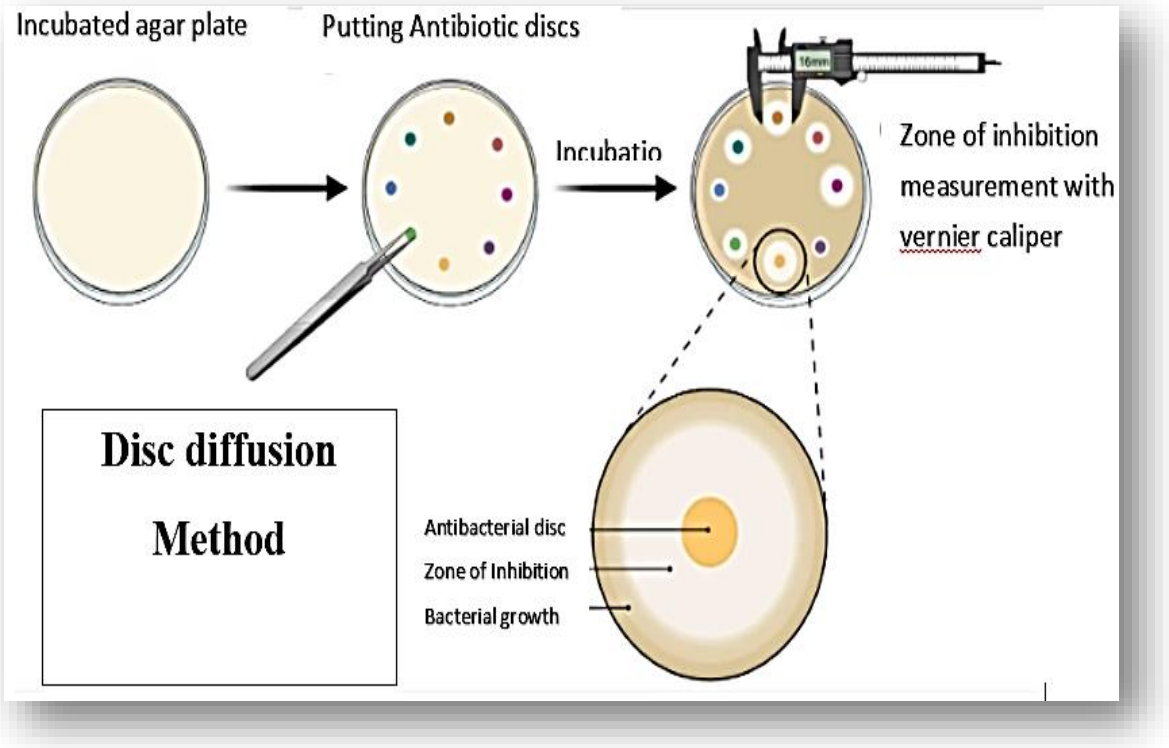


**Figure 9:** Antibacterial Activity by disc diffusion method [53]

### 3.3.9 Contact angle

The contact angle test is used for hydrogel membranes to investigate their surface wettability and interactions with liquids. It forms an angle at a certain point of contact between a liquid droplet and the surface of the hydrogel membrane. This angle provides an important data related to the hydrophilic or hydrophobic nature of the hydrogel membranes [57]. All those hydrogels that shows contact angle smaller than 90 degree are hydrophilic in nature [58].

### 3.3.10 Atomic Force Spectroscopy

The atomic force spectroscopy can be defined as a characterization technique that is useful in analyzing porosity, topography and smoothness of hydrogel membranes. It was done by using Atomic Force Microscopy, JOEL (JSPM-5200). Surface roughness of hydrogel membranes has been remained critical factor to analyze the functioning with respect to

biocompatibility. The 3-D images of hydrogel samples were captured. Furthermore, the surface of samples had contacted with the tip of AFM during the scanning period. There were a repulsive force between them which had provided 3-D images of membranes. For analyzing the roughness of membranes surface a 10 μm × 10 μm area was choose for scanning. Thus, the roughness was analyzed with the help of AFM software. The parameters such as “Ra”, “Rt” and “RMS” showed data related to mean roughness and root mean square roughness. This information is vital for designing and developing hydrogel membranes with improved performance for applications [59].

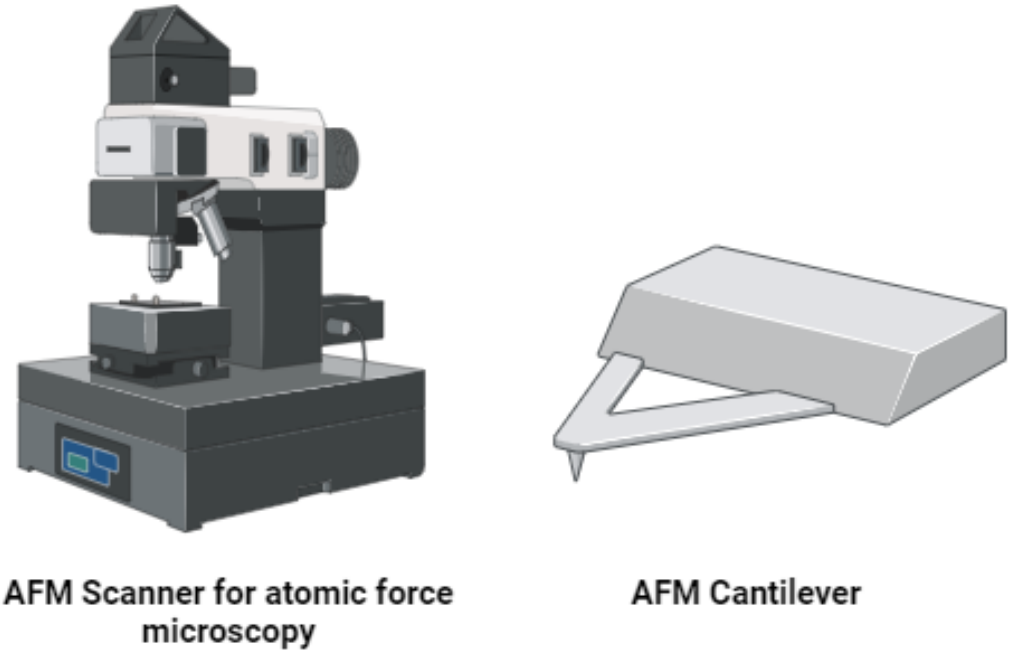


**Figure 10:** Atomic Force Spectroscopy [59]

### 3.3.11 Cytotoxicity

The cytotoxicity test was conducted to analyze that the material present in hydrogels could be harmful for the cells or not. In this cytotoxicity test, the cell culture was grown in which hydrogels were placed to investigate their impact on cells. The cells were taken from L929 fibroblast family and placed into a plate [28, 29]. The cells was supposed to expand in the

prepared liquid named DMEM medium at 37 ℃ for 1 day. Afterward, the pure liquid of DMEM medium was swapped by the extracts and the blank wells were used as control and their mediums were refreshed. After 1 day, 2 days and 3 days the CCK-8 had putted in the wells and incubated at 37 ℃ for 60 minutes. The procedure was repeated three times to ensure that results were accurate. The cell viability investigates against the different concentrations. Furthermore, the cells were analyzed after 3 days with the help of microscope. The healthy cells was showing green color while unhealthy cells were showing red color. The cell viability was noted with the support of formula given below [30].

$$\textbf{Cell Viability (\%)} = \frac{\mathrm{OD(sample)} - \mathrm{OD(positive)}}{\mathrm{OD(negative)} - \mathrm{OD(positive)}} \times 100 \qquad (3)$$

# Chapter 4

# Results and discussion

## 4.1 Characterization of Ag-ZnO Nanoparticles

The characterizations of polymer based hydrogel membranes were done and evaluated against different characterizations such as XRD, SEM, AFM and FTIR etc. The results of all characterizations are given below.

### 4.1.1 XRD

The X-ray diffraction can be defined as a technique which has been used to analyze the molecular and atomic structure of the sample. This technique has been remained useful because of its data providing ability. The main working principle of X-ray is that the rays from XRD machines comes and hit the sample then it scatter and provides information about the peaks occurring in the sample. The XRD technique has been working on the principle that when the x-rays focus on a crystalline sample the rays will start to scatter by the regular arrangement of atoms in the crystal structure. Moreover, this thing will result in of pattern that shows interference in its structure. This is also known as diffraction patterns. Furthermore, it has been detected by the detector. The XRD technique was used to investigate crystallographic structure of nanoparticles. The results of XRD of Ag and ZnO nanoparticles are shown in Fig.1 below. The Ag nanoparticles was shown peaks on 38.1°, 44.83°, 64.6°, 77.54°. The peaks of silver nanoparticles was sharp which shows that the high crystallinity of Ag nanoparticles. The peaks gives a significant information about the crystal size, crystallinity of structure and structural quality as well. Arrangement of bonds in silver nanoparticles was regular as shown in Fig.1 (a). The ZnO nanoparticles was shown peaks on 31.8°**,**34.3°,36.4°,56.5° and 63.4°. The peaks of zinc oxide nanoparticles were also sharp which shows its high crystallinity in Fig.1 (b). The sharp peaks of nanoparticles was occurred because of highly crystalline material where the molecular or atomic arrangement was ordered. Both nanoparticles were formed. The XRD peaks of both nanoparticles are sharp and providing detailed information about the formation of nanoparticles. The XRD technique has been remained important for hydrogel

membranes to analyze their crystal structure. We have done XRD of two different nanoparticles. The XRD was also done against two theta degree angle.

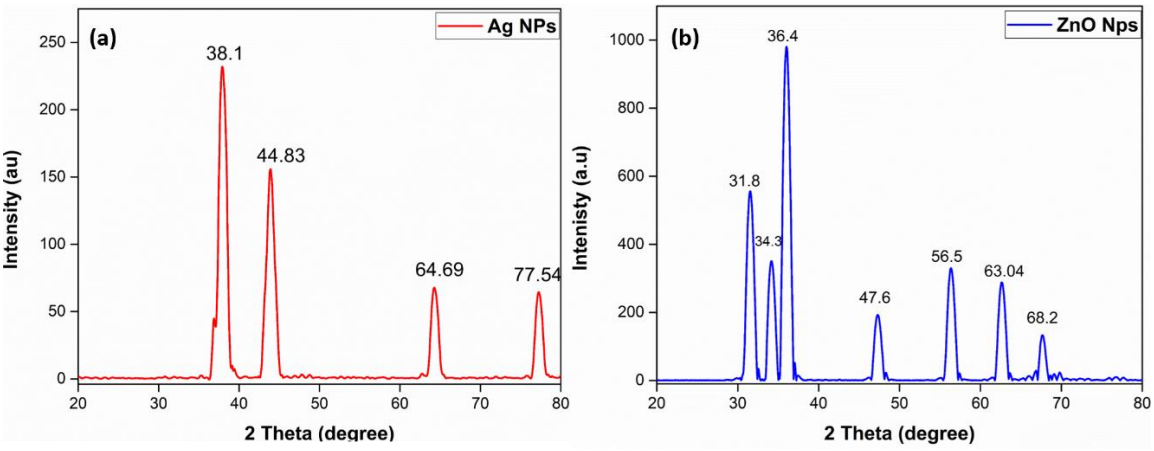


**Fig.11.** (a) XRD Pattern of Ag nanoparticles, (b) XRD Pattern of ZnO nanoparticles

### 4.1.2 Scanning Electron Microscopy

Scanning Electron Microscopy is a useful technique to investigate the morphology of a substance. It has been working on the principle that it produces high-energy bean electrons and these electrons attract the sample at its surface. After that, the electrons will start to disperse or scatter in various directions by giving data related to the sample morphology, composition, and other properties. Moreover, this attraction or intersection gives comprehensive information about the sample. The scanning electron microscopy was done for nanoparticles to analyze their surface morphology, size and shape of nanoparticles. The SEM images of zinc oxide nanoparticles were illustrated in Fig.2 (a). The average size of zinc oxide nanoparticles was 43.1nm. The surface morphology of zinc oxide nanoparticles was smooth and show uniform size distribution of nanoparticles. The shape of ZnO nanoparticles is spherical. This is because of the impact of crystallinity of nanoparticles structure and their size effect. The EDX analysis of ZnO nanoparticles is also demonstrated in Fig.2 (a). Moreover, the Ag nanoparticles SEM analysis is shown in Fig.2 (b). The EDX of silver nanoparticles is also displayed in Fig.2 (a). The silver nanoparticles shows agglomeration and their shape is non-spherical. The silver nanoparticles plays a significant role in wound healing application because of their

property to increase strength and antibacterial activity. While, zinc oxide nanoparticles are effective in enhancing wound healing rate by reducing inflammation in wounds. Both nanoparticles are formed and played an critical role in wound healing hydrogel membrane.

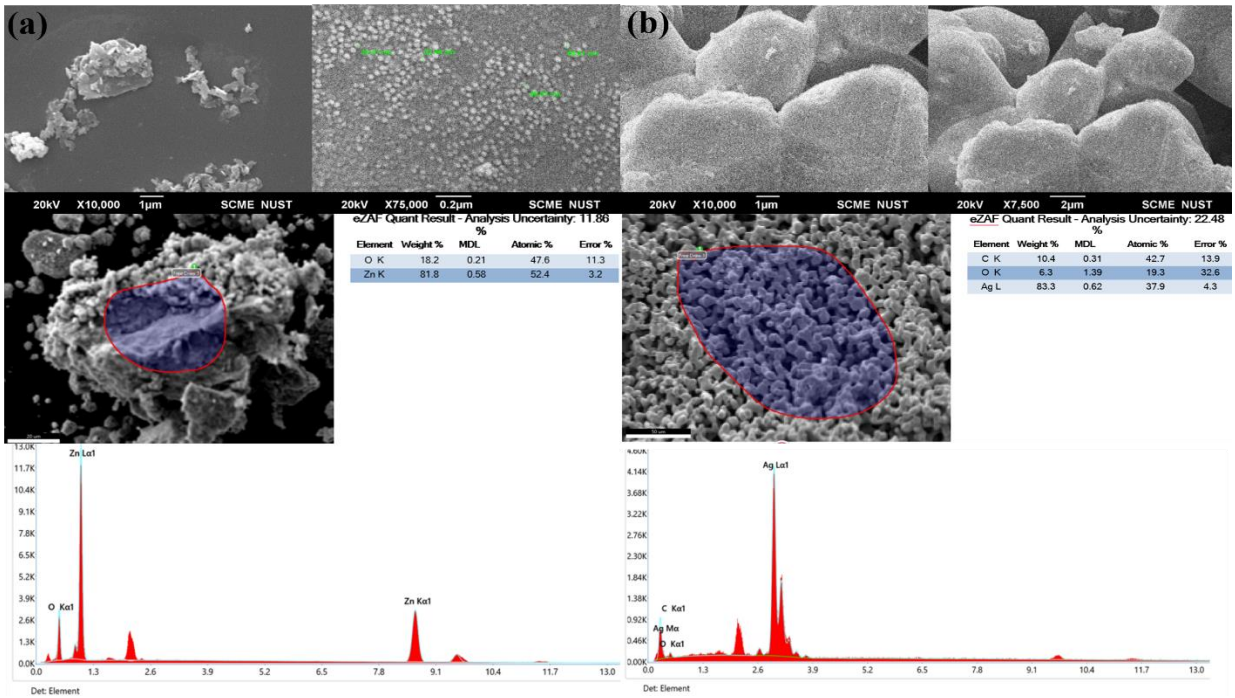


**Fig.12.** (a) SEM Images of ZnO nanoparticles, (b) SEM Images of Ag nanoparticles.

## 4.2 Hydrogel Membrane Characterizations

### 4. 2.1 X-ray Diffraction

The XRD pattern of hydrogel composed of sodium alginate and PEG incorporated with zinc oxide and silver nanoparticles is displayed in Fig.3. The SAPEG is representing the pristine hydrogel.

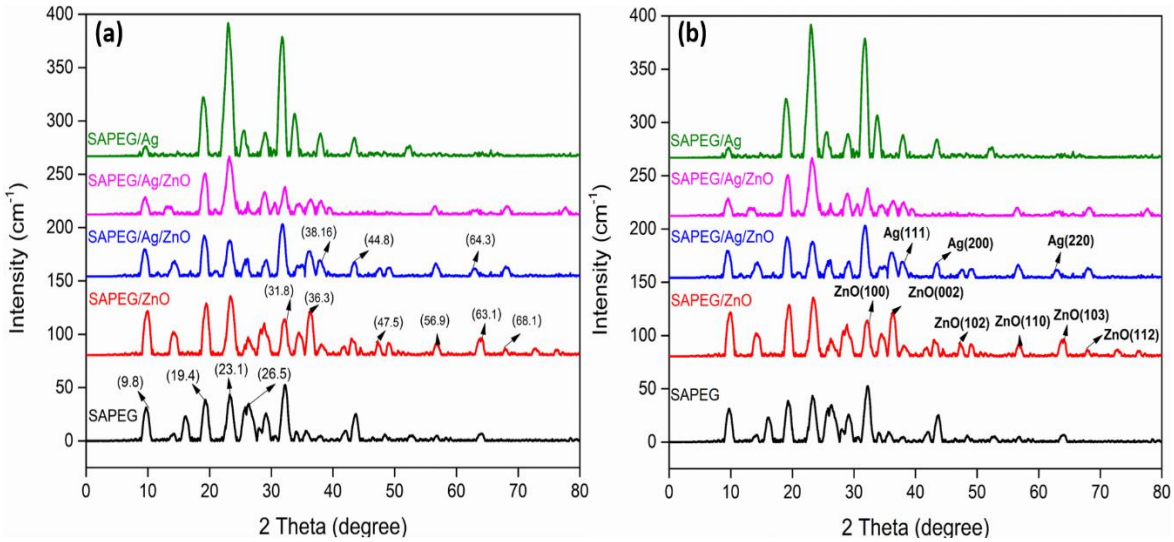


**Fig.13.** (a) XRD Pattern of Hydrogel Membranes, (b) XRD Phase coordinates of Hydrogel Membranes.

The XRD pattern of SAPEG hydrogel showed peaks 9.8°, 19.4°,23.1° and 26.5° were related to face centered structure of sodium alginate and PEG hydrogel. The SAPEG/0.030ZnO hydrogel was shown ZnO peaks 31.8°, 36.3°,47.5° ,56.9° and 68.1° related zinc oxide nanoparticles including SAPEG hydrogel membrane peaks. The SAPEG/0.01Ag/0.030ZnO hydrogel displayed major peaks of silver nanoparticles are 38.16°, 44.8° and 64.3° are illustrated in Fig.3 (a). The SAPEG/0.02Ag/0.01Zno hydrogel membrane had different composition of nanoparticles incorporated. The SAPEG/0.030Ag hydrogel with incorporated only silver nanoparticles. The size of the zinc oxide nanoparticles is approximately 43.41nm. All the peaks of hydrogel membranes was sharp and had high crystal structure due to regular pattern of bonds in them. The phase coordinates of synthesized hydrogel membranes is displayed in Fig.3 (b).

### 4.2.2 Scanning Electron Microscopy

The SEM of pristine SAPEG morphology is shown below in Fig.4. The mixing of polyethylene glycol solution into the sodium alginate solution was showing roughness on the surface of membrane and inhomogeneity. After adding 1g polyethylene glycol the SAPEG membrane showed morphology of two different phases. One phase was continuous that was of sodium alginate. However, the other phase was of polyethylene glycol that was dispersed phase. The phase separation between the polymers in membrane was seen. The SAPEG/0.01Ag/0.02ZnO NPs hydrogel membranes were porous in nature. It can be seen easily that the surface of pristine membrane which was composed of SAPEG was smooth then others. Moreover, the dispersions of nanoparticles in membranes is homogeneous as increased amount of nanoparticles in different samples can be seen on their membrane surface. As the amount of zinc oxide nanoparticles in sample 2,3 is more that can be seen on surface of membrane while the amount of silver nanoparticles is more in sample 5 which is SAPEG/0.030Ag that can also be seen on membrane surface. The surface of SA/PEG/0.02Ag/0.01ZnO samples are less smooth as compared to other samples of hydrogel membrane. It may be because of the polar nature of Sodium alginate and PEG. Furthermore, the hydroxyl polar group's existence in the SAPEG membrane structure had ability to develop hydrogen bonds. It enhances reliability of membrane [60]. The addition of ZnO and Ag nanoparticles increases the antibacterial property and tensile strength of synthesized hydrogel membrane. However, the addition of large amount of nanoparticles composite started to form agglomeration. This was the reason that particles with higher energy was showed on surface of membranes. This approach supports the affinity of nanoparticles to show agglomeration instead of dispersion in matrix. The SEM results of formulated membranes are useful in terms of their morphological analyses and size of hydrogel membranes had been analyzed.[61].

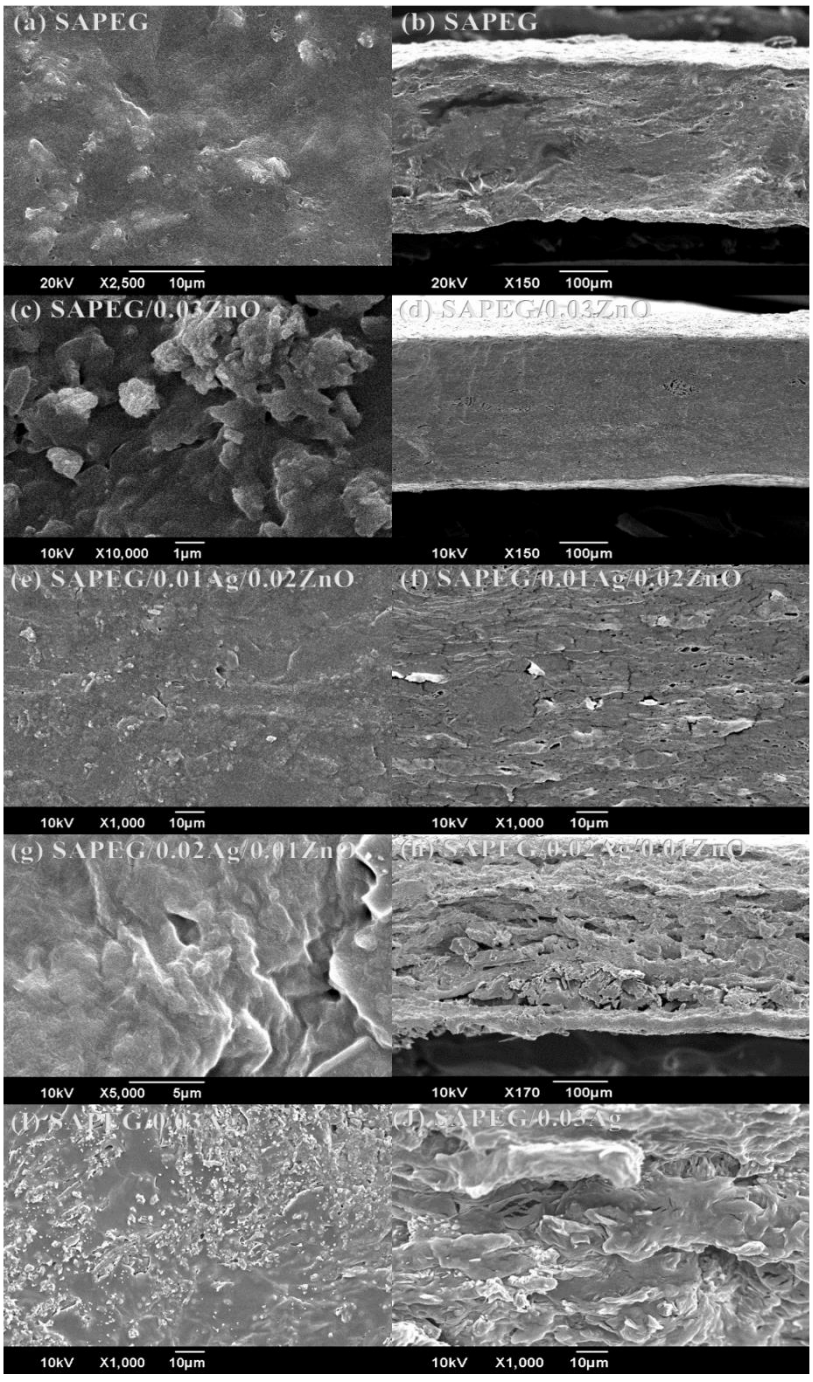


**Fig.14.** SEM Images of Synthesized Hydrogel Membranes showing surface and cross section analysis of membranes.

### 4.2.3 FTIR

Fourier Transform Infrared Spectroscopy (FTIR) has been working on the principle that the infrared ray's light will be hit on sample. Mainly, the rays light will attract the material bonds. After thus action the pattern will form in which the pattern converts into infrared spectrum. Now this created spectrum gives a data related to the material regarding its composition and structure. This technique has been remained helpful in analyzing that the chemical or material is fine or not. We can also use ftir to investigate the material characteristics and its functional groups. One of the main benefits of FTIR is that it also provides information about the nondestructive analysis of material existing in its original state. There is also a limitation of FTIR is that it cannot be done for dilute samples because the infrared rays will not be able to detect the spectrum. It is also not useful for the other materials or samples in which polar covalent bonds are involved.

The FTIR analysis of SAPEG nanocomposite hydrogel membranes with incorporated Ag-ZnO nanoparticles is displayed in Fig.15. (a). Because of the similarity of polymers and nanoparticles in all hydrogel membranes the spectrum of FTIR was similar. In all the hydrogel membranes given below the broad peak 3405 cm−1 is because of the presence of hydrophilic hydroxyl groups present in SAPEG/Ag/ZnO NPs hydrogel membrane. Furthermore, the band and expansion of $CH_2$ can be seen at 2923 $cm^{-1}$ in Fig.15. (a). It was seen at peak 1639 $cm^{-1}$ that acetated groups were existed in SAPEG. However, the existence of secondary alcohol in SAPEG/Ag/ZnO hydrogel membrane was seen at 1109 $cm^{-1}$ peak [62]. The peaks from 1106 $cm^{-1}$ to 1109 $cm^{-1}$ was showing hydroxyl groups existed in SAPEG. Moreover, the expansion near the 1044 $cm^{-1}$ was due to the crosslinking of SAPEG with glycerin. While, the peaks from 850–500 $cm^{-1}$ was because of the ring presence in sodium alginate [63]. In the end, the peak variations and change in intensities was due to the incorporation of nanoparticles in hydrogels.

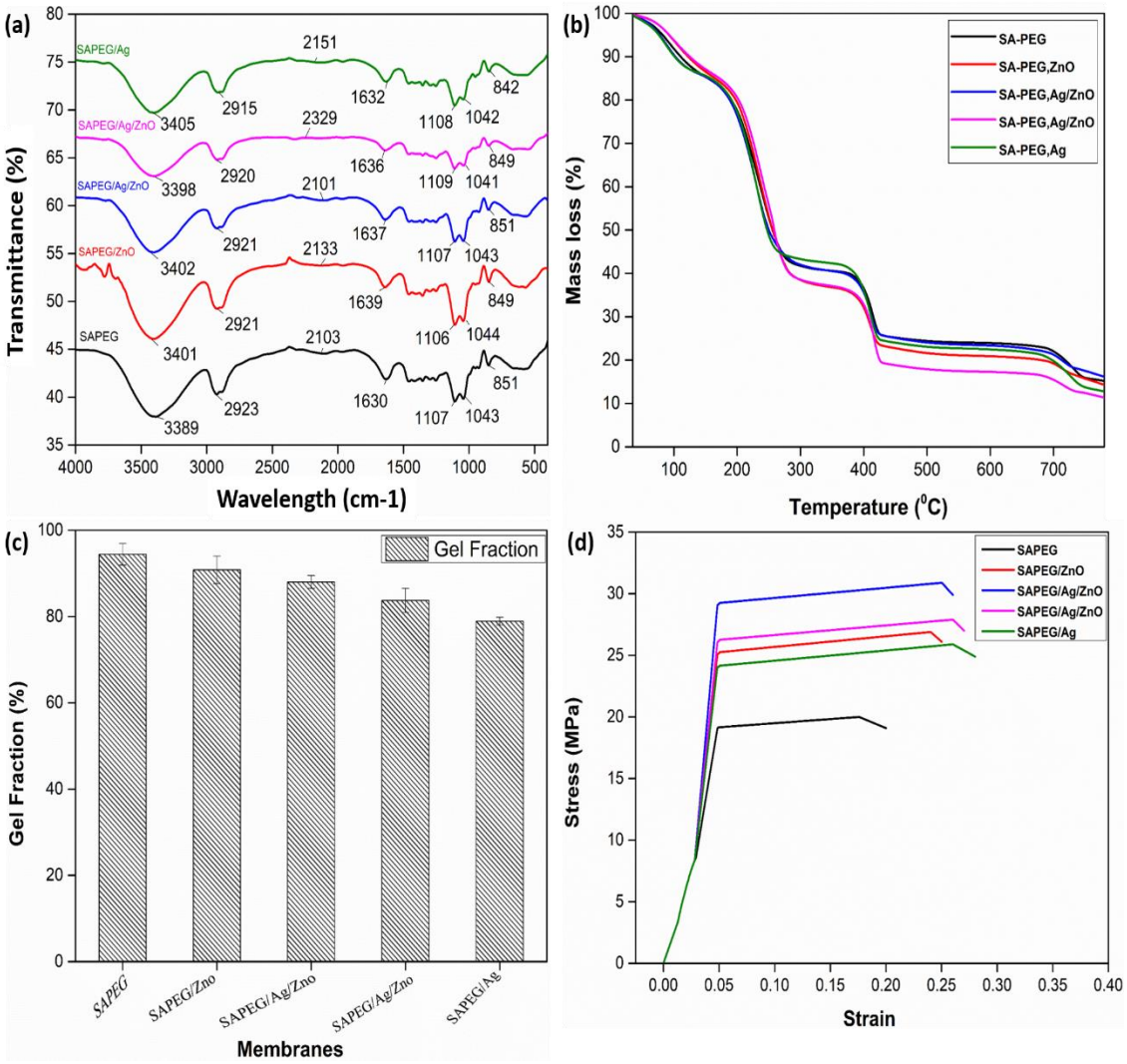


**Fig.15.** (a) FTIR pattern of hydrogel membranes, (b) Illustration of thermogravimetric analysis of hydrogel membranes, (c) Gel fraction measured of prepared hydrogel membranes, (d) Ultimate tensile strength of hydrogel membranes

### 4.2.4 Gel fraction

The principle of gel fraction is based on the principle that it finds the cross-linked polymer percentage that was turned into a solid and formed the three-dimensional structure. The solvent used in the gel fraction percentage test was water. Mainly, the calculation is done by the formula in which the initial dry and final wet weight of membranes is calculated as

the difference between the values. The best benefit of gel fraction percentage is that it measures the crosslinking of material and tells us whether the product is quality or not. Moreover, the procedure for determining the gel fraction is very time taking and a little complex. Sometimes the values vary due to weather conditions which causes inaccuracy and takes more time.

The gel fraction test was performed on all hydrogel membranes. All the results of gel fraction of hydrogel membranes is explained in Fig.5. (c). It can be seen that the gel fraction percentage is decreasing as the amount of silver nanoparticles increasing in hydrogel membranes. It shows less cross linking and ultimately high expansion of the hydrogel network. Moreover, the decrease in gel faction may also because of hydrophilic nature of hydrogel membranes. As the hydrophilicity of membranes increases its gel fraction percentages goes to decrease [64]. The gel fraction of pristine hydrogel SAPEG membrane is 93%. And the gel fractions of hydrogel with incorporated nanoparticles are illustrated in Fig.5(c). Furthermore, it has been seen that increase in water absorption weakened the structure of hydrogel and allows inhibition of water contents [65]. It has been seen that the decrease in the gel fraction percentages was beneficial for the Ag ions as the decrease in the gel fraction percentages allows Ag ions supports in the polymeric matrix. It was also helpful in the process of wound healing mechanism.

### 4.2.5 Thermal Gravimetric Analysis

The thermal gravimetric analysis was conducted for hydrogel membranes to investigate their mass loss against different temperature. All the formulated hydrogels were tested for thermal gravimetric analysis. The mass loss of pristine SAPEG hydrogel membrane is 100% at 31 degree Celsius while the mass loss is 15.5% at 743 degree Celsius. And the mass loss is increasing in those hydrogel membranes that were incorporated with nanoparticles. As there mass loss is occurring more at more temperature than pristine hydrogel membrane because nanoparticles move towards decomposition at higher temperature. The trend of graph is alike for all hydrogel membrane as their elements are same but concentrations are different. The investigated thermal gravimetric analysis results are shown in Fig.5. (b).

### 4.2.6 UV Spectroscopy

Ultraviolet (UV) spectroscopy works on the principle in which ultraviolet light interacts with the material and measures the absorbance trend of light or capacity of the material. The material absorbs ultraviolet light only when the electrons of the light are moving at higher rates of energy. After investigating the absorption pattern it becomes easy to find the concentrations in the sample. One of the main limitations of ultraviolet spectroscopy is that it does not give data related to the structure of the material. It only tells the absorption, concentration, and functional groups in the material. However, it does not give information as NMR. Moreover, one of the effective benefits of ultraviolet spectroscopy is that it investigates amounts of material. And it can easily detect small concentrations present in the material.

The ultraviolet visible spectroscopy test was conducted for formulated hydrogels membranes. The results of UV of hydrogel membranes are shown in figure Fig.6. The pristine SAPEG (S1) hydrogel membrane has shown absorbance 0.5198 at maximum wavelength 224nm. The SAPEG/0.03ZnO hydrogel membrane has shown absorbance 0.1876 at maximum wavelength 234nm. The SAPEG/0.01Ag/0.02ZnO hydrogel membrane has displayed absorbance 1.3528 at wavelength 234nm. The SAPEG/0.02Ag/0.01ZnO has shown absorbance 2.5652 at wavelength 235nm. The SAPEG/0.03Ag has shown absorbance 0.7738 at wavelength 233nm. So, as the incorporation of nanoparticles increases the absorbance of in hydrogel membranes increases.

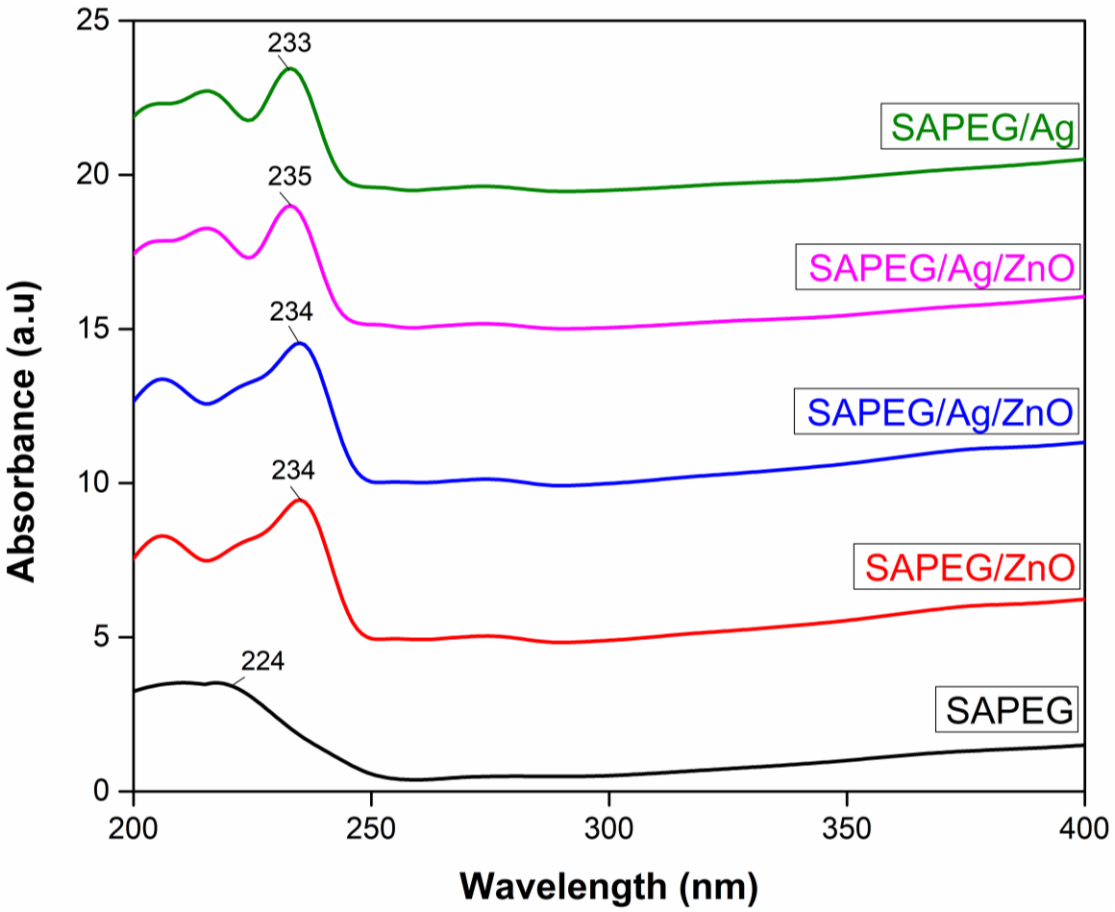


**Fig.16. Representation ultraviolet spectroscopy analysis of synthesized hydrogel membranes**

### 4.2.7 Tensile strength

The main aim of doing tensile testing is to get investigate the strength of hydrogel membranes. The tensile strength is measures by applying forces at specific intervals and force with units MPa. The tensile strength is useful to analyze that the prepared hydrogel will be good for human skin for wound healing or not. it applies forces to the material us until the sample breaks and we will analyses its stress rate, ductility and breakage point. Moreover, in order to become familiar with tensile strength one should also understand the difference between brittle and ductility. It is also helpful in analyzing the quality of material, strength, material selection characteristics and can provide an better results. Several natural and synthetic polymers are developed for treating wounds but less water absorption ability and weak mechanical properties have been limiting their use in tensile strength. All the synthesized hydrogels were analyzed against tensile strength. Mainly, the tensile strength of hydrogels shows the strength of hydrogels during the wound dressing. The synthesized hydrogels tensile testing graph was plotted between stress (MPa) and percentage elongation (%). The percentage elongation (%) is also strain. The hydrogels should hold enough strength to use for wound healing purpose without showing any damage. The tensile strength of SAPEG hydrogel was 20 MPa. The SAPEG/0.03ZnO hydrogel membrane has shown 26.8 MPa tensile strength. The SAPEG/0.01Ag/0.02ZnO hydrogel membrane has shown 30.8 MPa tensile strength. The SAPEG/0.02Ag/0.01ZnO hydrogel membrane has shown 27.9 MPa tensile strength. The SAPEG/0.03Ag hydrogel membrane has shown tensile strength 25.8 MPa. As the incorporation of nanoparticles increases in the hydrogel membranes the tensile strength of hydrogel membranes increases because nanoparticles are increasing the strength of hydrogel membranes. The tensile strength of the human skin is up to 11.5 MPa. The strength of the hydrogel should be higher than the skin as mentioned above [66]. With the incorporation of nanoparticles, the tensile strength increases. Hence, tensile strength starts to increase. All the results of tensile strength was shown in Fig.15 (d).

### 4.2.8 Antibacterial Activity

The synthesized hydrogels were tested against antibacterial activity by using disc diffusion method. There were two strains used in antibacterial activity such as Escherichia Coli and Staphylococcus aureus. The positive strain was S. aureus while the negative strain was E.coli. There were five hydrogels with different compositions was tested against antibacterial activity. The hydrogels were SAPEG, SAPEG/0.03ZnO, SAPEG/0.01Ag/0.02ZnO, SAPEG/0.02Ag/0.01ZnO and SAPEG/0.03Ag. Furthermore, the zone of inhibition was shown in Fig.17 (a). The images of Kirby-Bauer agar plates of SAPEG/Ag/ZnO NPs hydrogel membranes are depicted in Fig.17 (a). The clear and large zone of inhibition was observed against both the pathogenic strains. The positive control used was S.aureus, the diameter of zone of inhibition of SAPEG hydrogel membrane was 9 ± 0.37 and 7 ± 0.17. The diameter of zone of inhibition of SAPEG/ZnO hydrogel membrane against E. coli and S. aureus was 15 ± 0.67 and 26 ± 0.45 mm. All the results of hydrogels with incorporated nanoparticles was shown in Fig. 17 (a). The large zones of inhibition were obtained for S. aureus then E. coli. The highest zone of inhibition of SAPEG/0.01Ag/0.02ZnO 13 ± 0.24 and 27.25 ± 0.51 mm was attained against E. coli and S. aureus. [67]. The graph of zone of inhibition is illustrated in Fig. 17 (b).

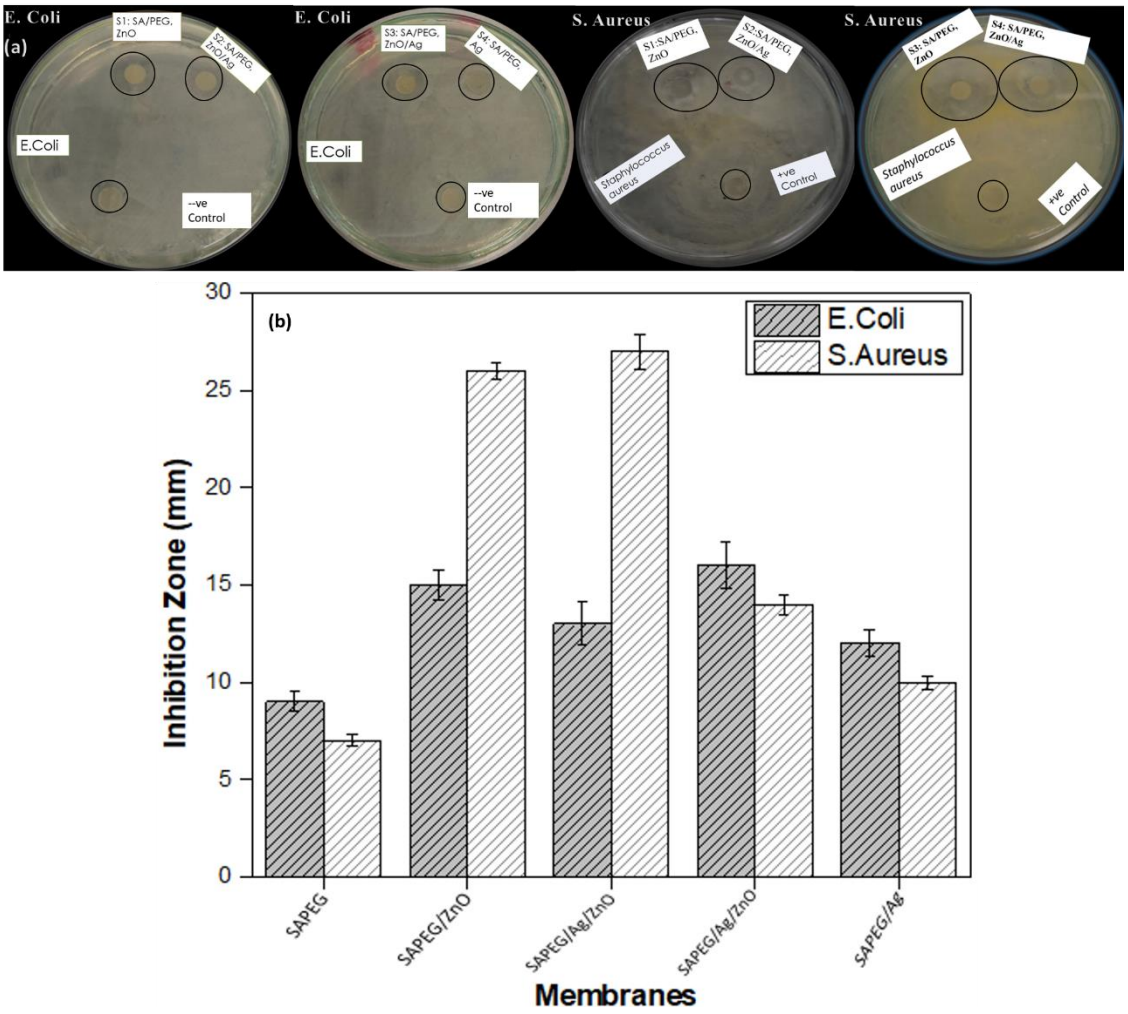


**Fig.17.** (a) Antibacterial activity of prepared hydrogel membranes is shown (b) Zone of Inhibition of hydrogel membranes is illustrated.

### 4.2.9 Cytotoxicity

The cytotoxicity test of synthesized hydrogel membranes was performed to check the ability of hydrogel membranes to regenerate tissues for wound healing. The test was conducted in the controlled environment after growing cell culture. The graph of cytotoxicity test was drawn between percentage of cell viability and concentration. The concentration was taken on x-axis and cell viability percentage was taken on y-axis. The effective results were obtained of hydrogel membranes such as SAPEG/0.030ZnO, SAPEG/0.01Ag/0.02ZnO and SAPEG/0.02Ag/0.01ZnO as described in Fig.8.

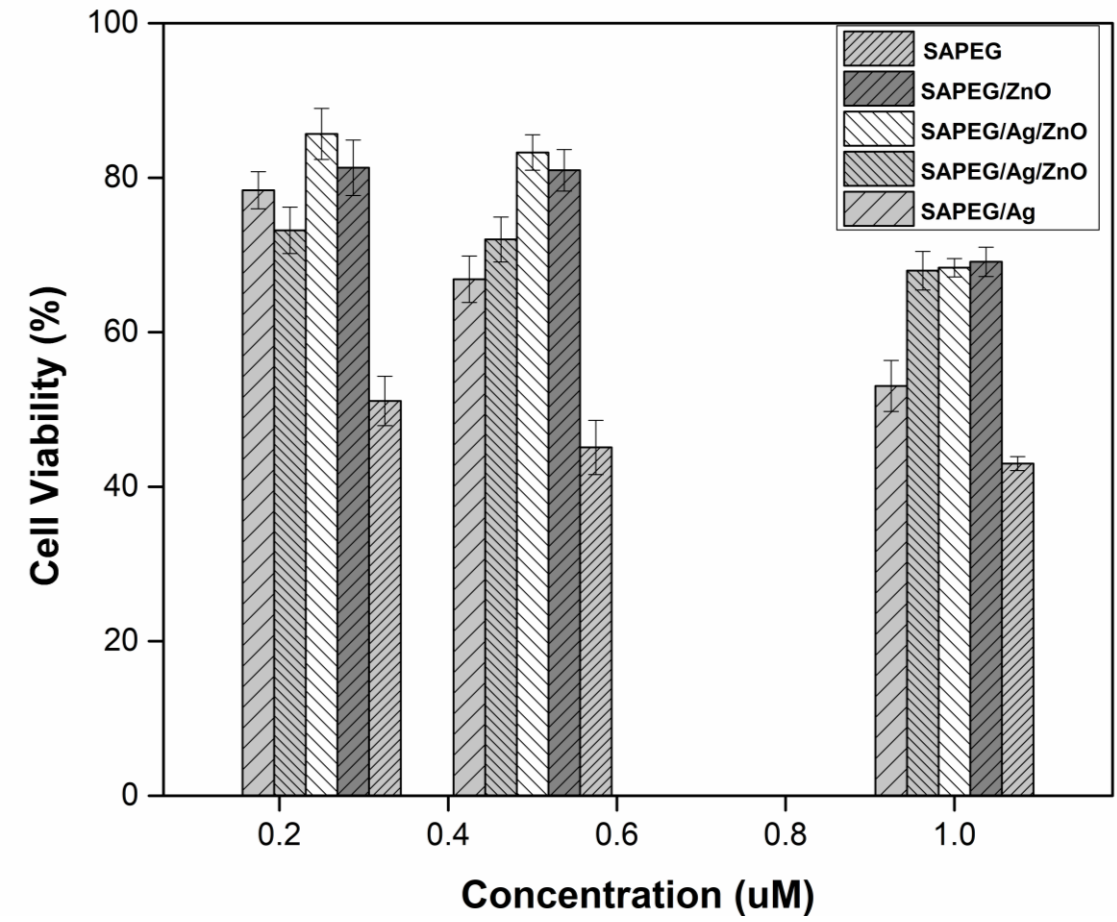


**Fig.18.** Cell viability (%) at different concentration is investigated for hydrogel membranes

### 4.2.10 Contact angle

The contact angle has been working on the principle that it calculates the wetting behavior of the liquid droplet on the surface of a solid sample. Basically, it related this principle to fluid dynamics. The contact can be defined as the angle that forms at the hitting point of the liquid and solid surface of the sample. It calculates the angles in degrees. It tells us the moisture rendition ability of membranes and if the angle is less than 90 the membranes will be hydrophilic. The hydrophilicity increases as the angles decrease and decreases as the angle increases. The efficient advantage of the contact test is that it gives the surface characteristics of the sample. It works only on idea conditions and will not give appropriate results if the sample have roughness.

The contact angle test was done for hydrogel membranes to investigate their nature of hydrophilicity. The prepared hydrogel membranes are hydrophilic in nature as all the membranes was shown less than 90°angle as depicted in Fig. 19. (a) [68]. The contact angle of pristine SAPEG hydrogel is 27.5° and the contact angle of other hydrogel membrane is decreasing. The contact angle of hydrogels was reduced due to the presence of different nomenclature groups including phenolic and hydroxyl groups as they developed bonding with water molecules which results in decrease contact angle[69]. As hydrophilic membrane is more good for wound healing because it gives more moisture retention property towards wound healing process [70]. The graphical representation of contact angle is shown in Fig.19. (b).

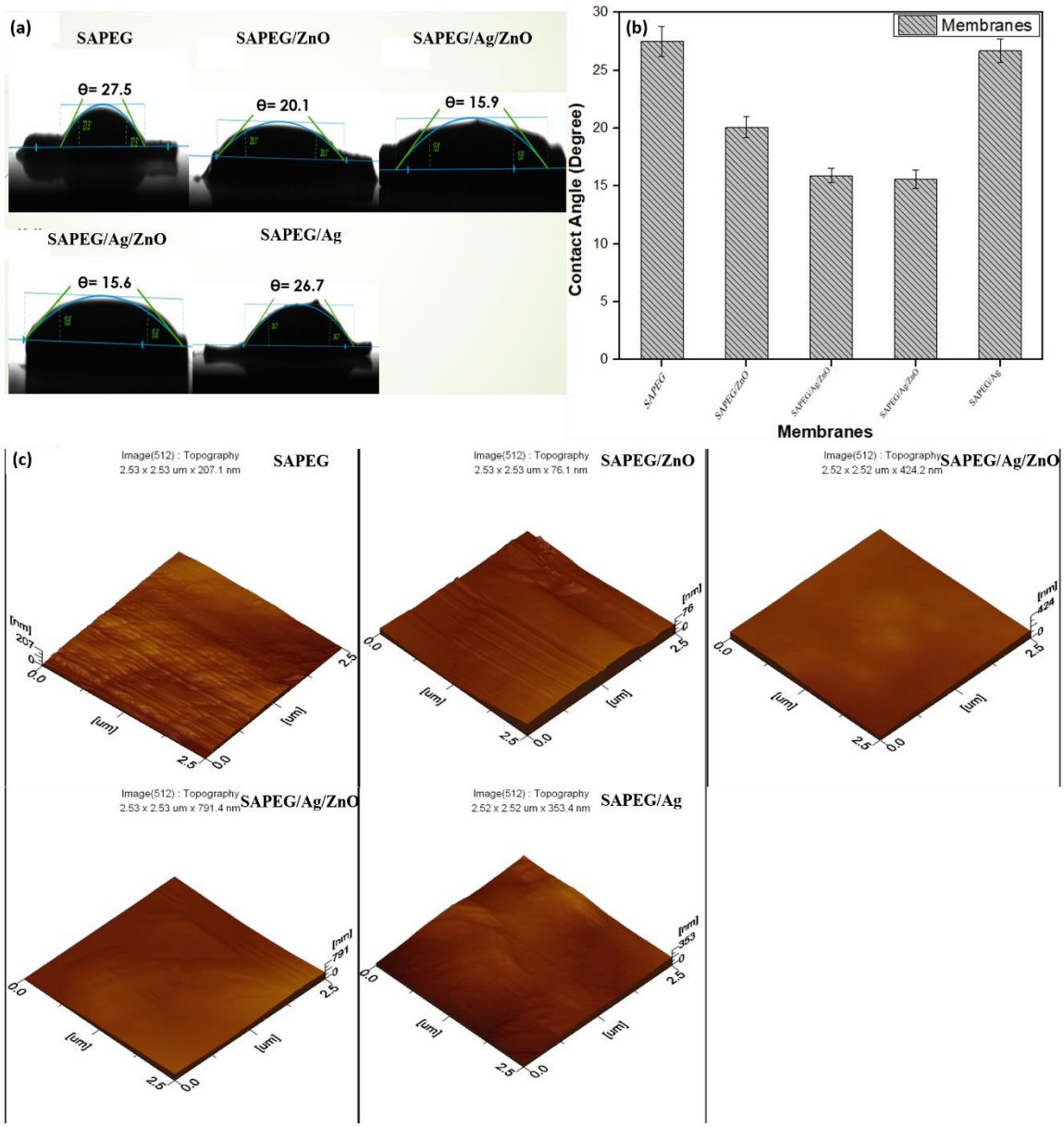


**Fig.19.** (a) Contact angle measurement of prepared hydrogel membranes (b) Graphical representation of contact angel of hydrogel membranes, (c) Atomic force spectroscopic illustration of hydrogel membranes at different composition of nanoparticles.

### 4.2.11 Atomic Force Microscopy

Atomic force microscopy has been based on the principle that its tip strikes with the material surface and uses Vander Waals forces and electrostatic attraction forces which cause beams to bend. This action will use uniform force and the tip will be in working to scan the whole material while recording the visuals of samples. The roughness, topography of samples, and high resolution of images can be obtained by using AFM. One of the effective advantages of AFM is that it gives high-resolution images so that the scientist can analyze the results and material very well. One of the limitations is that it does not test those samples that have moisture and also have slow imaging time.

The atomic force microscopy was conducted for prepared hydrogel membranes. The SAPEG synthesized hydrogels were investigated in “tapping mode” and results showed in Fig.19. (c). It was seen that the darkish region of AFM-tested hydrogel membranes was showing depression. However, the bright regions of tested hydrogels was showing height in three dimensional images. These images were showing topography of synthesized hydrogels. The results of pristine SAPEG hydrogel membrane showed little rough surface as compared to other hydrogel membranes. Furthermore, as the nanoparticles were incorporated in membranes the roughness of membranes was increased [71]. The roughness in hydrogel membranes decreases as the concentration of nanoparticles decreases.

# Conclusion

In this research study, we have prepared hydrogel membranes for wound dressing by making a sodium alginate blend with polyethylene glycol. The silver and zinc oxide nanoparticles were also incorporated into the blend to enhance antibacterial and mechanical properties of hydrogel membrane. The hydrogel membranes were formulated with constant composition of polymers but varying composition of incorporated nanoparticles. The prepared hydrogel membranes were also passed through characterizations such as XRD, SEM, TGA, FTIR and AFM etc. the characterizations were done to evaluate hydrogel membranes properties for wound healing. The XRD results have shown better crystalline structure in hydrogel membranes which is good for wound healing application while TGA results shows the enhancement of thermal stability of SA composites in terms of the onset of degradation and percentage of weight loss. The SEM Images had shown surface morphology of hydrogel membranes. The formulated hydrogel membranes was also assessed against in vitro study and showed efficient results. Thus, the formulated hydrogel membranes are effective for wound healing application.

# Future Recommendations

- In vivo study is essential for investigating formulated hydrogel membranes on living organisms for evaluating its wound healing efficiency.
- The interaction of such copolymers with different polymers can be studied since there are number of ways to add such copolymers into polymer matrix for increasing wound healing properties.
- The prepared hydrogel membranes can be investigated against different nanoparticles composite to investigate their response to other material.

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