PROJECT REPORT

On

"PREPARATION OF MEMBRANES FROM CHITOSAN/GELATIN POLYMER BLENDS AND EVALUATION OF THEIR PROPERTIES"

Submitted by

DORIS MARIA VALIYATHARA JOSEY
(AM23CHE005)

In partial fulfillment for the award of the
Post graduate Degree in Chemistry

DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

2024-2025

DEPARTMENT OF CHEMISTRY
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Year of Work : **2024-2025**

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Acceptable Maximum Limit	20
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Paper Title	"PREPARATION OF MEMBRANES FROM CHITOSAN/GELATIN POLYMER BLENDS AND EVALUATION OF THEIR PROPERTIES"
Similarity	6% AI - 18%
Paper ID	3383133
Total Pages	91
Submission Date	2025-03-06 17:22:23

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This is to certify that the work entitled "**PREPARATION OF MEMBRANES FROM CHITOSAN/GELATIN POLYMER BLENDS AND EVALUATION OF THEIR PROPERTIES**" is a bonafide record of project work carried out by **Ms. DORIS MARIA VALIYATHARA JOSEY under** my supervision for the partial fulfilment of degree of Master of Science in Chemistry of St. Teresa's College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam. It is further certified that the work is original and that no part of this work has been submitted elsewhere for the award of any other degree.

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I hereby declare that the project work entitled "**PREPARATION OF MEMBRANES FROM CHITOSAN/GELATIN POLYMER BLENDS AND EVALUATION OF THEIR PROPERTIES**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, Kerala is a record of an original work done by me under the guidance of **Dr. SARITHA CHANDRAN A., ASSISTANT PROFESSOR**, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam (Internal Guide) and **Dr. JORPHIN JOSEPH, ASSISTANT PROFESSOR,** Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology (External Guide). This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

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Acknowledgements

First and foremost, I thank the GOD Almighty for having made possible the successful completion of my project.

My sincere thanks and deep sense of gratitude to my guide, **Dr. Saritha Chandran A.,** Assistant Professor and Head, Department of Chemistry and the Centre for Research, St. Teresa's College (Autonomous), Ernakulam for her guidance, scientific freedom and cordial affection throughout the course of my study and research.

I would like to sincerely thank my external guide, **Dr. Jorphin Joseph**, Assistant Professor, Department of Chemical Oceanography, CUSAT, for his careful guidance, encouraging support, and invaluable suggestions throughout this project.

I am truly grateful to Ms. Ayona Joy, Research Scholar, Department of Chemical Oceanography, CUSAT for her significant assistance, generous guidance, invaluable insights, and unwavering moral support throughout this project.

I would like to express my heartfelt gratitude to **Dr. Alphonsa Vijaya Joseph**, Principal of St. Teresa's College, Ernakulam, as well as Manager

Rev. Sr. Nilima and Directors **Rev. Sr. Francis Ann** and **Rev. Sr. Tessa CSST** for their unwavering support and visionary leadership. Their efforts in creating a positive environment for learning and growth have been crucial to my journey.

Lastly, I would like to thank my family and friends for their unwavering support and understanding.

DORIS MARIA VALIYATHARA JOSEY

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INTRODUCTION

1.1. CHITIN AND CHITOSAN

Chitin is a natural polymer obtained from the exoskeleton and the internal structure of many invertebrates. Chemically it is made of 2-acetylamino-2-deoxy-D-glucose units linked by β-glycosidic bonds in the 1,4 position [1]. It is the most common reproducible biopolymer after cellulose found in nature. It is mainly obtained from shells of crabs, shrimps, and certain other microorganisms such as bacteria and fungi [2]. Chitin is found in two forms, referred to as the α and β allomorphs, which can be differentiated using techniques such as infrared spectroscopy, solid- state NMR spectroscopy, and X-ray diffraction. In its solid state, chitin chains are interconnected by hydrogen bonds, which play a crucial role in determining its solubility, swelling properties, and reactivity [3].

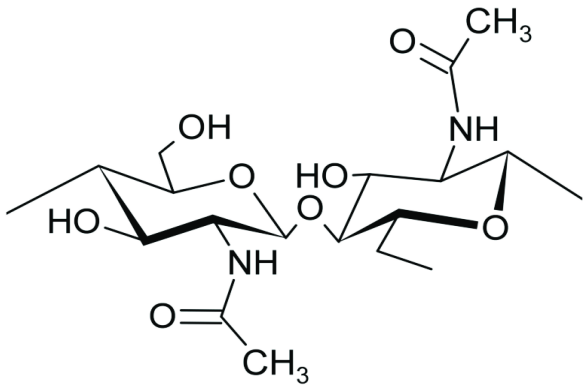

Fig. 1.1: Chemical structure of Chitin

The N-deacetylated derivative of chitin is known as Chitosan [4]. Chitosan is a linear copolymer made up of 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose, linked in a β- 1,4-configuration. Chitosan is mainly sourced from crustaceans, molluscs, insects, and fungi. The most significant industrial sources of chitosan are derived from shrimp and crab waste [5]. Chitin and chitosan are distinct from cellulose due to their nitrogen content, which ranges from 5% to 8%. In chitin, nitrogen is present as acetylated amine groups, whereas in chitosan, it appears as primary aliphatic amine groups. This nitrogen incorporation allows both chitin and chitosan to participate in typical amine reactions. Chitosan exhibits greater chemical reactivity than chitin, thanks to the presence of both primary and secondary hydroxyl groups in each repeat unit, along with an amine group in each deacetylated unit. These active groups are open to various chemical modifications, enabling adjustments to the mechanical and physical characteristics of chitosan [6].

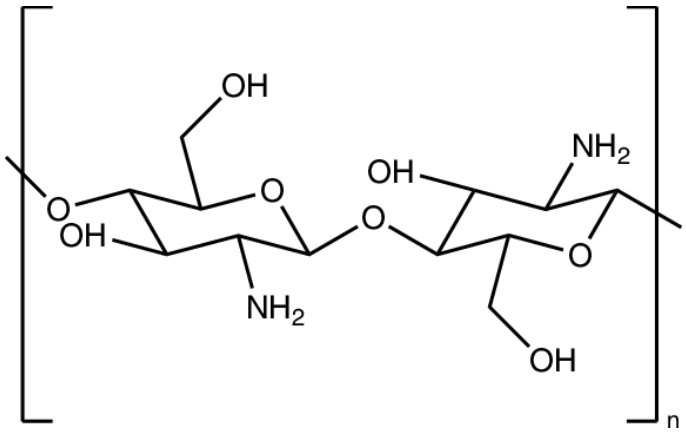

Fig. 1.2: Chemical structure of Chitosan

The molar percentage of monomeric glucosamine units in chitosan is determined by its degree of deacetylation (DD). The Degree of Deacetylation (DD) is a crucial factor for chitosan. Studies have indicated that chitosan with a greater DD shows enhanced biological effects and greater water solubility. This is primarily because a higher DD corresponds to a larger concentration of amino groups within the molecule. The protonation of the $-NH_2$ functional group plays a key role in activating chitosan's biological properties and increasing its solubility in water. Alongside DD, molecular weight (MW) is another vital factor that influences the bioactivity of chitosan. Typically, chitosan with a lower MW exhibits more pronounced bioactivities compared to chitosan with a higher MW [3]. A range of techniques has been reported in the literature for assessing the deacetylation degree of chitosan. These techniques include pH-potentiometric titration, infrared (IR) spectroscopy, proton (^1H) NMR spectroscopy, UV spectroscopy, colloidal titration, and enzymatic degradation. The molecular weight of chitosan is typically determined using viscosimetry or size exclusion chromatography [7].

The presence of amine groups in chitin and chitosan provides notable benefits, enabling specialized biological functions and allowing for different modification reactions. These polysaccharides possess remarkable qualities such as biocompatibility, biodegradability, bioactivity, non-toxicity, and effective adsorption properties. Consequently, they are regarded as valuable and essential biomaterials, drawing significant industrial interest as potential substitutes for synthetic polymers [6].

1.2. PREPARATION METHODS

Chitin and chitosan are derived from the shells of crustaceans like crabs, prawns, lobsters, and shrimp, as well as from certain insects and fungi such as Aspergillus and Mucor. The main industrial source for these biopolymers is waste from crab and shrimp shells, which aids in the recycling of by-products from marine food processing. These shells primarily comprise proteins, inorganic salts, chitin, and lipids. The extraction process occurs in two phases: initially, chitin is obtained from waste produced in the food industry, especially shrimp canning. In the second phase, chitin undergoes fermentation with alkali treatment to produce chitosan. The first step includes grinding the shells and using dilute hydrochloric acid to remove minerals. Following this, proteins are extracted with aqueous sodium hydroxide to avoid contamination. The chitin is then deacetylated in a 40–45% sodium hydroxide solution at a temperature of 160°C for 1 to 3 hours, yielding chitosan. The degree of deacetylation, which can range from 56% to 99% depending on the production conditions, affects how soluble chitosan is. For chitosan to be effectively soluble, it must have at least 85% deacetylation [6].

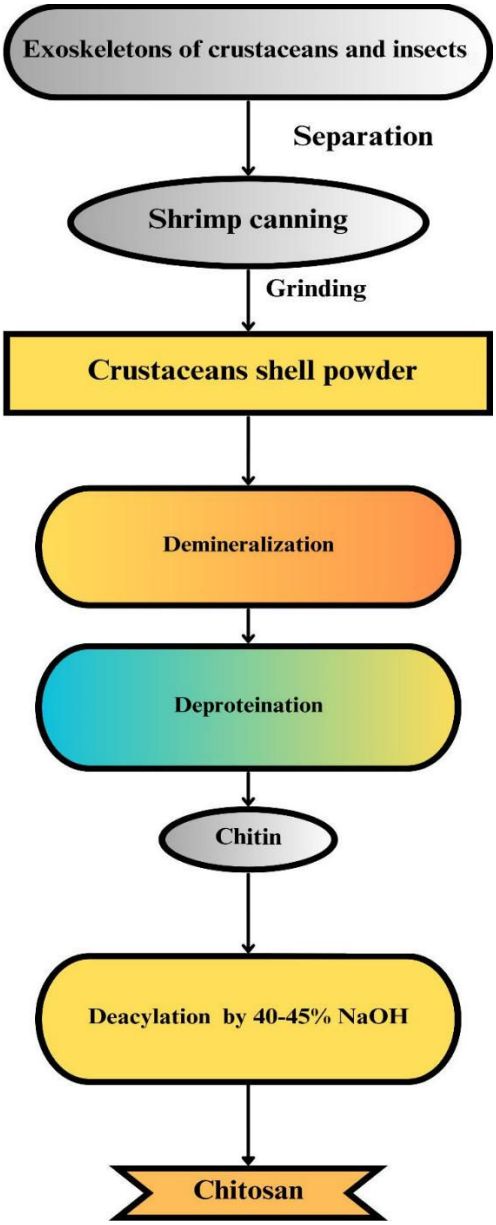

Fig.1.3: Flow chart representing the extraction of chitin and chitosan

1.3. PROPERTIES OF CHITOSAN

Chitosan has become the subject of research due to its unique properties and ease of modification. The physical, chemical, and biological properties of chitin and chitosan depend on parameters such as degree of deacetylation (DD), molecular weight distribution, and solubility which are dictated by the chitin sources and the preparation method.

1.3.1. Physico-Chemical Properties

1.3.1.a. Degree of Deacetylation

Chitosan is usually produced by partially deacetylating chitin. The resulting product is a copolymer that consists of N-acetylglucosamine units and D-glucosamine units. The proportion of N-acetylglucosamine units in the polymer chain is known as the degree of deacetylation (DD), which can be calculated with the following equation:

$$DD = 100 \times \frac{n_{Glen}}{n_{Glen}+n_{GleNAc}}$$

In this equation, $n_{\{GlcN\}}$ refers to the average number of D-glucosamine units, while $n_{\{GlcNAc\}}$ refers to the average number of N-acetylglucosamine units. Some research uses the term degree of acetylation (DA), which is calculated as follows:

$$DA = 100 - DD \text{ [8]}$$

The deacetylation process entails the removal of the acetyl group from chitin molecules, which influences the amount of free amine groups ($–NH_2$) present in chitosan. This procedure can reach deacetylation levels of up to 98%, but achieving complete deacetylation through this heterogeneous approach requires additional modifications. The degree of deacetylation (DD) indicates how much chitosan has been transformed from chitin, and it is affected by factors such as the concentration of NaOH, the reaction temperature, and the duration of the process. Depending on the method of production and the species used, the degree of deacetylation can vary between 56% and 99%. Nonetheless, a minimum of 85% deacetylation is necessary for chitosan to have good solubility [6][9]. Several analytical tools have been used to determine this ratio, including IR spectroscopy, pyrolysis gas chromatography, gel permeation chromatography, and UV spectrophotometry. Other methods employed include ^{1}H-NMR spectroscopy, ^{13}C solid-state NMR, thermal analysis, various titration techniques, acid hydrolysis, HPLC, separation spectrometry methods, and near-infrared spectroscopy [10].

1.3.1.b. Molecular weight

The molecular weight (MW) of chitosan is influenced by the quantity of monomeric units in the biopolymer. Viscosity and solubility are two key characteristics affected by the MW, making it vital to control, evaluate, and modify these properties. Chitosan's MW generally ranges from 20 to 1200 kDa. While light scattering and high-performance liquid chromatography can be used to determine MW, the viscosimetric method is the most widely used and straightforward approach [11].

The molecular weight plays a crucial role in determining the physicochemical and biological properties of chitosan. It varies depending on the source material and the methods of preparation and extraction. Chitosan can be classified into three categories: high, medium, or low molecular weight, based on its MW range. As the molecular weight increases, chitosan tends to become more viscous and less soluble, which can be problematic for many industrial applications. Therefore, low molecular weight chitosan is often favoured for biological and industrial applications due to its enhanced solubility and stability [12].

1.3.1.c. Solubility

Chitosan dissolves in acidic solvents but does not dissolve in neutral or alkaline conditions. Although chitin is typically insoluble in solvents, the deacetylation process converts chitin into soluble chitosan, which features primary amino groups with a pKa of 6.5. When chitosan is placed in an acidic solution, the amine groups become protonated and gain a positive charge, allowing it to stay soluble. However, if the pH reaches 6 or above, the amine groups lose their charge, leading to the insolubility of chitosan. The solubility of chitosan is also influenced by several factors, including molecular weight, the degree of deacetylation, temperature, and the crystallinity of the polymer [12].

1.3.1.d. Crystallinity

Crystallinity refers to a characteristic of chitosan that indicates the balance between its crystalline and amorphous components. This property is assessed through the crystallinity index (CI). Chitosan is a polymorphic biopolymer and is considered semi-crystalline in its solid state, which consists of an orthorhombic unit cell made up of two antiparallel chains devoid of water molecules.

The origin of chitosan and the techniques used for its preparation play a significant role in determining its crystallinity. The highest levels of crystallinity are found in chitin (which is not deacetylated) and in fully deacetylated chitosan. Measuring the crystallinity index is important because it affects various attributes of chitosan, such as its capability to swell, porosity, water absorption, and moisture retention. The correlations between the X-ray diffraction peaks can be employed to assess the crystallinity index [11].

1.3.2. Chemical Properties

Similar to many biopolymers, chitosan is amphiphilic, influencing its physical properties in solutions and solid forms. This is due to the combination of hydrophilic amino groups and hydrophobic acetyl groups in its molecular structure. The high concentration of amino groups imparts a strong positive charge to chitosan, distinguishing it from most polysaccharides.

Due to its amino and hydroxyl groups, chitosan exhibits a cationic nature, allowing for various chemical and physical modifications such as complexation, grafting, crosslinking, and blending. The presence of hydrogen bonding in its molecular structure gives chitosan rigidity, enabling it to be easily formed into films with significant mechanical strength when cast from solutions.

Moreover, chitosan is classified as a weak polyelectrolyte and is considered a very poor anion-exchanger. This property enables it to create films on negatively charged surfaces and to chemically interact with negatively charged entities like fats, cholesterol, proteins, and larger macromolecules [13].

1.3.3. Biological Properties

Chitosan is a natural, non-toxic product, making it suitable for use in the food industry for birds and fur-bearing animals. It can be broken down by certain enzymes in humans, especially lysozyme, and is regarded as biodegradable. Moreover, chitosan is biocompatible, enabling its application in various medical fields, such as topical treatments for the eyes, implants, or injections. It has antibacterial properties and promotes wound healing in both humans and animals, as well as aiding in hemostasis. Additionally, chitosan exhibits bio-adhesive characteristics due to its positive charges at physiological pH [13].

1.4. APPLICATIONS

1.4.1. Wound dressing

The use of chitin and chitosan as potential enhancers of wound healing has been studied for many years. Both chitin and its derivatives are safe for use in humans and animals [6]. Wound dressings made from chitosan exhibit several beneficial properties, such as hemostatic, biodegradable, and antibacterial effects, making them valuable in the healing process.

Chitosan displays antibacterial properties even at low concentrations against various pathogens, including *Escherichia coli* and *Staphylococcus aureu*s. It can be utilized in different forms, such as gels, films, or nanoparticles, leading to its widespread application in both medical and veterinary practices as a promoter of wound healing.

Studies indicate that chitosan plays a role in all phases of wound healing. In the early stages, it exhibits unique hemostatic functions and encourages the movement and infiltration of neutrophils and macrophages. This process cleanses wounds of foreign substances and supports the development of granulation tissue, which is crucial for forming fibrous tissue and re-epithelialization.

In instances of hypertrophic scars—resulting from the overproduction of collagen during the remodelling phase—chitosan can

help minimize scar tissue and facilitate effective re-epithelialization. Moreover, chitosan also affects the expression of growth factors that are vital for the healing process [14].

1.4.2. Tissue Engineering

Tissue engineering is a branch of science that unifies materials engineering and biomedicine–deals with the production of materials that can substitute and/or induce advanced regenerative processes in damaged tissue. The objective of tissue engineering is to repair, replace, maintain, or enhance the function of a particular tissue or organ. According to existing literature, there are several crucial requirements for the development of tissue engineering scaffolds. These scaffolds should not provoke an acute or chronic immune response, and they need to be biodegradable so that the new tissue can replace the biomaterial. Furthermore, they should have surface characteristics that promote cell attachment, differentiation, and proliferation. The mechanical properties of the scaffold should also be appropriate for handling and should resemble the properties of the damaged tissue. Lastly, they should be capable of being manufactured in various shapes.

Given its notable properties, chitosan stands out as a promising candidate for creating biomaterials that can replace missing or damaged tissues and organs [7].

1.4.3. Supercapacitors

Supercapacitors are innovative energy storage devices known for their high capacitance, excellent power density, long lifespan, and enhanced stability, as well as outstanding electrochemical properties. Given these impressive features, extensive research has been conducted to create these materials using sustainable resources.

1.4.4. Fuel cells

Fuel cells are eco-friendly devices utilized for energy conversion and electricity generation, making them highly promising options for zero-emission power sources. These devices operate electrochemically, converting chemical energy from a redox reaction directly into electrical energy.

A fuel cell comprises an electrolyte material sandwiched between two thin electrodes: a porous anode and a cathode. When the fuel comes into contact with the anode and oxygen interacts with the cathode, both are catalytically broken down into ions and electrons. The electrons flow through an external electrical circuit, generating power, while the ions migrate through the electrolyte to the oppositely charged electrode.

To achieve their desired performance, fuel cell membranes need to have several key characteristics, such as high proton conductivity, low electronic conductivity, impermeability to fuels (gases or liquids), and good mechanical toughness in both dry and hydrated conditions. These

characteristics are linked to various factors, including ion exchange capacity, morphology, and water uptake, all critical for evaluating the viability of new fuel cell membranes.

Chitosan, a widely available biopolymer, has been studied as a membrane material for applications like ultrafiltration, reverse osmosis, pervaporation, and lithium batteries. Membranes made from chitosan are simple to fabricate, exhibit high hydrophilicity, and possess good chemical and thermal stability. These favourable traits have led to increased interest in chitosan as a material for membrane electrolytes and electrodes in numerous fuel cell types, including polymer electrolyte fuel cells, alkaline polymer electrolyte fuel cells, direct methanol fuel cells, and biofuel cells [15].

1.5. GELATIN

Gelatin is a natural biopolymer that is produced through the partial hydrolysis of animal collagen from skins, bones, and tendons, using either acid (gelatin type A) or alkaline (gelatin type B) methods. The source of collagen and the preparation techniques result in varying physical properties and chemical compositions of gelatin. Despite these differences in production methods, gelatin is highly regarded for its many advantages, making it a multifunctional natural biopolymer.

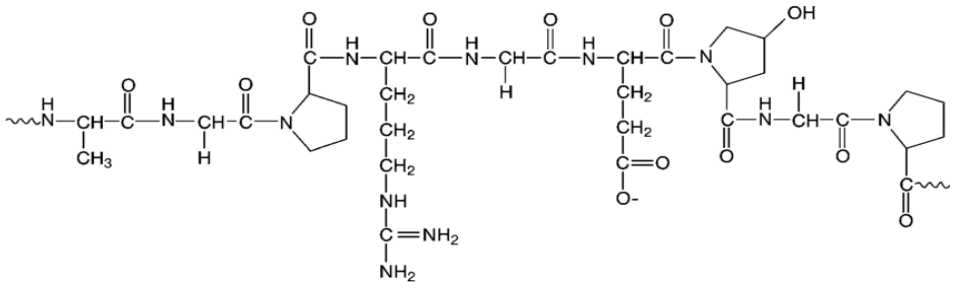

Fig.1.4: Structure of Gelatin

This substance has long been utilized in the food industry, cosmetics, pharmaceuticals, and medical fields due to its outstanding qualities. Gelatin comprises 19 different amino acids arranged in a partially ordered manner. Among these, four amino acids are particularly abundant: roughly 330 glycine, 132 proline, 112 alanine, and 93 hydroxyproline residues are found in every 1,000 amino acid residues, while the remainder consists of other amino acids [16].

Gelatin is a protein that is colourless, tasteless, and usually has a slight yellow tint. It has a fragile and translucent appearance and comes in various forms, including sheets, flakes, and powders. While it does not dissolve in organic solvents, it is soluble in glycerol, hot water, and acetic acid. Gelatin is classified as an amphoteric substance, which means its properties can change based on the characteristics of the solution it is placed in [17].

1.6. SOURCES OF GELATIN

Mammals, particularly pig skin and cow hides, are the primary sources of gelatin, making up 46% of global production. This is followed by other sources like bones (23%) and hooves (29%), with marine sources, such as fish, accounting for just 1%. Recently, there has been a growing interest in gelatin derived from fish and poultry by-products. While poultry waste is expected to become a significant source of gelatin in the future, its commercial production is currently limited due to low yield rates. Ongoing research is focused on extracting gelatin from fish skin and poultry waste as alternatives to traditional mammalian sources. The source of gelatin is essential in the context of packaging, as it significantly affects the physical, chemical, and functional characteristics of the films produced.

Poultry waste and byproduct gelatin possess strong film-forming abilities due to their higher bloom value and the presence of imino acid groups. Fish gelatin also shows effective film-forming properties; it is clear, almost colourless, soluble in water, and very stretchable. Common mammals used for gelatin production are cows, goats, buffaloes, and yaks. Mammalian collagen and gelatin are primarily sourced from hides or skin, tendons, skeletons, and cartilage [17].

1.7. MODIFICATION OF CHITOSAN

1.7.1. Chitosan Blends

Polymer blending is an effective technique used to create polymeric materials that combine the desirable properties of different components for specific uses. In recent times, blends of natural polymers have become increasingly important due to their potential to replace synthetic polymers in various applications. These natural polymers are advantageous as they are renewable, non-toxic, cost-effective, and lead to biodegradable waste.

Chitosan and its blends have gained notable attention among natural polymers because of their versatility and applicability across a wide range of fields. Chitosan's properties can be improved by blending it with both synthetic and naturally derived macromolecules, which has garnered significant interest in recent years.

Chitosan (CS) has recently been combined with synthetic polymers, offering an effective approach to creating new polymeric materials. These materials possess various desirable characteristics that meet specific application needs, which single polymers alone may not achieve—such as being biodegradable and having enhanced mechanical properties. One polymer that can be mixed with chitosan is polyvinyl alcohol (PVA). PVA is a safe, water-soluble, biodegradable, and biocompatible polymer recognized for its excellent physical and chemical qualities. It has a wide range of applications, including membrane fabrication, drug delivery

systems, polymer recycling, and textiles [18]. Chitosan has hydroxyl and amine groups, which allows it to potentially mix with polyvinyl alcohol (PVA) due to the creation of hydrogen bonds. To improve the physical characteristics of chitosan films, PVA was combined with chitosan using a solution blending technique [19].

The solution blending method entails dissolving chitosan in a solvent and subsequently evaporating the solvent. It is the most commonly utilized approach for preparing chitosan blends because of its simplicity and effectiveness in creating various forms, such as beads, microspheres, films, and fibres. In this process, chitosan is dissolved in a suitable solvent while continuously stirring at room temperature. Then, a specific amount of another polymer, also dissolved beforehand, is mixed in while stirring continuously. A crosslinking agent is often added to the chitosan blend solution to enhance mechanical properties. The solution is then filtered and cast onto a glass plate or petri dish to dry at room temperature or in an oven. Finally, the blend is washed with NaOH solution to eliminate any residual acetic acid [13].

The primary interactions observed in chitosan blends include hydrogen bonds, ionic and dipole bonds, π-electron interactions, and charge-transfer complexes. The blending of chitosan with other biopolymers presents an appealing strategy to develop new biomaterials with improved properties tailored to meet the specific requirements of various applications. The goals of chitosan blending can differ based on the demands of the applications, including:

1. Enhancing hydrophilicity
2. Improving mechanical strength
3. Increasing blood compatibility
4. Boosting antibacterial effectiveness [13]

1.7.2. Cross-linked Chitosan

The properties of chitosan after cross-linking are distinct compared to those achieved through chemical modification methods. Generally, the chemical properties of chitosan post-cross-linking are relatively stable and render it insoluble in both acids and bases. This stability allows chitosan to be used effectively as a carrier for adsorbing resins, drugs, immobilized enzymes, and heavy metal adsorbents [20].

Cross-linking modifications can occur either between molecules or within a single molecule. For example, Trimukhe et al. utilized hexamethylene diisocyanate and trimellitic anhydride to cross-link chitosan. They then compared the composition of metal complexes formed through the coordination of chitosan with various metal ions, including Hg, Gu, Cu, Pb, Zn, Cd, and Mn, both before and after cross-linking. The ability of chitosan to adsorb metal ions was ranked as follows: Hg > Gu > Cu > Pb > Zn > Cd > Mn [12].

Additionally, chitosan microspheres, obtained by cross-linking chitosan with tripolyphosphate, demonstrated significant adsorption capacity for metal ions and proved to be highly effective in wastewater

Treatment [21]. Zahid et al. employed tetraethyl orthosilicate as a cross-linking agent to create chitosan/PVA hydrogel composites, which exhibited a compact, uniform structure that is resistant to high temperatures and showed excellent performance in drug delivery systems [22].

1.7.3. Porous Chitosan Membranes

Porosity is a vital feature of polymeric membranes that significantly influences their separation capabilities. In the case of chitosan membranes, having a well-developed porous structure can enhance both permeability and the ability to adsorb contaminants. This improvement stems from decreased mass transfer resistance in the porous framework, a greater capacity for void adsorption, and improved water absorption characteristics [23].

Pore generators, commonly known as porogens, are additives utilized to develop porous matrices mainly via a selective dissolution process. Both organic and inorganic porogens, such as polyethylene glycol (PEG), polyvinyl pyrrolidone (PVP), polyethylene oxide, silica, alumina, zirconia, and inorganic salts like sodium chloride (NaCl), and calcium carbonate ($CaCO_3$), are examples used in the preparation of chitosan-based membranes [23].

The creation of porosity in chitosan membranes involves selectively dissolving a component from the polymer mixture. For example, pore-forming agents like PEG, PVP, or specific salts are soluble in water and

integrate well with chitosan. During the alkaline treatment and washing phases, these pore-formers dissolve, resulting in the formation of pores and cavities within the membrane [24].

1.7.4. Chemical Modifications

1.7.4.a. Sulfonation

The sulfonation of chitosan is an effective approach to enhance its water solubility. Recent developments in the sulfonation process have allowed for better control over reaction sites and the extent to which sulfonate groups are substituted in sulfonated chitosan. This modified form of chitosan demonstrates various biological activities, such as antiviral, antibacterial, anticoagulant, osteogenic, antioxidative, and anti-calcification effects. To improve human health, further investigation into its medical uses is crucial, especially regarding drug delivery systems that utilize alkylated chitosan [25].

The choice of sulfating reagent and reaction conditions can result in either selective or non-selective derivatives. Amino and hydroxyl groups can interact with electrophilic agents, such as alkyl halides, acids, or iso(thio)cyanides, leading to the creation of N- and O-modified chitosan (CS) derivatives. Furthermore, the sulfonation process may take place through different types of systems—heterogeneous, homogeneous, or pseudo-homogeneous—depending on the sulfonating reagent and the reaction medium, producing various sulfonated chitosan (SCS) derivatives.

It is indeed possible to generate different SCS derivatives from a single original CS following the sulfonation reaction. Although these SCS derivatives share a similar chemical composition, their structures vary, which may be attributed to regioselectivity influenced by the diverse reactivities of the three functional groups present in the parent polymer. Consequently, one can obtain mono-SCS derivatives, di-SCS compounds, or copolymers that incorporate both mono-sulfonated and disulfonated segments, either randomly or uniformly distributed along the polymer chain [26].

1.7.4.b. Carboxylation

The carboxy alkylation process introduces acidic groups into the polymer backbone. Due to the presence of native amino groups, chitosan variants can adapt to various environments. Like other amphoteric polymers such as polypeptides, these derivatives have an isoelectric point at which the overall polymer carries no net charge. While the polymer remains undissolved at the isoelectric point (pH), it can still be soluble in water when the pH is either above or below this point. Different reaction conditions result in either N-carboxyalkyl chitosan or O-carboxyalkyl chitosan, allowing for selectivity between the N and O modifications [25].

The carboxylation reaction mainly involves glyoxylic acid or chloroalkanoic acid interacting with the C-6–OH or C-2–NH_2 groups in chitosan, producing a −COOH group. Carboxylated chitosan shows good solubility in water and can dissolve in neutral and alkaline solutions. Furthermore, it possesses better properties for thickening, heat

preservation, film formation, flocculation, and kneading compared to chitosan. As a result, carboxylated chitosan finds wider applications across various sectors, including industrial, agricultural, medical, health, and biochemical fields [27].

1.7.4.c. Esterification

Chitosan can effectively react with aliphatic and aromatic acids because of the unique properties of the hydroxyl group at the C-6 position. This reaction enhances the water solubility of chitosan and retains the original amino groups, improving its antibacterial capabilities. A derivative of etherified chitosan has numerous applications in the pharmaceutical industry and is known for being non-toxic, bacteriostatic, water-soluble, and has excellent water retention properties.

When chitosan reacts with hydroxybutyl groups, it produces hydroxybutyl chitosan (HBC), a form of chitosan that is soluble in water due to etherification. Likewise, hydroxypropyl chitosan is created by interacting chitosan with propylene epoxide in an alkaline setting, resulting in a water-soluble etherified form of chitosan [25].

1.8. AIMS AND OBJECTIVES OF THE STUDY

The present investigation focuses on the synthesis of porous chitosan-gelatin membranes through a meticulously designed preparation strategy that ensures a uniform and well-defined porous structure. By selectively removing gelatin from the chitosan matrix, the process aims to produce membranes with minimal impurities and optimized porosity. This approach enhances the structural and physicochemical properties of the membranes, paving the way for their use in applications requiring precise performance standards.

1.8.1 Aim

The primary goal is to enhance the physicochemical properties of chitosan. This includes improving its solubility, biocompatibility, mechanical strength, and stability through structural modification. This will broaden the scope of applications for chitosan-based materials in areas such as drug delivery, tissue engineering, wound healing, and environmental remediation. Additionally, the aim is to optimize the properties of chitosan-based materials to meet the requirements of specific applications, such as controlled drug release, antibacterial activity, and adsorption capacity for pollutants. Finally, the aim is to explore sustainable and eco-friendly modification approaches that minimize the use of hazardous chemicals, reduce waste generation, and utilize renewable resources.

1.8.2 Objectives

- To identify suitable modification strategies. This involves reviewing and selecting appropriate chemical, enzymatic, and physical methods for modifying chitosan while considering factors such as reaction conditions, reagent compatibility, and scalability.
- To attempt comprehensive characterization of modified chitosan materials to understand their structure-property relationships. This includes analyzing changes in molecular structure, chemical composition, morphology, surface properties, and thermal stability.
- To optimize modification processes to ensure scalability, reproducibility, and cost-effectiveness, thus facilitating the translation of research findings into practical applications.

Chapter 2

LITERATURE SURVEY

Chitosan membranes are recognized for their versatility as platforms for drug delivery, especially through non-parenteral routes. In their research, Mohammed et al. underscored the distinctive characteristics of chitosan, focusing on its biodegradability and biocompatibility, which are critical for creating membranes designed for the safe delivery of therapeutic agents. By engineering chitosan membranes with mucoadhesive properties, they can stick to mucosal surfaces, enabling sustained drug release that is particularly advantageous for treating localized conditions like cancer and gastrointestinal diseases [28].

In addition, the integration of chitosan into mixed matrix membranes has shown promising results in improving drug release mechanisms. Caridade et al. discussed how chitosan membranes could enhance pharmacokinetics and therapeutic effectiveness, especially for anticancer and nucleic acid-based therapies. This emphasizes the role of chitosan membranes in overcoming the challenges posed by traditional drug delivery methods [29].

Regarding pH-responsive systems, Mohammadi et al. demonstrated that nanoparticles modified with chitosan could improve the stability and targeted delivery of antimicrobial agents, showcasing the adaptability of chitosan membranes in various drug delivery applications.

These membranes maintain stability in acidic conditions while becoming activated in neutral pH environments, offering an innovative way to target bacterial infections [30].

Chitosan membranes also play a significant role in guided tissue regeneration (GTR) and guided bone regeneration (GBR). Santos et al. highlighted their biocompatibility and biodegradability, which make chitosan ideal for membranes used in periodontal regeneration. The antimicrobial attributes of chitosan further support tissue healing by acting as a barrier to prevent non-target cell migration while fostering the regeneration of periodontal tissues [31].

The creation of free-standing films using chitosan and alginate for drug delivery systems showcases its promise in wound healing and tissue engineering. Kang et al. emphasized the importance of layer-by- layer assembly of these materials, which aids in manipulating membrane properties to manage drug permeation and enhance cell adhesion [32].

Chitosan membranes also have potential applications beyond the biomedical field, including water treatment and gas separation. The incorporation of chitosan into mixed matrix membranes for gas separation has shown increased selectivity and permeability, particularly in CO_2 separation, as demonstrated by Posocco et al.. This reflects the potential of chitosan membranes to address environmental challenges and promote sustainability [33].

In energy applications, Liu et al. reviewed the development of chitosan-based membranes for electrochemical uses, particularly in batteries and supercapacitors. Their findings highlighted the membranes' conductivity and electrochemical stability, indicating their potential in energy storage solutions [34]. Additionally, Thamphiwatana et al. proposed chitosan membranes as eco-friendly alternatives to conventional fuel cell membranes, with ongoing modifications aimed at improving their proton conductivity [35].

Gelatin is an essential component used as a porogen in the creation of hierarchically porous materials. It acts as a template or porogen to form structures with defined pore sizes and distributions, which are critical for applications in energy conversion, catalysis, and life sciences. Various methods for synthesis, such as freeze-drying and phase separation, enable the development of porous structures that boost the performance of materials by increasing surface area and improving accessibility for reactions or biological interactions by Yang et al. [36]. These results highlight the importance of porosity and structural hierarchy in achieving high-performance materials, thereby confirming the effectiveness of gelatin as a porogen in material science.

In biomedical engineering, gelatin's function as a porogen is particularly important for creating scaffolds that replicate the extracellular matrix. Research has demonstrated that gelatin can produce porous structures that promote cell growth and tissue regeneration. Recent advancements in 3D printing technology allow for precise manipulation of the porosity of gelatin-based materials, which is vital for nutrient and

oxygen transfer in tissue engineering was studied by Bello et al. [37]. Moreover, crosslinking techniques can improve the stability and functionality of gelatin scaffolds, making them applicable for various medical uses. These findings showcase the adaptability of gelatin in developing biomaterials that facilitate cellular processes and tissue repair.

Recent investigations have also looked into creative methods for using gelatin as a porogen in the production of porous hydrogels. For instance, Sun et al. employed gelatin beads as sacrificial porogens enabling the simultaneous encapsulation of cells while creating a porous structure, which enhances the transfer of nutrients and oxygen within tissue scaffolds [38].

The integration of chitosan and gelatin by Rodríguez-Rodríguez et al. has resulted in the creation of composite hydrogels and membranes with improved characteristics that are ideal for a range of biomedical applications [39]. The addition of gelatin to chitosan matrices significantly enhances mechanical strength, water absorption, and porosity—key factors for applications such as drug delivery systems and tissue engineering scaffolds. The porosity of chitosan membranes, which can be adjusted by incorporating gelatin as a porogen, is crucial for their functionality. Research done by Lan et al. indicates that the porosity of chitosan/gelatin composites can be fine-tuned to promote nutrient diffusion and cell migration, which are essential for effective tissue regeneration [40].

Furthermore, studies by Ahearne et al. have shown that the addition of gelatin improves the mechanical properties of chitosan membranes, making them more viable for load-bearing applications [41]. Moreover, the introduction of gelatin in chitosan membranes enhances both porosity and properties such as proton conductivity and gas diffusion. By applying structural modifications to chitosan membranes, comparable gains in power density and efficiency may be achievable.

Finally, the investigation into adding nanoparticles to chitosan composites by Ye et al. has revealed enhanced antimicrobial and wound-healing capabilities This indicates that incorporating additional functional materials alongside gelatin could further improve the properties of chitosan membranes, boosting their performance in both fuel cells and supercapacitors [42].

Chapter 3

MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Chitosan

Chitosan exists as white-coloured flakes having a molecular weight (of 1,50,000) used. It is purchased from Tokyo Chemical Industries (TCI) which is produced under a pressure range of 200-600 mpa.s at 0.5% in 0.5% ACH at 20°C. A catch no. 9012-76-4

Fig. 3.1: Chitosan polymer taken in a Petri plate

3.1.2. Gelatin

Gelatin purified. It is bought from MERK having batch no.ML9M593193

Fig. 3.2: The bottle of Gelatin

3.1.3. Glacial Acetic Acid

Chitosan was dissolved in 1% (v/v) aqueous acetic acid, purchased from Emplura Merk with an Assay of ≥99%.

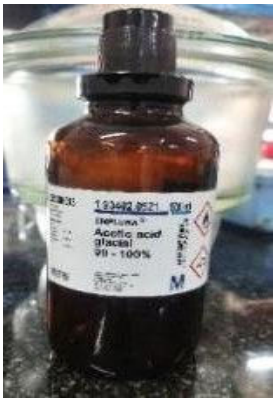

Fig. 3.3: The assay and the bottle of Acetic acid

3.2. METHODOLOGY

3.2.1. Preparation of Pristine Chitosan Membrane

Pristine Chitosan membrane was prepared by dissolving 0.2g of chitosan in 20 ml of distilled water. The solution was stirred continuously for 6 hours using a magnetic stirrer at 800 RPM at room temperature. After 6 hours the solution was poured into the casting surface of an area of 10 cm × 10 cm. The solution was spread evenly on the surface to ensure uniformity. The surface containing the solution was then kept in an oven at 50°C overnight for drying. After drying the chitosan membrane was carefully removed from the surface. It is labelled as CS and stored.

3.2.2. Preparation of Chitosan-Gelatin (CS/GN) Blend Membranes

A series of chitosan-gelatin blend membranes were prepared by varying the ratio of gelatin (10%, 20%, 30%, 40%, 50%). The 10% gelatin was dissolved in 16 ml distilled water and stirred for 30 minutes at 50°C using a magnetic stirrer. To this mixture, chitosan was added along with 4 ml of distilled water and stirred for 8 hours at room temperature. After 8 hours the solution was carefully poured into a casting surface of an area of 10 cm × 10 cm. The solution was spread evenly on the surface to ensure uniformity. The casting surface containing the solution was kept in an oven at 50°C overnight for drying. After drying the membrane was carefully removed from the casting surface. Its thickness was measured. The membrane was cut into two halves. One-half was subjected to neutralization and drying. The other half was labelled and stored. One-half

of the membrane was soaked in 1N NaOH solution for 2 hours to neutralize the acid. After 2 hours, the membrane was washed with distilled water to remove the NaOH adsorbed on the surface of the membrane. Then the membrane was soaked in distilled water and kept in a water bath at 60°C for 3 hours to remove the porogen from the membrane surface. After 3 hours it was kept at room temperature for drying and then kept in an oven at 50°C for a day to ensure it was completely dry. The membrane is labelled as CG_1 and stored. The process was repeated and membranes before the removal of the porogen are depicted as CG_{1B}, $CG_{2B,}$ CG_{3B}, CG_{4B}, and CG_{5B}, and the Blend Membranes after the removal of the porogen are depicted as CG_{1A}, CG_{2A}, CG_{3A}, CG_{4A}, and CG_{5A}.

3.2.3. Preparing Chitosan/Polyvinyl alcohol (CS/PVA) Blend Membrane

The CS/PVA blend membranes were fabricated using the solvent evaporation method. Blends with varying weight ratios of CS/PVA (90/10, 80/20, 70/30, 60/40, and 50/50) were prepared and labeled as CP_1, CP_2, CP_3, CP_4 and CP_5 respectively. The process began with accurately weighing the required amount of PVA, which was then dissolved in distilled water and stirred at 500 RPM for 30 minutes at 90°C. After the PVA was fully dissolved, the appropriate quantity of acidified chitosan and additional distilled water was added to the solution, forming a 1% (w/v) solution. The mixture was continuously stirred for 8 hours at 500 RPM to ensure complete dissolution and uniformity. Once a homogeneous solution was achieved, it was carefully cast into Teflon molds and incubated overnight in an oven at 50°C, leading to the formation of solid membranes.

To neutralize the acidic components within the membrane matrix, the membranes were treated with a 1M NaOH solution for 2 hours. Subsequently, the membranes were washed with distilled water several times to remove the NaOH adsorbed on the surface of the membrane. The membranes were then air-dried at room temperature and stored in a moisture-free environment, ready for further applications.

3.2.4. Water absorption capacity of CS/GN Blend Membrane

The water-absorption behaviour of CS-GN membranes in double-distilled deionized water at room temperature was examined. All the dried membranes, which measured 2 cm × 2 cm, were weighed beforehand and then submerged in deionized water for 24 hours. The membranes were removed and promptly wiped with tissue paper to remove any excess surface water, and then weighed in their wet state. The swelling ability, expressed as a percentage, was calculated using a formula that has been previously reported. (Fabrication of bioinspired chitosan/gelatin/allantoin biocomposite film for wound dressing application)

$$\text{Swelling } \% = \frac{(Wf - Wd)}{Wd} \times 100 \qquad (1)$$

Where, W_f is the weight of wet membrane after immersion, W_d is the weight of dry membrane before immersion.

3.2.5. Ionic Conductivity of CS/GN Blend Membrane

The ionic conductivity of chitosan-based membranes was assessed using electrochemical impedance spectroscopy (EIS). These measurements were conducted at 25°C with a potential amplitude of 10 mV. A two-electrode setup was utilized for the experiment. Before testing, the membranes were soaked for 24 hours in a 2 M aqueous solution of lithium acetate (LiOAc) to ensure proper swelling. The membranes were positioned between two flat electrodes within a test vessel during the measurements. The ionic conductivity was calculated using the conductivity formula (2)

$$\sigma = \frac{S_S}{A \times R} \qquad (2)$$

where, S_s represents the membrane thickness, A is the electrode surface area (2.8338 cm^2), and R corresponds to the resistance determined from the EIS data.

3.2.6. Ion-exchange capacity of CS/GN Blend Membrane

To determine how CS reacts with GN in blended form, we estimated the ion exchange capacity (IEC) of the blends. The IEC provides an idea about the extent of crosslinking by indicating the number of groups present before and after crosslinking.

To determine the IEC, we soaked specimens of similar weight in 150 mL of 0.01 N KOH solution for 48 hours at ambient temperature. After 48 hours, we decanted the KOH solution and washed the membrane with deionized water for 24 hours to remove any remaining KOH solution on the surface. Then, the membrane was soaked in 0.01N HCl solution for 48 hours.

Next, we back-titrated the HCl solution with a standardized 0.01 M NaOH solution using phenolphthalein as an indicator. We calculated the IEC using Equation (3)

$$\text{IEC (meq/g)} = \frac{(V_{NaOH} \times N_{NaOH})}{W_{dry}} \quad (3)$$

Where, V_{NaOH} is the Volume of NaOH used for the titration against HCL solution, N_{NaOH} is the Normality of NaOH used, and W_{dry} is the dry weight of the membrane.

3.3. CHARACTERIZATION METHODS

3.3.1. Fourier Transform Infrared Spectroscopy (ATR-FTIR technique)

Infrared spectroscopy relies on the interaction of molecules with IR radiation, which falls within the electromagnetic spectrum between visible light and microwave radiation. Each molecule exhibits distinctive vibrational modes, involving the motion of atoms within the molecule. Absorption of infrared radiation by a molecule leads to alterations in its vibrational energy levels, producing a spectrum that offers valuable insights into its structure and functional groups. The IR spectrum is commonly depicted as a graph showing absorbance (or transmittance) plotted against wavenumber, measured in reciprocal centimetres (cm^{-1}).

Attenuated Total Reflectance (ATR) serves as a sampling technique that involves the introduction of light onto a sample to obtain crucial structural and compositional information, making it a highly utilized method in the realm of FT-IR Spectroscopy. This method makes use of an optical sensor material characterized by a high refractive index, which facilitates internal reflection within the sensor, thus enhancing the accuracy of the results obtained. The manipulation of the number of back scatterings is made possible by precise regulation based on the refractive material used and the specific design of the sensor, showcasing a high level of control over the data collection process. The energy emitted during the analysis will effectively probe any material that makes contact with the sensor, allowing for a comprehensive examination of the sample under investigation. The intensity of the FTIR spectrum is intricately linked to various factors such as the number of reflections, the depth of penetration of the wave into the sample, the specific properties of the substances of interest, and their corresponding absorptivity characteristics. ATR methodology can capture a spectrum ranging from 10 microns to samples that are 10 cm thick, encompassing both liquid and solid substances, thereby demonstrating its versatility in analyzing a wide array of chemical reactions without being constrained by sample thickness. Moreover, the resilience of ATR sensors to abrasion and their ability to endure the harsh conditions often associated with diverse chemical reactions further solidify their utility in various scientific investigations.

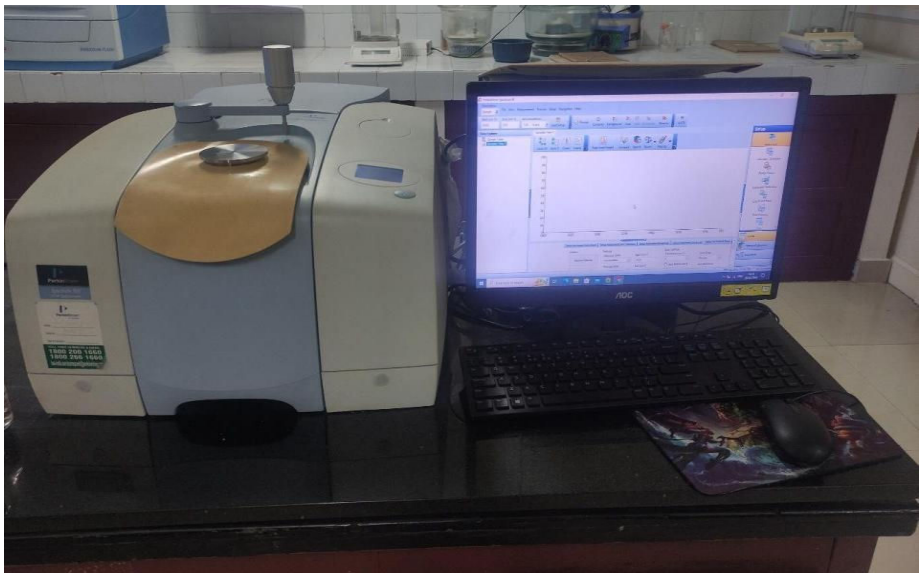

Fig. 3.4: Perkin Elmer ATR- FTIR Spectrometer

3.3.2. X-ray Diffraction (XRD)

X-ray diffraction (XRD) is a powerful analytical technique for determining a molecule's geometry or configuration through X-rays. The fundamental principles of XRD methods rely on the elastic scattering of X-rays from materials possessing a high degree of structural regularity and order over long distances. This scattering process allows for interrogating the atomic arrangement within a given material. When X-rays interact with a crystalline sample, they undergo diffraction since their wavelength is on the same order of magnitude as the inter-atomic distances present in the crystal lattice. This diffraction pattern provides valuable information.

about the spatial arrangement of atoms within the crystal structure, enabling researchers to elucidate the three-dimensional morphology of the material under investigation.

The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's law:

$$n\lambda = 2d\sin\theta$$

where, n is an integer, λ is the wavelength of the X-rays, d is the interplanar spacing generating the diffraction, and θ is the diffraction angle.

When the X-ray beam interacts with the ordered three-dimensional configuration of atoms within a crystal lattice, the majority of the X-rays undergo destructive interference, leading to their mutual cancellation; however, in certain defined orientations, the X-ray waves exhibit constructive interference, resulting in their mutual reinforcement. It is these intensified diffracted X-rays that give rise to the distinctive X-ray diffraction pattern essential for determining the crystal structure. This pattern serves as a critical tool in the field of crystallography, allowing scientists to analyze the arrangement of atoms within a crystal with high precision and accuracy.

3.3.3. Scanning Electron Microscope (SEM)

The Scanning Electron Microscope uses an electron beam instead of light for imaging specimens. The human eye can distinguish points 0.2 mm apart. The resolving power of the eye is the distance where points appear as one. Light microscopes use visible light and lenses to see small objects. Light microscopes have a magnification of 1000x and a resolution of 200 nm. Electron Microscopes were developed due to light microscopes' limitations. Electron Microscopes have higher magnification and resolution. The smaller wavelength of the light source gives better resolution in a microscope. The formation of a beam of electrons is achieved by utilizing the Electron Source, a device designed specifically for this purpose, which subsequently drives these electrons toward the specimen by applying a positive electrical potential. As the electron beam advances, it is precisely guided and concentrated utilizing a combination of metal apertures and magnetic lenses, meticulously arranged to ensure the production of a thin, focused, and monochromatic beam. Within this beam, the electrons engage in interactions with the atoms present in the specimen, resulting in the generation of signals that carry crucial information about the surface topography, composition, and various other electrical properties of the specimen under examination. These intricate interactions and ensuing effects are meticulously captured and discerned, ultimately

being translated into a comprehensible image that provides valuable insights into the characteristics of the specimen.

Fig. 3.5: Scanning Electron Microscopy Instrument

3.3.4. Thermal Gravimetric Analysis (TGA)

Thermal analysis, a methodological approach employed to assess alterations in the chemical, physical, and structural properties of a substance in response to fluctuations in temperature, holds paramount importance in scientific research. Temperature, being a fundamental state variable, exerts a profound influence on various chemical reactions, physical attributes, and structural modifications within materials. The overarching concept of thermal analysis encompasses any scientific or technological attempt aimed at characterizing a material by manipulating

temperature as an experimental parameter. Nevertheless, historically, the term "thermal analysis" has been primarily associated with specific methodologies about thermogravimetric and calorimetric phenomena. Contemporary understanding acknowledges that key techniques integral to thermal analysis include the detection of temperature differentials between a test sample and a reference material (DTA), the quantification of weight loss via thermogravimetry (TG) and its derivative (DTG), and the determination of heat flux through differential scanning calorimetry (DSC). Moreover, additional methodologies utilized for assessing thermal properties such as thermal conductivity, specific heat capacity, and thermal diffusivity often fall under the umbrella of thermal analysis. Depending on the specific objectives of the analysis, a diverse array of techniques or a combination thereof may be employed to evaluate material characteristics comprehensively. Notably, among these techniques, the TG/DTG method stands out as a compassionate approach extensively utilized for scrutinizing the thermal stability of natural fiber polymer composites, shedding light on the intricate interplay between composition and structure with thermal degradation behaviour.

Fig. 3.6: TGA Analysis Instrument

Chapter 4

RESULTS AND DISCUSSION

4.1. Chemical Structure Evaluation of CS/GN Blend Membrane

4.1.1. Before Porogen Removal

Attenuated Total Reflection Fourier Transform Infrared (ATR- FTIR) spectra of membranes were recorded with a Perkin Elmer Spectrophotometer, in a range of 4000-600 cm^{-1} with a resolution of 4 cm^{-1} and the no. of scans were 128.

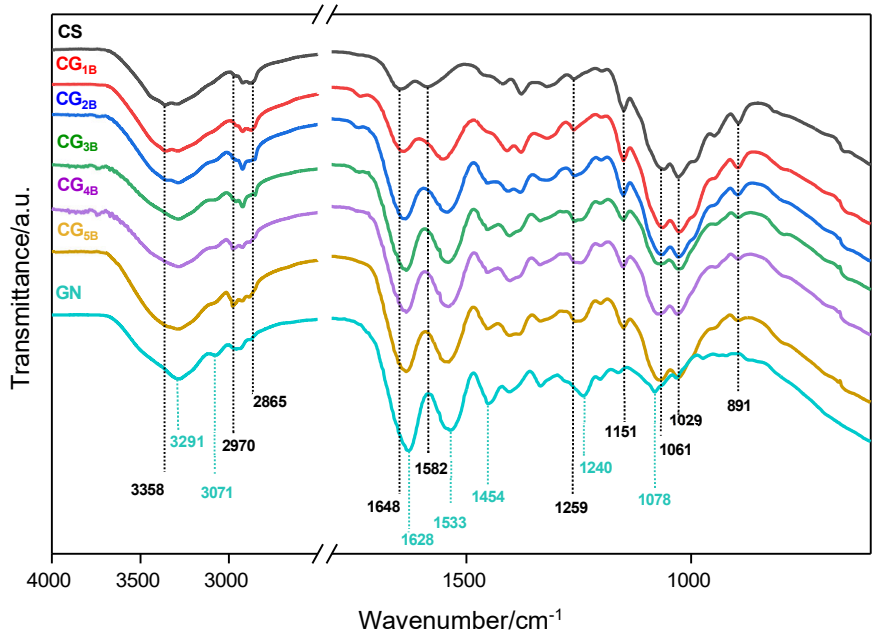

Fig.4.1: FTIR spectrum of CS/GN blend membranes before porogen removal

The FTIR spectrum of the pristine chitosan membrane (CS) exhibits characteristic peaks reflecting its functional groups. The broad peak in the range of 3400-3200 cm^{-1} corresponds to the hydroxyl group (-OH), while the peaks at 3363 cm^{-1} and 3286 cm^{-1} indicate the presence of amine groups ($-NH_2$), suggesting the presence of strong and weak intramolecular and intermolecular hydrogen bonds between -OH and $-NH_2$. Additionally, peaks in the range 2970-2800 cm^{-1} correspond to the stretching vibrations of the methyl group in $NHCOCH_3$, methylene group in CH_2OH, and methylene group in the pyranose ring. Furthermore, the peak at 1648 cm^{-1} is attributed to the amide I band, reflecting NH, CO(I) stretching as a result of incomplete deacetylation of chitosan. The 1582 cm^{-1} peak is often associated with the amide II band, arising from N-H stretching and bending vibrations. Lastly the peaks at 1151, 1061,1029 cm^{-1} correspond to bridge oxygen C-O-C) stretching bands, characteristics of CS saccharide structure

while the peak at 891 cm^{-1} reflects the presence of β-1,4-glycosidic bonds.

The FTIR spectrum of the pure gelatin membrane (GN) reveals significant peaks, with the amide A peak at 3291 cm^{-1} primarily associated with O-H and N-H stretching vibrations, indicating the presence of hydrogen bonds. The amide B peak at 3071 cm^{-1} is mainly attributed to N- H stretching vibrations. Peaks observed in the 2970-2800 cm^{-1} range correspond to the symmetric and asymmetric stretching of $-CH_2$ groups. The peak at 1628 cm^{-1} is linked to the C=O stretching of amide I, while the peak near 1533 cm^{-1} is associated with N-H bending vibrations of amide II. Additionally, the peak around 1454 cm^{-1} is attributed to C-O stretching in amide III, and the peak at 1240 cm^{-1} corresponds to N-H in- plane bending of amide III.

The spectrum of the blend membranes displays characteristic peaks from both chitosan (CS) and gelatin (GN), with slight variations in certain regions. Notably, the blend membranes exhibit a slight shift and alteration in peak structure, where the multiple stretching vibrations in chitosan decrease and the peak broadens in the 3400-3200 cm^{-1} range as the gelatin content increases. This change likely reflects modifications in hydrogen bonding, indicating inter- and intramolecular hydrogen bonds between the two polymers. The incorporation of GN into CS leads to conformational changes, with the peak at 1648 cm^{-1} in CS shifting to 1633 cm^{-1} in the blends, along with increased carbonyl stretching, suggesting alterations in the secondary structure of GN in the amide I region. Additionally, the introduction of GN into CS causes a shift in the amine bonds of CS from 1582 cm^{-1} to 1541 cm^{-1} in the blends, indicating the formation of a

hydrogen bonding network between polymer molecules within the polyelectrolyte complex. The variation in the intensity of amide III bands in the blend membranes, corresponding to the CS/GN ratio, suggests that the amino groups of CS interact with the carbonyl groups of GN through electrostatic interactions. The blend spectrum also shows a reduction in the C-O-C bridge stretching vibrations of CS at 1151, 1061, and 1029 cm^{-1}, indicating that the O-H groups of CS interact with the COOH groups of GN, leading to a decrease in the C-O stretching bands of the CS saccharide structure. As the GN content increases, the peaks become more distinct and broader, with increased intensity. The presence of both CS and GN peaks in the blend confirms the successful combination of these two polymers and verifies the ionic interactions between the positively charged CS and negatively charged GN.

4.1.2. After Porogen Removal

After the removal of porogen from the CS/GN blend membranes at various ratios specific observations were made from the FTIR spectra. The peaks between 3200-3400 cm^{-1}, associated with O-H and N-H stretching vibrations, remained unchanged, indicating the removal of gelatin and reaffirming that these peaks are primarily due to chitosan. The peaks at 1648cm^{-1} (amide I) and 1582 cm^{-1} (amide II) of chitosan remained constant in the blend membranes after washing, indicating the retention of the chitosan structure. Peaks at 1151, 1061 cm^{-1} (C-O stretching), 1029 cm^{-1} (skeletal C-O stretching), and 891 cm^{-1} (β-glycosidic linkages) remained unchanged in the blend membranes, indicating that these structural features were retained post-washing. A new peak at 796 cm^{-1} was observed in the blend membranes after washing, with its intensity increasing from CG_4 to CG_1.

After washing, the FTIR analysis of the chitosan-gelatin blend membranes shows that gelatin acts as a porogen, which helps retain important chitosan functional groups while possibly introducing new structural features after removing the porogen.

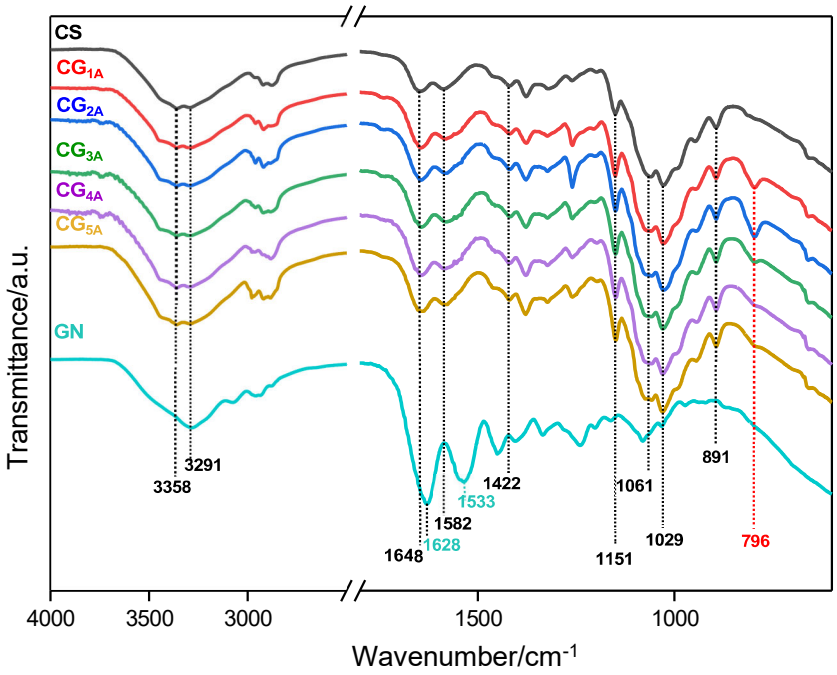

Fig. 4.2: FTIR spectrum of CS/GN blend membranes after porogen removal

4.2. Microstructure evaluation of the CS/GN Blend Membranes using XRD

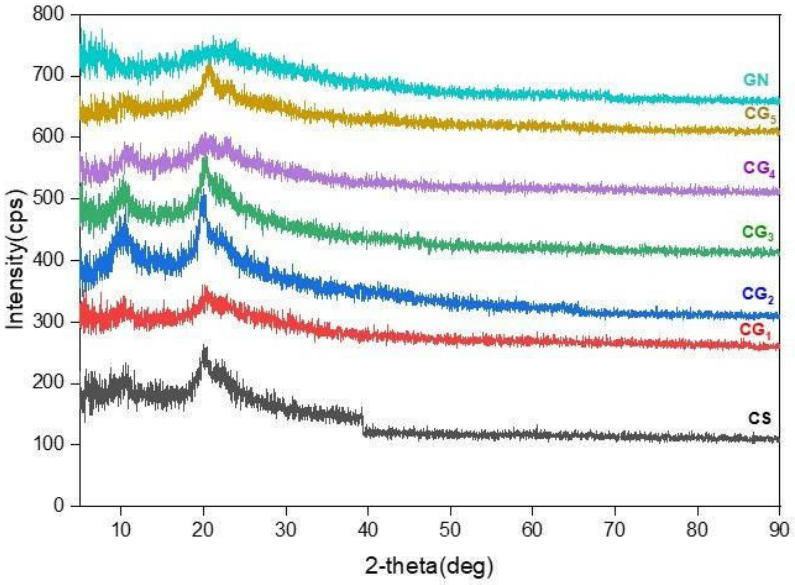

Fig. 4.3: Microstructure evaluation of XRD

The X-ray diffraction patterns of CS/GN blend membranes displayed in Fig.4.3. clearly show that the pristine chitosan membranes exhibited characteristic diffraction of about 2θ=10° and 20°, indicative of their crystalline regions resulting from the regular arrangement of polymer chains. However, the introduction of porogen, gelatin caused noticeable changes in the XRD patterns of the blend membranes. The diffraction peaks shifted, and the intensities decreased, accompanied by peak broadening. These modifications reflect a reduction in the crystalline regions, attributed to the disruption of the polymer matrix by the porogens. Such changes enhance the membrane's porosity.

4.3. Morphology evaluation of the CS/GN Blend Membranes using SEM

The SEM image of pristine chitosan clearly shows a featureless morphology as opposed to the chitosan membrane prepared by adding 20% gelatin as porogen and then removing the porogen using NaOH washing. The pores are visible in the CG_2 membranes indicating the removal of porogen and the generation of pores.

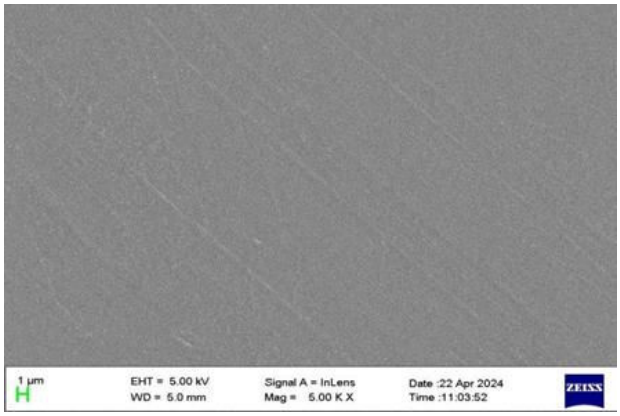

Fig. 4.4: SEM image of pristine chitosan film

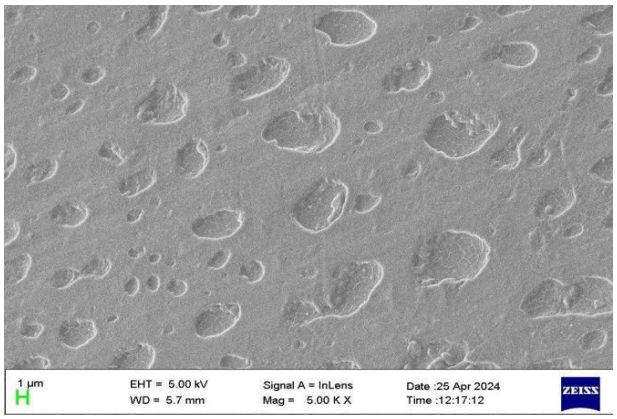

Fig. 4.5: SEM image of CS/GN blend membrane containing 20% gelatin after the removal of porogen-gelatin

4.4. Thermo-Gravimetric Analysis of CS/GN Blend Membranes

4.4.1. Before Porogen Removal

Thermogravimetric Analysis (TGA) on pure chitosan and chitosan-gelatin blends at different ratios were studied. Unfortunately, the TGA results for the CG_{1B} blend were not obtained due to a technical issue during the analysis. As a result, this data point was excluded from the study.

For pure chitosan, the decomposition typically occurs in two stages: the first stage between 50°C and 150°C due to moisture loss, and the second stage between 250°C and 350°C corresponding to the degradation of the polymer backbone. These temperature ranges provide insight into the thermal stability of the chitosan membrane.

The decomposition of chitosan-gelatin blend membranes typically occurs in multiple stages. The first stage, between 50°C and 150°C, involves moisture loss and initial polymer degradation. The second stage, from 200°C to 350°C, corresponds to the primary decomposition of both chitosan and gelatin, while the final stage, between 350°C and 500°C, involves further degradation and the formation of residual carbonaceous material. However, the results did not display a clear or consistent trend in thermal stability across the different ratios. This lack of a clear gradation in the decomposition temperatures suggests that further investigation is required to better understand the thermal behaviour of these blends and to ensure more consistent results in future analyses. Given the unexpected variability in the data, further refinement of the experimental process is necessary.

We plan to revisit the TGA analysis with a focus on improving accuracy and consistency across all blend ratios.

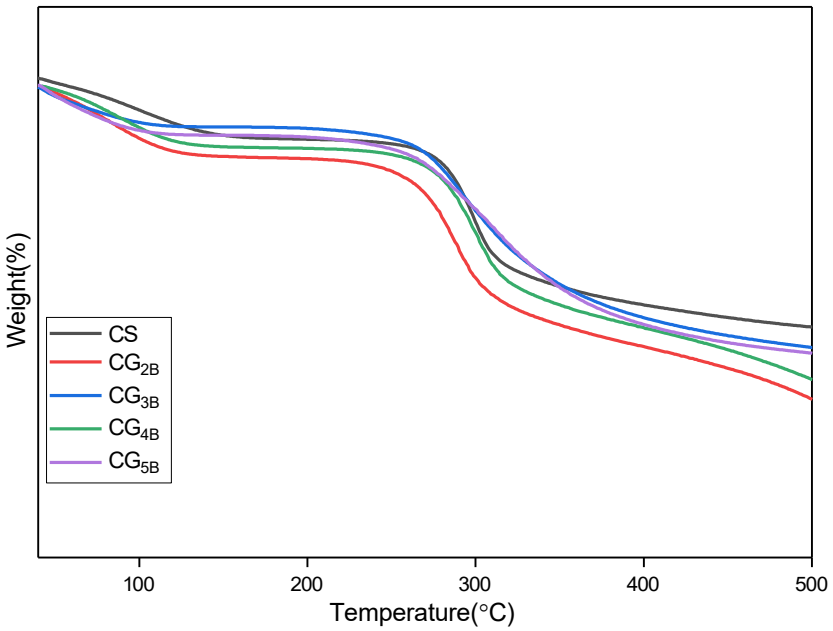

Fig 4.6: TGA CS/GN blend membranes before porogen removal

4.4.2. Thermo-Gravimetric Analysis of CS/GN Blend Membranes After Porogen Removal

After washing the blend membranes to remove GN from the CS matrix, the TGA results showed that the remaining membranes exhibited decomposition patterns identical to those of Pristine CS. Unfortunately, the data for the CG_{1A} after washing was also not obtained due to the same technical issue. This observation confirms that the chitosan component

retains its thermal characteristics even after the removal of gelatin from the blends.

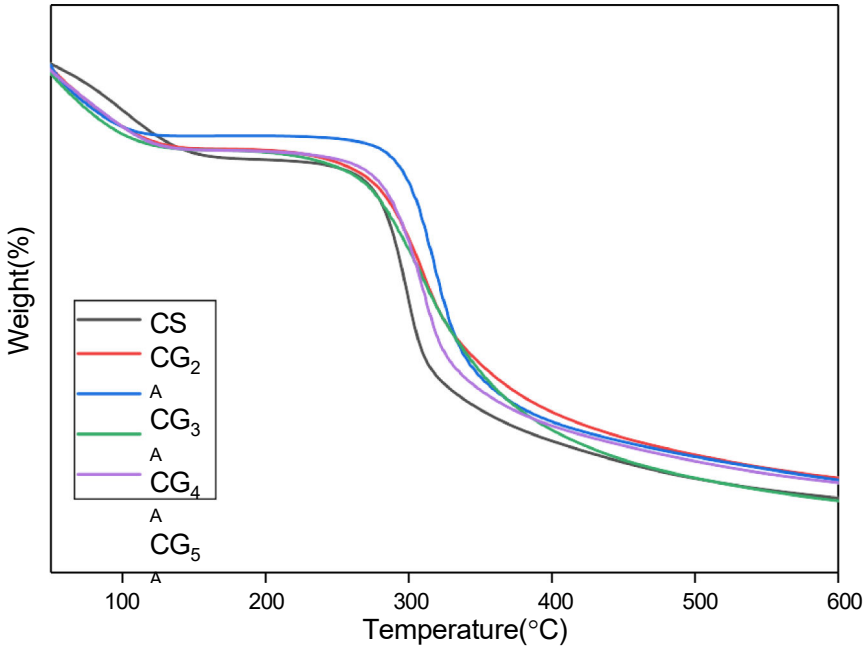

Fig 4.7: TGA of CS/GN blend membranes after porogen removal

4.5. Water Solubility of CS/GN Blend Membranes

The water solubility of chitosan-gelatin (CS/GN) blend membranes (**Table 4.1**) reflects their capacity to absorb water and is strongly influenced by their composition and structural properties. The pristine chitosan membrane (CS) displayed a water solubility of 74.53%, while the blend membranes prior to porogen removal (CG_{1B} to CG_{4B}) exhibited a progressive increase in water solubility, reaching 98.15% for CG_{4B}. This

behaviour can be attributed to the hydrophilic nature of gelatin, which enhances water molecule penetration in the membrane matrix. Conversely, after porogen removal, the blend membranes (CG_{1A} to CG_{4A}) demonstrated a significant reduction in water solubility, with CG_{4A} showing the lowest value of 53.42%. This reduction highlights the role of chitosan's hydrophobicity in disrupting water interactions and improving water resistance. These results suggest that porogen removal effectively modulates the hydrophilicity and water absorption characteristics of the membranes, balancing solubility and water resistance.

Table 4.1: Water Solubility of CS/GN membranes

Samples	Water Solubility
CS	74.53%
CG_{1B}	76.27%
CG_{2B}	84.62%
CG_{3B}	97.40%
CG_{4B}	98.15%
CG_{1A}	80.22%
CG_{2A}	79.81%
CG_{3A}	63.79%
CG_{4A}	53.42%

4.6. Ionic Conductivity of CS/GN Blend Membranes

The ionic conductivity of CS/GN blend membranes (**Table 4.2**) was analyzed to determine their ion transport efficiency. The pure chitosan membrane (CS) showed the lowest conductivity at 1.34×10^{-8} S/cm limited ion mobility due to its dense and less porous structure. In contrast, the blend membranes exhibited progressively higher ionic conductivities with increasing gelatin content, with CG_5 achieving the highest value of 1.36×10^{-5} S/cm. The enhanced conductivity can be attributed to the increased hydrophilicity and porosity imparted by gelatin, which promotes better ionic transport. This trend aligns with the water absorption results, where water solubility correlates with improved ionic conductivity, emphasizing the critical role of water content in facilitating ion mobility.

Table 4.2: **Ionic conductivity of the membranes soaked in 2M LiOAc aqueous solution for 24 h**

Sample	Ionic Conductivity (S/cm)
CS	1.34×10^{-8} S/cm
CG_1	1.41×10^{-7} S/cm
CG_2	2.23×10^{-6} S/cm
CG_3	3.67×10^{-6} S/cm
CG_4	4.25×10^{-6} S/cm
CG_5	1.36×10^{-5} S/cm

4.7. Evaluation of Ion Exchange Capacity of Chitosan/Gelatin Blend Membranes

The ion exchange capacity (IEC) (**Table 4.3**) provides insights into the membranes' ability to interact with ionic species and reflects their functional group availability. The pure chitosan membrane exhibited an IEC of 4.1928 meq/g, while the blend membranes showed slight variations in IEC values, ranging from 4.0550 meq/g (CG_1) to 4.2217 meq/g (CG_4). The incorporation of gelatin maintained a consistent level of ionizable functional groups, indicating that blending modifies the structure without significantly altering the membrane's ion exchange capacity.

Table 4.3: IECs of CS/GN blend membranes

CS/GN	Dry weight	Vol. of NaOH	IEC (meq/g)
CS	0.0610	27.8	4.1928
CG1	0.0633	27.9	4.0550
CG2	0.0619	28.2	4.1913
CG3	0.0614	27.8	4.1655
CG4	0.0608	27.9	4.2217

4.8.Chemical Structure Evaluation of Chitosan/PVA Blend Membranes Using ATR-FTIR

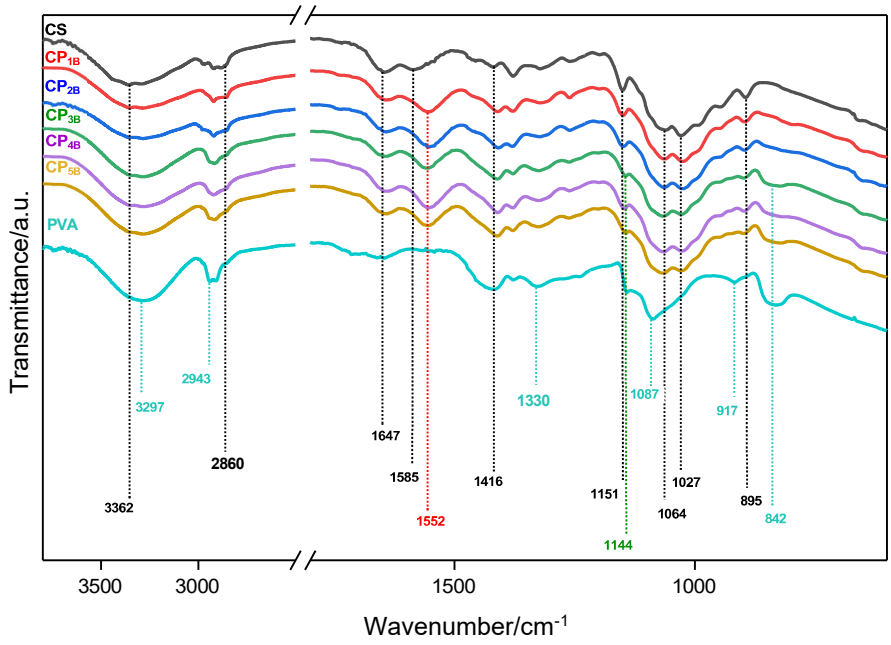

Fig 4.8: FTIR spectrum of CS/PVA blend membranes

Attenuated Total Reflection Fourier Transform Infrared (ATR- FTIR) spectra of membranes were recorded with a PerkinElmer Spectrophotometer, in a range of 4000-600 cm^{-1} with a resolution of 4 cm^{-1} and the no. of scans were 128. The CS/PVA blend membranes are depicted as CP_{1B}, CP_{2B}, CP_{3B}, CP_{4B}, and CP_{5B}.

The FTIR spectrum of the pristine chitosan membrane (CS) exhibits characteristic peaks reflecting its functional groups. The broad peak in the range of 3400-3200 cm^{-1} corresponds to the hydroxyl group (-OH), while the peaks at 3362cm^{-1} and 3286cm^{-1} indicate the presence of amine groups ($-NH_2$), suggesting the presence of strong and weak intramolecular and

intermolecular hydrogen bonds between -OH and -NH_2. Additionally, peaks in the range 2970-2800 cm^{-1} correspond to the stretching vibrations of the methyl group in $NHCOCH_3$, methylene group in CH_2OH, and methylene group in the pyranose ring. Furthermore, the peak at 1647 cm^{-1} is attributed to the amide I band, reflecting NH, $CO_{(I)}$ stretching as a result of incomplete deacetylation of chitosan. The 1585 cm^{-1} peak is often associated with the amide II band, arising from N-H stretching and bending vibrations. Lastly the peaks at 1151, 1064,1027 cm^{-1} correspond to bridge oxygen(C-O-C) stretching bands, characteristics of CS saccharide structure while the peak at 895 cm^{-1} reflects the presence of β-1,4- glycosidic bonds.

The FTIR spectrum of pure PVA reveals a broad band between 3550 and 3200 cm^{-1}, peaking at 3297 cm^{-1}, corresponding to O-H stretching due to intermolecular and intramolecular hydrogen bonds. The spectrum also shows a vibrational band in the 3000-2840 cm^{-1} range, indicative of C-H stretching from alkyl groups. A weaker band around 1330 cm^{-1} is associated with C-O-H bending vibrations. Additionally, the bands at 1144 and 1087 cm^{-1} are linked to C-O stretching vibrations of the acetyl groups on the PVA backbone. The band at 917 cm^{-1} corresponds to CH_2 rocking, while C-C stretching vibrations are observed near 842 cm^{-1}.

The FTIR spectrum of the blend membranes displays characteristic peaks from both CS and PVA. The O-H and N-H stretching vibrations appear as a broad and intense peak around 3400 cm^{-1} in the blend samples. This peak shifts to lower wavenumbers as the PVA content increases, suggesting the formation of hydrogen bonds between CS and PVA chains. As the PVA content increases, there is also a noticeable rise in the

intensity of the C-H group in the 2970-2800 cm^{-1} range. The presence of a peak at 1552 cm^{-1} in the blend membranes is attributed to the symmetrical deformation of $-NH_3^+$, resulting from the ionization of primary amino groups in an acidic medium. Additionally, the main absorption bands related to CS, such as the amine region at 1647 cm^{-1} and the C-O-C bridge stretching vibrations at 1064 and 1027 cm^{-1}, show a significant reduction in intensity as the CS content decreases from 100% to 50%. The band at 895 cm^{-1} disappears in the blend as PVA content increases, indicating changes in characteristic spectral peaks due to physical blending and chemical interactions between the two polymers. These observations suggest good miscibility between PVA and CS, likely due to the formation of intermolecular hydrogen bonds between the amino and hydroxyl groups in CS and the hydroxyl groups in PVA. The absence of new peaks in the CS/PVA blend membranes, compared to the pure membranes, indicates that CS is compatible with PVA.

Chapter 5

CONCLUSIONS

This study presents an in-depth investigation into the synthesis and characterization of chitosan-gelatin (CS/GN) blend membranes, focusing on the use of gelatin as a porogen to enhance porosity and tailor the membranes' properties. The incorporation of gelatin introduced significant structural and functional modifications to the chitosan matrix. FTIR analysis revealed the formation of hydrogen bonds and ionic interactions between chitosan and gelatin, while XRD analysis showed reduced crystallinity, corresponding to increased porosity. The removal of gelatin further emphasized chitosan's structural dominance, as observed in the retention of its key functional groups.

Morphological and thermal evaluations confirmed the success of the modification process. SEM images highlighted the presence of well-defined porous structures after the porogen removal, which are critical for applications in drug delivery and filtration. Meanwhile, TGA results demonstrated the thermal stability of both pristine chitosan and the blend membranes, affirming their suitability for use in high-temperature environments. These results collectively validate the efficacy of the blending and porogen removal strategies employed in this study.

Functional testing of the membranes further underscored their potential. The addition of gelatin enhanced the hydrophilicity and ionic conductivity of the membranes, as seen in the increased water solubility and

ion transport efficiency. After porogen removal, the membranes showed reduced water solubility due to the hydrophobic nature of chitosan, while their ion-exchange capacity remained stable, indicating the retention of functional groups and structural integrity during processing.

The findings highlight the versatility of CS/GN membranes for diverse applications. In the biomedical domain, these membranes can be employed as wound dressings, tissue engineering scaffolds, and controlled drug delivery systems due to their porosity, biocompatibility, and antimicrobial properties. Environmentally, their tunable porosity and ionic conductivity make them suitable for water filtration, gas separation, and energy storage applications, such as in fuel cells and supercapacitors. The eco-friendly nature of the materials also makes them a sustainable alternative to synthetic polymers.

Despite these promising results, challenges remain in optimizing the mechanical strength of the membranes for load-bearing applications and ensuring consistent performance in diverse environmental conditions. Future research could focus on incorporating nanoparticles or other functional agents to further enhance mechanical, thermal, and antimicrobial properties. Additionally, exploring advanced cross-linking and post-processing techniques, as well as alternative porogens, could expand the application scope and scalability of these membranes.

In conclusion, this research demonstrates the potential of chitosan-gelatin blend membranes as versatile, eco-friendly materials with tailored properties for a variety of biomedical and environmental applications. The study advances the field of biopolymer modifications, paving the way for innovative and sustainable solutions across multiple industries.

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