Conducting Bio IL's Modified Alginate Synthesis and Their Biomedical Applications

By

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MS THESIS WORK

V'he hereby recommend that the dissertation prepared under our supervision by: Asnia Gulzar, Regn No. 00000331169 Titled: Conduction Bio IL's Modified Alginate Synthesis and Their Biomedical Applications be Accepted in partial fulfillment of the requirements for the award of MS degree.

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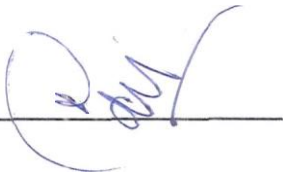
2. Name: DR. MUHAMMAD ADIL MANSOOR Signature: 

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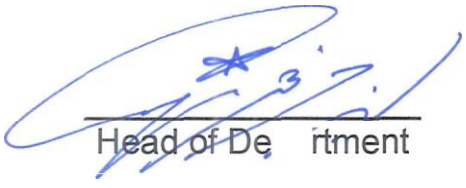

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Dedication

Dedicated to my Beloved Parents "Mr. Gulzar Ahmad and Khalida Perveen".

Statement of Original Authorship

I **Asma Gulzar** hereby state that my MS thesis titled **"Conducting Bio IL's modified Alginate Synthesis and their Biomedical Applications"** is my work and has not been submitted previously by me for taking any degree from this National University of Science and Technology or anywhere else in the country/ world.

At any time if my statement is found to be incorrect even after I graduate, the university has the right to withdraw my MS degree.

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ABSTRACT

Nowadays, biomedical science faces challenges like organ failure, tissue damage due to medical problems, or cancer treatment. So, tissue engineering is a hot topic nowadays to produce or synthesize an organ using the tissue of the donor, and for this purpose, conducting polymers that can be used in making scaffolds are of the most concern. Many conducting polymers are in use for tissue engineering but they have the limitations that they are not very much cytocompatible. And they do not possess the self-property of killing bacteria or pathogens, and they are mechanically less stiff and less strong. This can restrict their application in load-bearing tissues or environments that require structural integrity. In this thesis, BIL-based modified alginate was used to make conducting polymers that are highly cytocompatible and kill cancerous cells. Additionally, these are antibacterial and that's why can be incorporated into healing wounds. A series of choline-based ionic liquids were used to synthesize conducting polymers that are highly cytocompatible. Their synthesis was confirmed by FTIR, ^{1}HNMR, and SEM, their mechanical properties were determined, and conducting power was calculated. These polymers were subjected to test biocompatibility against healthy (3T3) which comes out to be (80-115%), and 30-of 40% of cancer cells (HEPG2) were killed and treated in wound healing rats.

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LIST OF ABBREVIATIONS

FTIR	Fourier transform infrared spectroscopy
^{1}HNMR SPEC	Proton nuclear magnetic resonance spectroscopy
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
BIL	Bio Ionic liquid
ALG	Alginate
CHO	Choline
SUC	Succinate
BEZ	Benzoate
GLY	Glycolate

CHAPTER 1

INTRODUCTION

1. Introduction

1.1 Background:

It is incredibly difficult to deal with resistant germs and defend animal cells from them. It is challenging to treat infections because resistant bacteria have created methods to counteract the effects of traditional medicines. Zoonotic transmission poses a risk to human health in addition to animal health. Alternative antimicrobial agents must be created, surveillance and diagnostic methods must be improved, stringent infection control procedures must be put in place, and responsible antibiotic usage must be encouraged. To battle resistant bacteria and protect animal cells, researchers are also pursuing the creation of novel therapeutics, such as bacteriophages or immune-based methods, and the investigation of synergistic combinations of antimicrobial drugs [1].

1.2 Problem statement:

The biomedical science field has various challenges when it comes to developing better drug-delivery systems and antibacterial wound healing material. Many challenges arise from biological complexity that makes this process more uncomfortable than ever before. Some of the critical issues identified as follows:

1) Drug delivery with minimal systemic side effects to targeted body parts remains a significant obstacle in biomedical practices [2, 3].

It necessitates overcoming various barriers like biological obstacles, and drug clearance rates which also depend on optimal release profiles. 2) Efficient controlled-strength medical agents maintain specific therapeutic concentration over an elongated period and need precise control of the release kinetics using complicated delivery systems over extended periods [4].

3) The global rise of antibiotic-resistant bacteria demanded urgent attention for the design of effective antibacterial products or wound-healing medical tools to combat bacterial infections effectively with improved health outcomes [5].

4) Safety and efficacy are paramount for any medicinal tool which requires biocompatibility & cytocompatibility assessments during medication practice; otherwise, it can hinder its acceptance among patients because it might result in harmful reactions such as discomfort or even damaging healthy tissues surrounding the spot. 5) Successful transition from lab-scale development needs scaling up manufacturing practices with adequate changes required at different stages until the clinical translation is possible through the various challenges. Therefore, ensuring acceptance becomes more comfortable at the clinical level emphasized reproducibility, reliability stability, and cost-effectiveness requirements [4] [6].

1.3 Properties and Nature of Hydrogels:

Hydrogels are cross-linked, three-dimensional networks of hydrophilic polymer chains that have a high absorption and retention capacity for water or biological fluids. Due to their outstanding qualities and many applications, these unique materials have attracted considerable interest and recognition in several sectors.

Odor: Hydrogels don't have a distinctive smell on their own. Depending on the particular ingredients or additives utilized in the hydrogel composition, such as scents or medicinal compounds, the aroma may differ [7].

Physical condition: In a gel-like state, hydrogels frequently contain a lot of water. They have a gelatin- or jelly-like texture that is soft and flexible. The physical properties of hydrogels can be

somewhat altered by varying factors including the water content, cross-linking density, and polymer composition.

Availability: Hydrogels are easily accessible and can be bought from many different places. They can be bought from chemical distributors, biotechnology firms, or specialized producers. Additionally, depending on the required features and applications, hydrogels can be synthesized or manufactured in research labs utilizing particular polymers and cross-linking agents [8].

It is significant to remember that the nature, smell, availability, and physical condition of hydrogels might change according to the formulation, makeup, and intended usage. Different hydrogels may have unique properties and can be customized for a variety of uses, from cosmetics to biomedical technology [9].

We'll first look at some of the fundamental properties of hydrogels. We shall examine their special structure, which allows them to expand while retaining the integrity of a solid. We will get insights into the mechanical, chemical, and physical aspects of hydrogels by comprehending the concepts of gelation and network creation.

The variety of applications that hydrogels provide will be our next focus. Hydrogels have revolutionized a wide range of fields, from biomedical engineering to environmental science. We will investigate their function in tissue engineering and regenerative medicine, where they serve as medication delivery mechanisms and scaffolding for cell growth. We'll also look into how they're used in cosmetics, contact lenses, artificial muscles, and wound healing [10].

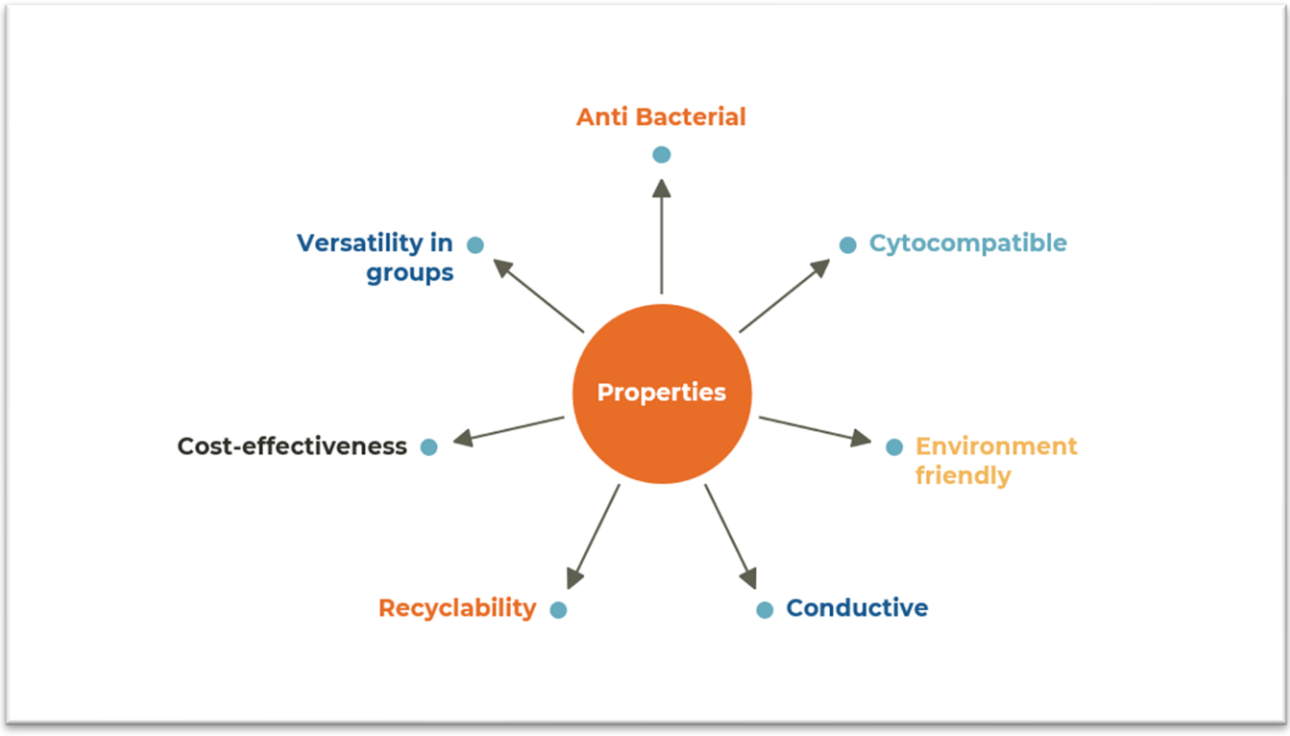


Figure 1.1 General properties of hydrogels.

We'll also examine how hydrogel technology has advanced and changed. As engineers and scientists work to enhance its properties and create new applications for it, hydrogel research is continually moving forward. We'll examine the development of hydrogels whose behavior can change in response to environmental factors like temperature, pH, or light. We will also discuss how the applicability of hydrogels can be increased by combining them with emerging technologies like 3D printing and nanotechnology [11, 12].

We will highlight the interdisciplinary aspect of hydrogels throughout this chapter since their study comprises chemistry, materials science, biology, and engineering. We will provide a thorough overview of the topic by highlighting significant research findings, noteworthy discoveries, and useful implications.

By the end of this chapter, deep learning to gain an appreciation for the unique properties and diverse applications of hydrogels will be achieved. Whether you are a student, researcher, or simply curious about the world around us, this chapter will provide you with valuable insights into one of the most exciting and promising materials of our time [13].

1.4 Biomedical Applications of Hydrogels:

1.4.1 Tissue Engineering:

One of the most promising materials for tissue engineering is hydrogel because of its unique properties that closely mirror the extracellular matrix (ECM) present in living tissue. To promote cell adhesion, development, and tissue regeneration, they offer a three-dimensional habitat. Numerous crucial applications for hydrogels in tissue engineering include:

a. **Scaffold for Cell Growth:** Hydrogels offer an excellent framework for seeding and growing cells in a three-dimensional environment. Infiltration and multiplication of cells are made possible by the porous nature of hydrogels, which promotes the growth of new tissues. The hydrogel scaffold imitates the natural ECM to offer mechanical support and signals for cell adhesion, migration, and differentiation [14].
b. **Organ and Tissue Regeneration:** Numerous organs and tissues, including bone, cartilage, skin, and blood vessels, have been regenerated using hydrogels. They can be designed to include growth factors, bioactive compounds, and ECM elements that promote tissue-specific differentiation and cell signaling. The regeneration of diseased or injured tissues is supported by hydrogels, aiding in functional recovery [15].
c. **Controlled Drug Delivery:** For localized and ongoing drug administration in tissue engineering applications, hydrogels are useful platforms. Therapeutic compounds can be

encapsulated, shielded against deterioration, and released gradually under controlled conditions. Growth factors, cytokines, and other bioactive substances can be delivered using this controlled release to encourage tissue regeneration and modify cellular behavior [16].

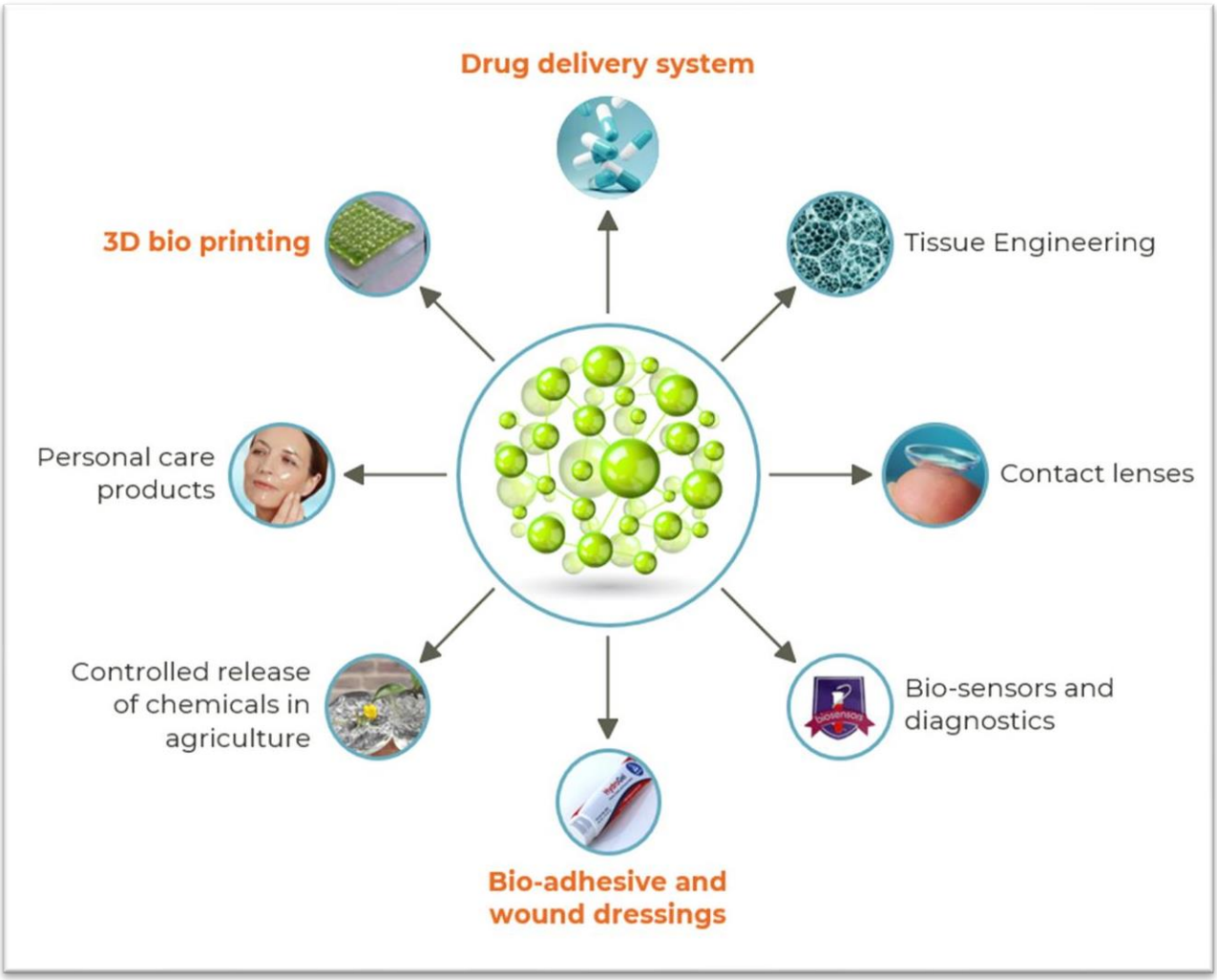


Figure 1.2. Biomedical and general applications of hydrogels.

d. Bioprinting and Tissue Fabrication: In the process of creating tissues and using bioprinting, hydrogels are widely used. They are the perfect bio-inks for building intricate,

three-dimensional tissue architectures due to their printable nature and capacity to preserve cell viability. Hydrogels can be accurately applied layer by layer to create tissues with complex structures and biological organization by adding cells and the required substances.

e. **Vascularization:** For synthetic tissues to survive and integrate and develop into working blood arteries, it is crucial. Hydrogels can create vascular networks by incorporating endothelial cells and promoting angiogenesis. By replicating the vascular environment and permitting nutrient supply, waste elimination, and tissue integration, hydrogels aid in the formation of functional blood vessels [17].

f. **Wound Healing:** In the medication delivery industry, hydrogels are often employed and have generated a lot of attention. They offer many advantages, making them perfect for this usage. The following list includes some crucial characteristics of hydrogel uses in medicine administration:

g. Drugs can be contained in hydrogels and released in a controlled and consistent manner over time. Drug molecules can become trapped in the hydrogel matrix, allowing for regulated release via diffusion or degradation over a prolonged period of time. This feature allows for the maintenance of medication concentrations at therapeutic levels, reducing the need for repeated doses and improving patient compliance [18].

1.4.2 Drug delivery system:

Hydrogels have received a lot of attention and are widely used in the pharmaceutical delivery business. They are ideal for this application due to their numerous benefits. The following are some major properties of hydrogel uses in medicine administration:

Drugs can be held and delivered gradually and steadily over time using hydrogels. Drug molecules could be held captive in the hydrogel matrix for a prolonged period, allowing for diffusion- or

degradation-controlled release. This feature permits medicine concentrations to be kept at their ideal therapeutic levels, reducing the need for recurring dosage and improving patient compliance [19].

1. **Targeted Drug Delivery:** Hydrogels can be developed to deliver drugs to precise locations within the body. They can selectively interact with certain cells or tissues by adding targeted ligands, such as antibodies or peptides, on the surface of the hydrogel. This focused method lowers off-target effects while increasing drug therapeutic index.
2. **Protection and Stability:** Drugs can be delivered to specific places within the body using hydrogels. They can selectively interact with certain cells or tissues by adding targeted ligands to the hydrogel's surface, such as antibodies or peptides. This targeted approach reduces off-target effects while enhancing drug therapeutic index [20].
3. **Combination Therapy:** The ability to simultaneously distribute many drugs using hydrogels simplifies combination therapy. This is especially useful for treating infections or diseases that necessitate the use of many therapeutic medications with complementing effects. Many drugs can be released under controlled conditions from a single platform using hydrogels, boosting therapy effectiveness and eliminating the need for recurrent administrations.
4. **Localized Drug Delivery:** Hydrogels can be used to provide localized medication distribution, which is very useful when personalized therapy is required. In the treatment of cancer, for example, hydrogels put near the tumor site can allow anticancer drugs to flow continuously into the tumor microenvironment. This focused method decreases systemic side effects while increasing pharmaceutical efficacy [21].

5. **Personalized Medicine:** By enabling the customization of drug delivery systems based on the unique requirements of each patient, hydrogels present prospects for personalized medicine. The mechanical strength, release kinetics, and degradation rate of hydrogels can all be adjusted to optimize medication delivery for particular individuals or medical situations [22].
6. **Combination with Imaging Agents:** Drugs and imaging agents, such as contrast agents or nanoparticles, can both be included in hydrogel formulations. Using imaging techniques, this integration enables simultaneous medication delivery and real-time drug distribution and release monitoring. It helps with therapy optimization by offering insightful information about the pharmacokinetics and pharmacodynamics of the medicine [23].

Overall, there are several uses for hydrogels in drug administration that have great potential to enhance therapeutic results. They are a flexible platform in the field of pharmaceutical sciences since they may give regulated release, target certain regions, protect pharmaceuticals, and enable combination therapy. Hydrogel-based drug delivery systems are being improved through ongoing research and development, providing creative answers for efficient and patient-specific treatments.

7. **1.4.3 Biosensors:**

Hydrogels have emerged as valuable components in biosensor technology, enabling sensitive and specific detection of various analytes and biomarkers. The unique properties of hydrogels make them well-suited for biosensing applications. Here are some key aspects of hydrogel applications in biosensors:

1. **Sensing Element:** Hydrogels can be functionalized with specific receptors, such as antibodies, enzymes, or DNA probes, to create a sensing component within a biosensor

[24]. These specifically focused interactions between the functionalized hydrogels and the target analytes result in a quantifiable signal response. The hydrogel expands or contracts when the target analyte is present, changing its optical, electrical, or mechanical properties and allowing for detection.

2. **Biocompatibility:** The very nature of hydrogels makes them compatible with biological samples. As a result, they can interact directly with biological systems like body fluids or living cells. With the incorporation of hydrogels into wearable or implanted biosensors, it is possible to continuously monitor biomarkers in real-time without endangering the user or causing them great discomfort.
3. **Enhanced Sensitivity and Selectivity:** Hydrogels' porous structure enables effective binding and diffusion of target analytes. This encourages applications for biosensing to be more sensitive and selective. The binding affinity and specificity for the target analyte can be optimized, leading to increased sensor performance, by adjusting the composition and characteristics of the hydrogel [25].
4. **Biomimetic Environment:** A biomimetic environment that closely replicates the natural microenvironment of the analyte or target biomarker can be produced via hydrogels. As a result, the hydrogel matrix's biological recognition components, such as enzymes or receptors, are more stable and active. Even in complicated biological samples, the biomimetic environment offered by hydrogels provides precise and dependable analyte detection [25].

5. **Versatile Signal Transduction:** Biosensors are given long-term stability and resilience by hydrogels. For consistent and dependable sensing performance, they can maintain their

structural integrity and functionality over a lengthy period. over continuous monitoring applications, where biosensors must function over extended periods without noticeably degrading, this stability is very crucial.

6. **Long-Term Stability:** Biosensors are given long-term stability and resilience by hydrogels. For consistent and dependable sensing performance, they can maintain their structural integrity and functionality over a lengthy period. over continuous monitoring applications, where biosensors must function over extended periods without noticeably degrading, this stability is very crucial [25].
7. **Multiplexed Detection:** Multiplexed detection is made possible by hydrogels, enabling the simultaneous measurement of several analytes or biomarkers on a single biosensor substrate. Multiple target molecules can be identified simultaneously by functionalizing various areas of the hydrogel with distinctive recognition elements, each one particular to a different analyte. The efficiency and throughput of biosensing tests are improved by this multiplexing capacity.

Numerous industries, including healthcare, environmental monitoring, food safety, and diagnostics, use hydrogels in biosensors. The performance of hydrogel-based biosensors is currently being improved by increasing sensitivity, selectivity, and miniaturization. Hydrogel-based biosensors have a lot of potential for enabling quick and precise analyte detection for a variety of real-world applications [26].

1.5. Conducting Hydrogels:

Hydrogels that conduct electricity sometimes referred to as conductive hydrogels or smart hydrogels, have the distinctive ability to conduct electricity while preserving the typical swelling and water-absorbing properties of ordinary hydrogels. This is accomplished by adding conductors to the hydrogel matrix, such as conductive polymers or nanoparticles. The hydrogel can conduct electricity or act as electrodes in electrical devices thanks to these conductive components.

Hydrogels acquire distinctive features and possible uses when conductive components are added to them. To be employed in the creation of sensors, actuators, and electroactive devices, conducting hydrogels must be able to respond to electrical or electrochemical stimuli. In tissue engineering, they may also be employed as scaffolds since electrical conductivity can be exploited to encourage cell proliferation and tissue regeneration. Conducting hydrogels have also been researched for use in biosensing, flexible electronics, and energy storage [27].

Bioelectronics and bioresponsive materials have advanced as a result of the creation of conducting hydrogels, which have created new opportunities for the fusion of biological and electrical systems.

The conductivity of the product may be increased by crosslinking bio-ionic liquids with hydrogels through two main mechanisms: ionic conduction and charge transfer.

Ionic Conduction: Compared to conventional ionic liquids, bio ionic liquids are more ecologically friendly since they are made up of organic ions produced from renewable resources. Charged species included in these bio-ionic liquids, such as cations and anions, can help ions conduct inside the hydrogel matrix [28].

Electric current may be conducted due to the presence of mobile ions in the hydrogel matrix, which gives the conducting hydrogel its conductivity. Bio ionic liquid molecules get immobilized within

the hydrogel network when they are crosslinked with hydrogels. This immobilization keeps the bio-ionic liquid's ionic properties intact, enabling ion flow across the hydrogel structure. Electric current may be conducted thanks to the presence of mobile ions in the hydrogel matrix, which gives the conducting hydrogel its conductivity.

Charge Transfer: When bio-ionic liquids and hydrogels are crosslinked, charge transfer between the molecules of the bio-ionic liquid and the hydrogel matrix is encouraged. The hydrogel's polymer chains can interact with the bio-ionic liquids, which often include conjugated or aromatic moieties, through π-π stacking interactions or other electrostatic interactions [29].

These interactions help the bio ionic liquid molecules and the hydrogel matrix transfer charges. The creation of charge-transfer complexes or the delocalization of electrons within the conjugated system of the bio-ionic liquid and the polymer network can both result in the transfer of charges. This charge transfer makes the hydrogel more electrically conductive, allowing current to flow across it.

Conducting hydrogels with improved conductivity may be created by connecting bio-ionic liquid characteristics with hydrogel structure. Potential uses for these conducting hydrogels include energy storage, bioelectronics, and biosensors.

Why Sodium Alginate?

A naturally occurring hydrogel formed from algae called sodium alginate is well-known for its ability to gel when in contact with divalent cations. Ionotropic gelation of sodium alginate creates a three-dimensional network structure that holds onto the water or biological fluids inside the hydrogel matrix. Since sodium alginate's gelation process is reversible, gel formation and dissolution can be manipulated and controlled. Numerous biomedical applications are possible for

sodium alginate hydrogel because it is biocompatible, readily gels, and can contain cells, medications, or bioactive substances. The nature of the polymer, the density of the cross-linking, and the amount of water present all have an impact on the mechanical properties of hydrogels, particularly sodium alginate hydrogels. The normal behavior of hydrogels is elastic and viscoelastic, allowing them to deform under tension and regain their original shape after the load has been removed. Due to their strong hydrophilicity and capacity for water absorption, sodium alginate hydrogels can significantly swell when exposed to moisture [30]. Sodium alginate hydrogels' mechanical moduli can change, however, they typically have a low modulus in comparison to other hydrogels. Sodium alginate hydrogels have a moderate degree of toughness, which can vary depending on the amount of alginate present, the density of the cross-links, and the presence of reinforcing chemicals. By varying the concentration, cross-linking density, and adding extra ingredients or additives, sodium alginate hydrogels' mechanical properties can be customized. These modifications allow for the customization of sodium alginate hydrogels to suit specific applications, ranging from soft and flexible to more rigid and load-bearing systems [30].

Chapter 2

Literature Review

2.1 Synthesis of Acrylic-modified Alginate

Synthesis of modified acrylates Alginate can be made by combining the acrylic group with acrylic anhydride or acrylic acid at various temperatures, solvents, and pH levels.

Lee et al. (2015) The purpose of this study was to develop and characterize acrylate alginate for use in tissue engineering applications. Acrylic anhydride and pyridine were utilized as catalysts in the acrylation process. The acrylate alginate was characterized using Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). The results showed that acrylated alginate had improved mechanical properties and higher thermal stability than untreated alginate. The researchers also observed that acrylated alginate increases cell development and proliferation, making it a good material for tissue engineering [31].

Shuibo Hua et al. (2015) By grafting acrylic acid onto sodium alginate with potassium persulfate as an initiator and N, N'-methylene bisacrylamide as a crosslinker, sodium alginate-g-poly(acrylic acid) was created. The reaction took place in an aqueous solution containing a constant ratio of sodium alginate to acrylic acid. After that, the solution was nitrogen-purged to remove any dissolved oxygen [32].

S. Thakur et al. The paper describes the fabrication of an acrylic acid-grafted sodium alginate-based TiO2 hydrogel nanocomposite. Acrylic acid-grafted sodium alginate was synthesized using graft copolymerization with potassium persulfate as an initiator and N, N'-methylene bisacrylamide as a crosslinker. The reaction occurred in an aqueous solution containing sodium alginate and acrylic acid in a predetermined sodium alginate to acrylic acid ratio [33].

Mao et al. (2018) The treatment of natural alginate with acrylic acid in this study results in a cross-linked hydrogel with improved mechanical strength and heavy metal ion adsorption capabilities. The synthesis starts with the creation of a sodium alginate solution in distilled water that is constantly stirred at room temperature. The solution is then polymerized in the presence of a catalyst (potassium persulfate) and a cross-linker (ethylene glycol dimethacrylate) by adding acrylic acid. The reaction is allowed to continue in an inert atmosphere for a defined amount of time to prevent oxidation and achieve optimal cross-linking density [34].

Işıklan and Küçükbalcı et al. (2016) The synthesis and characterisation of pH- and temperature-sensitive materials for drug delivery applications are described in this work. The following is a description of the acrylated alginate synthesis: To begin, a solution of sodium alginate is made by dissolving it in distilled water. The acrylic acid is then added to the solution and thoroughly mixed together. A cross-linking agent, ethylene glycol dimethacrylate (EGDMA), and a free radical initiator, 2,2'-azobis(isobutyronitrile) (AIBN), are added first. The mixture is stirred and heated to 60°C for a specific amount of time to allow the reaction to begin. The end result is acrylated alginate, which is subsequently washed and dried to yield a dry powder. [35].

Tally et al. (2015) This research discusses the use of microwave irradiation to create a pH-sensitive semi-interpenetrating network (semi-IPN) superabsorbent polymer (SAP) based on sodium alginate-g-poly(acrylic acid-co-acrylamide) and polyvinylpyrrolidone (PVP). The manufacturing of acrylated alginate involves two phases. Acrylic acid and acrylamide are added to the solution after dissolving sodium alginate in distilled water, along with potassium persulfate as an initiator. The mixture is then stirred for many hours at a constant temperature until the graft copolymerization reaction is complete [35].

Tally et al. (2015) The production of a pH-sensitive semi-interpenetrating network (semi-IPN) superabsorbent polymer (SAP) based on sodium alginate-g-poly(acrylic acid-co-acrylamide) and polyvinylpyrrolidone (PVP) is discussed in this paper utilizing microwave irradiation. Two stages are involved in the production of acrylated alginate. After dissolving sodium alginate in distilled water, acrylic acid and acrylamide are added to the solution, along with potassium persulfate as an initiator. The mixture is then agitated at a steady temperature for many hours until the graft copolymerization reaction is complete [36].

Agnihotri et al. (2019) This research discusses the use of microwave irradiation to create a pH-sensitive semi-interpenetrating network (semi-IPN) superabsorbent polymer (SAP) based on sodium alginate-g-poly(acrylic acid-co-acrylamide) and polyvinylpyrrolidone (PVP). The manufacturing of acrylated alginate involves two phases. Acrylic acid and acrylamide are added to the solution after dissolving sodium alginate in distilled water, along with potassium persulfate as an initiator. The mixture is then stirred for many hours at a constant temperature until the graft copolymerization reaction is complete [37].

Schuurmans et al. (2021) This article describes how to make hydrogels from hyaluronic acid (HA) and chondroitin sulphate (CS) methacrylate. Methacrylated HA and methacrylated CS are formed when the carboxylic acid groups on HA and the amine groups on CS interact with methacrylic anhydride. The degree of acrylation can be varied by adjusting the ratio of methacrylic anhydride to the amount of carboxylic acid or amine groups present in the polysaccharides [38].

Moore et al. (2019) The manufacture and applications of bioactive poly (ethylene glycol) (PEG) acrylate hydrogels for regenerative engineering are discussed in this article. PEG acrylate hydrogels are created by adding acryloyl chloride to PEG diols in the presence of a base catalyst,

resulting in the creation of PEG diacrylates. By altering the molar ratio of acryloyl chloride to PEG diol, the degree of acrylation can be adjusted [39].

Krafcik, M. J. et al. (2016) The production and characterization of superabsorbent hydrogels utilizing sodium acrylate and acrylamide monomers are described in this study. Hydrogels with high swelling capacity, mechanical strength, and crosslink density were discovered. The hydrogels were also discovered to be useful as an internal curing agent for mortar, increasing compressive strength and decreasing drying shrinkage. The study reveals the utility of hydrogels in a variety of practical applications, such as building materials [40].

2.2 Anion exchange of Choline chloride

Zhang et al. (2016) The synthesis and anion exchange of methacrylated choline chloride for the manufacture of ionic liquids are described in this work. The authors began by treating choline chloride with methacrylic anhydride to create methacrylated choline chloride. This chemical was then employed as a precursor for anion exchange with various carboxylic acids to produce diverse ionic liquids. The authors used a variety of techniques to characterize the resultant ionic liquids, including NMR spectroscopy, thermogravimetric analysis, and differential scanning calorimetry. The results demonstrated that the anion exchange reaction produced a variety of choline-based ionic liquids [41].

Wang et al. (2017) The authors used anion exchange with several carboxylic acids to create choline chloride-based deep eutectic solvents (DESs). They evaluated the effects of several parameters on the anion exchange process, such as reaction time and temperature. The authors used a variety of techniques to characterize the generated DESs, including NMR spectroscopy, thermogravimetric analysis, and differential scanning calorimetry. The results demonstrated that

the anion exchange reaction produced a variety of choline-based DESs with varying melting temperatures and viscosities.

Li et al. (2018) The authors evaluated the ability of choline chloride-based DESs to dissolve cellulose by anion exchange with various carboxylic acids in this research. The authors used a variety of techniques to characterize the generated DESs, including NMR spectroscopy, thermogravimetric analysis, and differential scanning calorimetry. The results demonstrated that the anion exchange reaction produced a variety of choline-based DESs with varying parameters like as melting point and viscosity. The authors also discovered that some of the DESs were capable of degrading cellulose.

Yu et al. (2019) The synthesis and anion exchange of choline chloride-based ionic liquids for carbon dioxide capture are described in this work. The authors began with choline chloride and then exchanged the chloride anion with various carboxylic acids to produce diverse ionic liquids. The authors used a variety of techniques to characterize the resultant ionic liquids, including NMR spectroscopy, thermogravimetric analysis, and differential scanning calorimetry. The results demonstrated that the anion exchange reaction produced a variety of choline-based ionic liquids with varying properties such as melting point and viscosity. The researchers also discovered that some of the ionic liquids were effective at carbon dioxide capture.

Zhao et al. (2021) In this study, the authors assessed the melting point, viscosity, and thermal stability of choline chloride-based DESs generated by anion exchange with various carboxylic acids. To characterize the created DESs, the authors used a range of techniques, including NMR spectroscopy, thermogravimetric analysis, and differential scanning calorimetry. The results showed that the anion exchange reaction created a wide range of choline-based DESs with variable properties.

Yukinobu et al. (2015) Although anion exchange of halide salts with metal salts or acids is routinely used to prepare ionic liquids (ILs), this method was not suitable for the production of choline-based ionic liquids (CILs) in this study. Instead, the scientists used a two-step anion exchange reaction to create Bio-ILs, with choline acetate being a typical example of the choline carboxylate synthesis technique. The first step was to make an aqueous solution of choline hydroxide ([Ch][OH]) by passing a choline iodide ([Ch][I]) water solution through an anion exchange resin-filled column. The second stage involved adding a small amount of acetic acid aqueous solution to the [Ch][OH] solution while it was cooling, agitating the combination at room temperature, evaporating the water, and washing the product many times with diethyl ether to remove the excess acetic acid.The second step involved adding a slight excess of acetic acid aqueous solution to the [Ch][OH] solution while cooling, stirring the mixture at room temperature, evaporating water, washing the product several times with diethyl ether to remove unreacted acid, and then dissolving it in dichloromethane containing 5% ethanol before passing the resulting solution through a short column filled with neutral activated alumina. The resultant product was then vacuum dried for 24 hours at 70°C to obtain choline acetate.

Schuurmans et al. (2021) This paper discusses the coupling of acrylated hydrogels. The covalent crosslinking of the (meth)acrylated polymers results in the creation of hydrogels from hyaluronic acid-methacrylate (HAMA) and chondroitin sulfate-methacrylate (CSMA). This is achieved through radical polymerization, which can occur either thermally or through light irradiation. The most typical method is light-triggered initiation, in which a small quantity of initiator is introduced to the polymer solution to create enough radicals for crosslinking upon UV irradiation. The efficiency of such reactions is determined by factors such as light wavelength and intensity,

photoinitiator type and concentration, and the concentration of oxygen or other radical scavenging molecules present in the sample [38].

2.3. Photo-crosslinking in Polymers

UV photo-crosslinking is a versatile technique employed in polymer chemistry to enhance the mechanical properties and stability of polymers. This method utilizes ultraviolet (UV) light to induce crosslinking between polymer chains, forming a three-dimensional network structure. Particularly, polymers containing -C=C- (carbon-carbon double bond) endings are suitable candidates for UV photo-crosslinking. The process involves the use of a photo-initiator, which absorbs UV light and initiates a series of chemical reactions leading to the formation of covalent bonds between adjacent -C=C- groups. This results in the creation of a network of interconnected polymer chains, offering improved strength, durability, and resistance to degradation. UV photo-crosslinking enables precise control over the degree of crosslinking and can be applied to a wide range of polymers, making it a valuable tool in various fields including materials science, coatings, adhesives, and biomedical applications.

Carvalho et al. undergone the self-crosslinking of ChMA (methacrylated chitosan) and crosslinking of ChMA and gelatin. The photo-crosslinking of -C=C- was carried out in the presence of photo-initiator Irgacure 2959 under UV light of 254 nm for 10 minutes and then ascorbic solution was added for the scavenging and elimination of free radicals. Photo-crosslinking was confirmed by constant monitoring through FTIR and UV-Vis spectroscopy at regular intervals [42].

Kuflet et al. studied the photo-crosslinking of chitosan modified with methacrylated group by using glycidyl methacrylate and VEGF (vascular endothelial growth factor) which was modified

with the vinyl groups to make them photocrosslinkable. These two modified moieties were crosslinked under UV light of 254 nm wavelength in the presence of Irgacure 2959 for 15 minutes. The synthesized products was used as 3D scaffolds in tissue engineering and regenerative medicine [43].

Joshi et al. In DPBS containing 2% (w/v) Irgacure 184 as a photoinitiator, 10% (w/v) GelMA and 10% (w/v) vacuum dried ChMA macromer was individually mixed until completely dissolved. The solution was then put into tiny aluminum dishes and exposed to UV light (365 nm) at ambient temperature for 5 to 10 minutes to photopolymerize it. For later testing, the produced crosslinked hydrogels were kept in distilled water. Figure 2 depicts the reaction pathway that produces crosslinked hydrogels by reacting modified polysaccharides with their end double bonds [44].

Krishnadoss et al. used GelMA and PEGDA to synthesize photocrosslinked hydrogel by adding 20% bio-ionic liquid i.e., acrylated choline bitartrate to each 25% solution of GelMA and PEGDA. To the mixture of methacrylated polymer and acrylated choline LAP (lithium phenyl-2,4,6-trimethylbenzoylphosphinate) was added to attain the photocrosslinking under visible light of 450 nm. The time duration for each polymer varies such as 120 s for GelMA and 60 s for PEGDA. The adhesive and physical properties of the synthesized hydrogel was studied for hemostatic activity [45].

Prasad et al. developed the fourth-generation therapeutic approach, known as gel-type autologous chondrocyte implantation (GACI) to address the drawbacks of earlier generation approaches, particularly issues with cell leakage during implantation and diminished chondrocyte functionality due to dedifferentiation of cells in culture and the formation of fibrocartilage. An injectable gel device serves as the cell carrier in GACI. However, this approach has significant difficulties in maintaining the morphology and redifferentiation of chondrocytes with acceptable bio-

functionality. In this work, we created a photocrosslinkable injectable hydrogel using polyethylene glycol diacrylate (PEGDA) and carboxymethyl cellulose-methacrylate (CMC-MA), and we assessed chondrocyte-matrix interactions and bio-functionality on various mix ratios of the gels with varied stiffness [46].

Zanon et al. investigated the preparation of 3D printable hydrogels based on methacrylated chitosan for tissue engineering applications. For this purpose, firstly dissolution of freeze dried ChMA was occurred in acetic acid and water solution. To this solution photoinitiator LAP was added and the solution was irradiated with visible light of 400 nm. Cell migration and proliferation, 3D printability, biocompatibility, and enzymatic degradability was studied for further use in tissue engineering [47].

Poon et al. developed the ionic hydrogels by blending photo-crosslinkable MA-OCMCh with PEG diacrylate. Photopolymerization occurred by pouring precursor solution consisting of O-CMCh, PEG diacrylate and Irgacure 2959 which was used as a photoinitiator in flat molds, then this homogenized solution was exposed to UV light of 365 nm for 15 minutes. Swelling analysis and biodegradation of the synthesized product was carried out to study the absorption of TGF-ß1 [48].

Araiza-Verduzco et al. studied the role of alginate based soft materials for the replacement of soft tissues. They introduced -C=C- into the polymer matrix of alginate using three different substrates that are glycidyl methacrylate, 2-aminoethyl methacrylate, and methacrylic anhydride. Then these were photopolymerized by using I2959 under UV light of 365 nm for continuous 1h. The mechanical properties of all the products were compared for biomedical applications [49].

Sun et al. Hydrogels made of photocrosslinked gelatin and methacrylic acid (GelMA) have raised a lot of questions in the biomedical community due to their high biocompatibility and adaptable

physicochemical characteristics. Here, many methods for creating GelMA were described, with specific emphasis on the common technique for crosslinking the methacrylated gelatin precursors using UV light. Additionally, the conventional and modern technologies used to describe the characteristics of GelMA hydrogels and GelMA prepolymer were reviewed and contrasted. Finally, some concluding observations were made after a summary of the uses of hydrogels made from GelMA in tissue engineering and cell culture, particularly in the load-bearing tissues (bone and cartilage) [50].

Zhou et al. Recently, natural polymer-based nanofibers have caught the attention of researchers as potential skin substitution materials. Here, methacrylated chitosan/poly (vinyl alcohol) (PVA) mats were electrospun and photocrosslinked in the presence of D2959 initiator and Hg lamp to create photocrosslinked nanofibrous scaffolds based on methacrylated chitosan (MACS). PVA was then removed from the nanofibers. We thoroughly investigated the intermolecular interactions between MACS and PVA constituents, the solution characteristics of MACS/PVA precursors, and the physical characteristics of MACS/PVA nanofibers. The results demonstrated that the MACS/PVA mass ratio regulated the fibre diameter and shape of the photocrosslinked methacrylated chitosan-based nanofibrous scaffolds, which exhibited extremely microporous architectures with many fibrils [51].

Chapter 3

Experimental Section

3.1 Chemicals and Materials

The following chemicals were used for the synthesis of Bio-modified hydrogels and the process of polymer synthesis. Sodium Alginate (Sigma Aldrich), Methacrylic anhydride (MAA) (Sigma Aldrich), Methacryloyloxy ethyl ammonium trimethyl chloride METAC (Sigma Aldrich), Sodium acetate (Sigma Aldrich), Sodium propanoate (Sigma Aldrich), Sodium succinate (Sigma Aldrich), Sodium benzoate (Sigma Aldrich), Sodium glycollate (Sigma Aldrich), Irgacure 2959 (Sigma Aldrich), Deionized water, Ethanol, Ethylacetate (Sigma Aldrich).

3.2 Glassware and Apparatus

3.2.1 Glassware

Distillation assembly, three-necked round bottom flasks, conical flasks, beakers, graduated cylinders, volumetric cylinders, spatula, reagent bottles, condenser, thermometer, pipettes, micropipettes, measuring cylinder, funnel, sample vials, rubber septa, nitrogen balloons, magnetic bar, syringes, oil bath, etc.

3.2.2 Apparatus

Magnetic stirrer hot plate, weighing balance, melting point apparatus, vacuum pump, pH meter, UV lamp, vacuum oven, stands, etc.

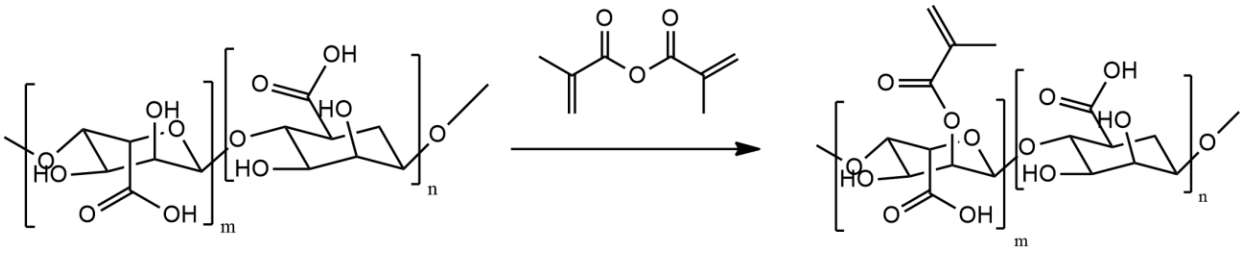
3.3 Instrumentation

Analytical balance ATY22d was used to weigh accurately all chemicals. FTIT-ATR model ALPHA 200d88 was used to check the reaction progress and identification of functional groups of synthesized compounds. TGA was performed for the polymer analysis and characteristics

determination. NMR 500 MHz was used for the confirmation of the structure. SEM and XRD analysis were used for confirmation of the modification of hydrogel. UV-VIS spectrophotometer was used for the reduction studies of the unsaturation.

3.4 Synthesis of Methacrylated Sodium Alginate

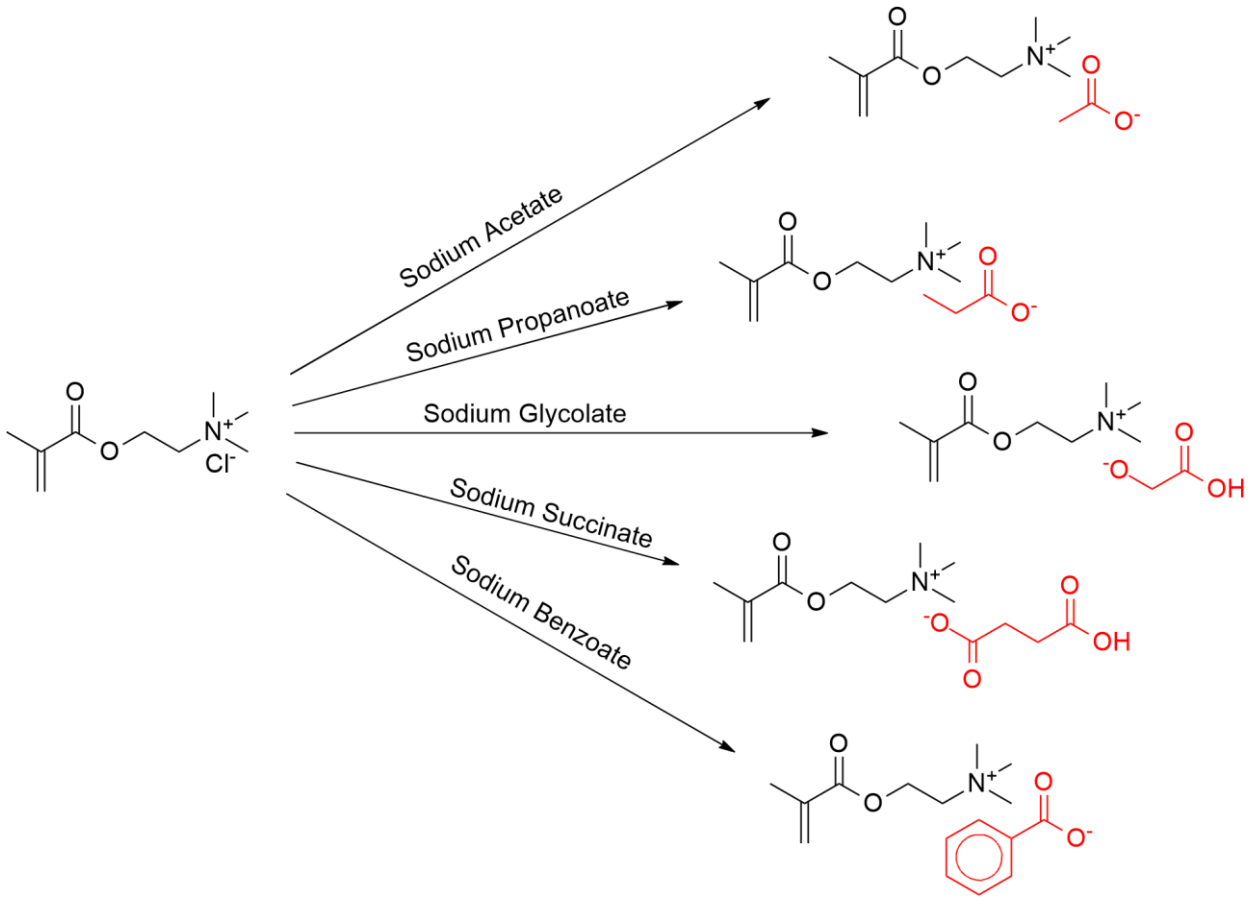
3 g of Sodium Alginate was taken and dissolved in 50 ml of DI water by proper stirring for about 30 minutes. To this solution, Methacrylic anhydride (MAA) was added at 2ml per hour with vigorous stirring. pH was maintained at 8 using the dilute solution of NaOH and the reaction mixture was kept on stirring at room temperature for 24 hours. After about one day, Ethanol was added in excess to the reaction mixture resulting in the precipitation of modified hydrogel. Methacrylic acid was also produced as a by-product that was separated by the washing of the gel using ethanol in centrifugation at 3000 rpm for about 5 minutes. Then gel was dried, weighed, and stored for further modification and reaction.



Equation 3.1: Modification of Sodium Alginate into Meth acryloyl Alginate

3.5 Anion Exchange of Methacryloyloxy Ethyl trimethyl ammonium chloride METAC

75 % solution of METAC from Sigma Aldrich was taken for the exchange of anions with 5 different organic anions to couple it further with modified hydrogel. All 5 anions were taken in the form of Sodium named Sodium acetate, Sodium propanoate, Sodium glycolate, Sodium succinate, and Sodium benzoate were dissolved in DI water in the equimolar ratio of METAC and stirred for about 48 hours at STP. After 2 days, ethyl acetate was added to the reaction mixture in excess separating the dissolved by-product NaCl that was removed by filtration separating the modified ionic liquid Methacryloyloxy Ethyl trimethyl ammonium acetate. And same method was performed for all the remaining 4 anions.

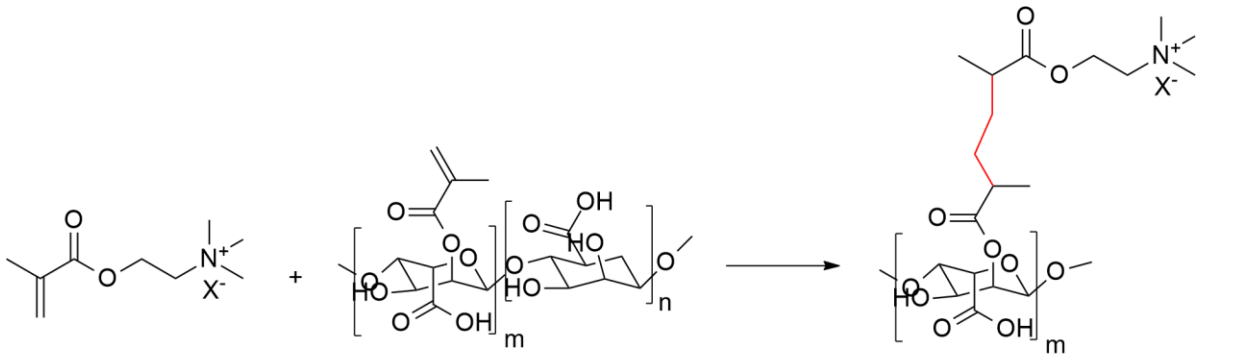


Equation 3.2: Anion exchange of METAC

3.6 Coupling of Meth acryloyl Alginate with METAC anions

The final step for the synthesis of a novel polymer is the coupling of acrylated groups of both the substrate i.e., ionic liquid with a modified hydrogel to form a polymer of totally different properties. The procedure to carry out this reaction is to take ionic liquid and polymer dissolved in water in a 1:1:10 and to this reaction mixture initiator was added. The initiator used for this coupling reaction was Irgacure 2959 which initiated the radical polymerization at 365 nm. For this purpose, 3 rods of 2 V were taken and installed in the lamp and the reaction mixture along with the initiator, properly stirred was placed inside the lamp for the duration of 12 hours. After

about 12 hours, thin films of polymers were obtained that were removed by using a scraper and stored in an inert atmosphere.



X= Acetate, Propanoate, Glycolate, Succinate, Benzoate.

Equation 3.3: Schematic representation of the formation of Bio-ionic liquid-modified polymer

Chapter #04

Results & Discussion

4.0 Results and Discussion

This chapter includes the characterization and analysis of novel synthesized BIL polymers namely Choline-based alginate chloride polymer **(Alg Cho Cl)**, Choline-based alginate acetate polymer **(Alg Cho Ac)**, Choline-based alginate Propanoate **(Alg Cho Pr)**, Choline-based alginate Glycolate polymer **(Alg Cho Gly)**, Choline-based alginate Succinate **(Alg Cho Suc)**, and Choline-based alginate Benzoate **(Alg Cho Ben)**. The main characterization analysis includes:

- FTIR
- ^{1}H NMR
- SEM
- XRD
- TGA
- Mechanical analysis

The polymers synthesized were further subjected to perform their Biomedical applications i.e., Antibacterial and cytocompatibility against healthy and cancerous cells, and animal modeling were performed.

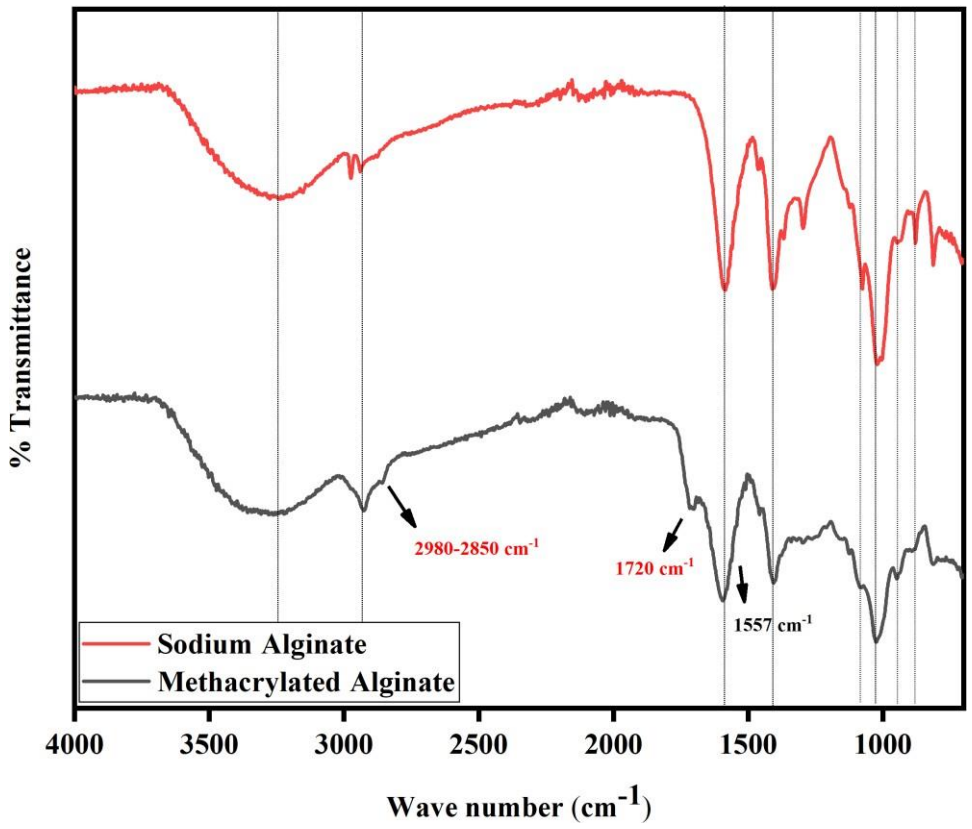
4.1. Fourier transform infrared spectroscopy (FTIR)

Fourier transforms infrared spectroscopic (FTIR) analysis was performed on the ATR-alpha FTIR instrument to identify functional groups present in synthesized materials.

4.1.1. MA alginate and sodium alginate

FTIR spectrum of sodium alginate and MA alginate is shown in Figure 4.1 which shows the characteristic peak of functional groups present. Comparing the peaks of functional groups present

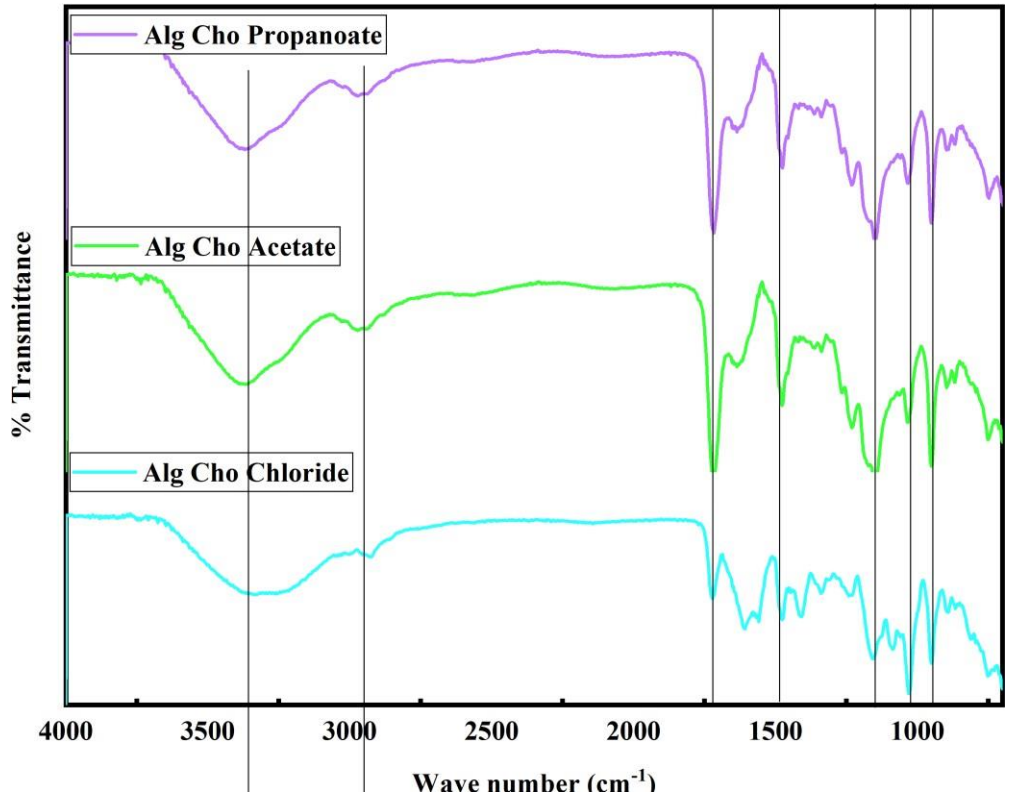
in the starting compound and newly synthesized modified hydrogel indicates the introduction of the methacrylic group. For example, growing peaks of -CH proton at 2980-2880 cm-1 indicates the more methyl part in comparison to the starting material, and at 1720 cm-1 addition of -C=O indicates anhydride part that is now the group in alginate hydrogel.



4.1 FTIR of sodium Alginate and MA sodium alginate.

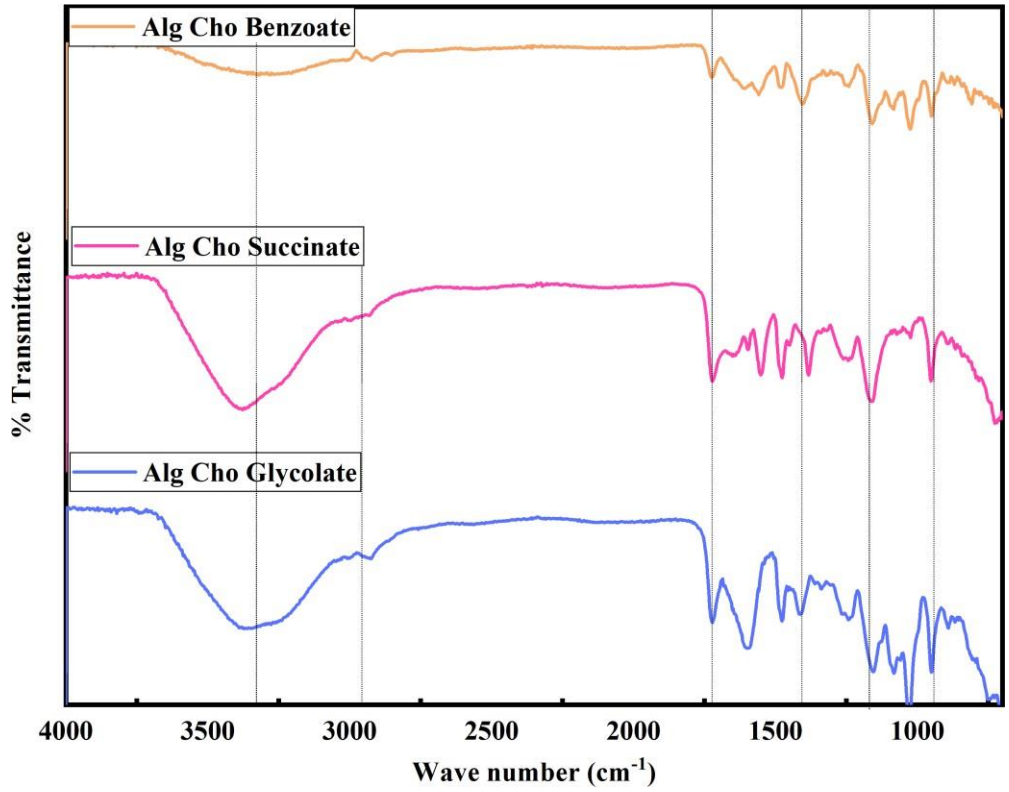
4.1.2. Choline-based Alginate polymer

Comparison of FTIR spectrum of newly synthesized polymer indicates the bands for the introduction of new anionic part and dissolution of double bond indicating the new C-C bond formation. Hence, the band at 1720 cm-1 disappeared.



4.2 FTIR of Alg Cho cl, Alg Cho Ac, and Alg Cho Pro.

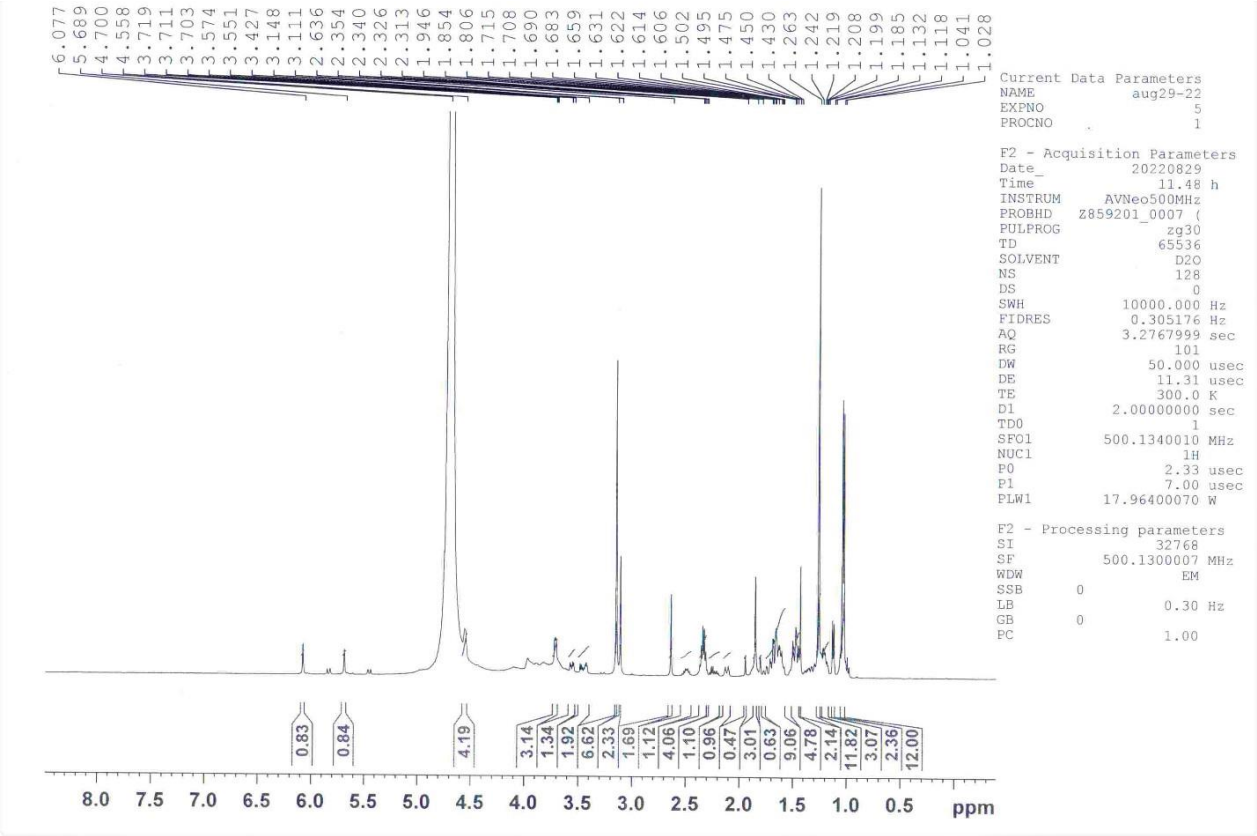
For the acetate and propanoate anions, a band around 2900-2800 cm-1 appears indicating more C-H stretching but in chloride and benzoate polymers, this band is not visible as expected.



4.3 FTIR of Alg Cho Gly, Alg Cho Suc, and Alg Cho Ben.

4.2 ^{1}HNMR of MA alginate

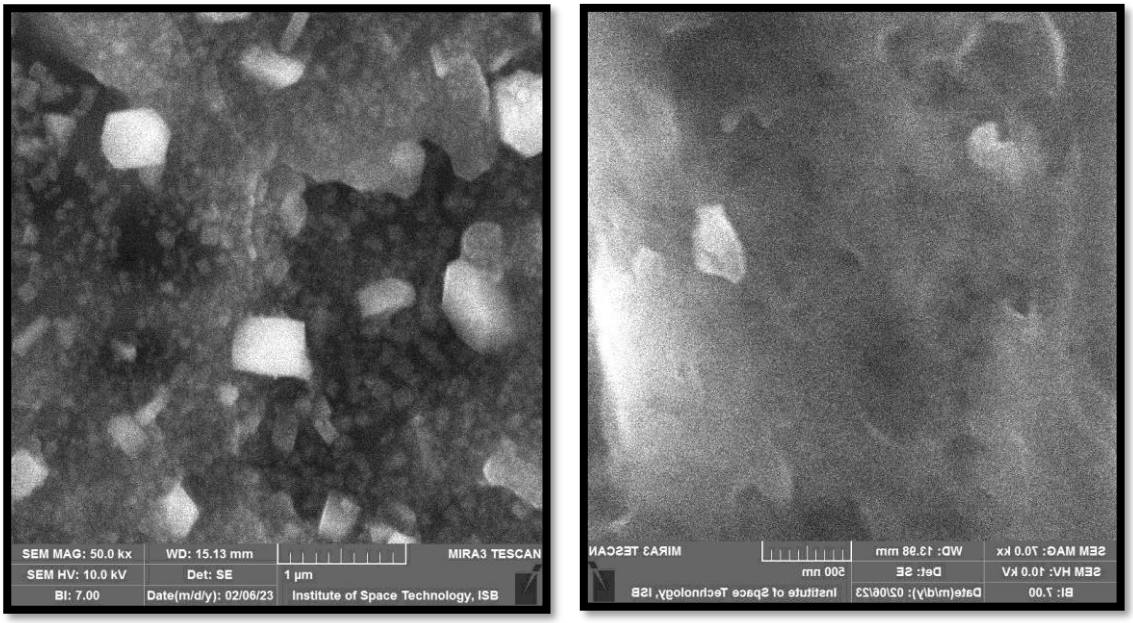
^{1}HNMR analysis of MA alginate indicates the same peak as in the sodium alginate except for 3 peaks. The peak at 1.8 for the 3 protons indicates the introduction of a new methyl group for the Methacrylic part. And the two small peaks of the integral value of 1 for each around 5.5-6.2 ppm are for the $=CH_2$. Normally, these kinds of protons show coupling but, in this case, low resolution makes it a non-coupling proton.



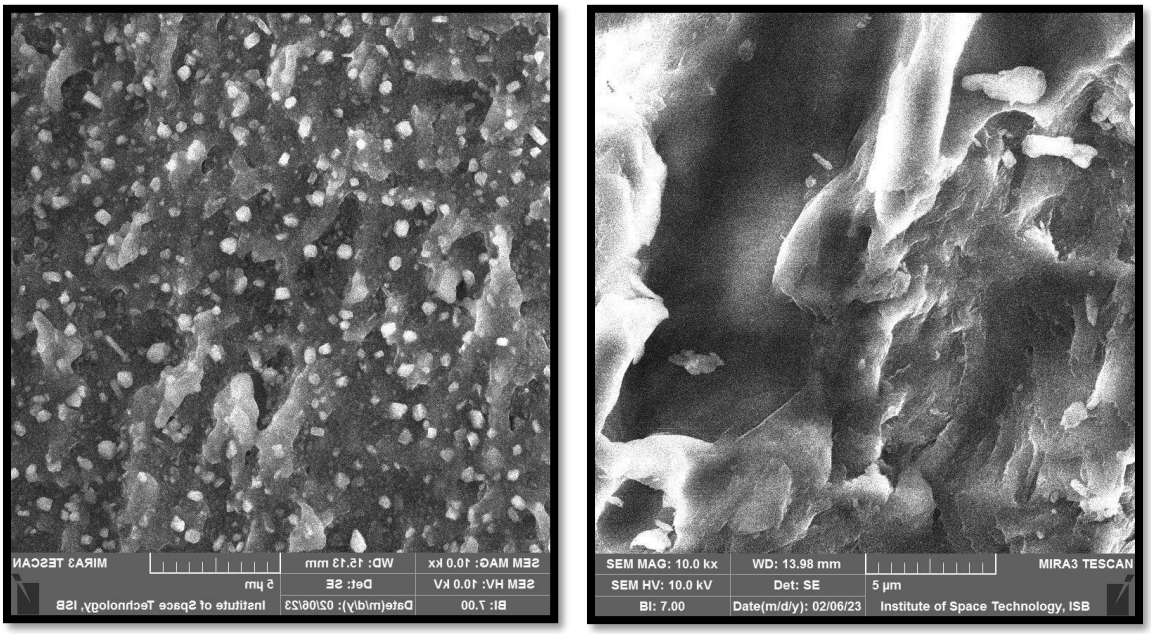
4.4 ^{1}H NMR of intermediate MA Alginate.

4.3 Scanning electron microscopy (SEM)

The morphologies of the prepared materials were determined by using SEM (Nova-Nano) at different resolutions (500 nm, 1000 nm) with 10-50kV working voltage. SEM analysis of simple alginate film and the comparison of BIL-modified films. Scans at different resolutions indicate the uniform distribution of BIL on the surface of the modified hydrogel indicating the coupling of the Choline-based BIL-modified Alginate polymer formation with a variety of polymers.



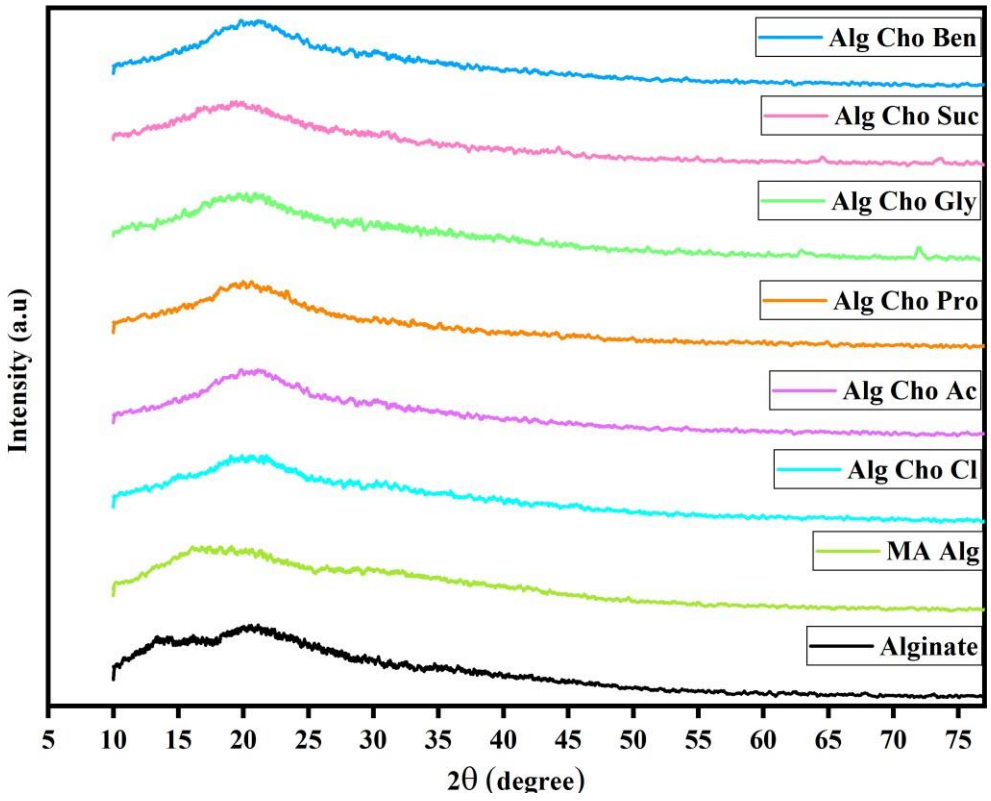
4.5 SEM images of Alg Cho Succinate and comparison with starting Sodium Alginate.



4.6 SEM images of Alg Cho Succinate and comparison with starting Sodium Alginate.

4.4 XRD analysis of Choline-modified Alginate anions

The p-XRD analysis of all synthesized materials was performed on Advanced Brucker D8, x-ray diffractometer (Germany) with Cu Kα x-ray source (λ = 1.5405A˚) in 2θ range of 5-80˚. XRD analysis determine that starting material which is sodium alginate is a more amorphous structure and after coupling with ionic liquid a new polymer formed having the same amorphous structure for all the 6 anions.



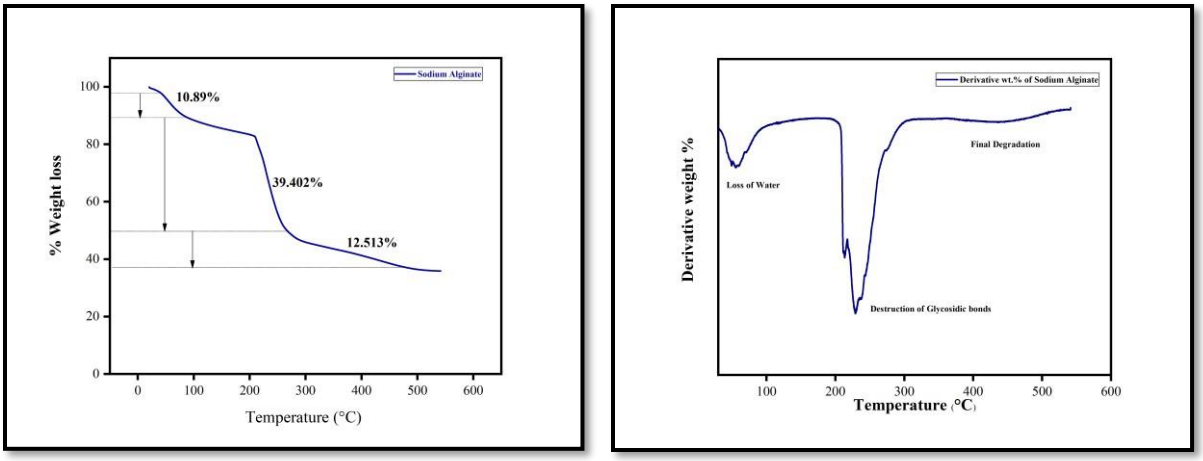
4.7 XRD analysis of starting material and final product of 6 anions.

4.5 Thermogravimetric Analysis

4.5.1 Sodium Alginate

TGA analysis of Sodium Alginate indicates that 10 % of total mass loss takes place between 80-120 ºC and at 250 ºC 39.462 % mass loss takes place due to the glycosidic bond breakage that further leads to more 12.53 % loss to final degradation and almost 38 % residue mass left.

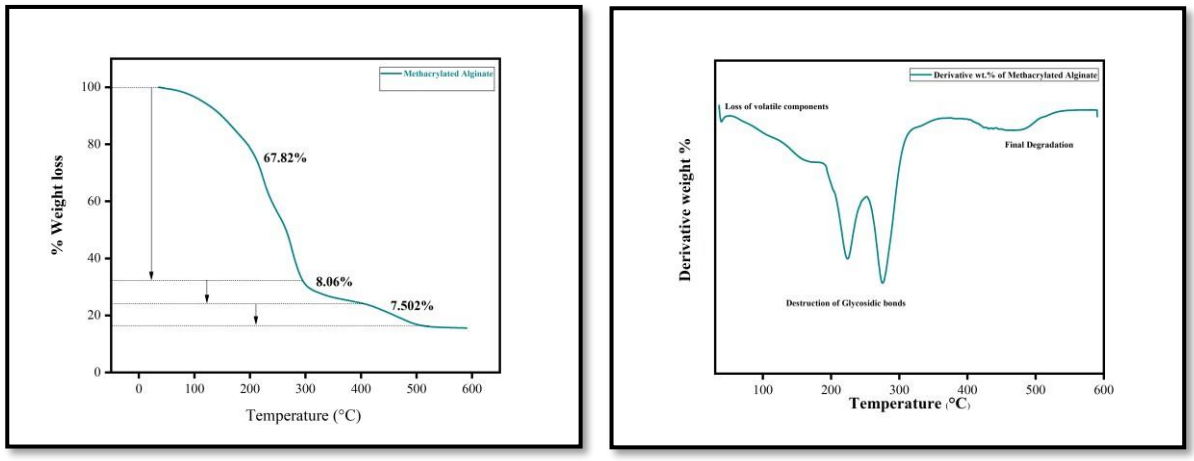
The DTA graph shows 3 peaks. The first is below the 100 ºC indicates water loss and the second between 200-250 ºC, the major peak indicates the glycosidic bond breakage and the third is broader than the other two, indicating final degradation.



4.8 TGA and DTA Analysis of Starting Material Sodium Alginate

4.5.2 Methacrylic Alginate

MA Alginate is the intermediate formed by the acrylation of the Methacrylic group and this intermediate when subjected to thermal degradation undergoes breakdown and residue mass of least around 18 %. MA alginate is less stable than sodium Alginate and hence its degradation is more visible in the graph.

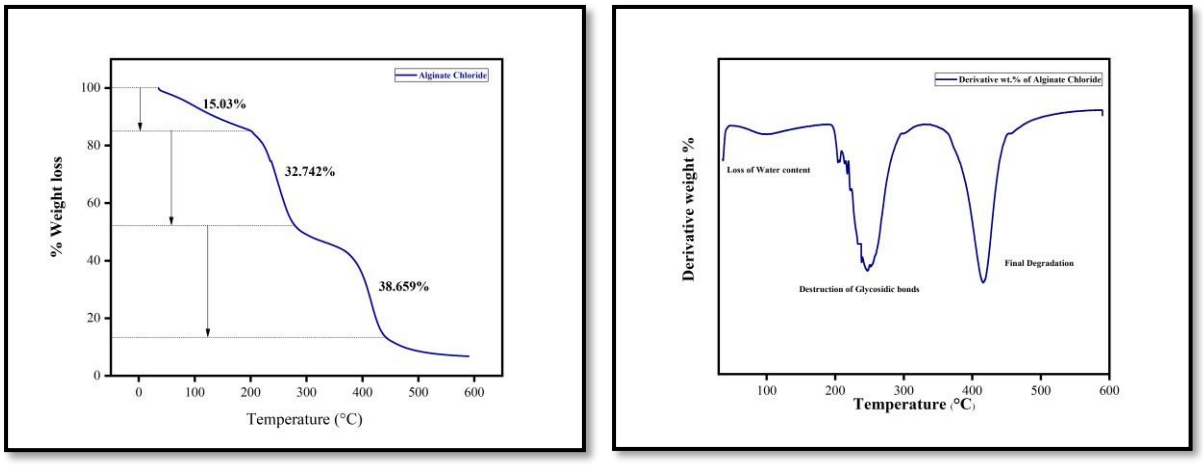


4.9 TGA and DTA Analysis of Intermediate MA Alginate

4.5.3 Alginate Choline Chloride

TGA analysis of Alginate Choline Chloride indicates that 15 % of total mass loss takes place between 80-120 ºC and at 250 ºC 37.62 % mass loss takes place due to the glycosidic bond breakage that further leads to more 12.53 % loss to final degradation and almost 38 % residue mass left.

The DTA graph shows 3 peaks. The first is below the 100 ºC indicates water loss and the second between 200-250 ºC, the major peak indicates the glycosidic bond breakage and the third is less broad than the other two, indicating final degradation.

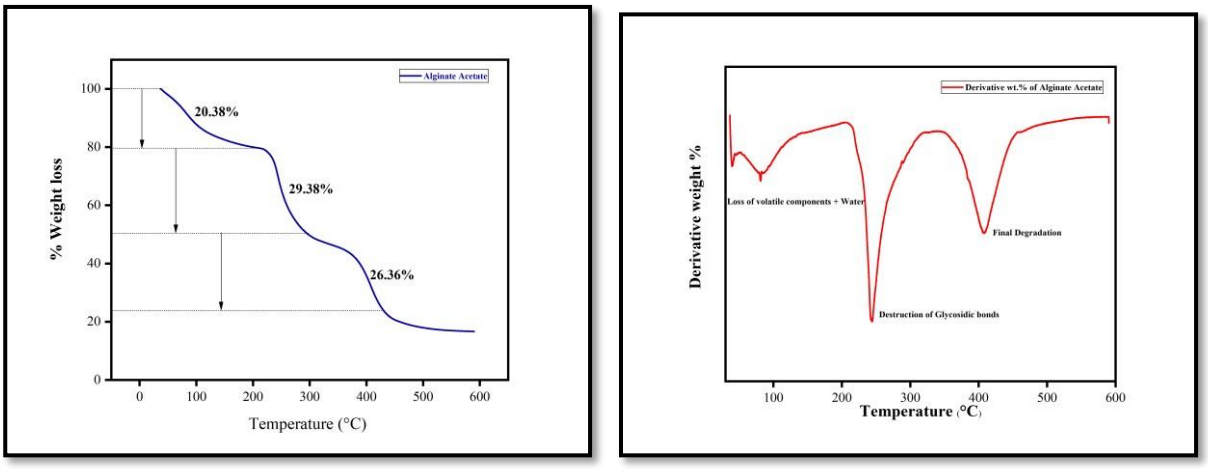


4.10 TGA and DTA analysis of Alginate modified Choline chloride.

4.5.4 Alginate Choline Acetate

TGA analysis of Alginate Choline Acetate indicates that 20 % of total mass loss takes place between 80-120 ºC and at 250 ºC 29.462 % mass loss takes place due to the glycosidic bond breakage that further leads to more 26.53 % loss to final degradation and almost 18 % residue mass left.

The DTA graph shows 3 peaks. The first is below the 100 ºC indicates water loss and the second between 200-250 ºC, the major peak indicates the glycosidic bond breakage and the third is less broad than the other two, indicating final degradation.

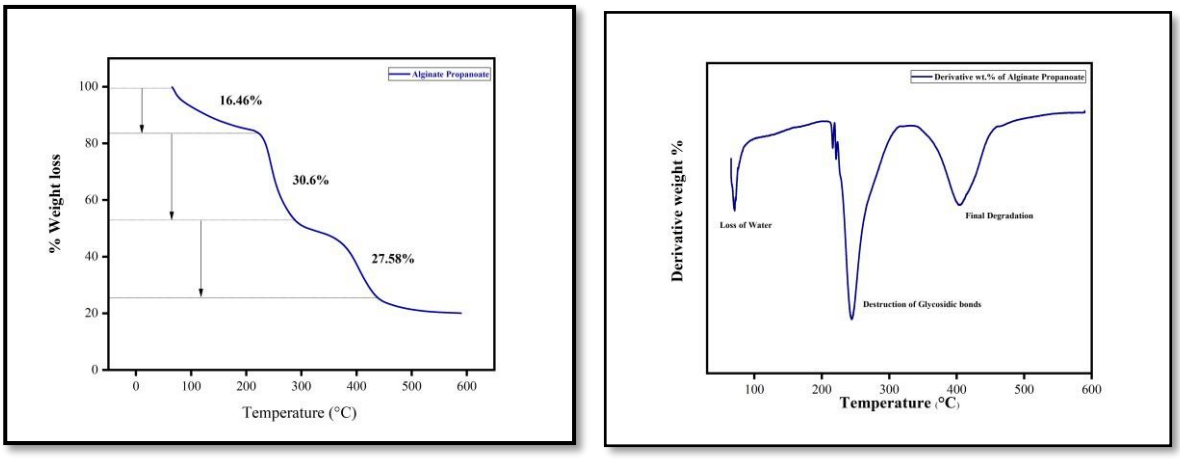


4.11 TGA and DTA analysis of Alginate modifies Choline Acetate.

4.5.4 Alginate Choline Propanoate

TGA analysis of Alginate Choline Propanoate indicates that 20 % of total mass loss takes place between 100-120 ºC and at 250 ºC 29.462 % mass loss takes place due to the glycosidic bond breakage that further leads to more 26.53 % loss to final degradation and almost 18 % residue mass left.

The DTA graph shows 3 peaks. The first is below the 100 ºC indicates water loss and the second between 200-250 ºC, the major peak indicates the glycosidic bond breakage and the third is less broad than the other two, indicating final degradation.

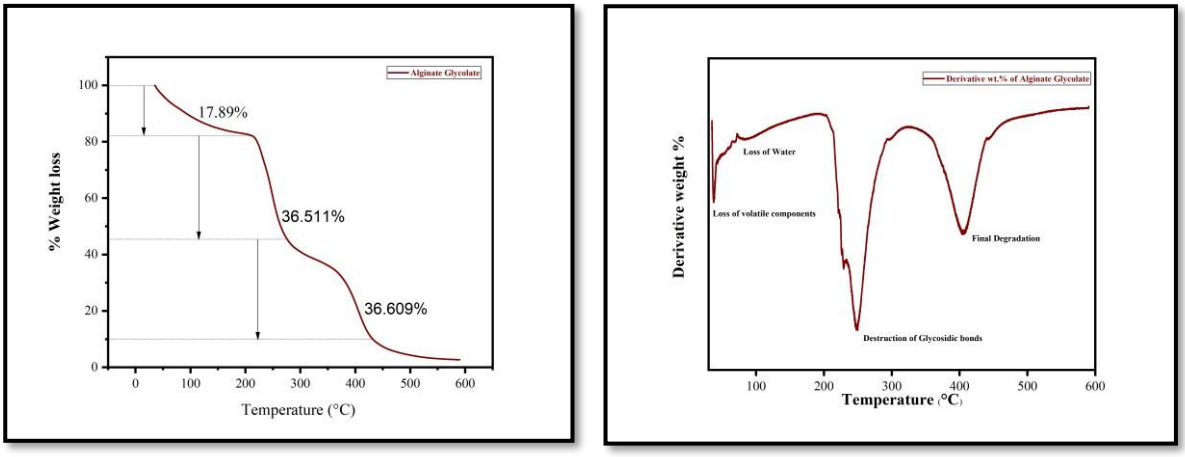


4.12 TGA and DTA analysis of Alginate modifies Choline Propanoate.

4.5.4 Alginate Choline Glycolate

TGA analysis of Alginate Choline Glycolate indicates that 17 % of total mass loss takes place between 80-120 ºC and at 250 ºC 36.9 % mass loss takes place due to the glycosidic bond breakage that further leads to more 36.53 % loss to final degradation and almost 8 % residue mass left.

The DTA graph shows 3 peaks. The first is below the 100 ºC indicates water loss and the second between 250-300 ºC, the major peak indicates the glycosidic bond breakage and the third is less broad than the other two, indicating final degradation.

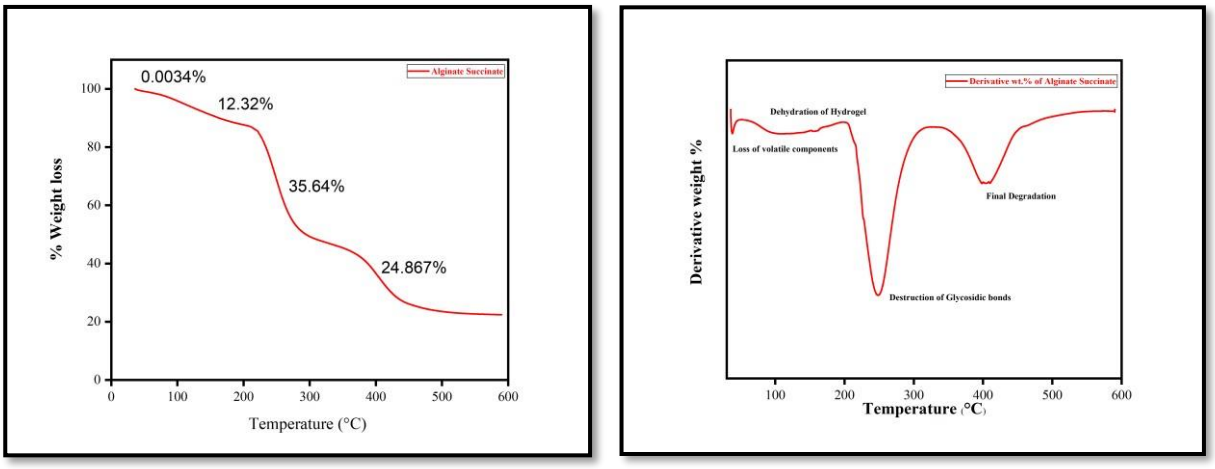


4.13 TGA and DTA analysis of Alginate modifies Choline Glycolate.

4.5.5 Alginate Choline Succinate

TGA analysis of Sodium Alginate indicates that 12 % of total mass loss takes place between 100-120 ºC and at 250 ºC 35 % mass loss takes place due to the glycosidic bond breakage that further leads to more than 24.53 % loss to final degradation and almost 18 % residue mass left.

The DTA graph shows 3 peaks. The first is below the 100 ºC indicating water loss and the second is between 200-250 ºC, the major peak indicates the glycosidic bond breakage and the third is less broad than the other two, indicating final degradation.

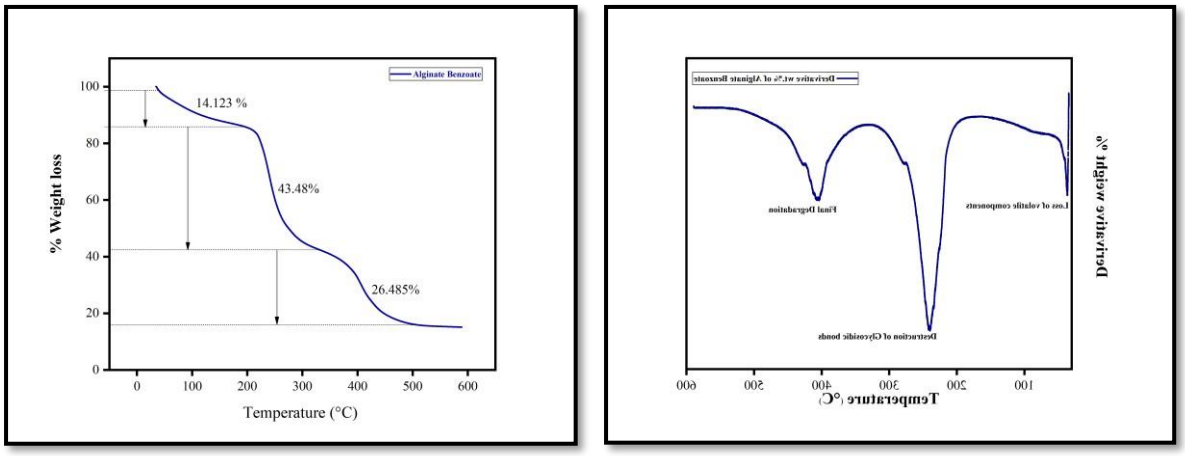


4.14 TGA and DTA analysis of Alginate modifies Choline Succinate.

4.5.6 Alginate Choline Succinate

TGA analysis of Alginate Choline Benzoate indicates that 14 % of total mass loss takes place between 100-120 ºC and at 250 ºC 43 % mass loss takes place due to the glycosidic bond breakage that further leads to more 26.53 % loss to final degradation and almost 18 % residue mass left.

The DTA graph shows 3 peaks. The first is below the 100 ºC indicating water loss and the second is between 200-250 ºC, the major peak indicates the glycosidic bond breakage and the third is less broad than the other two, indicating final degradation.



4.15 TGA and DTA analysis of Alginate modifies Choline Benzoate.

Polymer	TGA curve Temperature (°C)	Residue mass (%)
Sodium Alginate	211	35.90
MA Alginate	220	16.06
Alg Chloride	207	8.9
Alg Acetate	243	19.82
Alg Propanoate	244	21.7
Alg Glycolate	229	5.1
Alg Succinate	249	23.4
Alg Benzoate	240	15.5

Table 1 TGA and DTA analysis of Choline modified Alginate polymers.

4.6 Mechanical testing of Choline modified Alginate polymers

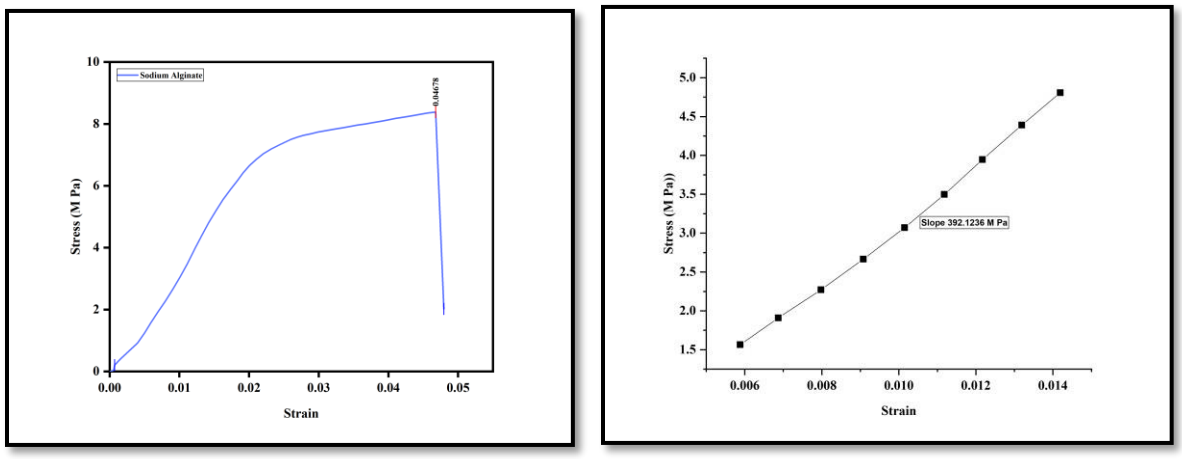
A polymer film's mechanical nature relates to its mechanical properties, which include stiffness, strength, flexibility, and toughness. A variety of experimental procedures can be used to determine these qualities. Here are a few popular methods for determining the mechanical characteristics of a polymer sheet:

- Tensile Testing: Tensile testing is a typical method for determining a film's mechanical properties. Stretching a film specimen until it breaks is required, followed by measuring the applied force and the resulting elongation. Tensile strength, elastic modulus, elongation at break, and yield strength are all measured in this test.
- Bending testing measures a film's ability to survive unexpected collisions or shocks by applying a bending force to a supported film specimen and measuring the resulting deformation. It is done by striking a film specimen with a pendulum or a falling weight and measuring the amount of energy absorbed or the amount of deformation caused.
- • Hardness testing determines a film's resistance to indentation or scratching. The hardness of a polymer film surface can be determined using Shore hardness and Rockwell hardness.
- Dynamic Mechanical Analysis (DMA): Dynamic Mechanical Analysis (DMA) is a technique for assessing the viscoelastic behavior of polymers at different temperatures and frequencies. It includes information on the storage modulus, loss modulus, damping properties, glass transition temperature, and other aspects of a film.

Rheological testing is used to determine a film's flow behavior and viscosity under various shear circumstances. These tests provide information about the melt flow index, melt viscosity, and shear rate dependency of the film.Among other things, these procedures aid in characterizing the mechanical properties of a polymer film and determining its applicability for various applications.

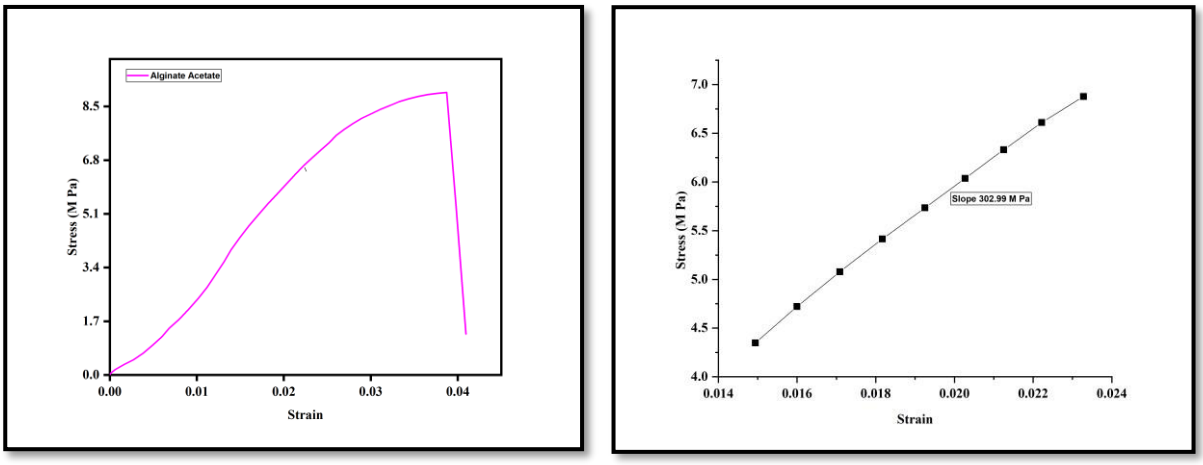
It is vital to note that the testing technique chosen is determined by the attributes of interest as well as the unique requirements of the film.

Flexural testing, often known as three-point or four-point bending tests, is used to measure a film's flexural strength and stiffness.



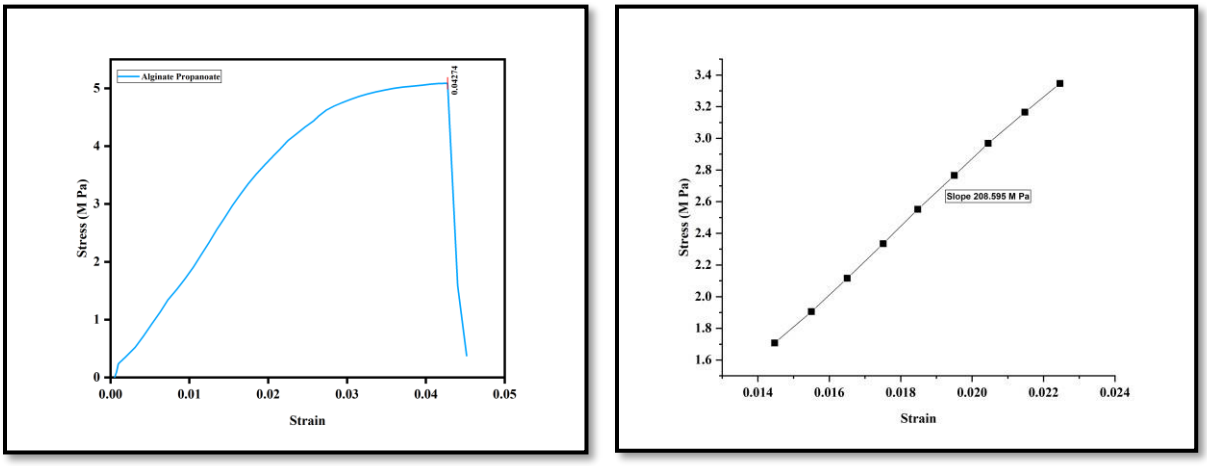
4.16 Stress-Strain curve and Young's modulus of Sodium Alginate

By comparing the Stress-strain curve of starting material and newly formed polymers, it is observed that by adding the ionic liquid into the hydrogel, the flexibility of the hydrogel is compromised. This caused the increased ionic character of the newly formed polymer.



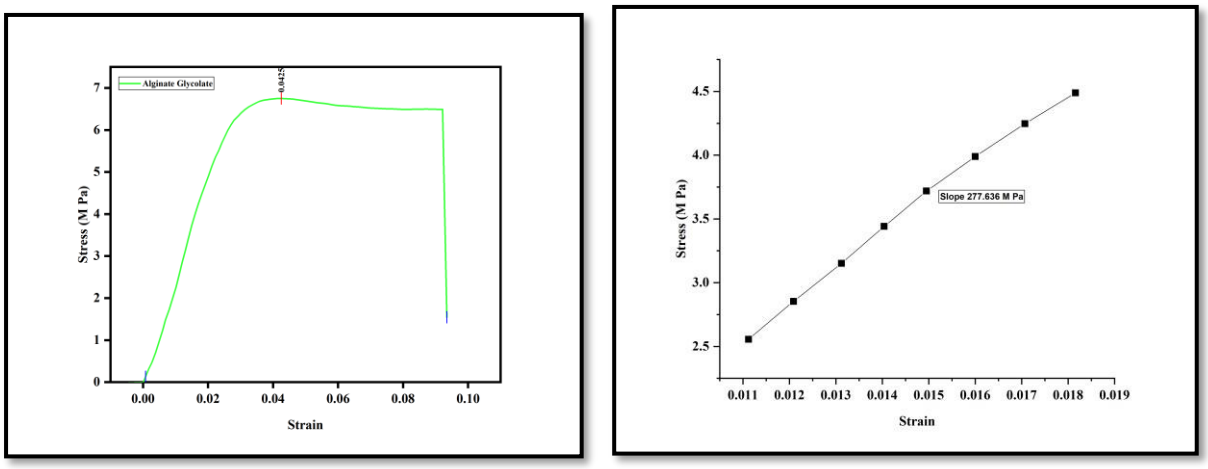
4.17 Stress-Strain curve and Young's modulus of Alg Cho Acetate

Young's modulus also decreased in the polymers in comparison to the starting material. The less the Young's modulus more the stiffness of the material and this was the expected outcome.

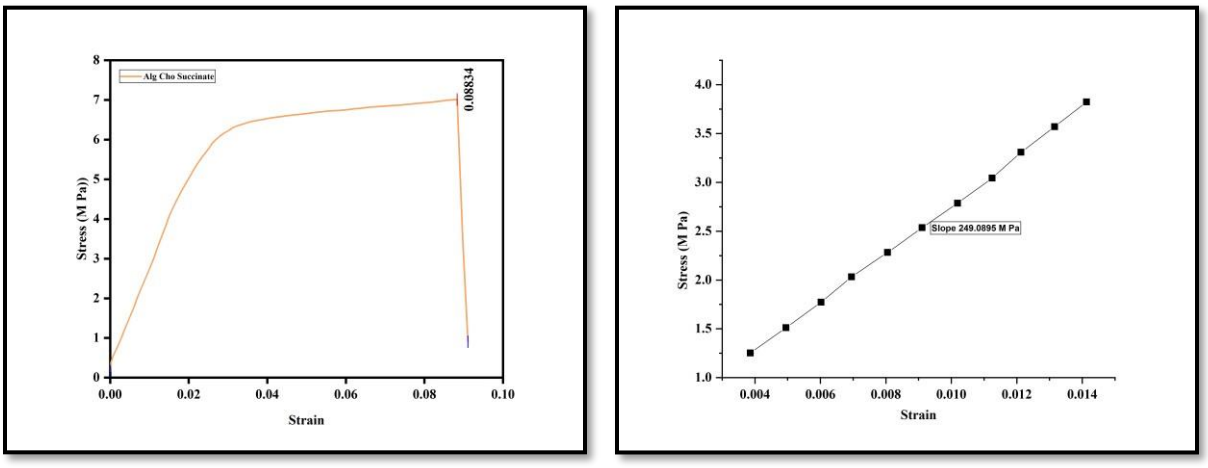


4.18 Stress-Strain Curve and Young's Modulus of Alg Cho Propanoate.

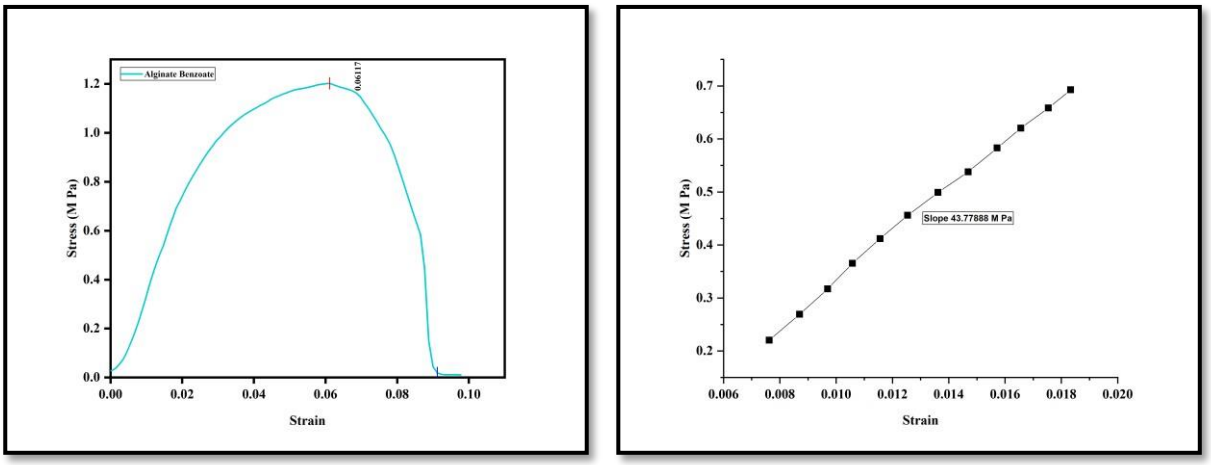
The ultimate tensile strength of some polymers increased in comparison to the starting material.



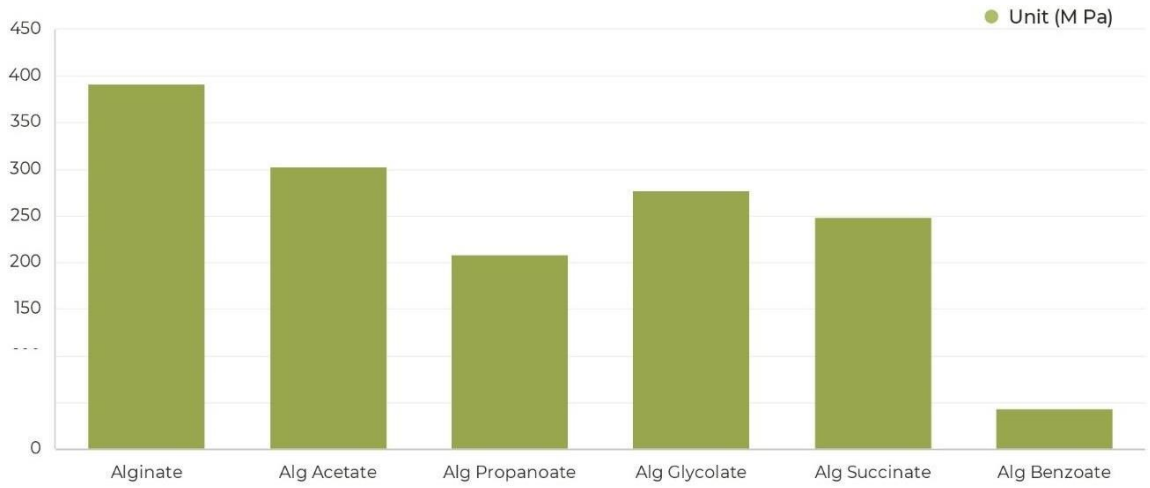
4.19 Stress-Strain Curve and Young's Modulus of Alg Cho Glycolate.



4.20 Stress-Strain curve and Young's modulus of Alg Cho Succinate.

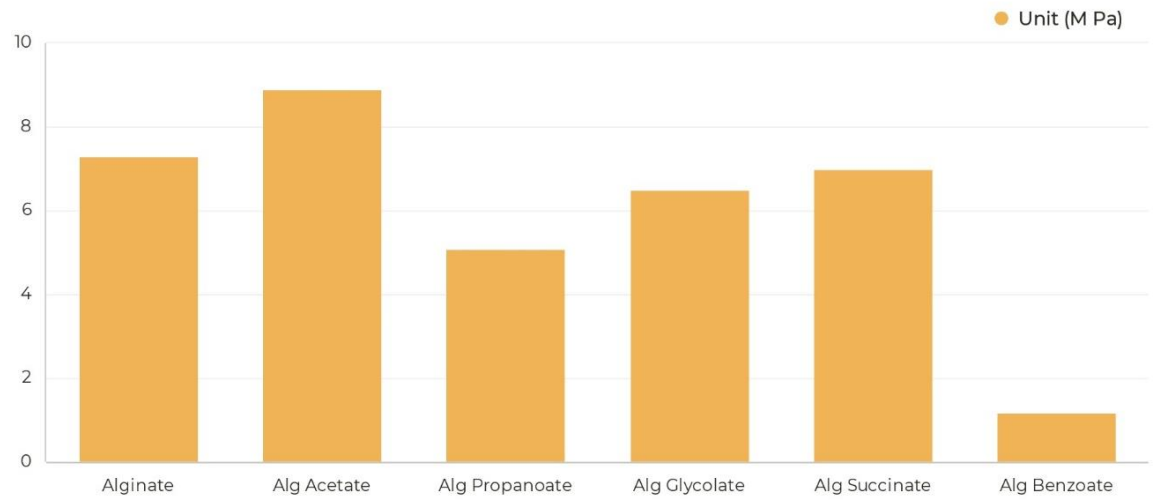


4.21 Stress-Strain curve and Young's modulus of Alg Cho Benzoate.



4.22 Young's modulus comparison of polymers with starting material.

Alginate Cho Acetate and Alg Cho Succinate show a remarkable improvement in the UTS in comparison to the starting material Sodium Alginate.

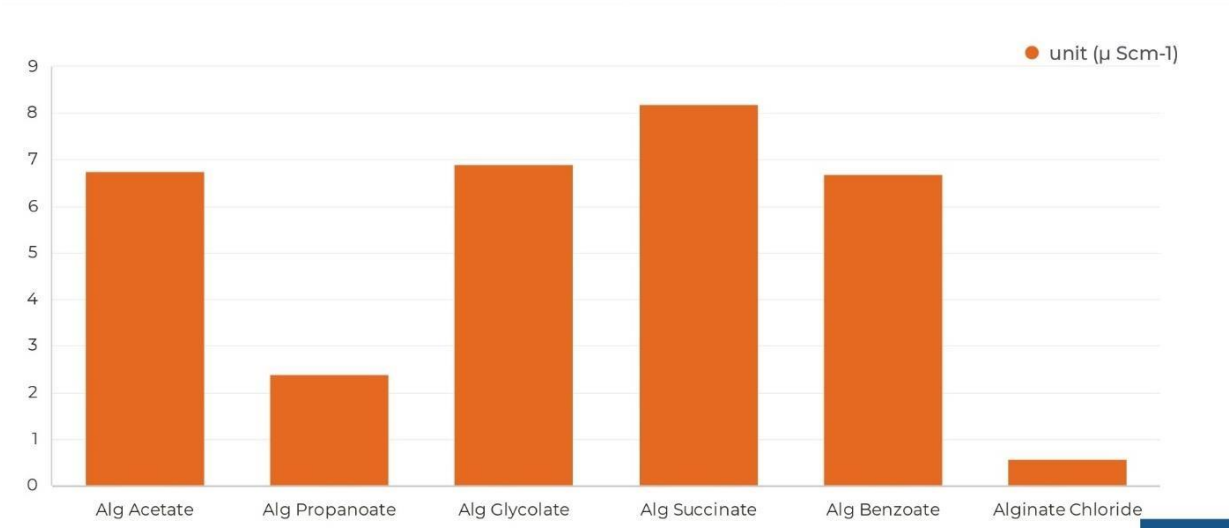


4.23 UTS point of newly synthesized polymers with starting material.

4.6 Conductivity of newly synthesized polymers

Polymer conductivity varies greatly based on its chemical composition, structure, and production circumstances. Polymers, in general, are electrical insulators, which means they have low electrical conductivity. Certain forms of polymers, however, exhibit electrical conductivity and are referred to as "conductive polymers." These conductive polymers have distinctive features that make them ideal for use in electronics, sensors, and energy devices.

Comparing the properties of the newly synthesized polymer with the starting material, it is observed that Sodium alginate is not a conducting polymer. But newly synthesized polymer has the power of conduction due to its anions that are reason to conduct electricity.



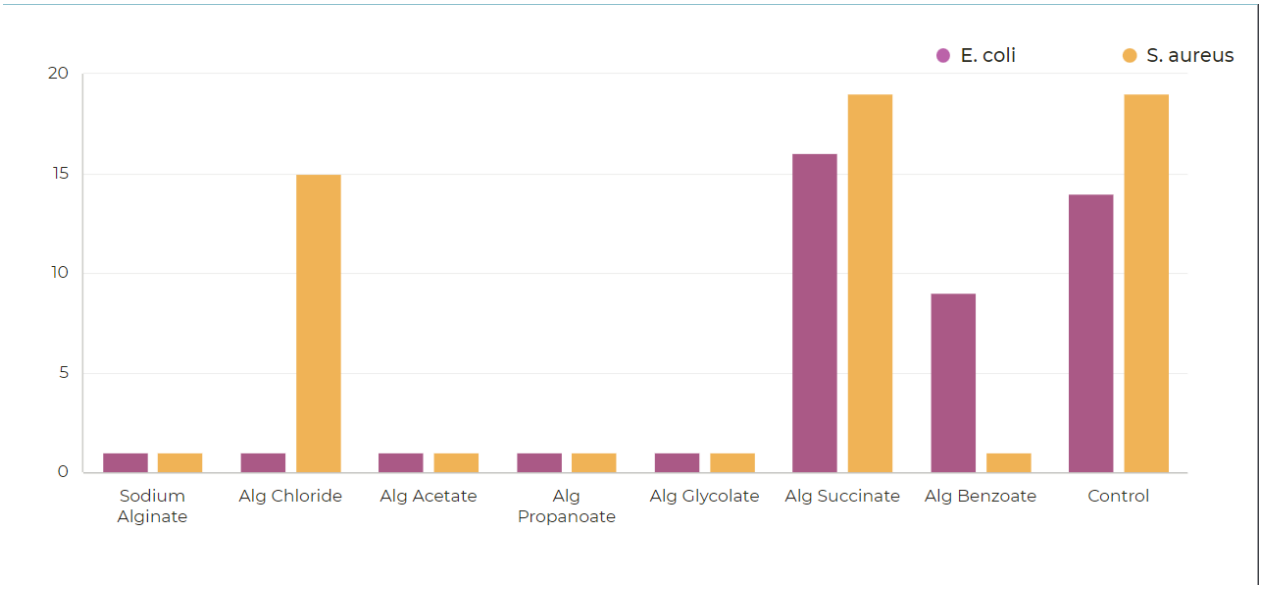
4.24 Conductivity of newly synthesized polymers.

Among these, Alginate Cho Succinate is the most conducting polymer and Alginate Chloride is the least conducting polymer overall.

4.7 Antibacterial activity of newly synthesized polymers

Newly synthesized polymers were found to be more anti-bacterial in nature. Zones of inhibition were determined against 2 strains of bacteria. One was gram-positive (S. aureus) and the other was gram-negative (E. coli). The experiment began with the preparation of nutritional agar plates in Petri dishes using sterilized agar media. The agar was allowed to solidify, providing bacteria with a suitable growth substrate. Using a sterile inoculating loop or brush, a bacterial culture was then injected onto the surface of the agar. The culture was distributed evenly throughout the agar surface with care. Antibiotic discs were placed on the agar surface and were impregnated with specific antibiotics. To ensure contact with the agar, gentle pressure was applied. The agar plates were then incubated for 24 hours at an acceptable temperature, typically 37°C (98.6°F). This allowed the germs to multiply and the antibiotics to take effect.

To perform this experiment, two types of antibiotics were used. Gentamicin and erythromycin were used, and zones of bacterial inhibition were then compared about the antibiotic zone.



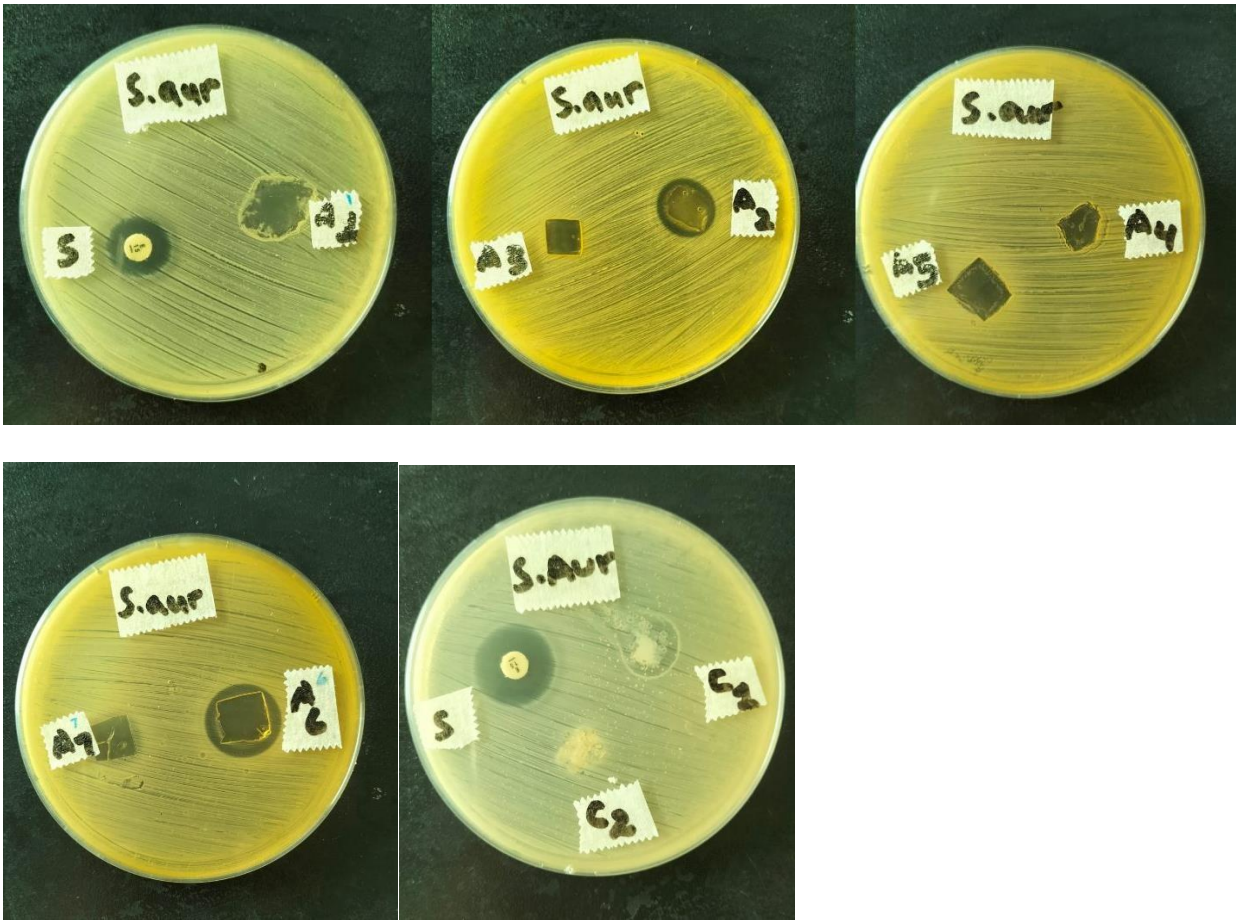
4.25 Zones of inhibition against 2 strains of bacteria for newly synthesized polymers.

Among all the polymers synthesized, Alginate Choline Succinate, Alginate Choline Chloride, and Alginate Choline Benzoate show remarkable antibacterial properties.

Antibacterial activities of alginate-based ionic liquid polymers are achieved by a variety of methods that inhibit bacterial growth and survival. These polymers, which are mostly composed of alginate with the addition of an ionic liquid, provide a promising technique for fighting bacterial infections. Membrane disruption, electrostatic interactions, oxidative stress, and the suppression of critical cellular processes are some of the methods by which alginate-based ionic liquid polymers kill bacteria.

For starters, these polymers can disrupt and increase the permeability of bacterial cell membranes. This disturbance can cause cellular contents to leak, eventually leading to bacterial

cell death. Furthermore, because alginate-based ionic liquid polymers are charged, they can interact electrostatically with bacterial surfaces. These interactions damage the cell membrane's integrity, resulting in cell death. Furthermore, when some alginate-based ionic liquid polymers come into contact with microorganisms, they produce reactive oxygen species (ROS). ROS causes oxidative stress within bacterial cells, causing damage to biological components and contributing to cell death. Furthermore, these polymers can disrupt important cellular functions including enzyme activity and protein synthesis, reducing bacterial growth and survival even further.



4.26 Petri dishes pictures during antibacterial.

Depending on the formulation of the alginate-based ionic liquid polymer and the targeted bacteria, the specific mode of action may differ. These polymers provide a diverse and successful strategy for antibacterial applications by combining the unique features of alginate with ionic liquids. Continued research and development in this subject bodes well for the creation of novel techniques to battle bacterial diseases and enhance public health.

4.8 Cytocompatibility of Healthy and cancerous cells

4.8.1 Fibroblastic (skins cells) of rats (3T3)

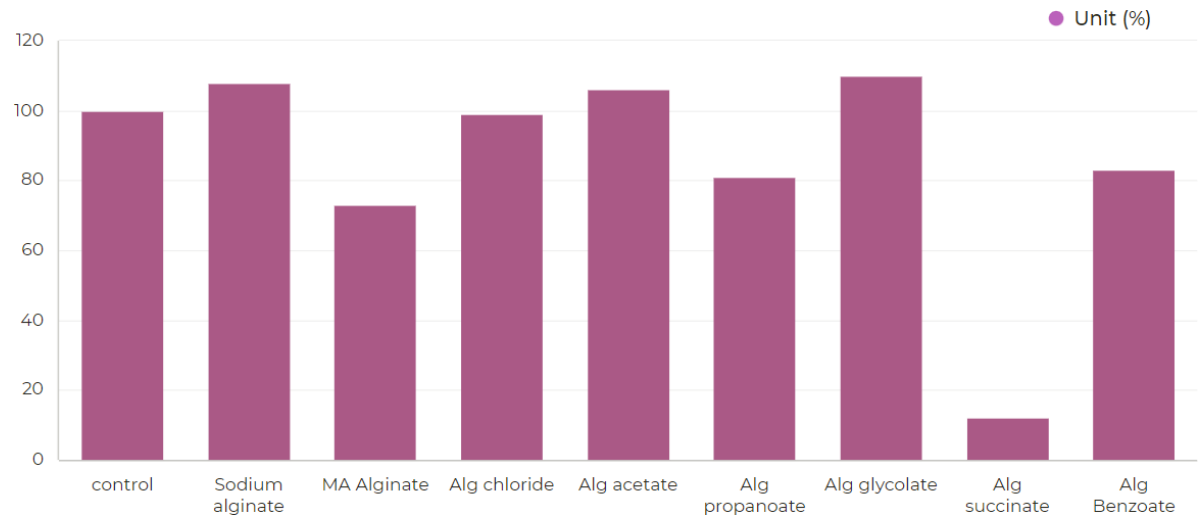
An indirect method was performed to evaluate the cytocompatibility of water-insoluble polymer. The indirect approach to testing healthy cell cytocompatibility is examining the impact of a material or substance on cell viability and function without direct physical contact. Cells are cultivated in a medium that contains the material under test, allowing for indirect interaction between the cells and the material or its components. Various processes, including as diffusion, might cause the material to leak soluble components, ions, or degradation products into the culture media during the culture. These components subsequently diffuse into the surroundings and make contact with the cells. In turn, the cells respond to the substances in the culture media, which might alter their behavior and functionality.

Cell response can be assessed using a variety of tests and measurements, including cell viability, metabolic activity, gene expression, protein synthesis, and cellular morphology. Researchers can determine the cytocompatibility of a material or substance by observing and analyzing certain factors.

When direct contact between the material and the cells is not desired or practicable, the indirect method provides a valuable tool for testing cytocompatibility. It provides insight into the

material's possible effects on cell behavior through its influence on the surrounding environment. This method is very beneficial for determining the biocompatibility and safety of materials planned for use in tissue engineering, drug delivery systems, and medical implants.

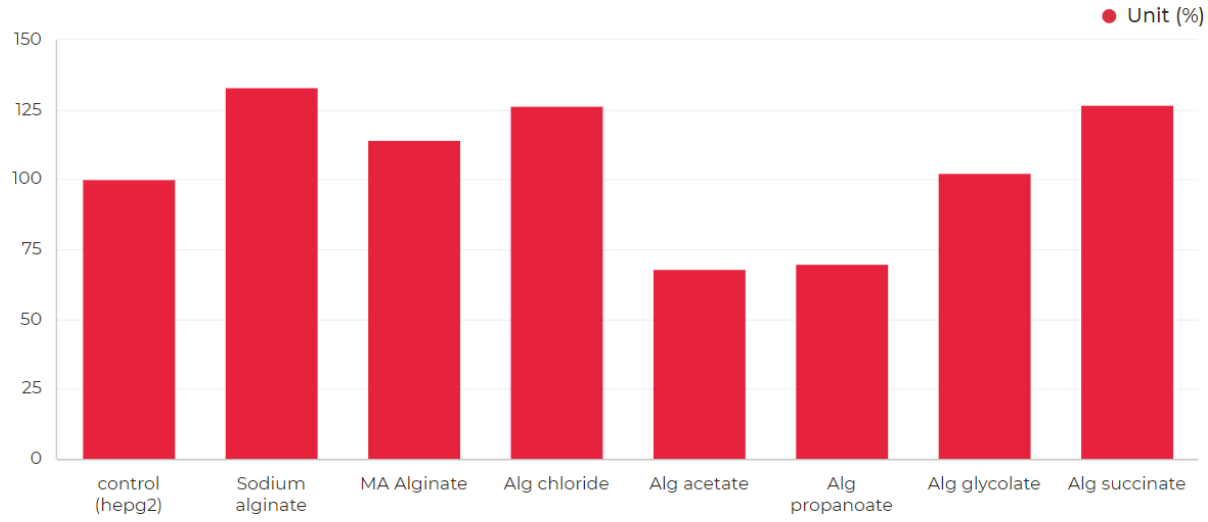
Results extracted here indicate that all of the modified anionic polymers are highly cytocompatible, especially for skin cells indicating that this can be used in wound healing and wound dressing as this can help in making bandages also.



4.27 Cytocompatibility of polymers against skin cells of rats (3T3).

4.8.2 Liver cells of rats (HEPG2)

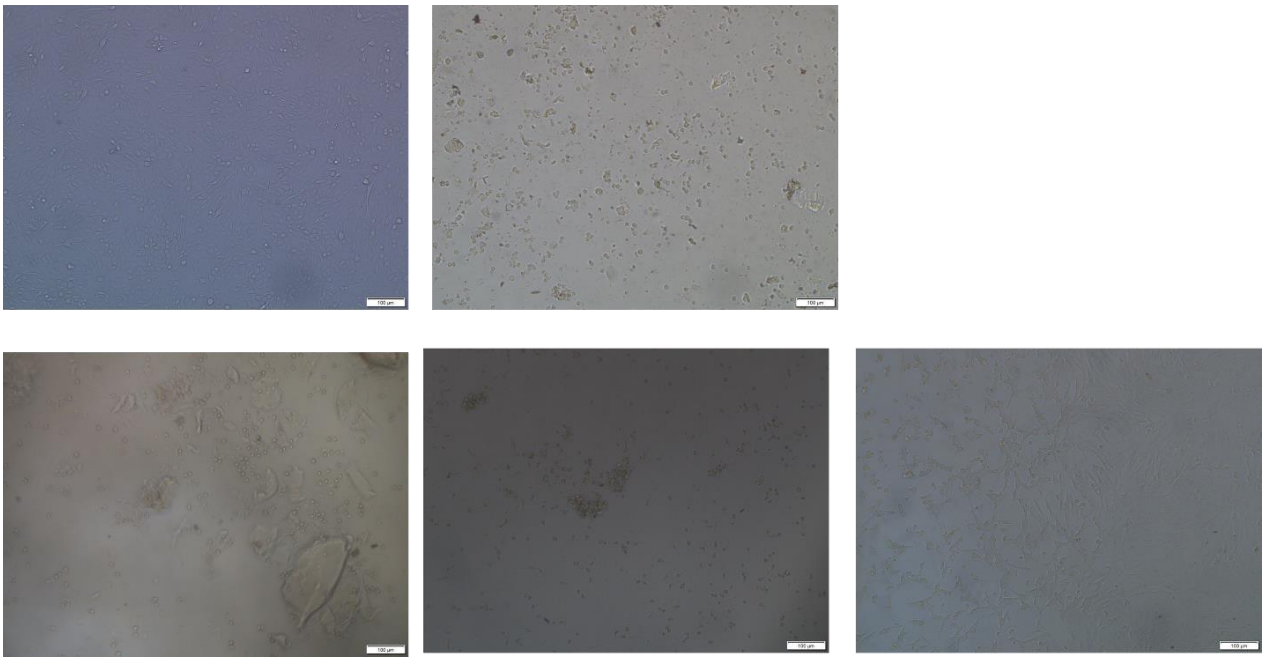
The cytotoxicity of the newly formed polymers was also checked for being anti-cancerous. Many polymers like Alginate Choline Propanoate, Alginate Choline Acetate, and MA Alginate are comparatively better at killing cancer cells. This can also be used in drug delivery, especially for cancer cells.



4.28 Cytotoxicity of polymers against liver cells of rats (HEPG2).

Control-hepg2	100
Alig-propionate	69.93865
Alig-glycolate	102.454
Alig-succinate	126.9939
Alig-chloride	126.3804
Alig-acetate	68.09816
Alig-MA	114.1104
Alig-SM	133.7423

Table 2 Cytocompatibility of newly synthesized polymers on hepg2 cells.



3.29 Cytocompatibility photos of 3T3 skin cells of rats.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In conclusion, this work reported the modification of Sodium Alginate and coupling with BIL choline that can be used in various biomedical applications. A variety of novel polymers were synthesized due to the anionic exchange of choline that features the specific properties of the polymers. This synthesis was then confirmed by FTIR and 1HNMR. XRD and SEM evaluation confirms the physical contact of the BIL due to the uniform depositing of the BIL on the surface of the hydrogel. To check the nature of the polymer, TGA and mechanical analysis were performed indicating the thermal stability of novel hydrogels in comparison to the starting material and synthesis of novel BIL-modified Alginic polymers. A variety of such polymers were reported and evaluated against various biomedical applications that were found to be good antibacterial in comparison to their starting material and their cytocompatibility against healthy and cancerous cells were checked. Among these, all the polymers were found to be highly cytocompatible against skin cells, and cell growth of cancerous cells was limited by them making them good polymers to be used in drug delivery and tissue engineering and leading to be used in wound treatment and healing of wounds.

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