PROJECT REPORT

On

"PREPARATION AND CHARACTERIZATION OF CHITOSAN BASED HYDROGELS"

Submitted by
ANJANA B ANI, AB16CHE011
ANU P THOMAS, AB16CHE012
SHALOM VARGHESE, AB16CHE024

In partial fulfillment for the award of the B. Sc Degree in Chemistry

POST GRADUATE AND RESEARCH DEPARTMENT OF CHEMISTRY

ST. TERESA'S COLLEGE (AUTONOMOUS) ERNAKULAM

2018-2019

POST GRADUATE AND RESEARCH

DEPARTMENT OF CHEMISTRY

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

B.Sc. CHEMISTRY PROJECT REPORT

Name : **ANJANA B ANI, ANU P THOMAS, SHALOM VARGHESE**

Register Number : **AB16CHE011, AB16CHE012, AB16CHE024**

Year of Work : **2018-2019**

This is to certify that the project "**PREPARATION AND CHARACTERISATION OF CHITOSAN BASED HYDROGEL**" is the work done by **ANJANA B ANI, ANU P THOMAS, and SHALOM VARGHESE.**

Dr. Ushamani M
Head of the Department

Safalya A S
Staff-in-charge

Submitted to the Examination of Bachelor's Degree in Chemistry

Date: ……

Examiners : …….

: ……

DECLARATION

I hereby declare that the project work entitled "PREPARATION AND CHARACTERISATION OF CHITOSAN BASED HYDROGEL" submitted to Department of Chemistry, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by me under the guidance of SAFALYA A S, Assistant Professor, Department of Chemistry, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfillment of the requirements for the award of the degree of Bachelor of Science in Chemistry.

ANJANA B ANI
ANU P THOMAS
SHALOM VARGHESE

Acknowledgements

First of all, I am highly Grateful to Almighty God for his continuous blessings for the successful completion of my project.

I wish to express my profound sense of gratitude to Mrs. Safalya A S, my guide and Assistant Professor of Department of Chemistry, St.Teresa's College for her valuable guidance, personal attention, meaningful suggestion, help and encouragement.

I take this opportunity to convey my gratefulness to Dr. Remya K R, Sree Chitra Tirunal Institute for Medical Science & Technology for the advice and help received from her.

I am thankful to Miss. Georgina Grace, Assistant Professor of Department of Chemistry, St.Teresa's College, Ernakulam.

I am also thankful to Dr. Ushamani. M, Head of Department of Chemistry, St.Teresa's college, Ernakulam.

I would like to express my gratitude to all our teachers and non- teaching staff for the whole hearted help throughout our project.

I am grateful to the Principal Dr. Sajimol Augustine and Director Rev. Sr. Dr. Vinitha, St.Teresa's college, Ernakulam for being the pillars of support and providing good infrastructure for the study and development of students.

I thank STIC, Cochin University of Science & Technology, Kalamassery for the facilities provided for the analysis of my sample.

Last but not the least, I am grateful to my loving family and friends for the care and concern they provide to follow my passion.

Place: Ernakulam

Date:

ANJANA B ANI

ANU P THOMAS

SHALOM VARGHESE

Contents

Objectives of the present work

The objective of the work is the preparation and characterisation of chitosan based hydrogel for wound healing application.

Chapter 1

Introduction

Every year millions of people are exposed due to hot water, f1lames, accidents, and boiling oil which may result in major disabilities and sometimes it may lead to drastic death also. Burn wounds due to fire are considered as one of the 15 leading causes of death in India. Global statistics shows that nearly 2, 65,000 deaths occur due to burn injuries and of this, nearly 1, 40,000 deaths occur in India alone (WHO Report, 2014). Therefore we need proper wound dressing to avoid excessive fluid loss and bacterial invasion, and these can be achieved by proper wound dressing materials, that includes traditional as well as modern dressing materials. In order to improve the efficiency of wound healing, chitosan based hydrogels are considered as an ideal material for enhancing wound healing owing to their biodegradable, biocompatible, non-toxic, antimicrobial, biological adhesive, biological activity and homeostatic effect. The unique biological properties of chitosan based hydrogel enable it to serve as both a wound dressing and as a drug delivery system (DDS) to deliver antibacterial agents, growth factors, stem cells and so on, which could further accelerate wound healing. For various kinds of wounds, chitosan-based hydrogels are able to promote the effectiveness of wound healing by modifying or combining with other polymers, and carrying

different types of active substances. In this review we will take a close look on the application of chitosan based hydrogel for wound healing.

Burn injuries are characterised by the non-viable tissue, body fluid, serum and blood and colonised by potentially pathogenic bacteria which may significantly result in the skin loss, fluid loss and infections and that may eventually lead to death. Therefore we need proper wound management to prevent excessive fluid loss and bacterial invasion, and these can be achieved by appropriate wound dressing material. And it may include traditional as well as modern dressing. Traditional wound dressing material includes cotton wool, natural and synthetic bandages. And gauze is a type of traditional wound dressing material which possess high absorption capacity. Due to their highly absorptive nature, wounds become dehydrated easily and may adhere to the wound, which in turn creates problems during dressing removal. And sometimes it may cause infections also. And other one is the modern dressing, which includes hydrocolloids, alginate dressings, foam dressings, and hydrogel dressings.

1.1 HYDROGELS

Hydrogels are three-dimensional networks composed of hydrophilic polymers cross linked through covalent bonds or held together via physical intermolecular attractions. Hydrogels can absorb large amounts of water or biological fluids, from 20% up to several thousand % and swell readily without dissolving. The high hydrophilicity of the hydrogels is mainly due to the presence of a number of hydrophilic moieties such as amino, carboxyl, amide and hydroxyl group. The three dimensional polymeric network can either formed by chemical or physical crosslinking

of hydrophilic polymer chains. In chemical gels, they are formed by covalent bond where as in physical gels; they are formed by non-covalent bonds such as van der Waals interaction, ionic interaction, hydrogen bonding, hydrophobic interaction etc. Different synthetic, natural and modified natural polymers, including chitosan are used to form hydrogels. Hydrogel based on natural polymers are currently receiving a great deal of interest, and are notable for controlled drug delivery and tissue engineering.

1.2 CLASSIFICATION OF HYDROGELS

Hydrogels are classified into natural or synthetic polymeric based networks. Natural hydrogel are often made of polysaccharide or protein chains. Polysaccharides have hydrophilic structure which is a favourable property of hydrogel preparation. Some examples of polysaccharide based hydrogels are hydrogels made of alginate, cellulose, chitin, chitosan, dextran, hyaluronic acid, pectin, starch and xanthan gum. Collagen, silk, keratin, elastin, resilin and gelatin are protein chains that form natural hydrogel lattices. Synthetic polymers such as poly vinyl alcohol, polyacrylamide, poly ethylene oxide and poly ethylene glycol have been used for hydrogel formation. Natural polymers usually have higher biocompatibility compared to synthetic polymers, as they undergo enzyme controlled biodegradation by human enzymes like lysozyme and produce biocompatible by-products. On the other hand, synthetic polymers are chemically stronger than natural ones, because of hydrolysable moieties with slower degradation rate. This feature provides more prolonged lifetime in human body.

1.3 FORMATION OF HYDROGELS

Hydrogels can be prepared via chemical (permanent bonds) or physical cross-linking. Methods for chemical cross-linking of hydrogels include radical polymerization, photo polymerization, enzymatic reactions, and covalent cross-linking via linkers such as aldehydes. In contrast, physical cross-linking forms a non-permanent network with physical interactions such as hydrogen or electrostatic bonds, physical entanglements (and crystal formation). So the physically cross-linked hydrogels can be formed via ion interactions, using graft copolymers, crystallization and stereo complex formation.

1.4 APPLICATION OF HYDROGELS IN BIOMEDICAL FIELD

Hydrogels are widely used in agriculture, food industry and pharmaceutical fields. In biomedical area, they are applied for systemic and localized drug delivery and tissue engineering.

1.4.1 DRUG DELIVERY

Hydrogels are used as platforms for both drugs and gene delivery. Hydrogels can encapsulate macromolecule drugs especially proteins into their polymeric chains. Polymeric network of hydrogels protects drugs from fast dissolution and control release rate from matrices. Hydrogels can be administered via oral, ocular, nasal, vaginal and subcutaneous routes. Hydrogels are also utilized extensively in tissue repairs. Hydrogels have also been developed as artificial cartilages, contact lenses, artificial

corneas, biosensors and surgical aids. Synthetic materials are also applied as alternative to extracellular matrix.

1.5 CHITOSAN BASED HYDROGELS

1.5.1 CHITOSAN

Chitosan is naturally occurring polymer. The effects of degree of deacetylation on properties like solubility and antimicrobial activity have been studied in several articles. Chitosan is widely used in diverse fields such as waste management, medicine, food and agriculture. And it includes properties like biocompatibility, antimicrobial, antibacterial, mucoadhesion, anticholesterolemic, and permeation enhancement effect. And these properties led to increased utility in specific application such as antibacterial/antibiofouling coating, controlled release coating and microcapsules, nano filterates, drug delivery hydrogel, gene delivery and tissue engineering scaffolds. The polysaccharide structure of chitosan is made of glucosamine and N-acetyl glucosamine. . Glucosamine is generated from glucose in body and it can produce glucosaminoglycans (GAGs), which is a part of extracellular matrix and cartilage tissue.

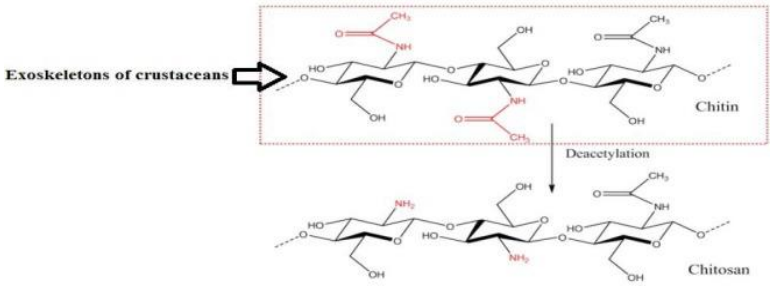


Fig: Chitin is extracted from crab shell from which chitosan is made by N-deacetylation.

The charge density of chitosan depends on degree of deacetylation which represents the amino group density. Indeed, pH of the chitosan solution represents the quantity of ionized amino groups.

Chitosan is a weak base with pk_a 6.5 which can be dissolved in dilute acidic medium. Because of the presence of amine and hydroxyl groups, chitosan molecules can form hydrogen bonds leading to the crystalline structure of the polymer.

Chitosan exists in different molecular weights and degree of acetylation. The average molecular weight of chitosan lies between 50-2000 KD. Hydrophilic polymers such as chitosan may undergo systemic absorption in human body, so the polymers should have proper molecular weight to eliminate by renal filtration. In vitro studies showed that chitosan can be degraded via several enzymes such as β-N-acetylhexosaminidase, chitosanase, chitinase and chitin deacetylase. In human body, chitosan can be biodegraded by lysozyme, acid, gastrointestinal enzymes and colon bacteria.

1.5.2 HYDROGEL PREPARATION VIA CHITOSAN CROSSLINKING

The intermolecular forces between polysaccharide chains of chitosan are Hydrogen bonding, hydrophobic and ionic interactions. These interactions are influenced by molecular weight and ionic strength.

Cross-linking of chitosan polymers is necessary to improve chitosan properties such as stability and durability for the aim of wound healing. Chitosan based hydrogel networks are categorized based on the method of chitosan cross-linking and preparation.

1.5.3 PREPARATION CHITOSAN HYDROGELS VIA CHEMICAL CROSSLINKING

Chemically cross-linked hydrogels are formed by covalent linking of the chitosan macromers, where the bond formation is irreversible. Chemical cross-linked hydrogels are found in four states of formation, a) chitosan cross-linked system, b) hybrid polymer networks (HPN), c) interpenetrating polymer networks (IPN), and d) semi interpenetrating polymer networks (SIPN).

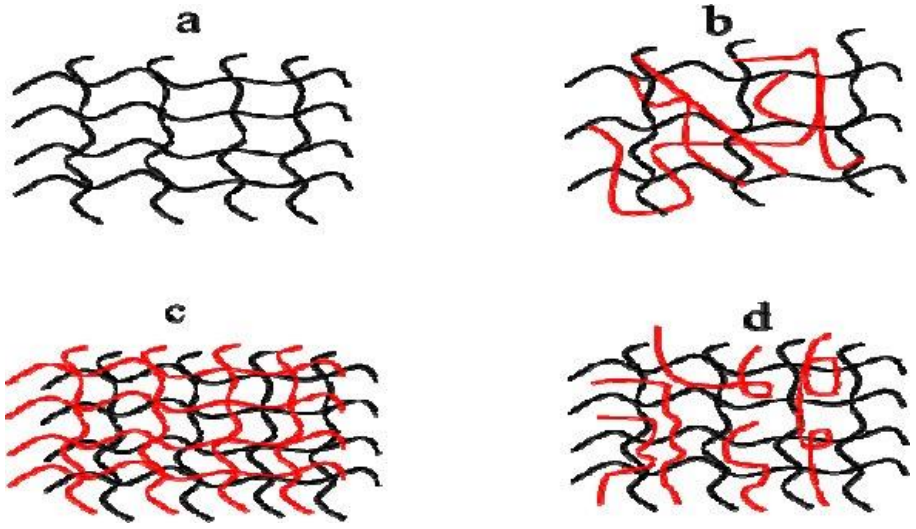


Fig: schematic representation of a) chitosan cross-linked system, b) hybrid polymer networks (HPN), c) interpenetrating polymer networks (IPN), and d) semi interpenetrating polymer networks (SIPN).

Structure of chitosan-based hydrogel prepared by covalent cross-linking. Chitosan-based hydrogels include a; only chitosan chain cross links, b; chitosan is cross linked via a different polymer, c; chitosan and another polymer are entangled and each polymer type is cross-linked, d; another polymer entangles with chitosan, where chitosan macromers are crosslinking.

The simplest type of chemical hydrogel formation occurs when chitosan undergoes cross-linking reaction with another polymeric chain of its own. Second chain can be similar to or different from first structural unit in derivation.

Amines and hydroxyl groups situated on chitosan chains are responsible for chemical cross-linking. Chemical cross-linking can occur via cross-linkers or photo polymerization reaction.

1.5.4 PREPARATION OF CHITOSAN HYDROGELS VIA PHYSICAL CROSSLINKING

Physical cross-linking to form chitosan-based hydrogel networks are another class of crosslinking. Physical interactions can be ionic interactions, as in ionically cross-linked chitosan hydrogels and polyelectrolyte complexes, or can be secondary interactions such as networks named grafted chitosan hydrogels and entangled chitosan hydrogels. Since chitosan is a cationic polyelectrolyte polymer with ionisable amine groups, anions are often employed as ionic cross-linkers to engineer ionically cross-linked chitosan hydrogels.

1.5.5 CHEMICAL VERSUS PHYSICAL CROSSLINKING

Type of cross-linking determines the stability of hydrogels. Covalently cross-linked hydrogels with covalent cross-linkers have permanent feature that show resistance to environmental variables. However these systems need extra process of purification to remove toxic unreacted cross-linkers.

Physically cross-linked hydrogels are more biocompatible due to the lack of chemical cross-linkers and well tolerated compared to covalently systems.

However they may have not high mechanical stability and they may react to environmental changes such as pH, temperature or ionic strength.

1.5.6 CHITOSAN BASED HYDROGEL APPLICATIONS

Chitosan is known to be biocompatible and biodegradable and its degradation products are non-toxic and non-immunogenic. Chitosan is bio adhesive and bacteriostatic, acts as chelating agent, hemostatic agent and antioxidant. This polymer can control bleeding via incorporating a procoagulant that helps accelerated clotting. Chitosan has found attention in many different fields including pharmaceutical, medical, cosmetics, agricultural and food industries. The pharmaceutical applications of chitosan include drug and gene delivery, wound dressing, tissue repair, and tissue engineering.

1.5.6.1 DRUG DELIVERY

Hydrogels based on chitosan and its chemical modified forms are investigated in several drug delivery applications. Chitosan has cationic nature due to the presence of amine group and mucosal glycoproteins are

negatively charged. Therefore, it can adhere to negatively charged biological surfaces as a bio adhesive material. The use of bio adhesive polymers like chitosan prolongs the residence time of drug-loaded system and provides localized drug delivery. Chitosan also mediates paracellular transportation of drugs that greatly influences efficiency of drug delivery systems. As chitosan is a biocompatible and biodegradable with a structure which could be modified easily, it has been used as drug carrier for different routes of administration.

1.5.6.2 WOUND HEALING

Chitosan in topical form is used for wound healing. The probable mechanism of healing is infiltration of inflammatory cells such as polymorphonuclear leukocytes, secretion of inflammatory mediators like tumours necrosis factor-α, migration of macrophages and increase in the amount of collagen. The binding of GlcNac (N-acetyl-D-glucoseamine), a part of chitosan, to specific receptors in body increases macrophage activation that results in further events such as release of biological mediators. Additionally, chitosan activates the complement system and stimulates fibroblasts to release IL and other cytokines.

The major use of chitosan hydrogels for wound healing is using these systems as wound dressing and hemostatic agent to promote the process of wound healing. One of the commercially available chitosan based hemostatic products is HemCon bandage. HemCon can stop severe bleeding by attaching to negatively charged cells of tissue as well as attracting negatively charged red blood cells and forming a tight seal over the wound.

1.5.6.3 TISSUE ENGINEERING

Chitosan hydrogels were used as scaffolds for tissue engineering in the past two decades. The foundation of these systems relies on two components, cells and polymeric chains of hydrogel. Biodegradability is amongst advantages of chitosan as a scaffold. Chitosan can be degraded with human enzymes like lysozyme. Additionally, chitosan can be modified via N-acetylation to optimize biodegradability and biocompatibility properties needed in tissue engineering applications. Chitosan with high deacetylation degree near to 100 is reported to have higher rate of degradation, cell biocompatibility and higher opportunity for cell adhesion. The biodegradation rate of scaffold should conform to the time that malfunction tissue requires to be repaired.

For scaffolds used in tissue engineering, porosity of chitosan-based hydrogels presents a huge impact on properties such as swelling, cell adhesion and cell proliferation rate that are of importance in tissue growth. There are methods of forming porous hydrogels for tissue regeneration including i) freeze drying, ii) gas foaming and iii) salt leaching. The method of high pressure CO_2 employs CO_2 gas as a foaming agent and reduces the need of organic solvents. Channels formed in the hydrogel allow host cells migration and proliferation into the injured tissue and finally replacing the malfunction organs.

Chitosan scaffolds can be used for regeneration of various tissues such as bone, cartilage, skin and nerves. The treatment of central nervous system disorders is challengeable because neural cells have lower ability for regeneration. Nerve tissue engineering requires neural stem cells such as embryonic, foetal or adult stem cells.

Chapter 2

Materials and Methods

2.1 Introduction

This chapter comprehends on the materials and methods used for the synthesis of hydrogel. The properties of the polymer 'chitosan' used, the method of preparation of the hydrogel is also discussed. The various studies done on the hydrogel is also summarised.

2.2 Preparation of hydrogel

2.2.1 Materials

Reagents for the preparation of hydrogel such as chitosan (DA above 80%), was provided as gift sample from India Sea Foods, Cochin, glutaraldehyde was procured from Merck, India, the solvent acetic acid used for the study was analytical grade.

2.2.2 Method

2.2.2.1 Preparation of uncross linked chitosan hydrogel

1 g of chitosan was dissolved in 45 ml of 1% aqueous acetic acid at room temperature with continuous stirring to obtain a pale yellow viscous chitosan solution. The viscous solution was cast into Petri dish and dried to obtain hydrogels. The semi dried hydrogels were further dried in an oven at 45°C for 12 hours to completely remove the residual solvent.

2.2.2.2 Preparation of cross linked chitosan hydrogel

1g of chitosan was dissolved in 45 ml of 1% aqueous acetic acid at room temperature with continuous stirring to obtain pale yellow viscous chitosan solution. 0.1% aqueous glutaraldehye solution was added to amount of 5 ml was added to the samples of clear pale-yellow chitosan solutions to obtain solutions with crosslink density. The solutions were stirred for 30 minutes at room temperature as they become increasingly viscous and with denser colour. The solution were then cast into Petri dish and dried to obtain cross linked chitosan hydrogel. The semi dried hydrogels were further dried in an oven at 45°C for 12 hours to completely remove residual solvent.

2.3. Characterisation of hydrogels

2.3.1. Fourier Transform Infrared (FTIR) spectral analysis:

Infrared transmission spectra of the hydrogel are carried using Perkin-Elmer FTIR spectrophotometer (model 200) in KBr disc from 4000 to 400 cm^{-}. This FTIR study is carried out to the functional groups in the hydrogels.

2.3.2. **Morphology:**

Morphology of the hydrogel is carried out by scanning electron microscope (**SEM**).

Chapter 3

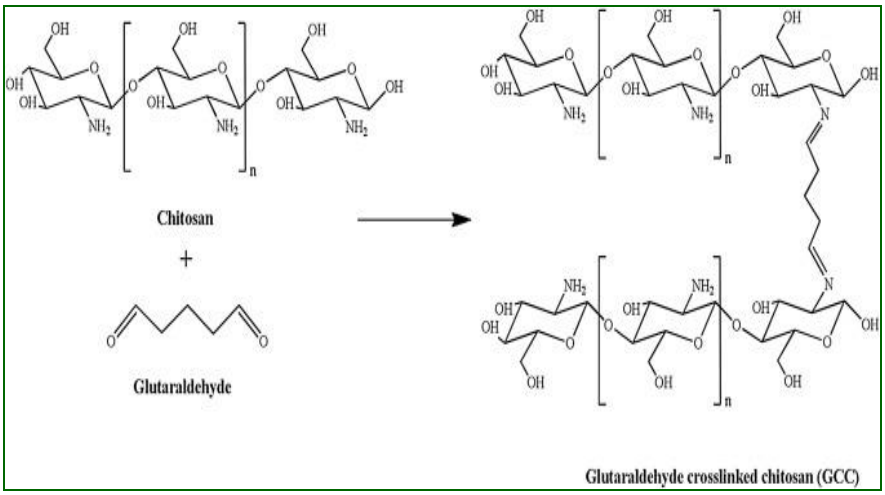
Results and discussion

This chapter comprehends on the results obtained from the present study. Hydrogels based on chitosan was prepared by chemically crosslinking with gluteraldehyde. The hydrogels were further characterized for its morphology by scanning electron microscope and chemically by Fourier transform infrared spectroscopy.

Due to the polyfunctionality of chitosan, several types of smodification reactions can be carried out on it to obtain different materials with different characteristics. The advantages of modifying chitosan are that modification improves the properties or gives it new properties, the sbackbone of chitosan is not affected and chitosan retains its properties after modification.

3.1 Preparation of Hydrogels

The natural biopolymer chitosan was chemically cross linked with the crosslinking agent gluteraldehyde (1%) to form hydrogels. The cross linker glutaraldehyde has been extensively used for chemical cross-linking of chitosan. The cross-linking of chitosan is necessary to improve its properties such as stability and durability especially if they are intended to use for drug delivery applications. The free amino group in chitosan reacts with aldehyde group in gluteraldehyde to form cross linked hydrogels. The mechanism of the hydrogel formation is represented in scheme 1.



3.2 Characterisation of Hydrogels

3.2.1. FTIR analysis of hydrogels

FTIR spectra of chitosan and cross linked chitosan are represented in Fig 2a and 2b respectively. The main peaks for chitosan can be assigned as follows: 3452 cm^{-1} (N–H and O–H stretching vibration), 2921cm^{-1} and 2851 cm^{-1}(CH_3symmetric stretch), 1642 cm^{-1} (C=O stretching vibration), 1452 $cm^{-1}$and 1418 cm^{-1} (C–N stretching vibration), 1384cm^{-1} (CH_3 bending vibration), 1260cm^{-1} (C–O–C bending vibration), and 1046 cm^{-1} (C–OH stretching vibration).However, some major changes have been observed in the spectrum of cross linked chitosan by comparing the spectral differences in the 4000–500 cm^{-1} region of FTIR spectra between chitosan and cross linked chitosan. The FTIR spectrum of cross linked chitosan revealed that the N–H and O–H stretching vibration at 3452 cm^{-1} shifts to 3442 cm^{-1}, the CH_3 symmetric stretch at 2921 cm^{-1} and 2851 cm^{-1} shifts to 2925 cm^{-1} and 2854 cm^{-1} the C=O stretching

vibration at 1642 cm^{-1}shifts to 1663 cm^{-1}, the C–N stretching vibration at 1418 cm^{-1} shifts to1407 cm^{-1}.

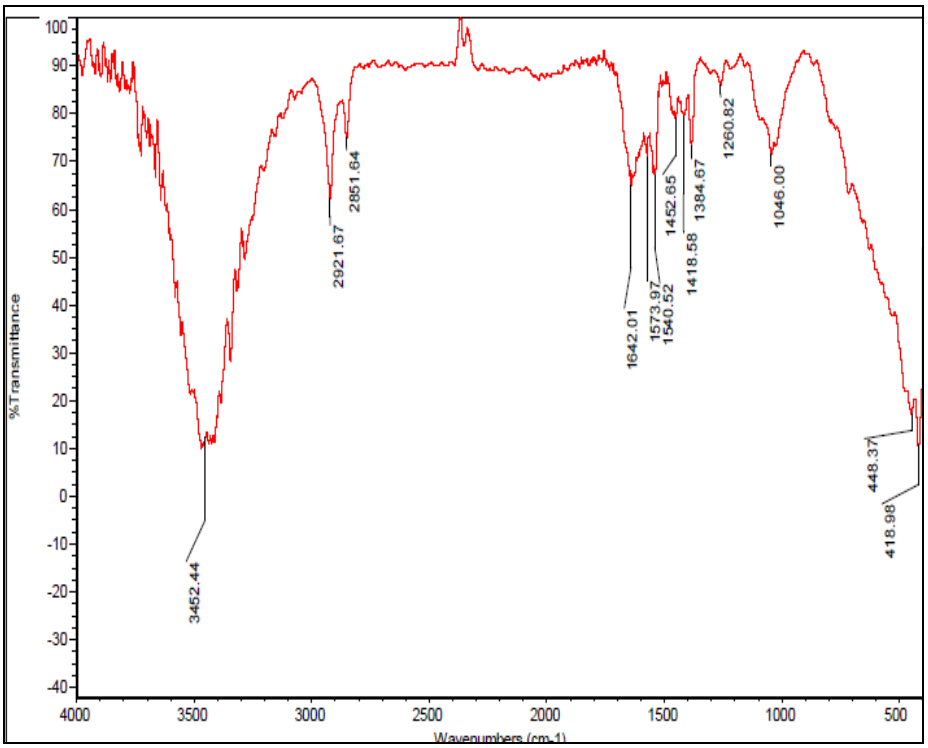


Fig 2a: FTIR spectra of cross linked chitosan

Fig 2b: FTIR spectra of cross linked chitosan

3.2.2. SEM analysis of hydrogels

One of the most important properties that must be considered is hydrogel microstructure morphologies. The surface morphology of the hydrogels was investigated by scanning electron microscopy. Fig 3a and 3b shows the SEM images of chitosan and cross linked chitosan at different magnification (1500x and 6000x) magnifications. Both chitosan and crosslinked hydrogel exhibited fractured surface with numerous pores. In general, this results clearly indicated that there is not much difference in

surface microscopic morphology between chitosan and cross linked chitosan.

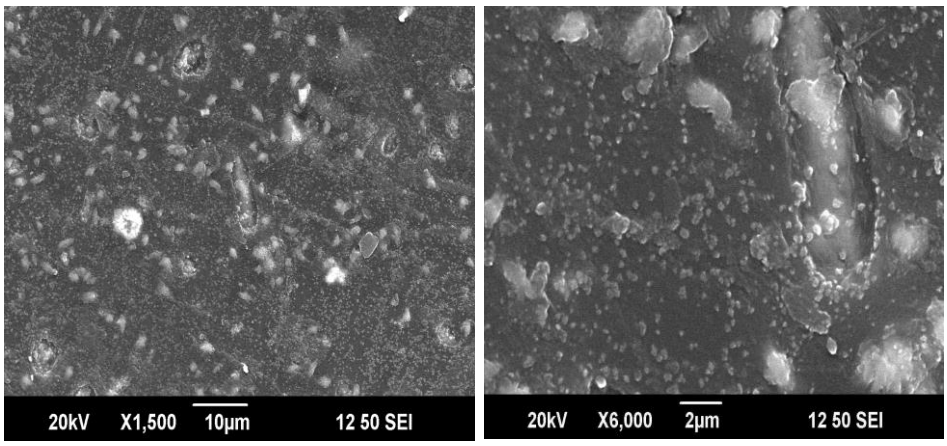


Fig3a: SEM images of chitosan at different magnifications (1500x and 6000x)

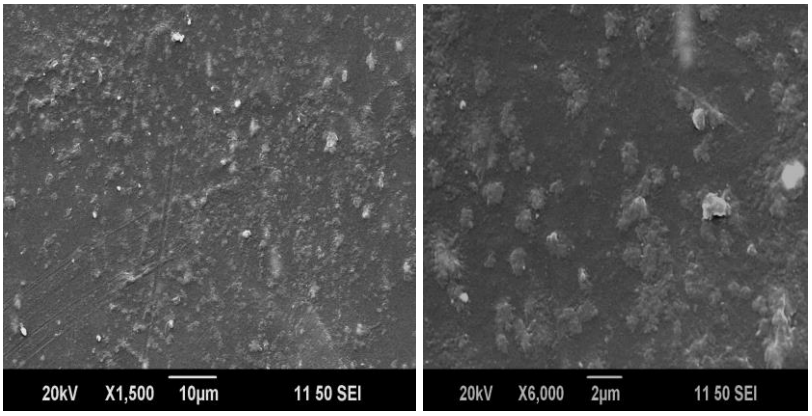
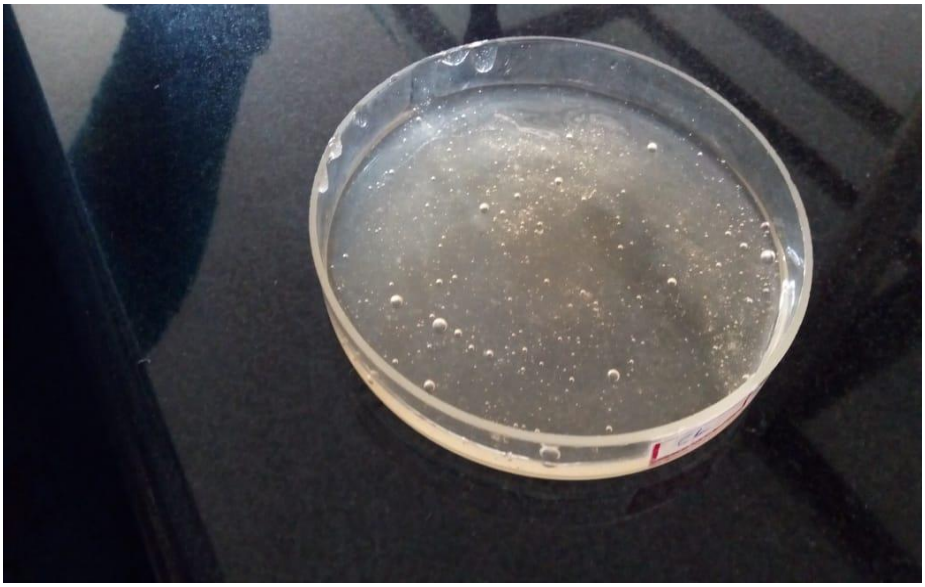
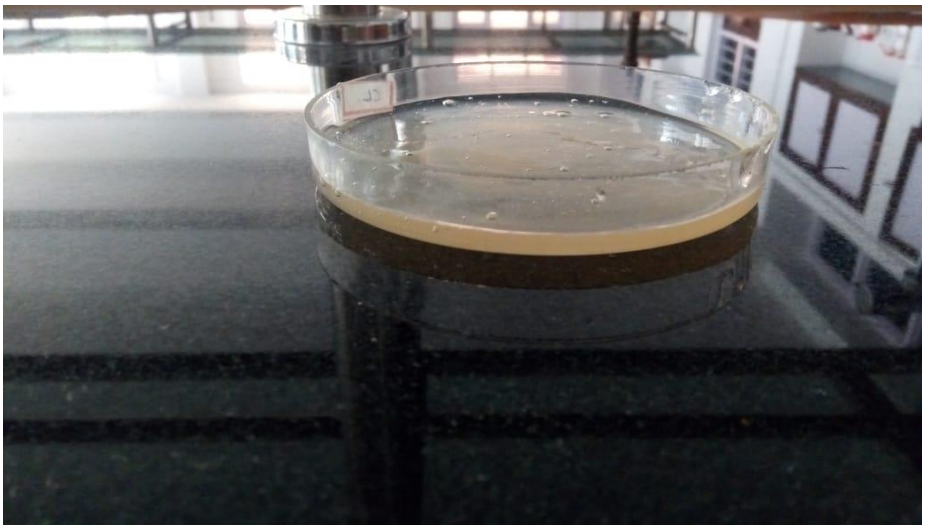


Fig 3b: SEM images of cross linked chitosan hydrogel at different magnifications (1500x and 6000x)

3.2.3 Visible observations





Chapter 4

Conclusions

Hydrogels have widespread applications in many areas such as industrial, medical and analytical field of chemistry. Chitosan was chosen for the present study owing to its low cost, non toxicity, inherent biocompatibility, biodegradability and antimicrobial property. Hydrogel based on chitosan was prepared by chemically crosslinking with gluteraldehyde. The prepared hydrogels were evaluated for its morphology and chemical structure. The results denoted the formation of hydrogels.

The present study shows that dual-cross linked polysaccharide hydrogels are in wound care management. It is synthesized by dissolving 1g of chitosan in 45 ml of 1% aqueous acetic acid and for the synthesis of cross liked hydrogel 5mL 0.1% aqueous glutaraldehyde solution was added as cross linking agent. Then it is stirred for 30 minutes at room temperature and cast into Petri dish and further dried at 45°C for 12 hours.

The characterisations were done using FTIR and SEM analysis. The FTIR spectrum of cross linked chitosan revealed that the N–H and O–H stretching vibration at 3452 cm^{-1} shifts to 3442 cm^{-1} due to hypsochromic shift the CH_3 symmetric stretch at 2921 cm^{-1} and 2851 cm^{-1} shifts to 2925 cm^{-1} and 2854 cm^{-1} the C=O stretching vibration at 1642 cm^{-1}shifts to 1663 cm^{-1}, the C–N stretching vibration at 1418 cm^{-1} shifts to1407 cm^{-1}.One of the most important properties that must be considered is

hydrogel microstructure morphologies. The surface morphology of the hydrogels was investigated by SEM studies. The wound healing application of hydrogel is considered as the future studies.

FUTURE SCOPE

Chitosan hydrogels have enormous applications in biomedical field especially in the area of wound healing, drug delivery etc.. To make these hydrogels suitable for such applications, specific tests should be done to evaluate its performance. These includes

- Swelling study
- Mechanical property evaluation
- Antimicrobial properties
- Biocompatibility studies

References

1. Mirzaei B E, Ramazani SA A, Shafiee M, Danaei M. Studies on glutaraldehyde crosslinked chitosan hydrogel properties for drug delivery systems. International Journal of Polymeric Materials and Polymeric Biomaterials. 2013 May 20;62(11):605-11.

2. Kildeeva NR, Perminov PA, Vladimirov LV, Novikov VV, Mikhailov SN. About mechanism of chitosan cross-linking with glutaraldehyde. Russian journal of bioorganic chemistry. 2009 May 1;35(3):360-9.

3. Argüelles-Monal W, Goycoolea FM, Peniche C, Higuera-Ciapara I. Rheological study of the chitosan/glutaraldehyde chemical gel system. Polymer Gels and Networks. 1998 Dec 1;6(6):429-40.

4. Rohindra DR, Nand AV, Khurma JR. Swelling properties of chitosan hydrogels. The South Pacific Journal of Natural and Applied Sciences. 2004;22(1):32-5.

5. Dai T, Tanaka M, Huang YY, Hamblin MR. Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. Expert review of anti-infective therapy. 2011 Jul 1;9(7):857-79

6. Azuma K, Izumi R, Osaki T, Ifuku S, Morimoto M, Saimoto H, Minami S, Okamoto Y. Chitin, chitosan, and its derivatives for wound healing: old and new materials. Journal of functional biomaterials. 2015 Mar 13;6(1):104-42.

7. Kumar A. A Comparative Study on the Efficacy of Chitosan Gel Formulation and Conventional Silver Sulfadiazine Treatment in Healing Burn Wound Injury at Molecular Level. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm. 2017 Oct 11;11(03).