PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF CHITOSAN-SILVER NANOPARTICLE BY GREEN APPROACH

A Major Project report submitted towards the partial fulfilment of the requirement for the award of the degree of

MASTER OF TECHNOLOGY

IN

POLYMER TECHNOLOGY

Submitted by

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Certificate

It is to certify that dissertation entitled **"Preparation, characterization and antibacterial activity of chitosan silver nanoparticle by green approach"** submitted by Ms. Reena Solanki to the Delhi Technological University, for the award of the degree of "Master of Technology" in Polymer Technology is a record of bonafide work carried out by her. Ms. Reena has worked under my guidance and supervision and fulfilled the requirements for the submission of this project.

To the best of our knowledge and belief the content therein is her original work and has not been submitted to any other university or institute for the award of any degree or diploma.

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Prof. R.C. Sharma
(Head of the Department)

Declaration

I, Reena Solanki hereby declare that the thesis entitled "**Preparation, characterization and antibacterial activity of chitosan silver nanoparticle by green approach**" is an authentic record of research work done by me under the supervision of Dr. D. Kumar, professor Delhi Technological University, Delhi. This work has not been previously submitted for the award of any degree or diploma of this or any other University/Institute.

Date:

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Acknowledgement

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(Reena Solanki)

ABSTRACT

The concept of treating an infected wound has been addressed for centuries, long before the knowledge of microbes was known to human. A wide range of attributes such as spider web, seaweed, honey, leaches etc. has been used in the past decades. Also, nobel metals have been used to fight various pathogens of wounds. Historically, silver (Ag) has been used in the field for long but it gained ground as an antiseptic in 1960's. A wide range of silver wound dressings has been introduced since then.

A Silver based dressing functions through the release of silver ions (Ag^+) from elemental silver or silver compounds incorporated into the dressing. Silver ions (Ag^+) then act as an antimicrobial agent by binding to the walls and DNA of the cells, thus interfering with cell division and replication. The presence of moisture is a prerequisite for Ag^+ to be released. For this reason most test methods in the field involve completely wetting of products prior to measuring antimicrobial effects.

Chitosan and silver nanoparticles (AgNPs) have shown wound healing properties individually and combination of both of these two may show improvement in wound healing activity. Thus, the composite films prepared in this project were evaluated for various *in vitro* evaluation tests. The AgNPs films have shown a significant difference in water absorption capacity and antibacterial activity as compared to the blank films.

The aim of this project work is to study and investigate properties and characteristics of chitosan-silver nanocomposite film. The chitosan-silver nanocomposite films were prepared by mixing silver nitrate ($AgNO_3$) with distilled water and acetic acid to prepare a 100 ml solution. Different concentrations of $AgNO_3$, viz., 0%, 2%, 3% were prepared in 100ml of aqueous chitosan solution. The different mixtures were then poured into separate petri dishes and kept aside for cooling. A thin layer of Chitosan-silver nanocompositefilm was formed and extracted from the petri dish. These composite films were then characterized by Scanning electron microscopy (SEM), X-ray diffraction (XRD), Universal testing machine (UTM), and Fourier transform infrared (FTIR) spectroscopy techniques. Further, the water absorption and antimicrobial properties of the composite films were subsequently studied and reported in the present work.

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Besides many tasks a physician performs for the wellbeing of a patient, wound healing being one of the physician's most important task. Historically, attributes used for wound healing purposes include spider web, seaweed, honey and leeches. For today's senerio the skin and wound infections cases are increasing worldwide. Recent reports suggest that approximately 1% of all wound patients die from microbial infections. Silver has been used as an antimicrobial agent for centuries and has been proven effective against a broad range of pathogens. The antimicrobial effect is exerted by silver in the form of Ag^+ which binds to the wall and DNA of the cells, thus interfering with the cell division and replication. Over the past decade, silver wound dressings have gained popularity and today there is a large range of silver dressings that exert microbial action.

Chitosan, which is obtained from a natural polymer, i.e., chitin has antibacterial feature. As a polycationic polymer, chitosan is also an environmentally friendly material because of its biodegradability. Features like nontoxicity and antibacterial feature of chitosan makes it usable in many areas related to human health. Due to the functional groups in chitosan structure as NH_2 and OH, chitosan is used as an excellent chelating agent [1].

Silver (Ag^+) ion has been used for a long time as anantibacterial agent due to its strong inhibiting effect on bacteria. Recently, nanoparticle Ag has taken considerable attention to providing maximum bactericidal effect with appropriate amount of Ag.

Chitosan is used as metal nanoparticle-chitosan material in biomedical applications because of its advantages mentioned above, i.e., biodegradability, antibacterial properties and excellent chelating agent. And as Both of Ag and chitosan are antibacterial agents so the chitosan-Ag nanocomposite material has a better antibacterial effect.

Comparative studies show that chitosan-Ag nanocomposite is much more efficient than pure chitosan. Chitosan is also used as a stabilizer instead of a chemical reducing agent for protecting the Ag nanoparticles from agglomeration. In these studies, characterization of chitosan-Ag nanoparticles by spectroscopic methods and antibacterial effects of these materials was investigated. The chitosan-silver nanoparticle films were prepared by mixing $AgNO_3$ with distilled water and acetic acid to prepare a 100 ml solution with chitosan at different concentrations such as 0%, 2%, 3% to 100ml $AgNO_3$ and distilled water solution, the mixture

was then poured into a Petri dish and kept aside for cooling. A thin layer of composite material was formed and extracted from the dish.

1.1 Wound

A **wound** is a type of injury on skin which may happens relatively quickly in which the skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). Pathologically, it specifically refers to a sharp injury which damages the dermis of the skin.

Types of wound :

1. Contusion – bruising or haemorrhage caused by a blow from something blunt
2. Abrasion – caused by skin being scraped along a hard surface
3. Incision – clean cut/surgical. Skin, soft tissues and muscle may be severed
4. Laceration – jagged edges, e.g., from teeth, claws, barbed wire
5. Puncture – small entry. May have some internal damage and can become infected
6. Tear/Avulsion – skin and soft tissue partially or completely torn away
7. Cavity – chronic, open wound.

1.2 Wound Dressing

The main aim of any wound dressing is to control bleeding and protect the wound from infection.

General principles for applying dressings are: One should wash hands before putting on clean disposable gloves so that the person who is giving the first aid doesn't get infected and the person who seeks medical attention needs to a germ free environment as well. Use a sterile dressing that extends about 2cm past the edges of the wound so that it covers the wounded area completely also it doesn't get stuck in the injured area and the dressing changing is also easy. Precaution such as not touching the surface that will contact the wound should be taken. If the wound is minor, one should clean the injured area with clean water or with some antiseptic before applying the dressing. We should change or replace atleast once a day any dressing which becomes wet or soiled. Changing dressing daily will help the wound to recover fast. When the dressing is done, wash hands after removing gloves.

Types of Dressing

1. Adhesive dressings- generally used for minor wounds.
2. Non – adherent dressings – these are used for any type of injury but these are especially used for burns and abrasions where the injury is mainly on the surface of the skin and it is required to prevent blood and other fluids from sticking to the dressing.
3. Combine and BPC dressings – these combine a bandage and pad dressings in one unit and are used for large or deep wounds. Because they are made of layers of gauze and cotton wool, their bulk is useful for controlling bleeding and for absorbing discharge.
4. Bandages – any material used to wrap or cover a wound. Bandages are used to:

- keep dressings in place

- control bleeding

-protect a wound from infection

-give support and pain relief

-restrict movement and minimise swelling

-immobilise limbs.

1.3 Wound Healing

Wound healing is a dynamical process which consists of a number of well-balanced and precisely regulated activities which are sequentially arranged for proper healing results. The interaction between cytokines, growth factorsand proteases is crucial in the process to repair and rebuild new tissue.

If the process is disturbed for any reason or the wound healing is interrupted, the consequence is lengthened healing time or even the development of a non-healing wound, i.e., a hard-to-heal wound. Hard-to-heal wounds have been reported to comprise 60-80 % of all human infectious disease. Usually, an infected wound is defined as a wound of 105 bacteria per gram of tissue or greater. [2, 3]

The wound healing process is divided into three overlapping phases.

1) The Inflammation phase: It appears somewhere around 3-4 days after the injury occurred and is identified by symptoms such as redness, temperature increase and pain in the wound. For hard-to-heal wounds this phase is active during most of the healing process. Immediately after the injury occurs, there is an intense contraction of surrounding blood vessels which promotes hemostasis and thus healing.
2) The Proliferative phase: It lasts for approximately 3-4 weeks in acute wounds. Fibroblasts produce collagen, which constitutes 70-80 % of the dermis, along with extracellular matrix. These are essential for the formation of new tissues.
3) The Remodeling phase: It occurs 1-3 weeks and may continue for years. The tissue is now rebuilt by synthesis of collagen.

1.4 Nanotechnology

Nanotechnology is expected to open some new aspects to fighting and preventing diseases using atomic scale tailoring of materials. The ability to uncover the structure and the function of biosystems at the nanoscale stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in-vitro biomedical research and applications.

The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. In all the nanomaterials with antibacterial properties, metallic nanoparticles and metallic oxides are the best. Nanoparticles increase chemical activity due to the crystallographic surface structure with their large surface to volume ratio. The importance of bactericidal nanomaterials study is all because of the increase in new resistant strains of bacteria against most potent antibiotics.

Here in this project work silver nanoparticles and chitosan nanoparticles were characterised and a composite film was produced using these nanoparticles.

Nanoparticles

A Nanoparticle is a particle with atleast one dimension less than 100 nm. Nanoparticles have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface-to-volume

ratios and an increased percentage of the atoms at the grain boundaries. The ongoing worldwide nanotechnology revolution is now predicted to impact several areas of biomedical research and other science and engineering applications. Nanoparticle-assisted drug delivery, cell imaging and cancer therapy are among important biomedical applications of nanotechnology. Progress in utilizing inorganic nanoparticles for biomedical applications has advanced rapidly as a result of the extensive amount of work done in the field of synthesis and modification of the particles. The advantage of using the inorganic oxides for biomedical applications is that they contain mineral elements essential to humans and exhibit potent activity even when administered in small amounts.

1.5 SILVER NANOPARTICLES

Nanoparticles (NPs) are defined as the particles having one or more dimensions in the order of 100 nm or less. Silver NPs (Ag NPs) have been shown to possess unique physical, chemical and biological properties. The effectiveness of Ag NP-containing dressings has been widely tested in vitro, and much research work has been published recently demonstrating that these dressings have a fast and broad spectrum antibacterial activity. [4]

Silver nanoparticles are nanoparticles of silver of between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a significant percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used are spherical silver nanoparticles but diamond, octagonal and thin sheets are also popular. Their high surface area permits the coordination of a vast number of ligands. The properties of silver nanoparticles applicable to human treatments are under investigation in the laboratory and animal studies, assessing potential efficacy, toxicity, and costs.

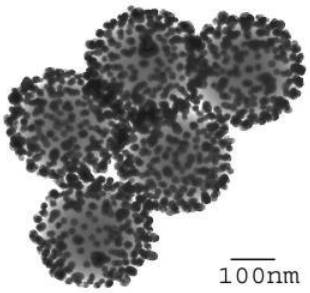


Fig 1 Silver nanoparticles

GREEN SYNTHESIS OF SILVER NANOPARTICLES

Numerous methodologies are invented to synthesize noble metal nanoparticles of a particular shape and size depending upon specific requirements, because the properties of metallic nanoparticles dependents on size and shape are of interest for applications ranging from catalysts and sensing to optics, antibacterial activity and data storage. The surface-to-volume ratio of nanoparticles is inversely proportional to their size. Thus, the biological effectiveness of nanoparticles can increase proportionately with an increase in the specific surface area due to the increase in their surface energy and catalytic reactivity.

Many methods have been used for the synthesis of silver nanoparticles, like chemical and photochemical reduction and electrochemical techniques and radiolysis methods.

However, in most of the methods hazardous chemicals, low material conversions and high energy requirements are used for the preparation of nanoparticles. So, there is a need to develop high-yield, low-cost, non-toxic and environmentally friendly procedures. In such a situation, the biological approach appears to be very appropriate. A natural material like plants, bacteria, fungi, yeast is used for the synthesis of AgNPs.

Tageteserecta (Marigold) is an ornamental plant belonging to the family Asteraceae. Flowers of this plant are used in garlands for many social and religious purposes in most of the countries. It is native to Mexico and widely distributed in South East Asia including Bangladesh and India. The flowers can be bright yellow, brownish-yellow or orange. Different parts of this plant including flower are used in folk medicines. It has been used for skin diseases, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, haemorrhoids, duodenal ulcers, etc. The flowers are primarily employed to cure eye diseases, colds, conjunctivitis, coughs, ulcer, bleeding piles and to purify the blood. Repellent and

biocide activities of essential oils of *T. erecta* against mosquito species have been reported. Antimicrobial activity of gold nanoparticles of flower extract is reported.[5]

In this project, an attempt has been made to synthesize silver nanoparticles using aqueous flower extract of *T. erecta*. And the characterization was done using several spectral analyses.

Fig 2 Marigold flower (*Tageteserecta plant*)

Silver Nanoparticle Applications

Silver nanoparticles are being used in numerous technologies and are also incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive, and antibacterial properties.

Diagnostic Applications: Silver nanoparticles are used in biosensors and numerous assays where the silver nanoparticle materials can be utilized as biological tags for quantitative detection.

- **Antimicrobial Applications:** Silver nanoparticles are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics for their antibacterial properties, air and water purification, films, food preservation.
- **Conductive Applications:** Silver nanoparticles are used in Conductive adhesives, high-intensity LEDs, touch screens, LCDs, conductive inks and integrated into composites to enhance thermal and electrical conductivity.
- **Optical Applications:** Silver nanoparticles are used to efficiently harvest light and for enhanced optical spectroscopies including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS), Optical limiters, medical imaging, solar cells, Surface-enhanced spectroscopy and surface plasmonic devices.
- **Chemical and thermal applications:** Chemical vapor sensors, catalysts silver compounds and ions have been significantly used for both hygienic and healing

purposes, beacuase of their strong bactericidal effects, as well as a broad spectrum antimicrobial activity [4, 5].

1.5.1 Silver in wound healing

Because of the advantage of its bactericidal properties, various silver-containing preparations have been used for the treatment of chronic wounds. In the 17th and 18th centuries, silver nitrate was already used for ulcer treatment, and in 1960, it was introduced specially to manage the burns. After a decrease in the use of silver salts consequent to the introduction of antibiotics in 1940, in more recent years there has been a renewed interest in silver, due to its increased resistance of bacteria to antibiotics and also due to improvements in polymer technology. This has resulted in a significant number of silver-containing dressings being available in the market. At the end of the 1990s, several Ag-containing dressings manufactured by different manufacturers appeared in commerce. Silver-based dressings are now available as a variety of fibres or polymeric scaffolds impregnated or coated withan Ag salt or metallic Ag in nanoparticulate form. They all exhibit fast and broad spectrum antibacterial activity against both Gram-positive and -negative bacteria.

Benefits of silver dressing: It seems that silver showed multilevel antibacterial effects, due to blockage of respiratory enzyme pathways, as well as alteration of microbial DNA and the cell wall. Silver has been demonstrated to be effective also against multidrug-resistant organisms, while maintaining a low systemic toxicity. Clinically, several studies have confirmed silver dressings to be safe but the concerns about their cytotoxicity on fibroblasts and keratinocytes have not been confirmed.

Mechanism: Silver can be present in four oxidation states; AgO, Ag+, Ag^{2+} and Ag^{3+}, the most common being the monovalent form. Metallic silver (AgO) is inherently stable and can produce Ag ions by oxidation in the presence of moisture. First, silver oxide is produced which may then release silver ions. The oxidation of elemental silver is a slow reaction, and the rate is determined by the surface area of the exposed metal. The antimicrobial ability of silver is exerted in the form of Ag^{+}. The exact mechanism is not entirely defined but silver ions are thought to bind to the bacterial cell membrane which results in the disruption of the membrane and finally the death of the bacteria. In addition to this, Ag^{+} is believed to bind and interfere with enzyme activity as well as bind to bacterial cell DNA and thus interfere with cell division and replication.

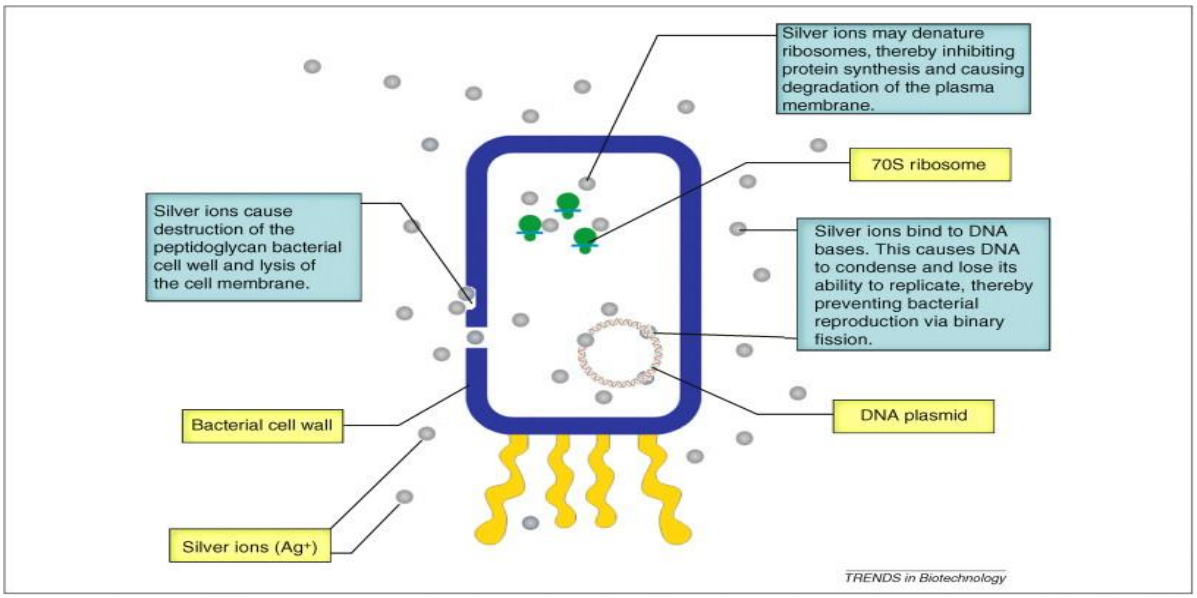


Fig 3 Functioning of silver ions

The silver ion is highly reactive and reacts with a variety of anions found in the biological fluids to form relatively insoluble complexes and precipitates. Evidently, Ag^+ does not only bind to the cell membrane and DNA of bacteria but also to proteins and nucleic acids available in wound fluid. In-vitro studies show that the choice of test media can have a significant impacton antimicrobial effect. Inorganic and organic material in a given test mediabinds and deactivate free Ag^+, thus negating its antimicrobial attributes.

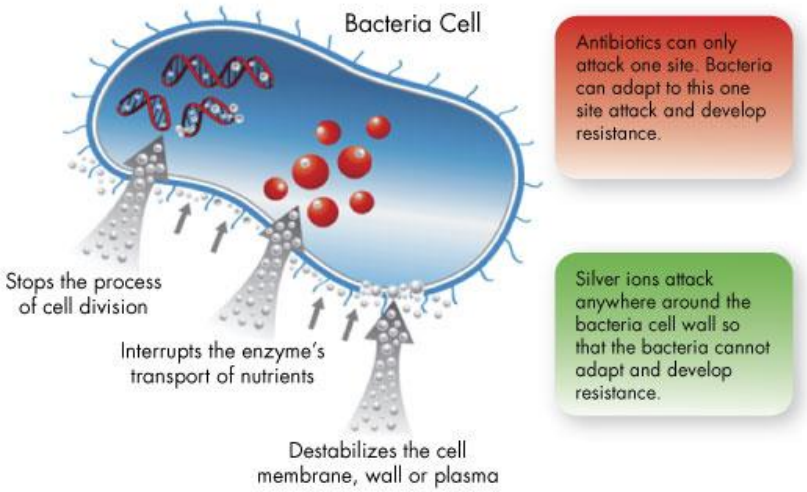


Fig 4 Mechanism of silver ions

1.6 Chitosan

Chitosan is an attractive polymer that has been used extensively in the medical field. It is either partially or fully deacetylated chitin (for example in fungal cell walls and in crustacean as chitin occurs naturally as shells), chitosan is a fully biodegradable and biocompatible natural polymer, and can be used as an adhesive and as an antibacterial and antifungal agent.

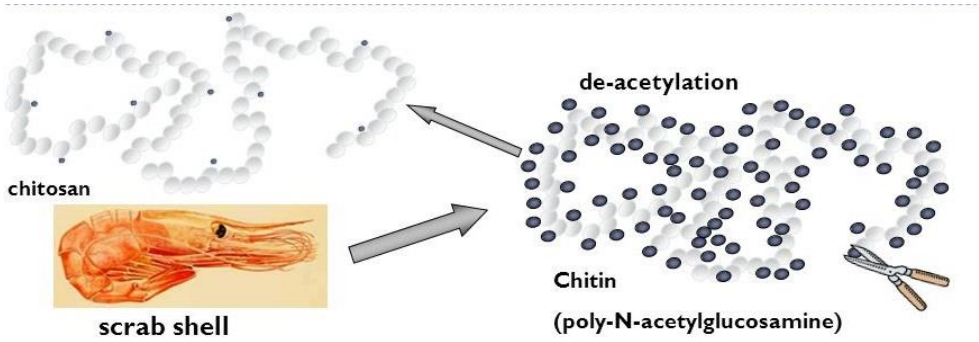


Fig 5 Extraction of chitosan

Chitosan has been investigated significantly as a potential drug carrier, due to its biocompatible properties. Some studies have suggested using chitosan to coat nanoparticles made of other materials to reduce their impact on the body and to increase their bioavailability.

The degree of deacetylation and the molecular weight of chitosan can be modified to obtain different physico-mechanical properties. The elemental composition of the chitosan polymer is carbon (44.11%), hydrogen (6.84%) and nitrogen (7.97 %). The viscosity average molecular weight of chitosan is ~$5.3x10^5$ Daltons.

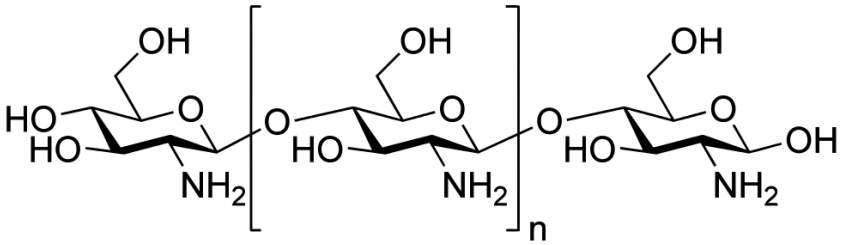


Fig 6 Chemical structure of chitosan

Chitosan, a polycation biopolymer, is a non-toxic, biocompatible, and biodegradable polysaccharide derived from naturally occurring chitin. Chitosan is commercially produced by deacetylation of chitin by using sodium hydroxide (NaOH). The degree of deacetylation of chitosan will be in the range of 60-100%. Positively charged chitosan is soluble in the acidic aqueous solution below pH 6, while insoluble in neutral conditions and most organic solvents.

Chitosan has many useful and advantageous properties such as hemostatic activity, wound healing ability, reducing scars, antimicrobial activity, as well as inhibition of a broad range of bacteria.

Chitosan was found to enhance the functions of polymorphonuclear leukocytes, macrophages and fibroblasts. As a result, chitosan promotes granulation and organization, and therefore it is beneficial for open wounds; certain polymorphonuclear functions are enhanced, such as phagocytosis and the production of chemical mediators. The peculiarity of chitosan is the ability to promote sufficient granulation tissue formation accompanied by angiogenesis and regular deposition of thin collagen fibres.

Manufacture and properties

Chitosan is commercially produced by deacetylation of chitin. Chitin is the structural element in the exoskeleton of Crustaceans (such as crabs and shrimps) and cell walls of fungi. The degree of deacetylation (%DD) can be determined by NMR spectroscopy and the degree of deacetylation (%DD) in the commercial chitosan ranges from 60 to 100%. On an average the molecular weight of the above mentioned commercially produced chitosan is between 3800 to 20,000 Daltons. A standard method for the synthesis of chitosan is deacetylation of chitin using NaOH in an excess as a reagent and water as a solvent. The reaction occurs in two stages under first-order kinetic control. Activation energy for the first step is higher than the second step. Its value is an estimated 48.76 kJ/mol at 25-120^{o} C.[6] This reaction pathway, when allowed to go to completion (complete deacetylation) yields up to 98% product.

The amino group in chitosan has a pKa value of ~6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the %DA-value. Due to this the chitosan is both a water soluble and a bioadhesive which readily binds to negatively charged surfaces such as mucosal membranes. Chitosan also enhances the easy transport of drugs like polar drugs across epithelial surfaces, and is biodegradable and biocompatible. It is not approved by FDA for drug delivery though. Purified quantities of chitosans are available for biomedical applications. [4,5]

Applications of Chitosan Nanoparticles

The applications of chitosan nanoparticles are:

- As antibacterial agents, gene delivery vectors and carriers for protein release and drugs.
- Used as a potential adjuvant for vaccines such as influenza, hepatitis Band piglet paratyphoid vaccine
- Used as a novel nasal delivery system for vaccines. These nanoparticles improve antigen uptake by mucosal lymphoid tissues and induce strong immune responses against antigens.
- Chitosan has also been proved to prevent infection in wounds and quicken the wound-healing process by enhancing the growth of skin cells.
- Chitosan nanoparticles can be used for preservative purposes while packaging foods and in dentistry to eliminate caries.
- It can also be used as an additive in antimicrobial textiles for producing clothes for healthcare and other professionals.
- Chitosan nanoparticles show effective antimicrobial activity against *Saprophyticus* and *E.coli*
- These materials can also be used as a wound-healing material for the prevention of opportunistic infection and for enabling wound healing.
- The nanoparticles have also been proven to show skin regenerative properties when materials were tested on skin cell fibrocblasts and keratinocytes in the laboratory, paving the way to anti-aging skin care products.[7]
- A number of pharmaceutical application of chitosan is also there which makes it more useful and a competitive chemical in the pharmaceutical world. Various examples are shown in figure 7.

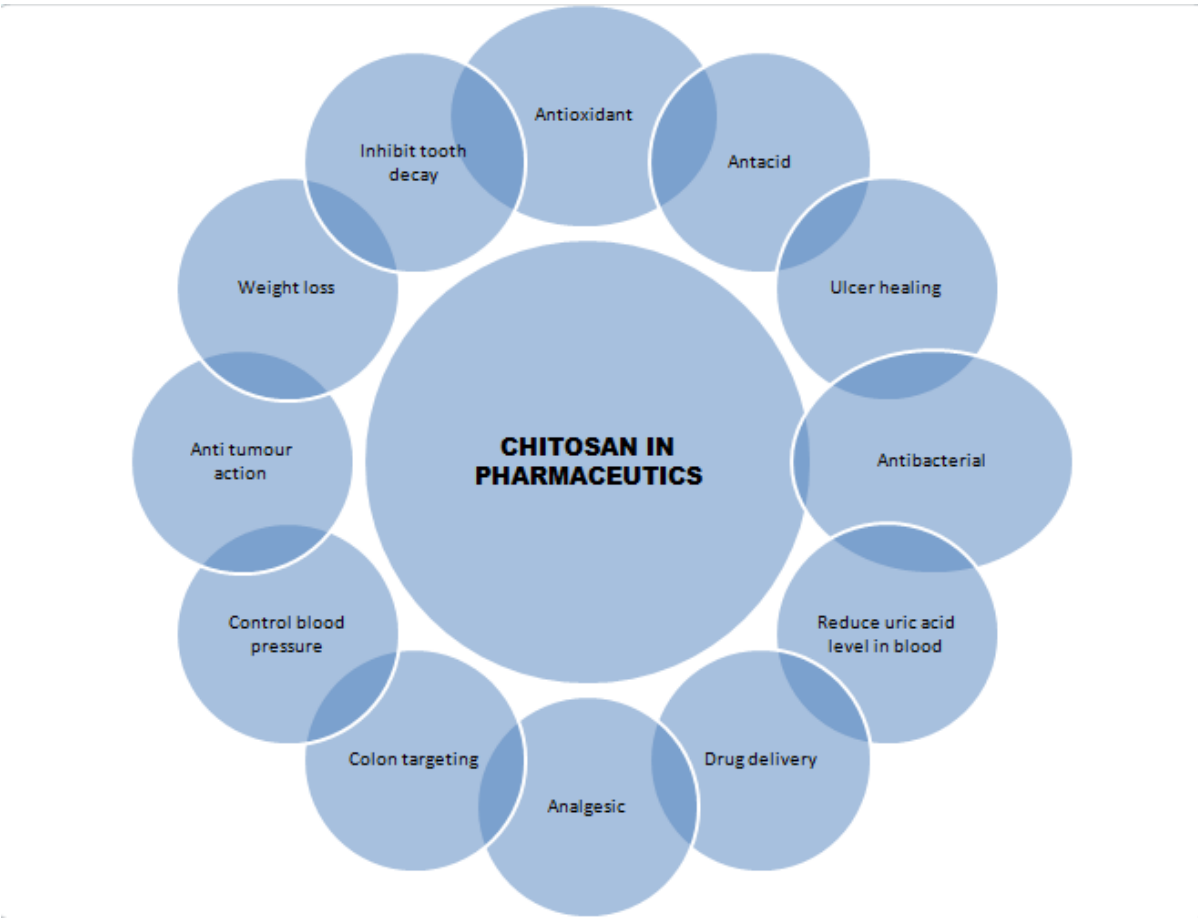


Fig 7 Applications of chitosan in pharmaceutics

Application of chitosan and its derivatives

A number of areas use chitin, chitosan and its derivatives such as it is used in water treatments, agriculture, textile and paper, biotechnology, food/ health supplements, cosmetics and in biomedical. In the table below (Table 1) specific use of these is listed along with their application area.

Table 1: Applications of chitosan and its derivatives

APPLICATION AREA	SPECIFIC USE
Water treatment	- Coagulating/ flocculating agents for polluted waste waters - Removal/ recovery of metal ions from aqueous waste water
Agriculture	- Plant Elicitor - Antimicrobial agents - Plant seed coating - Fertilizer
Textile and paper	- Fibres for textile and woven fabrics

	- Paper and film
Biotechnology	- Chromatography packing - Enzyme immobilizing materials
Food and health supplements	- Natural thickeners - Food additives including pet food - Food processing (e.g., Sugar refining) - Filtration and clarification - Hypocholesterolemicagent (slimming agent)
Cosmetics	- Ingredients for hair and skin care (conditioner)
Biomedical	- Burns and wounds dressings for humans and animals - Biomaterial (e.g., Absorbable sutures) - Anticoagulant or antithrombogenic materials - Hemostatic agents - Drug delivery - Gene delivery

Limitations of chitosan at neutral pH: At neutral pH, chitosan do not show antimicrobial activity; in order to impart that activity, it is necessary to incorporate some antimicrobial agents into it. Antibiotics can be used to perform this function. But the drug resistance of microbes against these antibiotics and specificity of antibiotics towards microbes limits the use of antibiotics. Hence, people started to look at various antimicrobial particles which were prepared based on nanotechnology.

1.6.1 Chitosan in wound healing

Wound dressings are used to protect wounded skin from contamination and infection. A chitin-based wound dressing protects the wound by –

- Increasing dermal regeneration
- Accelerating wound healing
- Preventing bacterial infiltration
- Avoiding water loss
- Chitin surgical threads are adamant, flexible and decomposes after the heals.
- ANTICOAGULATION: anticoagulation is essential for open heart surgery and kidney dialysis. Preventing blood from clotting during surgery
- Sulphated chitin derivatives have good anticoagulant activity.

Benefits of chitosan-based wound dressing

Chitosan-based wound dressing have a numerous benefits few of them are listed below.[8]

- Minimizes the scaring.
- Strengthens new tissue
- Provides proteins for healing
- Absorbs fluids from inflammations
- Blocks nerve endings to reduce pain
- Encourages natural blood clotting
- Forms barrier against infections
- Provides scaffold for cell growth

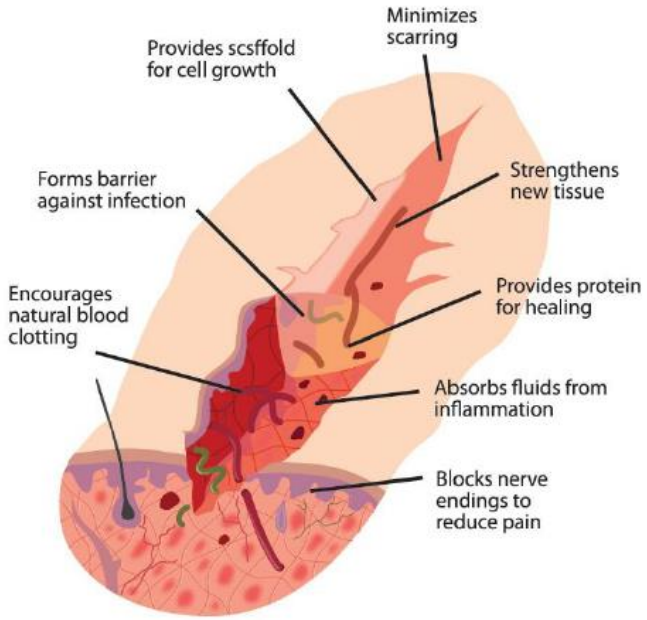


Fig 8 Schematic representation of the benefits of chitosan wound dressing

CHAPTER 2

MATERIALS AND METHOD

2. MATERIALS AND METHOD

2.1 Materials

Chitosan $(C_6H_{11}NO_4)_n$ was obtained from Sisco Research laboratories Pvt. Ltd. Silver nitrate ($AgNO_3$) was obtained from Sigma Aldrich Chemicals Pvt. Ltd., Glacial acetic acid 99.5% extra pure (Ethanoic acid CH_3COOH) was obtained from Loba Chemie Pvt. Ltd. Distilled water was used throughout the study. Fresh flower leaves of marigold flower (Tageteserecta plant) were taken from University (Delhi Technological University) premises.

2.2 Method

2.2.1 Preparation of pure chitosan film

The chitosan film was prepared by dissolving the 1gm of chitosan powder into 50 ml of 2% (w/v) acetic acid aqueous solution. The solution was poured into Petri dishes and kept perfectly in a horizontal manner in an electric oven at 60^o C. When water was completely evaporated, dry films were removed from Petri dishes and repeatedly washed with distilled water and then dried again.

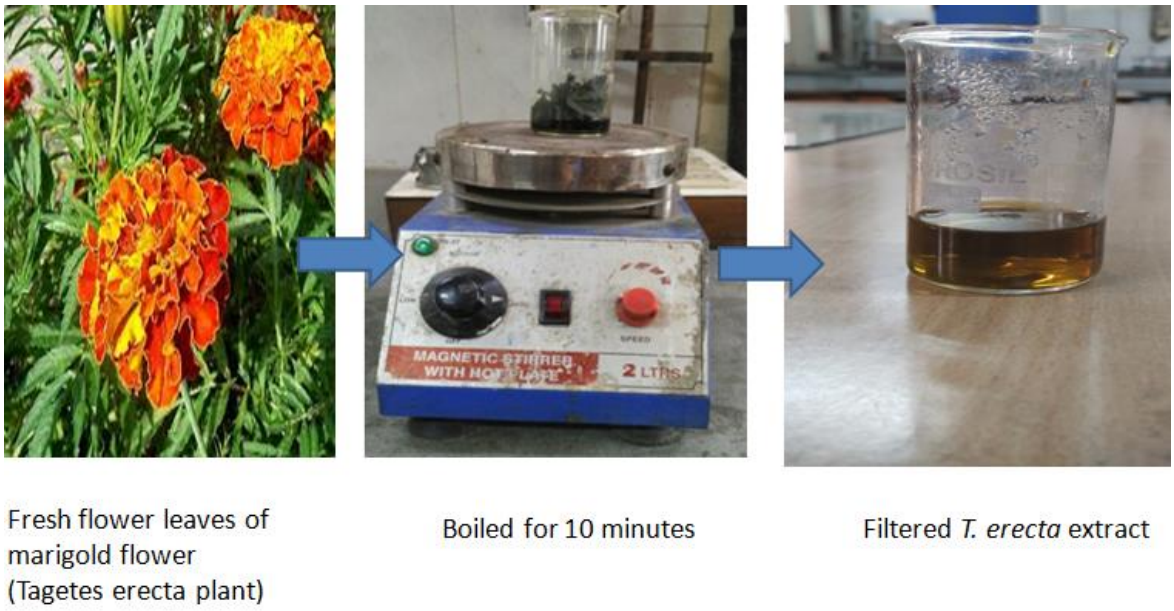


Fig 9 Extraction of *T.erecta* from marigold leaves

2.2.2 Preparation of the extract for synthesis of silver nanoparticles

Fresh flower leaves were thoroughly washed with tap water, followed by double distilled water and cut into small pieces. 5 g of cut flower leaves were boiled for 10 min in 100 ml ultra-pure

water and filtered through Whatmann No. 1 filter paper. The filtered *T.erecta* extract was used for the synthesis of silver nanoparticles.

2.2.3 Preparation of nanosilver loaded chitosan film

Firstly we prepared 10^{-3}µ $AgNO_3$ solution using distilled water. Chitosan powder of 2% (w/v) was dissolved into 10^{-3}µ $AgNO_3$ solution in the presence of 2% (v/v) acetic acid. The resulting solution was kept in sunlight for 1 h. The yellow solution started turning red, then brown and finally brownish black as shown in Fig 10.

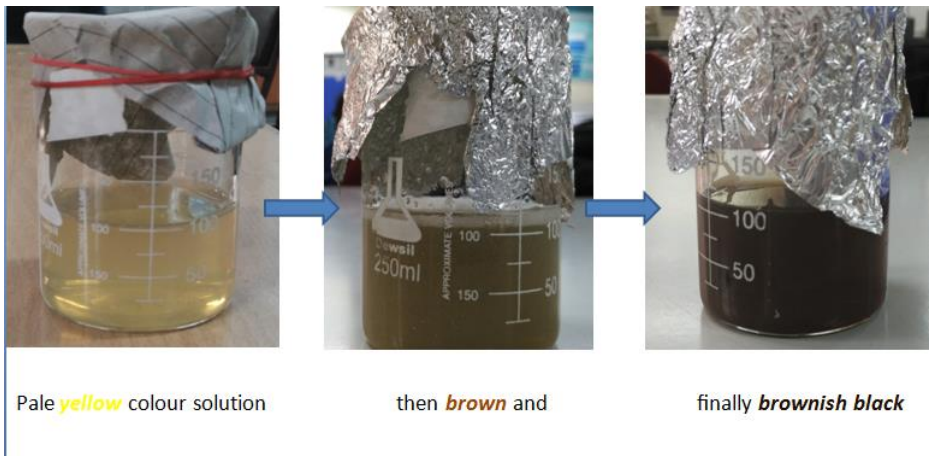


Fig 10 Colour change of the solution

The solution was poured into Petri dish and kept overnight. The silver nanoparticles containing film formed was extracted and then washed with distilled water and then dried in an electric oven at 60^0 C.

Fig 11: Composite film casted in Petri dish

CHAPTER 3

CHARACTERISATION TECHNIQUES

3 CHARACTERISATION TECHNIQUES

3.1 Structural characterization

3.1.1 Fourier transform infrared spectroscopy (FTIR)

The structural conformation of chitosan and chitosan-silver nanocomposite films after dissolution in BMIMAc was determined by Thermo Scientific Nicolet 380 Spectrometer, Japan, in reflection mode at 4 cm^{-1} resolution using 64 scans in the spectral range 2000 to 800 cm^{-1}.

Infrared spectroscopy has been a work horse technique for materials analysis in the laboratory for over seventy years or may be above. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the same infrared spectrum. Therefore, infrared spectroscopy can result in a **identification** (qualitative analysis) of every different kind of material. Also, the size of the peaks in the spectrum is a direct indication of the **amount** of material present. With modern software algorithms, an infrared is an excellent tool for quantitative analysis. Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is allowed to pass through a sample. Some of the incident radiation is absorbed by the sample and some of it is passed through or say transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. And just like any human fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis like it helps to identify unknown materials, determine the quality or consistency of a sample, determine a number of components in a mixture.

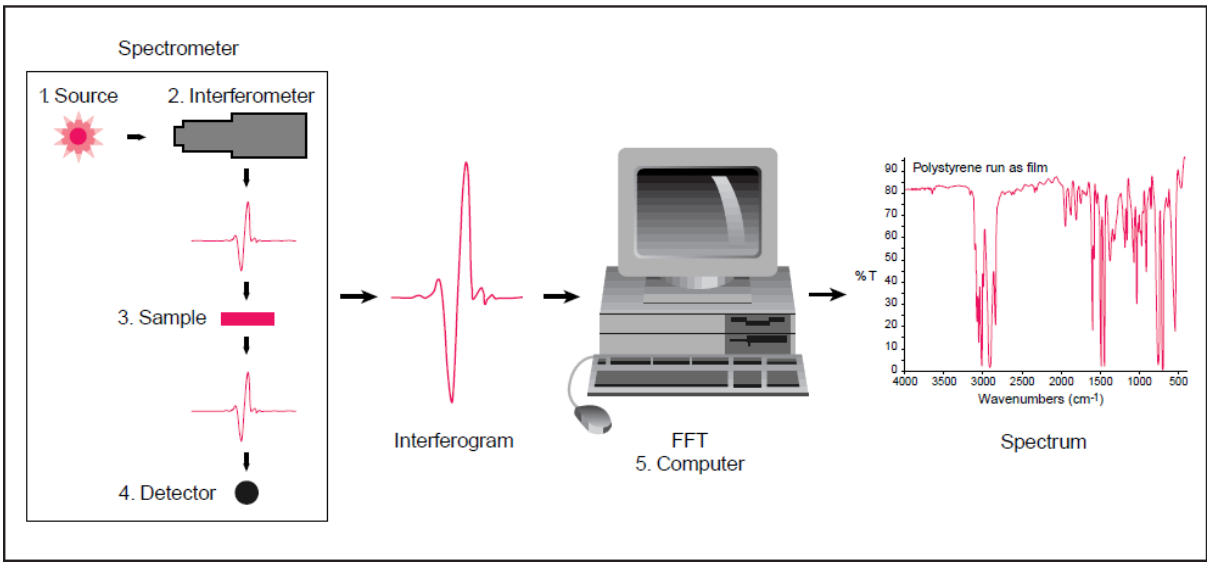


Fig 12 Pictorial view of FTIR

The normal instrumental process is as follows:

1. The Source: Infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample (and, ultimately, to the detector).

2. The Interferometer: The beam enters the interferometer where the "spectral encoding" takesplace. The resulting interferogram signal then exits the interferometer.

3. The Sample: The beam enters the sample compartment where it is transmitted through or reflectedoff of the surface of the sample, depending on the type of analysis being accomplished. This iswhere specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

4. The Detector: The beam finally passes to the detector for final measurement. The detectors usedare specially designed to measure the special interferogram signal.

5. The Computer: The measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

Because there needs to be a relative scale for the absorption intensity, a **background** spectrum must also be measured. This is normally a measurement with no sample in the beam. This can becompared to the measurement with the sample in the beam to determine the "percent transmittance."This technique results in a spectrum which has all of the instrumental characteristics removed.Thus, all spectral features which are present are strictly due to the

sample. A single background measurement can be used for many sample measurements because this spectrum is characteristic of the **instrument** itself. [9]

3.1.2 X-ray Diffraction (XRD)

The atomic planes of a crystal cause an incident beam of X-rays to interfere with one another as they leave the crystal. The phenomenon is called X-ray diffraction.
The crystalline structure of chitosan and chitosan silver nanocomposite films was determined using X-ray diffractometer (Bruker) with CuKα radiation (λ = 1.54 Å). The scanning of samples was carried out in the range of 7 to 30° at the speed of 0.04°/s.

Crystals are regular arrays of atoms, and X-rays can be considered waves of electromagnetic radiation. Atoms scatter X-ray waves, primarily through the atoms' electrons. Just as an ocean wave striking a lighthouse produces secondary circular waves emanating from the lighthouse, so an X-ray striking an electron produces secondary spherical waves emanating from the electron. This phenomenon is known as elastic scattering, and the electron (or lighthouse) is known as the *scatterer*. A regular array of scatterers produces a regular array of spherical waves. Although these waves cancel one another out in most directions through destructive interference, they add constructively in a few specific directions, determined by Bragg's law:

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

Here d is the spacing between diffracting planes, θ the incident angle, n is any integer, and λ is the wavelength of the beam. These specific directions appear as spots on the diffraction pattern called *reflections*. Thus, X-ray diffraction results from an electromagnetic wave (the X-ray) impinging on a regular array of scatterers (the repeating arrangement of atoms within the crystal).

X-rays are used to produce the diffraction pattern because their wavelength λ is typically the same order of magnitude (1–100 Å) as the spacing d between planes in the crystal. In principle, any wave impinging on a regular array of scatterers produces diffraction, as predicted first by Francesco Maria Grimaldi in 1665. To produce significant diffraction, the spacing between the scatterers and the wavelength of the impinging wave should be similar in size.

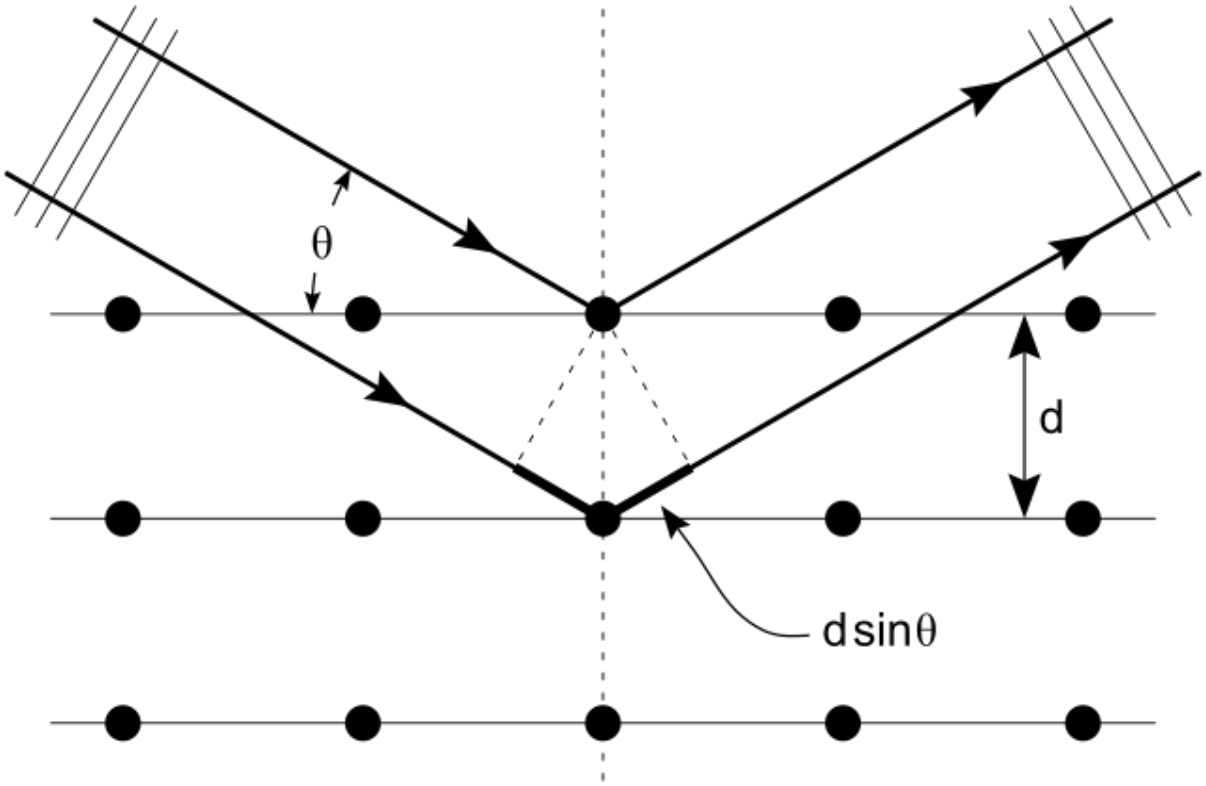


Fig 13 Working principle of XRD

Working principle of XRD: In the figure above (fig 13) it is shown that the incoming beam (coming from upper left) causes each scatterer to re-radiate a small portion of its intensity as a spherical wave. If scatters are arranged symmetrically with a separation *d*, these spherical waves will be in sync (add constructively) only in directions where their path-length difference 2*d* sin θ equals an integer multiple of the wavelength λ. In that case, part of the incoming beam is deflected by an angle 2θ, producing a *reflection* spot in the diffraction pattern [10].

3.2 Morphological characterization

Chitosan and chitosan silver nanocomposite films were sputter coated with gold and analyzed by scanning electron microscope (Hitachi3700 N).

Fig 14 Scanning electron microscope (Hitachi3700 N)

The main components of a typical SEM are electron column, scanning system, detector(s), display, vacuum system and electronics controls (fig. 15). The electron column of the SEM consists of an electron gun and two or more electromagnetic lenses operating in vacuum. The electron gun generates free electrons and accelerates these electrons to energies in the range 1-40 keV in the SEM. The purpose of the electron lenses is to create a small, focused electron probe on the specimen. Most SEMs can generate an electron beam at the specimen surface with spot size less than 10 nm in diameter while still carrying sufficient current to form acceptable image. Typically the electron beam is defined by probe diameter (d) in the range of 1 nm to 1 μm, probe current (ib) – pA to μA; and probe convergence (α) – 10^{-4} to 10^{-2} radians[11].

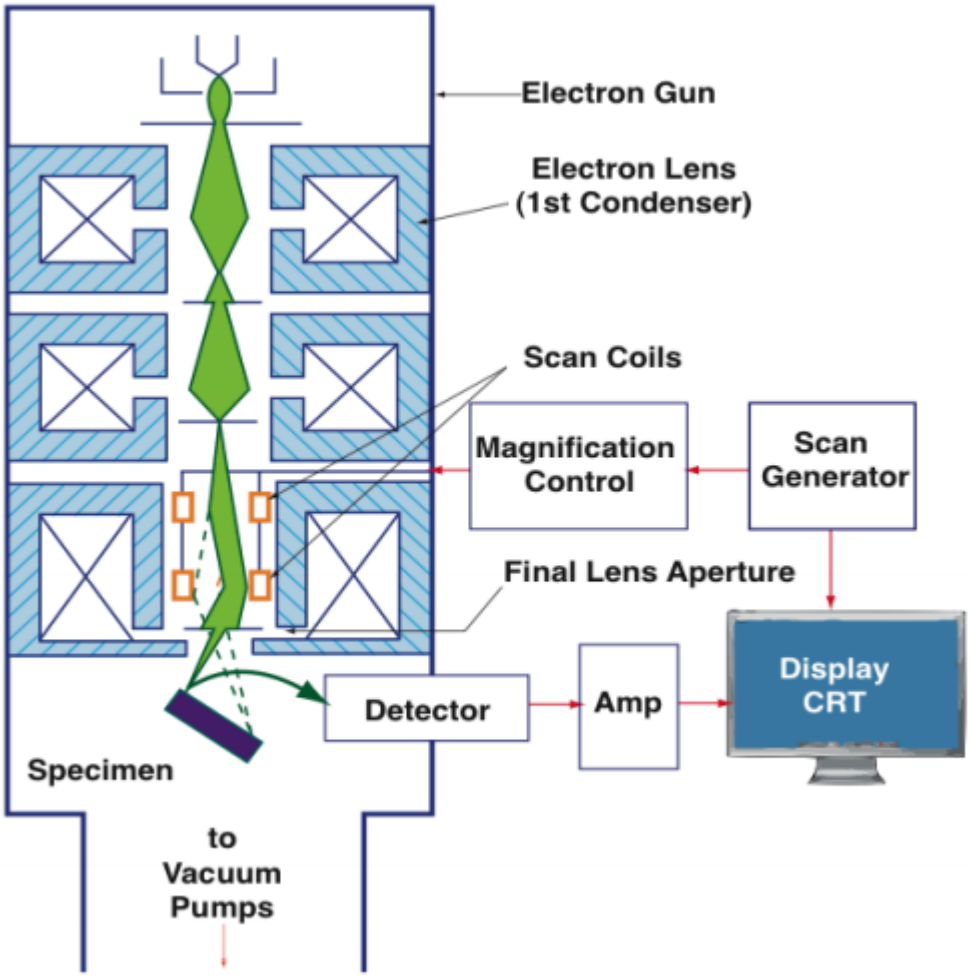


Fig 15 Components of SEM

In order to produce images the electron beam is focused into a fine probe, which is scanned across the surface of the specimen with the help of scanning coils (fig. 15). Each point on the specimen that is struck by the accelerated electrons emits signal in the form of electromagnetic radiation. Selected portions of this radiation, usually secondary (SE) and/or backscattered electrons (BSE), are collected by a detector and the resulting signal is amplified and displayed on a TV screen or computer monitor. The resulting image is generally straight forward to interpret, at least for topographic imaging of objects at low magnifications. The electron beam interacts with the specimen to depth approximately 1 µm. Complex interactions of the beam electrons with the atoms of the specimen produce wide variety of radiation. The need of understanding of the process of image formation for reliable interpretation of images arises in special situations and mostly in the case of high-magnification imaging. In such case

knowledge of electron optics, beam-specimen interactions, detection, and visualization processes is necessary for successful utilization of the power of the SEM.

3.3 Mechanical testing

The mechanical properties of samples were determined by using Universal Testing Machine (Instron-2700) in tension mode as per ASTM D 882-02. The samples were cut into strips with dimensions of 70 × 10 mm and then fixed at the jaw of tensile tester. The strain rate and gauge length were 50 mm/min and 50 mm, respectively. At least five samples were tested and their stress-strain curves were recorded.

Fig 16 Pictorial view of UTM

UTM machine is used to test specimens for tensile strength, compressive strength, and shear strength and to perform bend test along other important laboratory tests. The primary use of the testing machine is to create the stress strain diagram (fig: 16).

Components of UTM

It consists of two main parts, called:

1. **Loading Unit**
2. **Control Unit**

Loading unit

In this unit actual loading of the specimen takes place - consists of three cross heads namely upper head, middle head and lower head. Using appropriate cross heads tensile, compressive, shear, bending load with the help of different attachment can be applied. Loading unit of a UTM consists of:

1. **Upper cross head** - To clamp testing specimen from top
2. **Lower cross head** - To clamp testing specimen from below
3. **Table** - to place the specimen, used for compression test

Control Unit

The load is applied and recorded by this unit. The load is applied with control valve and released by release valve. The load is applied with the help of hydraulic pressure.

USE: The specimen is mounted in the machine between the grips and an extensometer, if required can automatically record the change in gauge length during the test. If an extensometer is not fitted, the machine itself can record the displacement between its cross heads on which the specimen is held. However, this method not only records the change in length of the specimen but also all other extending / elastic components of the testing machine and its drive systems including any slipping of the specimen in the grips. Once the machine is started it begins to apply an increasing load on specimen. Throughout the tests the control system and its associated software record the load and extension or compression of the specimen.

Tensile strength = Break load / Strip Cross- Sectional area

3.4 Water Absorbance

Water absorption of chitosan and chitosan-silver nanocomposite films was determined by the procedure as reported by Wharram et al.[12] For this study the samples werecut in specific size (20 mm × 20 mm) and weight after that the samples were immersed in deionized water and incubate at 37 °C. After, definite time intervals, the samples were taken out and gently wiped with tissue paper until the residual water was observed on the sample surface. Water swollen sample was weight at definite time interval till saturation has to be maintained. The percent water absorption was determined by the equation given below:

%water absorption =$(W_t - W_2)/W_2 \times 100\%$
Where, W_1 = Dry weight
W_2= Wet weight

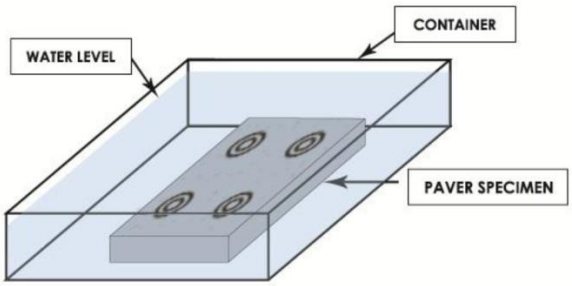


Fig 17 Water absorption test

3.5 Antimicrobial activity

The antimicrobial activity of Chitosan and chitosan silver nanocomposite films was investigated by agar diffusion assay (AATCC 30) against E. coli (gram–ve) and S. aureus (gram+ve) [13]. The Chitosan and chitosan silver nanocomposite films were cut in the form of circular disc of diameter 6 mm and sterilize under UV light in a laminar air flow for 24 h. The sterile sampleswere placed on agar plateswhich had been culturedwith E. coli and S. aureus and pressed lightly to ensure close contact with the surface. Plates were incubated at 37 °C for 24 h in an incubator. After incubation, the results were evaluated by determining the size of zone of inhibition produced around the sample to assess the efficacy of antimicrobial loaded films. In all the cases duplicate specimenswere tested.

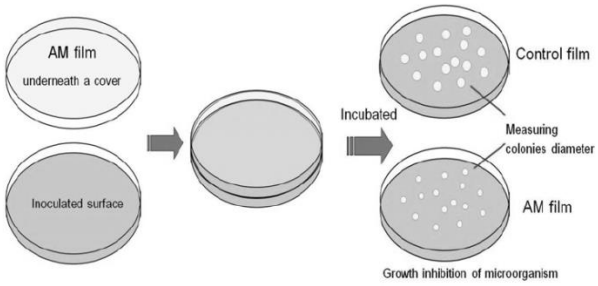


Fig 18 Antimicrobial activity

CHAPTER 4
RESULTS AND DISCUSSION

4.Results and discussion

4.1Fourier transform infrared spectroscopy (FTIR)

The IR spectra of chitosan and chitosan silver nanocomposite films are shown in the figures below fig 19 and fig 20, respectively. In the IR spectra, a broad band of 3500-2500 cm^{-1} is observed. A characteristic absorption bands of amino saccharide at 3430cm^{-1} is due to overlapping of –OH and $–NH_2$ stretching, band exhibited at 2920 cm^{-1} is due to CH_2 stretching and 1650 cm^{-1} is for carbonyl stretching (C=O) band of amide I and amide II, peak at 1380cm^{-1} is assigned to –C–O stretching mode of $–CH_2–OH$ groups and 1070 cm^{-1} stretching vibration is for C–O–C in glucose circle of chitosan.

Figure 20, exhibits characteristic IR bands of the functional group corresponding to pure chitosan and additional band at 571 and 813 cm^{-1} revealing to the Ag-O stretching. This reveals the formation of complex between surfaces charged silver nanoparticles (AgNPs) and cationic chitosan matrix, indicating the formation of chitosan/AgNPs nanocomposite. This indicates that the AgNPs nanoparticles are bonded with chitosan macromolecules and indicates the formation of chitosan/AgNPs nanocomposite. These changes may be a result of the reducing action of chitosan and the precipitation of metallic silver.

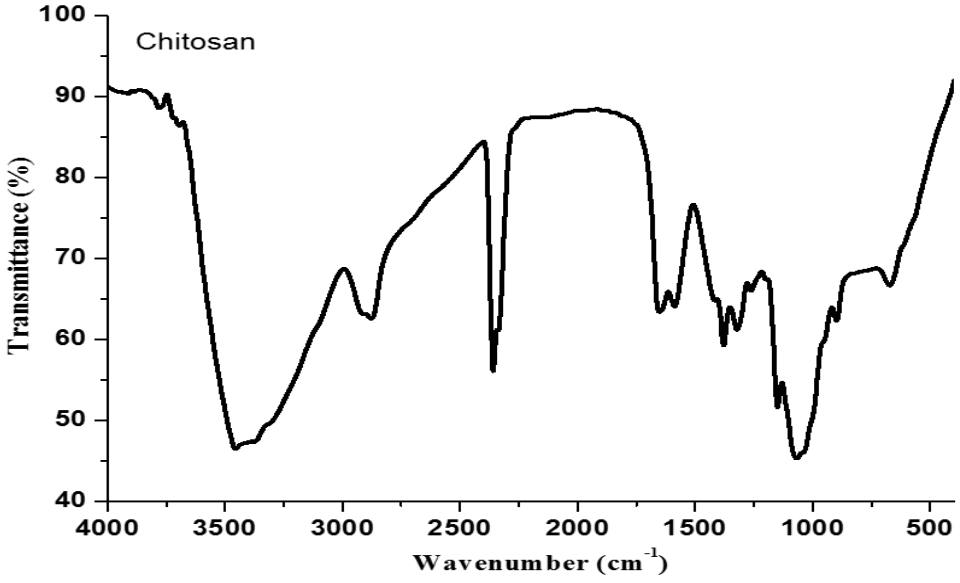


Fig 19 FTIR spectra of chitosan film

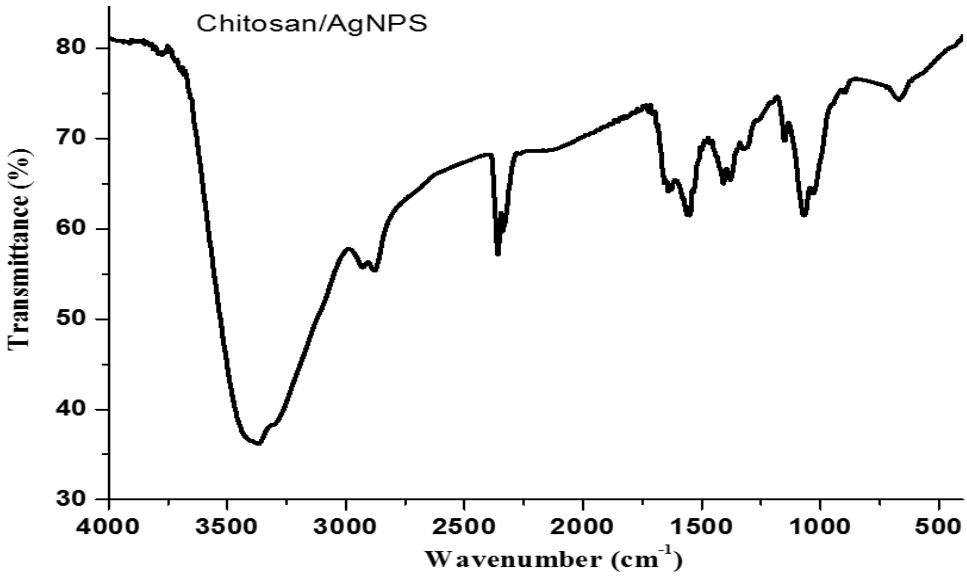


Fig 20 FTIR spectra of chitosan silver nanocomposite film

4.2 X-ray Diffraction (XRD)

The structural properties of the chitosan and chitosan-silver nanocomposite films were analyzed using the XRD technique. The obtained XRD pattern for chitosan is shown in Fig.21. The characteristic peaks appeared at 2 θ values of 12.5º, 22.5º and 32º which approximately matches well with the literature values (Wang et al. 2004; Kong et al. 2005). The broadening is noticed in the peaks which are due to the amorphous nature of the polymer (Rhim et al. 2006). Also there is no impurity peaks observed in the XRD pattern. The XRD pattern of chitosan silver NPs composite film shows chitosan as well as silver peaks in Fig.21 which clearly indicates the formation of silver in a single phase. Peaks were obtained at 2 θ values of 12.5º, 35º and 39º this seems to be in agreement with the values reported in the literature (Rhim et al. 2006). The peak value appeared at 2 θ value of 39º represents presence of silver nanoparticles.

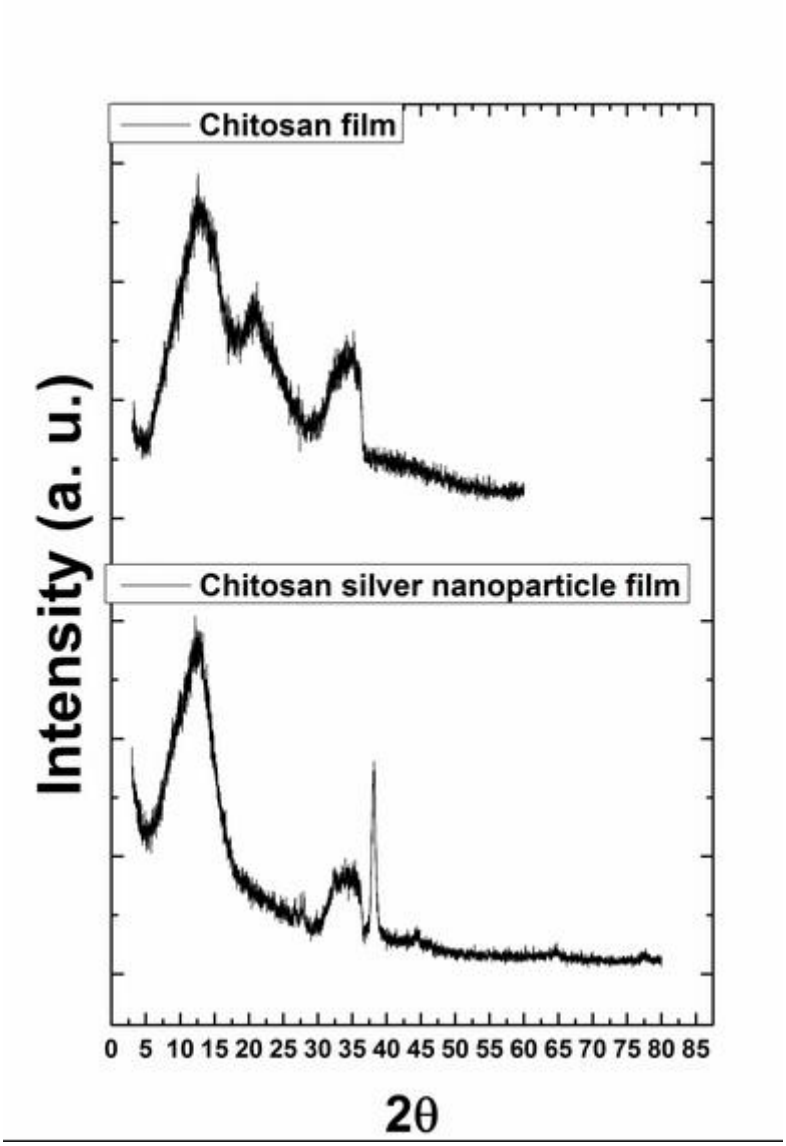


Fig 21 X- ray diffractograms of chitosan and chitosan-silver nanocomposite film

4.3 Scanning electron microscope (SEM)

The surface morphology of chitosan and chitosan-silver nanocomposite films is shown in the Fig 22(a-d). The size of the particles is seen to be almost uniform. The SEM image of Chitosan silver nanocomposite film shows a mixture of chitosan and silver wherein the silver nanoparticles are seen to be enveloped by the chitosan polymer. Low dispersibility and high stability is observed. From the figure, it is evident that pure chitosan film has smooth surface texture as compared to the Chitosan silver nanocomposite film. High rough texture was reported for Chitosan silver nanocomposite film. Higher surface roughness of chitosan silver nanocomposite film would favour higher attachment of cells as compared to pure chitosan films.

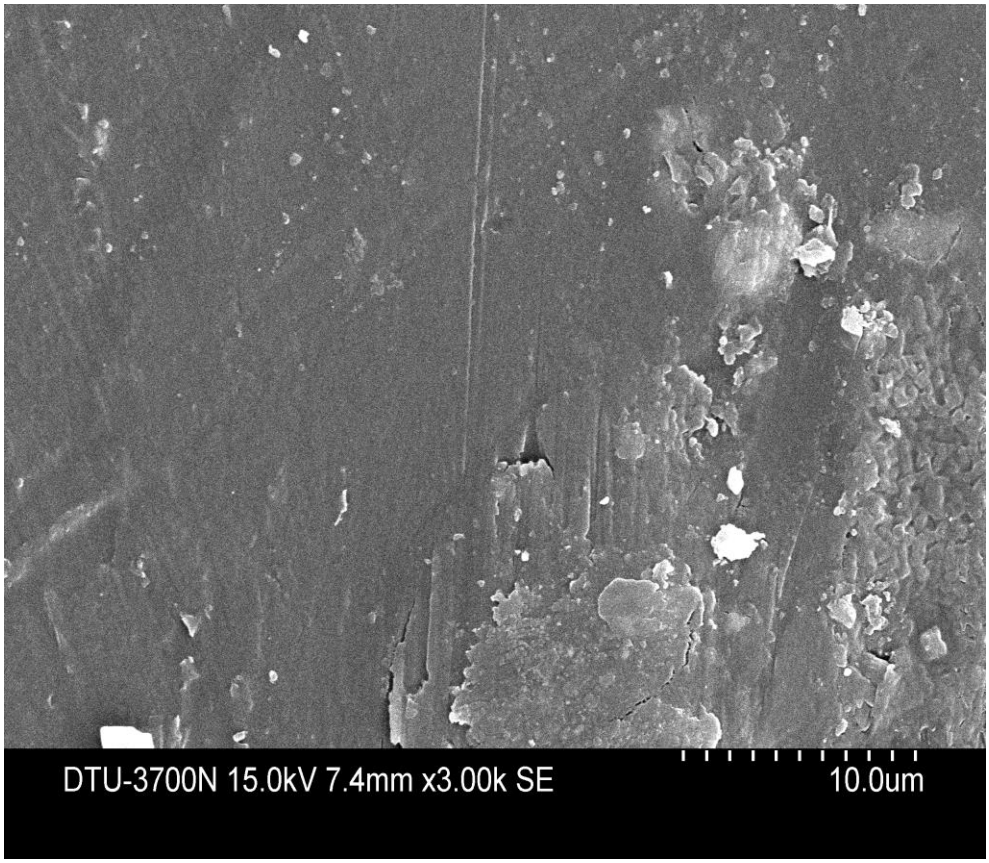


Fig 22(a) SEM micrograph of chitosan film

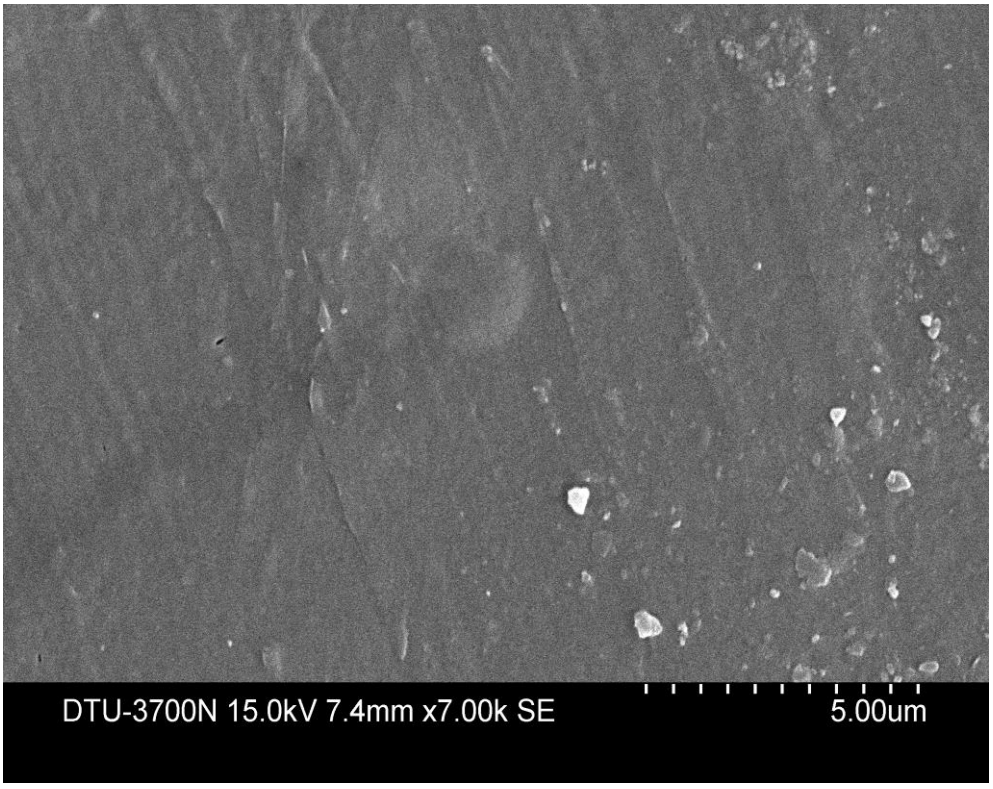


Fig 22(b) SEM micrograph of chitosan film

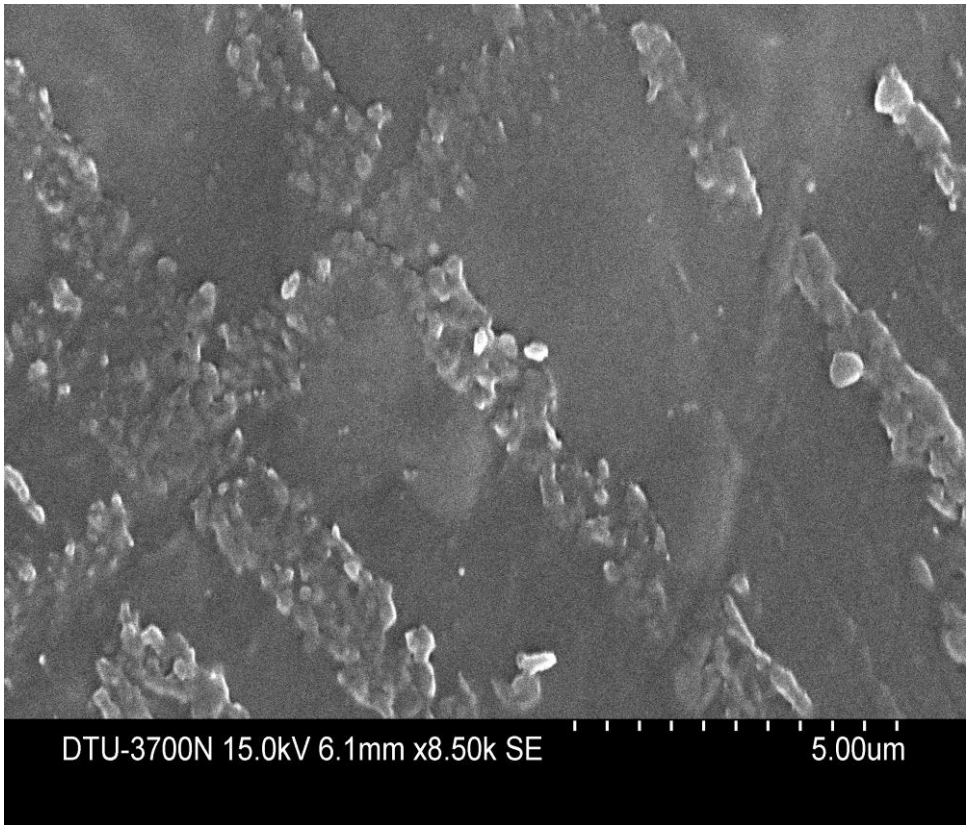


Fig 22(c) SEM micrograph of chitosan-silver nanocomposite film

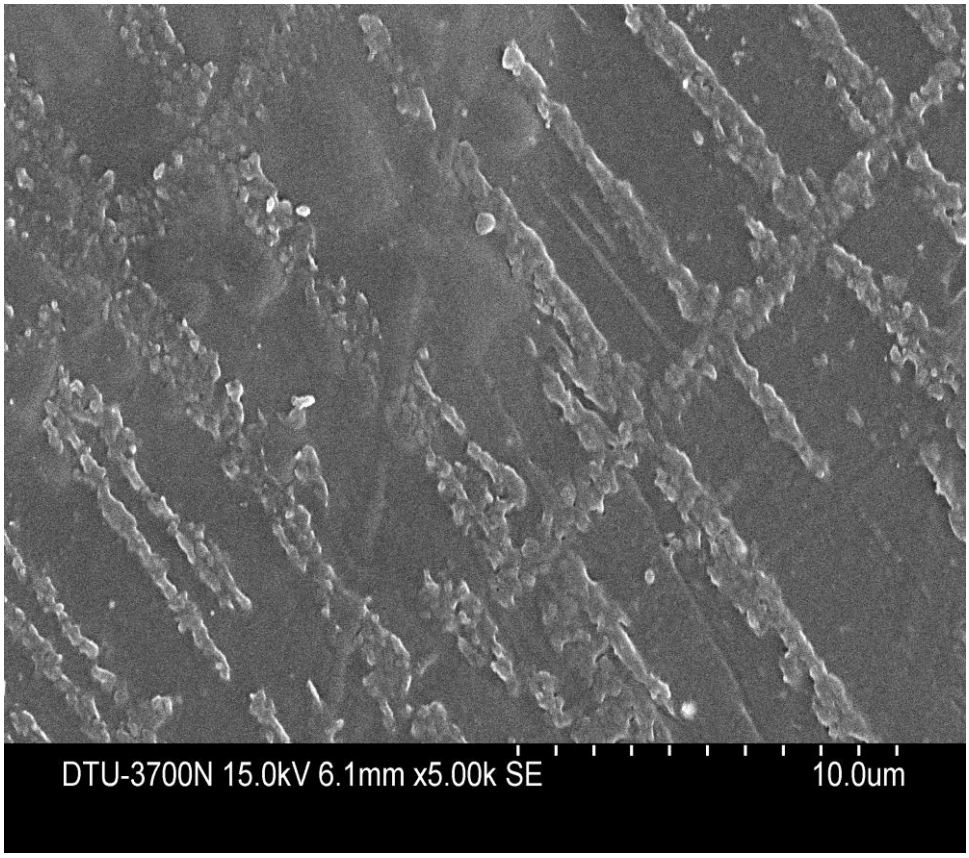


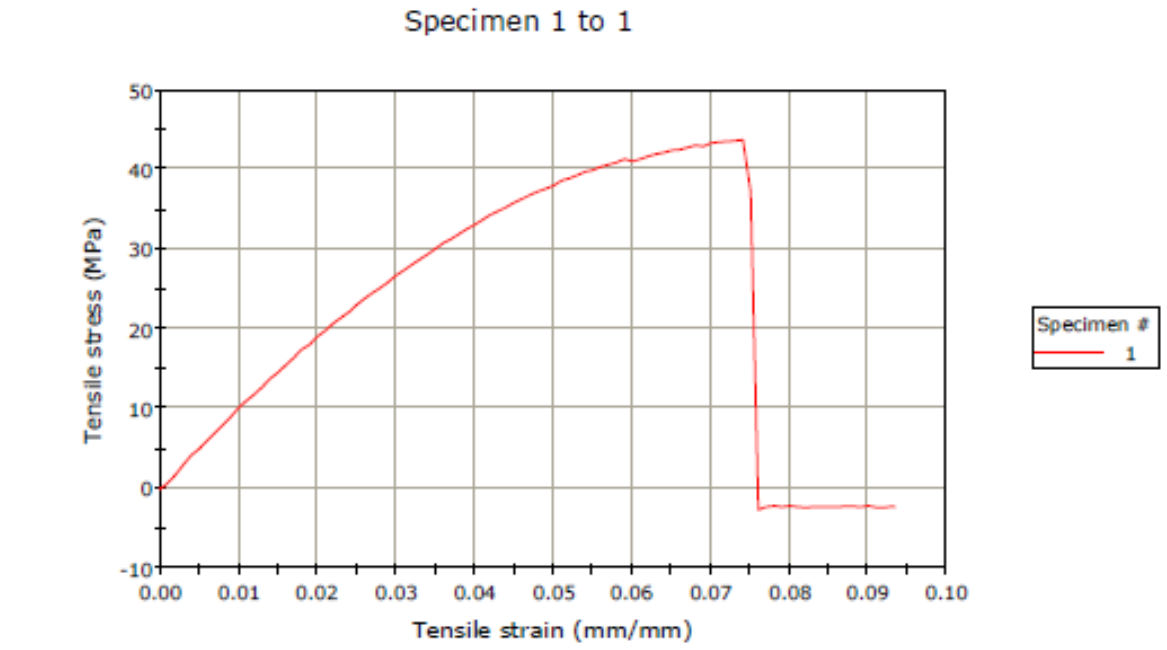
Fig 22(d) SEM micrograph of chitosan-silver nanocomposite film

4.4 Universal Testing Machine (UTM)

The mechanical properties of chitosan and chitosan-silver nanocomposite films are critical for their successful application in wound dessing. The appropriate mechanical strength of films is a prerequisite requirement for functioning of soft tissue substitute in close proximity of neotissues. Beside, the biocompatibility, high tensile strength and flexibility (fig 23(a-b) are prerequisite condition for films used as wound dressing.

DELHI COLLEGE OF ENGG.

Graph 1



Results Table 1

	Maximum Load (kN)	Tensile extension at Maximum Load (mm)	Tensile stress at Maximum Load (MPa)	Length (mm)
1	0.08735	3.70407	-2.28958	50.00000

	Thickness (mm)	Width (mm)	Area (mm^2)	Extension at Break (Standard) (mm)
1	0.20000	10.00000	2.00000	4.65016

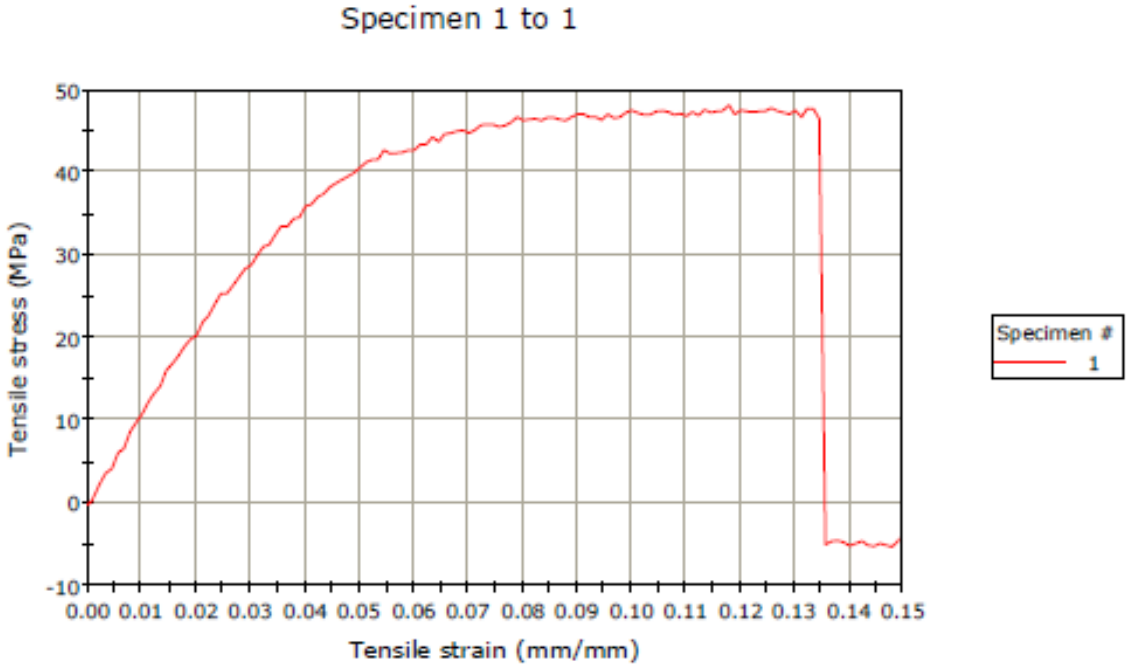
	Tensile strain at Break (Standard) (mm/mm)	Maximum Extension (mm)	Load at Maximum Extension (N)	Modulus (Automatic) (MPa)
1	0.09308	4.66812	-4.57916	983.81551

	Modulus (Automatic) (MPa)	Final area (cm^2)
1	983.81551	0.03600

Fig 23(a) Stress-strain curve of pure chitosan film

DELHI COLLEGE OF ENGG.

Graph 1



Results Table 1

	Maximum Load (kN)	Tensile extension at Maximum Load (mm)	Tensile stress at Maximum Load (MPa)	Length (mm)
1	0.02403	5.30804	-4.28257	45.00000

	Thickness (mm)	Width (mm)	Area (mm^2)	Extension at Break (Standard) (mm)
1	0.05000	10.00000	0.50000	6.70484

	Tensile strain at Break (Standard) (mm/mm)	Maximum Extension (mm)	Load at Maximum Extension (N)	Modulus (Automatic) (MPa)
1	0.14918	6.70484	-2.14129	1045.94698

	Modulus (Automatic) (MPa)	Final area (cm^2)
1	1045.94698	0.03600

Fig 23(b) Stress-strain curve of chitosan-silver nanocomposite film

It is noted that chitosan-silver nanocomposite films showed higher mechanical properties as compared to the pure chitosan film. From the above figures fig:23(a-b), it is evident that the tensile stress at maximum load (MPa), Modulus (MPa) and extensional break (mm) value of

pure chitosan film were 2.28 (MPa), 983 (MPa) and 4.6 (mm), respectively. On the other hand, for chitosan-silver nanocomposite film the value of tensile stress at maximum load (MPa), Modulus (MPa) and extensional break (mm) were 4.28 (MPa), 1045 (MPa) and 6.7 (mm), respectively.

4.5 Water Absorbance

The figure below (fig 24) shows the water absorbance ability of pure chitosan and chitosan-silver nanocomposite films over the time. From fig 24, it is clear that water absorbance capacity of pure chitosan and chitosan-silver nanocomposite films increases with time and saturate after 48 h. The chitosan-silver nanoparticle film showed higher water absorbance capacity (50%) as compared to pure chitosan film (45%). The water absorbance value obtained for chitosan-silver nanoparticle film is higher as comparable to pure chitosan film which is also reported by *Ahamed et al*. Water holding/absorbance ability of a material to be used as wound dressing is very important to provide moist environment and that would favour regeneration of neotissues to heal the wound.

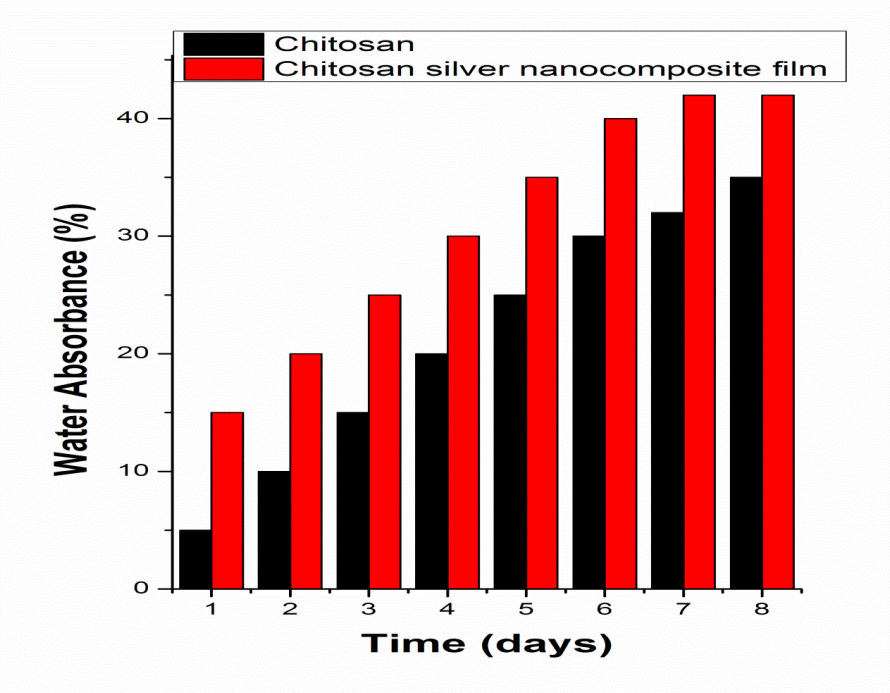


Fig 24 Water absorption of chitosan and chitosan - silver nanocomposite film

4.6 Antimicrobial activity

As the wounds are highly prone to bacterial infections, so the success of material to be used as wound dressing depends on whether it contains an active ingredient to reduce bacterial infections and promote the regeneration of the wound. Antimicrobial activity of chitosan and chitosan-silver nanocomposite films was determined by agar disc diffusion assay, and the results are shown in the fig 25(a-d).

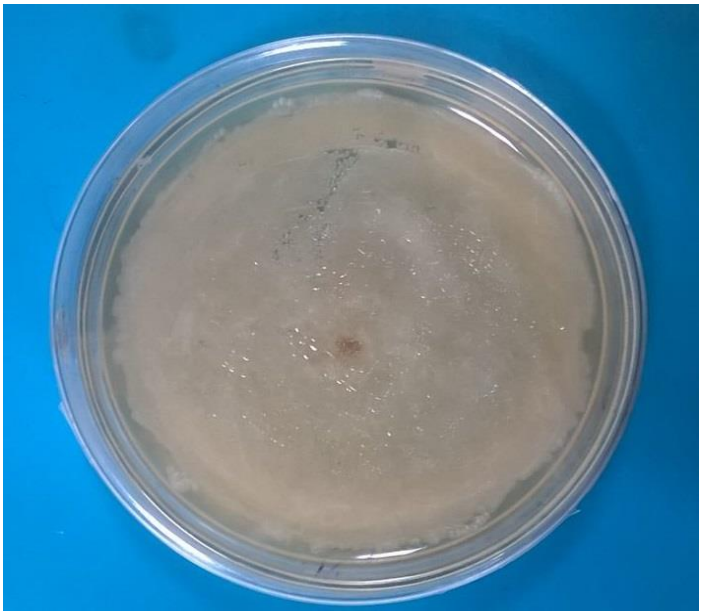


Fig 25(a) No zone formation of antimicrobial activity of pure chitosan film for *E.coli*

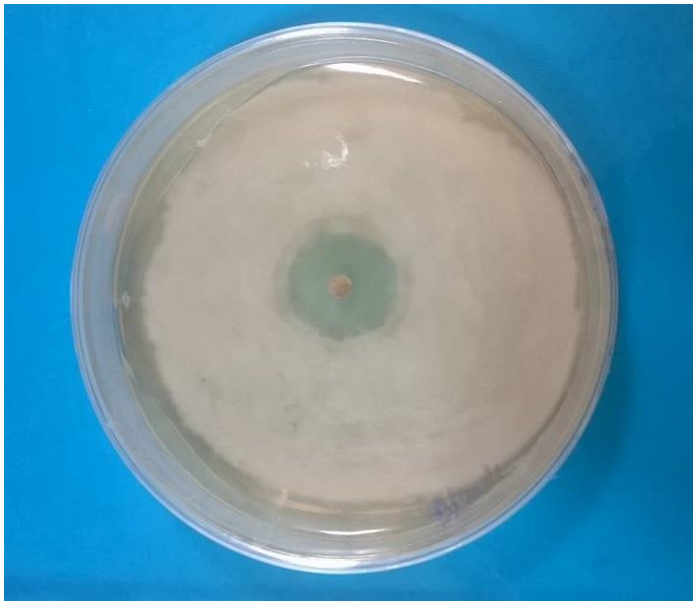


Fig 25 (b) Visible zone of antimicrobial activity of chitosan-silver nanocomposite film for *E.coli*

At neutral pH, chitosan does not show antimicrobial activity to impart that activity, it is necessary to incorporate some antimicrobial agents into it like silver nanoparticle in this case. At physiological pH, chitosan amine group does not protonate, so it does not show any antimicrobial activity. On the other hand, chitosan-silver nanocomposite films exhibit very strong antimicrobial activity due to the release of silver nanoparticles against both E. coli (gram-negative) and S. aureus (gram positive) strains. The mean diameter of zone of inhibition (ZOI) for chitosan-silver nanocomposite film was 25 and 20 against E. coli (gram negative) and S. aureus (gram positive) strain, respectively.

So this type of silver nanoparticle loaded chitosan composite films would behave as ideal wound dressing material.

Fig 25(c) No zone formation of antimicrobial activity of pure chitosan film for *S. aureus*

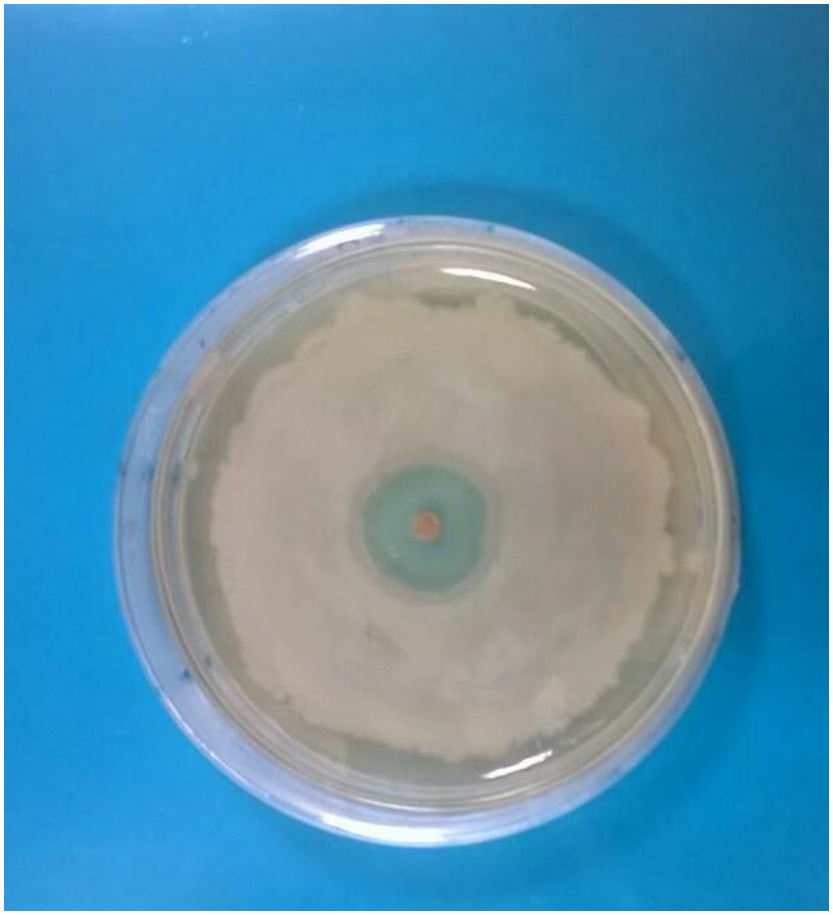


Fig 25(d) Visible zone of antimicrobial activity of chitosan-silver nanocomposite film for *S. aureus*

CHAPTER 5

CONCLUSION

5 CONCLUSION

Green synthesis of AgNPs and chitosan-silver nanocomposite films was successfully carried out by using the simple chemical method. The XRD patterns of pure chitosan and chitosan-silver nanocomposite films are found to be free from impurity peaks and the respective XRD patterns clearly indicates the formation of chitosan-silver nanocomposite film. From the SEM images, it is evident that the size of the particles is almost uniform. The SEM image of chitosan-silver nanocomposite film shows that the silver nanoparticles are seen to be enveloped by the chitosan polymer. The shift in the peaks in FTIR spectra of chitosan indicate the formation of chitosan-silver nanocomposite film. Higher tensile strength and flexibility were recorded through the mechanical testing of the chitosan and chitosan-silver nanocomposite films using the universal testing machine. High tensile strength and flexibility are prerequisite conditions for films to be used as wound dressing. Water holding/absorbance ability of a material to be used as a wound dressing is also crucial to provide a moist environment that would favour regeneration of new tissues to heal the wound. From the water absorption test, it is gathered that the chitosan-silver nanocomposite film showed higher water absorbance capacity (50%) as compared to pure chitosan film (45%). Due to the limitation of chitosan at neutral pH, chitosan does not show antimicrobial activity, and to impart that activity, it is necessary to incorporate some antimicrobial agents into it like AgNPs in this case. At physiological pH, chitosan amine group does not protonate, so it does not show any antimicrobial activity. On the other hand, the chitosan-silver nanocomposite films exhibit very potent antimicrobial activity due to the release of the silver nanoparticles against both *E. coli* (gram-negative) and *S. aureus* (gram positive) strains. Successful green synthesis and characterization were carried out to show that chitosan-silver nanocomposite film can be used as an ideal wound dressing material.

6 REFERENCES

1. E. I. Rabea, M. E.-T. Badawy, C. V. Stevens, G. Smagghe, and W. Steurbaut, “Chitosan as antimicrobial agent: applications and mode of action,” Biomacromolecules, vol. 4, no. 6, pp. 1457–1465, 2003.
2. The McGraw Hill Companies, Inc (2008) “Current Diagnosis & Treatment: Emergency Medicine” 6th Edition
3. St. John Ambulance Australia “Australian First Aid” 3rd Edition, edited by Shirley Dyson, pages140-448, 1998.
4. Wikipedia(en.wikipedia.org)- silver nanoparticles
5. Arabian journal of chemistry (September 2015) “Green synthesis of silver nanoparticles from marigold flower”- Volume 8, Issue 5, September 2015, Pages 732–741
6. Ahlafi, Hammou; et al. (2013). "Kinetics of N-Deacetylation of Chitin Extracted from Shrimp Shells Collected from Coastal Area of Morocco" . Mediterranean Journal of Chemistry. Vol. 2, no3, 2013
7. University of Iowa “Chitosan for biomedical applications” edited by Aiman Omar Mahmoud Abbas, December 2010, pages 176-259.
8. Chitosan and alginate wound dressings: A short review. Paul, W., and Sharma, CP., Trends Biomater. Artif. Organs , 18 (1), (2004) 18-23.
9. “Principles of Instrumental Analysis, 4th Ed.”, D.A. Skoog and J.J. Leary. Harcourt Brace Jovanovich. Philadelphia, PA, 1992. Chapter 12.
10. “50 Years of X-ray Diffraction”,(1999) edited by P. P. Ewald, chapter-6, published by The International Union of Crystallography.
11. “Brief introduction to Scanning Electron Microscopy (SEM)”- Published by Central facility for advanced microscopy and microanalysis- University of California, Riverside, chapter 1, vol.1 (1998).
12. S.E. Wharram, X. Zhang, D.L. Kaplan, S.P. McCarthy, Electrospun silk material systems for wound healing, Macromol. Biosci. 10 (2010) 246–257.
13. S.W. Ali, R. Purwar, M. Joshi, S. Rajendran, Antibacterial properties of Aloe vera gel finished cotton fabric, Cellulose 21 (2014) 2063–2072.