Physiochemical behaviour of chitosan/polyvinyl alcohol binary film for food packaging

A Major Project Report submitted in partial fulfilment for the Award of the degree of

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POLYMER TECHNOLOGY

Under the supervision of

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Submitted by

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CERTIFICATE

This is to certify that ***Tafadzwa Ziyambi (2K13/PTE/15),*** *has Satisfactorily Completed this Major project entitled "****Physiochemical behavior of chitosan/polyvinyl alcohol binary film for food packaging****" in the Partial Fulfillment for the Award of the Degree of Master of Technology in Polymer Technology at Delhi Technological University, Delhi during the Academic Session 2014-2015.*

To the Best of my knowledge and Belief, this Work has not been submitted to any other University or Institutions for the Award of any Degree or Diploma.

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UNDERTAKING

I declare that the work presented in this thesis titled "Physiochemical behaviour of chitosan/polyvinyl alcohol binary film for food packaging", submitted to the Department of Applied Chemistry & Polymer Technology, is an authentic record of my own work carried out under the supervision of Prof. D Kumar and Dr. Jay Singh, Department of Applied Chemistry and Polymer Technology, Delhi Technological University, Delhi.

This report does not to the best of my knowledge contain part of my work which has been submitted for the award of any other degree either of this university or any other university without proper citation.

Date:

Place: DTU, Delhi Signature of the candidate

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Taking the opportunity of this column, I would like to express my sincere gratitude to all those who directly or indirectly helped me in successful completion of my project.

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ABSTRACT

The aim of this study is to prepare, characterize and further develop films of polyvinyl alcohol (PVA) and chitosan. Blends which comprise of synthetic and natural polymers embody a fresh group of materials and have attracted considerable attention especially in bio application when used as a biomaterial. The resulting properties of the blends mainly rely on the properties of the constituent polymers and on the phase morphology that has been developed during blending. These antimicrobial packaging films have been of much concern due their ability to slow or kill pathogenic microorganisms that tend to reduce the shelf life of food products. Thus chitosan based films have been studied because of their effectiveness in food preservation. Chitosan is well known for its excellent biocompatibility and biodegradable properties. It also has got a high modulus of elasticity which is due to high glass transition temperature and also its crystallinity. PVA is known to be a semi crystalline, excellent chemical resistance water soluble, better film and fiber forming, non-toxic, good mechanical properties, biocompatible and biodegradable synthetic polymer. The present work describes the structural characterization, thermal study, surface morphology and bioactive behavior of edible cross-linked chitosan-PVA film. An edible film is prepared by blending chitosan and poly (vinyl alcohol) (PVA) with glutaradehyde as cross-linker. The presence of hydrogen bonding between ─OH of PVA and ─OH or ─NH_2 of chitosan has been ascertained by IR study. TGA thermogram of chitosan-PVA film has provided a fair idea about the thermal degradation and the energy required to vaporize the water present in the film. Antimicrobial assessment has shown the positive inhibitory effect against *E. coli.* When chitosan and PVA are blended there is improved strength as well as flexibility of the films. Adding a cross linking agent will further improve the tensile strength but decrease the elongation properties of the films. As the PVA content in the film increase the water uptake increases due to an increase in the hydroxyl groups thus water uptake can be reduced by minimizing the amount of PVA, adding a cross linking agent and adjusting the pH of the solution. Thus bulk and surface hydrophilicity of the films is improved. The findings of the antimicrobial activity has demonstrated that chitosan-PVA solution may be used for the preservation of fruits and vegetables.

CHAPTER 1

1. INTRODUCTION

Food packaging is useful because it provides protection, tampering resistance, and vital physical, chemical, or biological needs. It may also contain nutrition facts label and other information about food that is available for sale [1].

Food packaging has several functions which are:

- Physical protection - This includes protection from the outside environment to things like temperature and bacteria. Food may also need protection from shock and vibrations to improve on their shelf life.
- Barrier protection - This also act in a similar way as the physical protection by preventing the penetration of oxygen, water vapor and dust. Some packages are designed in a way that they have oxygen absorbers which help them to better their shelf life while other packages may contain atmospheres that are controlled to enable that the food stuff is kept fresh for a long period of time [2].
- Containment or agglomeration - The best way to handle small items is by putting them together in one packages so that they can be handle well. Other things such as liquids, powders, and granular materials also require proper containment for efficient handling.
- Information transmission - These packages are very important because they also provide information on how best the end user will use, transport, recycle or even ways of disposing the product or package.
- Marketing - Packages and labels can be used to improve the rate of sales of a particular product thus the way in which the package is designed attracts a buyer to buy the product. Package design has been a very vital and ever changing phenomenon for several decades [3].
- Security - Packaging has also a key part in lowering the security risk when shipment is being done an important role in reducing the security dangers during shipment. Packages thus can be made with more efficient tamper resistance to eradicate tampering and can also contain some tamper-evident features to help in observing any items that are tampered with. Packages can be designed to help lower the risks of package pilferage;

some package constructions are made in way such that they are more resistant to pilferage while some have pilfer-indicating seals on them. Packages may include some form of authentication seals which will help ii investigating whether the package and contents are not compromised. Packages can also have extra security features like anti-theft devices which can prevent casualties such as retail loss. Tags can also be put on packages which can be detected whenever you pass through exits points and these tags will need sophisticated tools to make that they are disabled

- Convenience - Packages may also an important role by aiding convenience in conveying a message during handling, sale, reuse and distribution
- Portion control - Single-serving packaging has a stipulated volume of contents to manage usage. Bulk commodities can be categorized into packages that are manageable by individual households. It also help in the controlling of inventory.

1.1 Scope and significance of the present work

The spoilage of foods each year all over the world incurs great economic losses. In order to preserve the food from microorganisms, various antimicrobial based products and processes have been developed. In particular, polymeric bioactive films laced with assortment of antimicrobial agents have been found very effective and practical in applications. Till date, a number of review articles have been published describing the nature of different materials used in making such films and their effectiveness in food preservation [4-13].

1.2 What is a biopolymer?

Biopolymers can be defined as polymers that are biodegradable. The starting materials/ingredients for the synthesis of these polymers can be either renewable (mainly from agricultural produce or animal products) or purely synthetic [14].

There are basically four main types of biopolymer which are:

- Cellulose
- Synthetic materials
- Starch
- Sugar

Recent and future activities in biodegradable polymers [15] and renewable input materials emphasis mainly on the scaling-up of manufacturing and addition of value to the product properties. A bigger scale production will result in an increase in the availability and reduce prices.

Recent studies have shown that either renewable or synthetic starting materials can be used to produce polymers that are biodegradable. There are two main ways which can be used in synthesizing a polymer. The first one is to make up the polymer structure from a monomer using chemical polymerization. Another alternative is to use a naturally occurring polymer then bring the desired properties by chemically modifying it. A major drawback in chemically modified polymer is that the ease of biodegradability of the polymer may be unfavourably affected. Therefore there is need to moderate between getting the required properties of the material and biodegradability.

Polymers exhibit properties which makes them very useful in protecting products from things like moisture, extended shelf-life and ease of product dispense becomes very easy.

All biopolymer have their own material-specific properties, for example barrier properties such as the permeability of oxygen. These barrier properties are important to the choice of biopolymers during the packaging of certain products. Bioplastics have very encouraging prospects in pesticide soil pins, dairy products and for packaging in-flight catering.

1.2.1 Starch based polymers

Thermoplastic starch can't be used for packaging liquids. It can't sustain long periods in contact with water only although it has excellent oxygen barrier properties. Starch is a common natural polymer which usually occurs as granules in the plant tissue from where it can easily be recovered in very large quantities. Starch is mainly obtained from potatoes, wheat, maize, tapioca and similar sources. It can be modified in a manner in which it can be heated until it melts and at the same time deformed thermoplastically. The material that is obtained is thus suitable for processes such as extrusion and injection moulding.

1.2.2 Sugar based biopolymers

Polylactides decompose without causing any harm in the human body and have therefore been used for medical applications for long. Examples may include surgical implants which basically do not need operative removal. These polylactides were not used for packaging because they are very expensive.

• The initial material for Polyhydroxibutyrate is from starch or sucrose with a process called bacterial fermentation. Changing nutrient composition of the bacteria results in differences in the end product. Hence this makes it possible to adjust the properties of the material, for example its moisture resistance. Another way of making the polymer is by injection, blowing, vacuum forming and extrusion [15-17].

• lactic acid polymers are usually made from lactic acid, which is mainly obtained from lactose (or milk sugar) which is found from sugar beet, maize, wheat, potatoes. Polylactides are purely water resistant and can be made by injection moulding, vacuum forming and blowing.

1.2.3 Cellulose based biopolymers

Well known uses of cellophane include packaging cigarettes and confectionary. The material is slowly falling out of preference, however because of its very high price. Other cellulose polymer materials for example cellulose film have also been commercially abundant for so many years but are now losing market ground share to newly emerging polymers such as polypropylene. The use of cellulose for preparing packaging material such as cellophane has been long established now. This material is transparent and has got good folding properties as well. Whether it's in the form of pure cellulose or just a nitrocellulose coating, the material is purely biodegradable and can be composted by the existing waste processing plant [18].

1.2.4 Synthetic based biopolymers

The high price of biodegradable polymers of synthetic materials, such as aliphatic aromatic copolyesters has stopped them from reaching a very large scale market. The well-known use is for making substrate mats. Synthetic compounds that are derived from petroleum can also be used as a starting point for biodegradable polymers, for example aliphatic aromatic copolyesters. These polymers usually exhibit technical properties resembling those of low density

polyethylene (LDPE). Regardless of the fact that these polymers are produced from synthetic materials as their starting material, they are wholly biodegradable and compostable [18].

1.2.5 Benefits of using biopolymers

Besides the fact that they are available on a sustainable basis, biopolymers have many economic and environmental merits. Biopolymers could also turn out to be an asset to waste processing. Plastic scraps occurring in compost can be eliminated by replacing the polyethylene that is used in coated papers by a biopolymer.

Consumers have an overwhelming interest in biopolymers. Most conventional plastics are known to be environmentally unfriendly. These sustainable plastics could also help in providing an image advantage.

The main advantage of biodegradable packaging is its ease of being composted. The biodegradability of raw materials or starting materials does not certainly reveal that the product itself or package made from them for example coated paper is itself compostable.

Although biopolymers have advantages for waste processing coated paper which contain polyethylene is a main problem product for composting. Such materials are usually banned from being incorporated in organic waste under separate collection plans, a couple of them usually end up in the mix. What is observed is that the papers decompose but in the compost small scraps of plastic are left. The need to adopt biopolymers for this purpose would surely solve the problem.

The widespread interest for biopolymers in consumers now emanate from the fact that conventional plastics are environmentally unfriendly to the public view. These environmental benefits of biodegradable packaging should be seen in cost advantages, if wide use applications are to become more feasible. It would be more desirable to communicate the functional merits of biodegradable packaging preferably than its compostability.

1.3 Why binary films?

Blends which comprise of synthetic and natural polymers embody a fresh group of materials and have attracted considerable attention especially in bio application when used as a biomaterial. The triumph of synthetic polymers as biomaterial depends mainly on their large range of

mechanical properties, transformation processes which allow an assortment of different shapes to be easily achieved and very low production costs. Biological polymers [19-21] also possess very good biocompatibility but their mechanical properties are most of the time poor, the need of preserving biological properties make if difficult on their process ability and their rate of production costs is very high. It is most favorable that at least intermolecular interaction exists between two polymeric species. Hydrophilicity of the synthetic polymers also have a great influence on the blend preparation and resulting properties. Surface and bulk hydrophilicity of the blended polymers have an effect mainly on their biological performance. Bulk hydrophilicity of polymers is usually examined by the water uptake ratio, and also surface hydrophilicity could be measured using water contact angle and surface tension. Chitosan contains hydroxyl and amine groups and PVA is a water-soluble polymer which has got hydrophilic properties.

The resulting properties of the blends mainly rely on the properties of the constituent polymers and on the phase morphology that has been developed during blending. Chitosan is known as the second most abundant natural polysaccharide which comes next to cellulose and is also well known for its excellent biocompatibility and biodegradable properties [22]. Chitosan also has got a high modulus of elasticity which is due to high glass transition temperature and also its crystallinity. Chitosan also has got very good film forming capacity, excellent gas barrier properties [23] at dry conditions and pleasant smell (aroma). PVA is known to be a semi crystalline, excellent chemical resistance water soluble, better film and fiber forming, non-toxic, good mechanical properties, biocompatible and biodegradable synthetic polymer which is mainly used in the biomedical field [24]. The most stable state of the polymer is its crystalline state.

1.4 Polyvinyl alcohol

Polyvinyl alcohol is a water-soluble[16], non-toxic, synthetic polymer that has good physical and chemical properties and also film-forming ability .The wide use of this polymer is key in many applications such as in the controlled drug delivery systems, recycling of polymers and packaging and also membrane preparation. Researches on the mechanism of dissolution and typical changes in the crystallinity and also swelling behaviour of polyvinyl alcohol and its physical gel-forming capabilities, have also been carried out. PVA exhibit bio inertness and it thus has found many uses in medical uses such as hemodialysis, artificial pancreas, synthetic

vitreous, implantable medical device and nano-filtration. Polyvinyl alcohol can be used as an emulsion polymerization aid [25] or as a protective colloid to make polyvinyl acetate dispersions. It finds its largest market application in China and in Japan its major use is in vinylon fiber production.

Uses of polyvinyl alcohol include:

- Paper adhesive and solid board production
- Thickener and also as a modifier in polyvinyl acetate glues
- Textile sizing agent
- Paper coatings
- As a water-soluble film
- As a biodegradable plastic backing sheet in feminine hygiene
- Carbon dioxide barrier usually in (PET) bottles
- Water transfer printing process
- As a form release because there are materials such as epoxy which do not stick to it
- Children's play putty or slime when it is combined with borax
- Used in eye drop and also in hard contact lens solution mainly as a lubricant
- PVA fiber can be used as reinforcement in concrete
- Raw material to polyvinyl nitrate
- It can be used as a surfactant for the development of polymer encapsulated nanobeads
- In protective chemical-resistant gloves
- As a fixative for specimen collection
- When it is doped with iodine PVA can be used in the polarization of light
- As an embolization agent
- Carotid phantoms for application as synthetic vessels in the Doppler flow testing
- Used in 3D printing mainly as a support structure that can then be easily dissolved away.

PVA is commonly used in freshwater when doing sport fishing. Small bags produced from PVA are packed with oil based bait and put on the hook or make sure the baited hook is positioned inside the bag and put into the water. When this bag gets on the lake or river bottommost it disintegrates leaving the hook bait encircled by ground bait and pellets. This technique helps entice fish to the hook bait. On the other hand anglers use a string made of PVA for the reason of

making provisional attachments. For instance holding a length of line that is in a coil might tangle when the cast is being made.

1.4.1 Preparation of PVA

Unlike most of the vinyl polymers, PVA is not prepared by the polymerization of corresponding monomer. The monomer which is vinyl alcohol is very unstable with respect to the acetaldehyde. PVA is prepared by firstly polymerizing vinyl acetate and then the resulting polyvinylacetate is converted to PVA [25]. Some other precursor polymers are usually used with formate, chloroacetate groups instead of the acetate. The conversion of these polyesters is often carried by base-catalysed transesterification using ethanol:

$$[CH_2CH(OAc)]n + C_2H_5OH \rightarrow [CH_2CH(OH)]n + C_2H_5OAc$$

The properties of the resulting polymer rely on the amount of residual ester functional groups present. The worldwide consumption of polyvinyl alcohol is very high with over one million metric tons in 2006. The biggest manufacturers comprise of Kuraray and Sekisui Specialty Chemicals but mainland China managed to installed a couple of very large production facilities over the past decade and currently accounts for at least 45% of the world capacity. North Korean-manufacture fiber Vinalon likewise is produced from polyvinyl alcohol. In spite of its inferior features as clothing fiber it is similarly produced for the self-sufficiency reasons as no oil is necessary to make it.

1.4.2 Structure and properties of polyvinyl alcohol

Polyvinyl alcohol is also known to be an atactic material that also exhibits crystallinity. [6] The microstructure of PVA is composed mostly of 1, 3-diol linkages [-CH_2-CH(OH)-CH_2-CH(OH)] but a lesser quantity of 1,2-diols [-CH_2-CH(OH)-CH(OH)-CH_2-] are therefore subject to the conditions necessary for polymerization of vinyl ester precursor. Moreover polyvinyl alcohol has excellent film forming, emulsifying as well as adhesive properties. PVA is also resistant to oil, solvents and grease. It also has great tensile strength and the flexibility is high also coupled by good aroma barrier properties and oxygen barrier properties. All these properties are dependent on humidity which means with higher humidity more water is also absorbed. The water which now acts as a plasticizer will result in a reduction in its tensile strength but at the same time cause a rise in elongation and tear strength.

Furthermore PVA has a got melting point in the region of 230 °C and 180–190°C for the fully hydrolysed and partially hydrolysed types respectively. It decomposes rapidly at a temperature above 200 °C as it can undergo pyrolysis at very high temperatures. PVA is close to being incompressible [25].

1.5 Chitosan

Anti-thrombogenicity, cell compatible Chitosan is a well-known natural polysaccharide formed through the deacetylation of chitin in an alkaline condition [26]. It also comprises of an unbranched chain consisting mainly of β-(1, 4)-2- amino-2-deoxy-D-glucopyranose which is a very distinctive basic linear polysaccharide. Also the hydrophilicity of the polymer is due to the amine functionality in most repeat units and it's the one that makes this polymer more soluble in dilute acidic solutions. Chitosan is mostly used in food and pharmaceutical industry as well as in biotechnology. This unique polysaccharide has been studied in the area of biomaterials and as a result of its biological properties bioactivity, biodegradability and biocompatibility it has drawn considerable attention. Polymeric blending is one of the most beneficial methods to have new material with desired properties and there have been a great scientific and commercial achievement in the area of polymeric blends. This was aided by the realization that it's not always that new molecules are required to meet the necessity for different materials and that blending can also be implemented more efficiently, rapidly and economically than opting for the development of new materials.

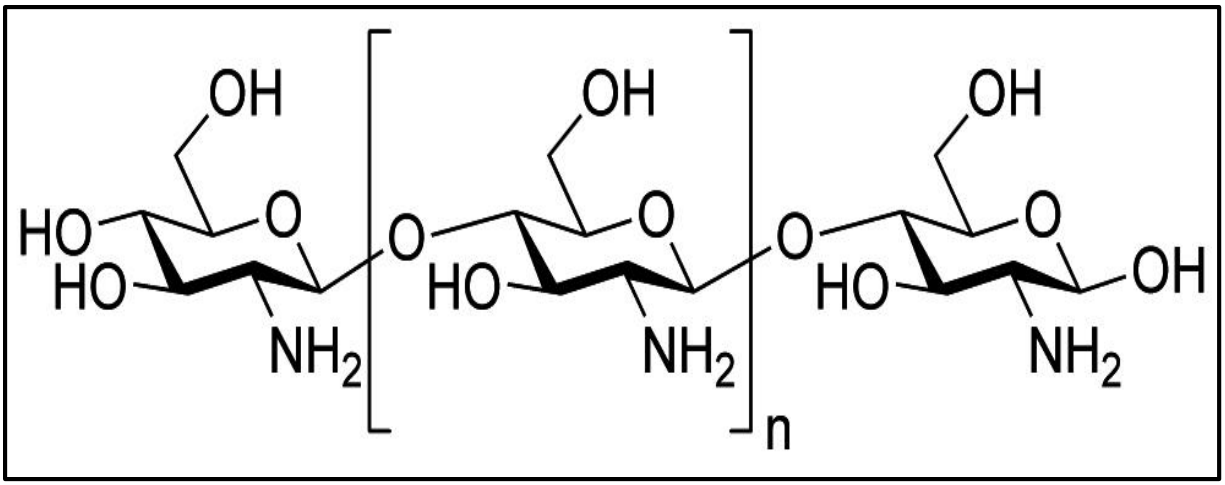

Fig 1.1: Chemical structure of chitosan

Chitosan is a known linear polysaccharide composed mainly of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).Usually it is made by treating shrimp and crustacean shells with sodium hydroxide alkali.

Chitosan has a wide number of commercial and also biomedical uses. Chitosan can be used in agriculture to function as a seed treatment and bio-pesticide thus helping plants to avoid fungal infections. In the process of winemaking it can be incorporated as a fining agent which helps to prevent spoilage. In many industries it can be used as a self-healing polyurethane paint coating. It also has medicinal applications were it can be valuable in bandages to lessen bleeding and also as an antibacterial agent or can be used to help delivery of drugs through the human skin.

In ongoing researches chitosan has been proclaimed to have applications in limiting absorption of fat, which would make it essential for dieting but there is proof against this. Other applications of chitosan that have been studied which comprise application as a soluble dietary fiber.

1.5.1 Manufacturing and properties of chitosan

Chitosan is mainly produced commercially by the process of deacetylation of chitin [27] which is the main structural element in the exoskeleton of all crustaceans which includes crabs and shrimp and also the cell walls of fungi. The degree of deacetylation can be determined by the NMR spectroscopy and also in commercial chitosan usually ranges from between 60 to 100%. The

molecular weight on average of a commercially produced chitosan is around 3800 and 20,000 Daltons. The most common method for synthesizing chitosan is by deacetylation of chitin using an excess sodium hydroxide as the reagent and water as the solvent. This reaction pathway usually yields 98% of the product when it is allowed to go to completion.

The amino functional group in the chitosan has a pKa value of approximately 6.5 which usually leads to a protonation in an acidic to neutral solution with the charge density dependent mainly on pH and the degree of deacetylation. This causes chitosan to be water soluble and a bio-adhesive which easily binds to negatively charged surfaces like mucosal membranes. Chitosan also enhances the transport of polar drugs across the epithelial surfaces and is known to be biocompatible and biodegradable although it has not approved by FDA for the drug delivery. Purified amounts of chitosan are also available for biomedical applications.

Chitosan and its derivatives like trimethylchitosan (where the amino functional group has been trimethylated), have also been used in nonviral gene delivery. These derivatives Trimethylchitosan, or quaternised chitosan has been observed to transfect breast cancer cells with an increased degree of trimethylation increasing the rate of cytotoxicity at approximately 50% trimethylation, thus the derivative is most efficient at gene delivery. On the other hand oligomeric derivatives (3-6 kDa) are also relatively nontoxic and have very good gene delivery properties.

1.5.2 Uses of chitosan

The agricultural and horticultural applications for chitosan primarily for the plant defense and yield increase are mainly based on how this glucosamine polymer influences biochemistry and molecular biology of a plant cell. The chief cellular targets are the nuclear chromatin and plasma membrane. Subsequent some changes occur in, chromatin, cell membranes, MAP Kinase, DNA, reactive oxygen species, calcium, callose pathogenesis-related (PR) genes, oxidative burst and phytoalexins.

In the agricultural sector chitosan is used primarily as a plant growth enhancer and natural seed treatment and also as an ecologically friendly bio-pesticide substance that usually boosts the innate ability of plants so as to defend themselves against things like fungal infections. This natural bio-control active ingredient (chitin/chitosan) is found in shells of crustaceans such as

crabs, lobsters, and shrimp and other organisms including insects and fungi. It is among one of the most abundant biodegradable materials available in the world.

Some degraded molecules of chitin/chitosan exist either in water or soil. Chitosan's uses for crops and plants are usually regulated by the EPA and the other board USDA National Organic Program regulates the use on organic certified farms and crops. EPA also approved biodegradable chitosan products which are allowed for outdoors and indoors use on crops and plants grown commercially and also by consumers.

The natural bio-control capability of chitosan should not be mistaken with the effects of fertilizers or pesticides on plants or the environment. Recent studies show that chitosan active bio-pesticides represent a new class of cost-effective method for biological control of crops [28] in horticulture and agriculture. The bio-control mode on the action of chitosan succeeds natural innate protection responses within plants to fight pathogens, insects and soil-borne diseases when it is applied to the soil or foliage. Chitosan therefore increases the rate of photosynthesis, enhances and promotes plant growth, increases germination, stimulates nutrient uptake and boosts plant vigor. When it is used as seed curing or seed coating, seed potatoes, corn, soybeans, tomatoes, wheat, sugar beets and plenty other seeds, it usually elicits an innate immunity response in the developing roots which in turn destroys parasitic cyst nematodes without causing damage to beneficial nematodes and organisms.

Agricultural uses of chitosan can decrease environmental stress due to drought and also soil deficiencies, improve stand quality, increase yields, strengthen seed vitality and decrease fruit deterioration of vegetables, fruits and also citrus crops. Horticultural uses of chitosan increases blooms and increases the life of cut flowers. The US Forest Service did carry a research on chitosan to control the pathogens in pine trees and also increase resin pitch outflow which tends to resists pine beetle infestation.

Chitosan can be used in water processing engineering during the filtration process. Chitosan also causes the finer sediment particles to bind to one another and is thus subsequently removed with the sediment during the sand filtration. It removes phosphorus, oils from the water and heavy minerals. Chitosan is also an important additive during the filtration process. Sand filtration evidently can take away up to 50% of the turbidity, while the chitosan with sand filtration also

removes up to 99% of the turbidity. Chitosan has other functions which is to precipitate caseins from bovine milk and also cheese making.

Chitosan is useful in other filtration [27, 28] situations where it may be necessary to remove suspended particles in a liquid solution. When it is combined with bentonite, silica gel, gelatin, isinglass or some other fining agents it can be used to clarify wine, beer and mead. When it is added late during the brewing process chitosan usually improves flocculation and removes fruit particles, yeast cells and other detritus that might try to cause hazy wine. Chitosan when combined with colloidal silica becomes a fining agent for white wines due to the fact that chitosan does not need acidic tannins (found primarily in some red wines) with which to flocculate.

Chitosan containing objects have been recycled with the view that the dye will be introduced and then discarded in each recycling step thus facilitating the reuse of the polymer freely of any colorants. This process usually differs from that of the conventional plastics whereby the colorant would be covalently bonded to the structure plus also different plastics are sorted in terms of colors before reuse. Unlike the other plant-based bio-plastics such as cellulose and starch the major natural sources of chitosan is marine environments and they do not compete for land or any other resources required for basic human needs. The method usually employed in the manufacturing with chitosan is dependant on the rate of reproduction of a natural hierarchical design with natural molecules that are associated to it. The fabrication of very large objects with chitosan became the first example for the production of functional objects using this technique and it represents the next iteration of Shrilk, which is a material composed mainly of fibroin protein from silk and chitin from shrimp shells that replicates the unique features of living insect cuticle.

Chitosan has got a very long history for application as a fining agent during winemaking. Fungal source chitosan has also shown a rise in the settling activity, reduction of oxidized polyphenolics in wine and juice, chelation and removal of copper, and also in controlling the spoilage yeast Brettanomyces.

Scientists have managed to develop a polyurethane coating that works by healing its own scratches when it is exposed to sunlight thus offering the promise of a possibility of scratch-free cars, packaging, furniture and drugs. The self-healing coating uses chitosan that has been

incorporated into traditional polymeric materials such the ones used in coatings on cars enable to protect paint. When a scratch harms the chemical arrangement, the chitosan reacts to sunshine by forming chemical chains that link with other materials in the substance ultimately smoothing the scratch. This procedure can take barely less than an hour. Thus the polymer can only restore itself in the similar spot once and it would not work after recurring scratches. Whether this kind of technology can be incorporated to industrial materials however relies on a number of parameters such as long-term perseverance of the stiffness, repair and heat resistance of the coating.

The property of chitosan allow it to speedily clot blood and has lately gained endorsement in the United States and Europe for application in bandages and also other hemostatic agents [18-20]. Chitosan hemostatic products have been publicized in testing by the U.S. Marine Corps to rapidly stop bleeding and to lessen blood loss and effect 100% endurance of otherwise lethal arterial injuries in swine. The hemostatic products of chitosan reduce blood loss in contrast to gauze dressings and rise patient survival. Chitosan hemostatic produces have been retailed to the U.S. Army and are presently used by the UK military. The US and UK have previously used the bandages on the battlegrounds of Iraq and Afghanistan. Chitosan is known to be hypoallergenic and has got some natural antibacterial properties which auxiliary support its application in field bandages.

Chitosan hemostatic agents [28] are frequently chitosan salts made by mixing chitosan by means of an organic acid like lactic or succinic acid. The hemostatic agent functions by contact between the negative charged cell membrane of erythrocytes and the positive charged protonated chitosan leading to participation of platelets and prompt thrombus creation. The salts of chitosan can be mixed with other materials to brand them more absorbent or to differ the rate of solubility and bio-absorbability in the chitosan salt. The chitosan salts are biodegradable and biocompatible making them beneficial as absorbable homeostats [29]. The positively charged protonated chitosan is fragmented down by lysozyme in the human body to glucosamine and also the conjugate base of an acid are those substances that are naturally found in the body. Chitosan salt may also be placed on an absorbable support. The absorbable support may be synthetic that is it can be made from existing absorbable suture materials such as Tephaflex polymer or it can be natural such as cellulose or gelled/solidified honey.

Chitosan's features also permit it to be used in applications such as transdermal drug delivery [30] and it is mucoadhesive in its nature thus reactive and can be synthesized in many different ways and most notably carries a positive charge in acidic conditions. This positive charge emanates from protonation of its free amino functional groups. Nonexistence of a positive charge show that chitosan is insoluble in both neutral and basic environments. Nonetheless in acidic environments protonation of the amino functional groups results to an increase in solubility. The inferences of this are very essential to biomedical applications [31]. This molecule will preserve its structure in a neutral environment but in the case of an acid environment it will solubilize and degrade. This proves that chitosan can be used to convey a given drug to acidic environments a place where the chitosan packaging will end up degrading and thus releasing the drug to the preferred environment. An example of this type of drug delivery is the transport of insulin.

1.6 Glutaraldehyde

Glutaraldehyde is a known organic compound that has the formula $CH_2(CH_2CHO)_2$. It is a pungent colorless oily liquid which is usually used to disinfect medical and dental apparatus. It is as well used during industrial water treatment and also as a preservative. It is mostly available in the aqueous solution state [32] and in all these solutions the aldehyde functional groups are hydrated.

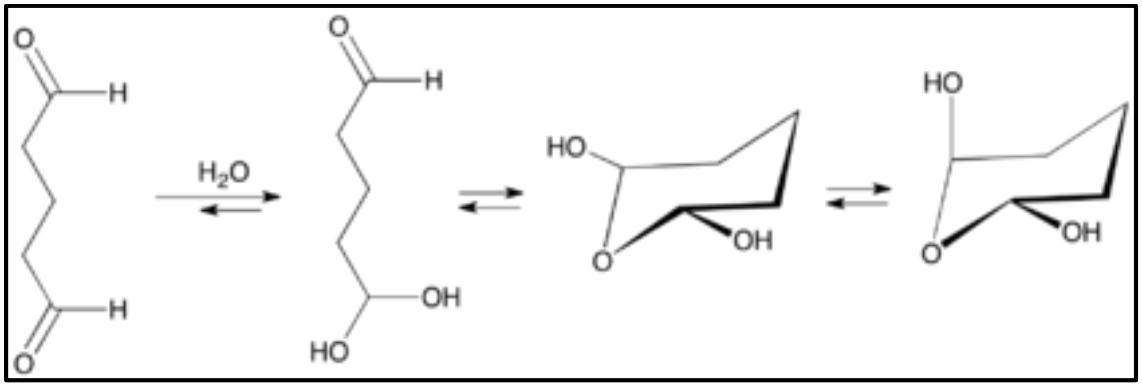

Fig 1.2: Synthesis of glutaraldehyde

Glutaraldehyde is manufactured industrially by oxidation of cyclopentene, the Diels-Alder reaction containing acrolein and methyl vinyl ether and then followed by hydrolysis. Identical

other dialdehydes such as glyoxal, it does not appear as a dialdehyde but as the hydrate. Also these hydrates embrace several structures. Monomeric glutaraldehyde can also polymerize by the aldol condensation reaction producing alpha, β -unsaturated poly-glutaraldehyde. Most of the time this kind of reaction frequently occur at alkaline pH values.

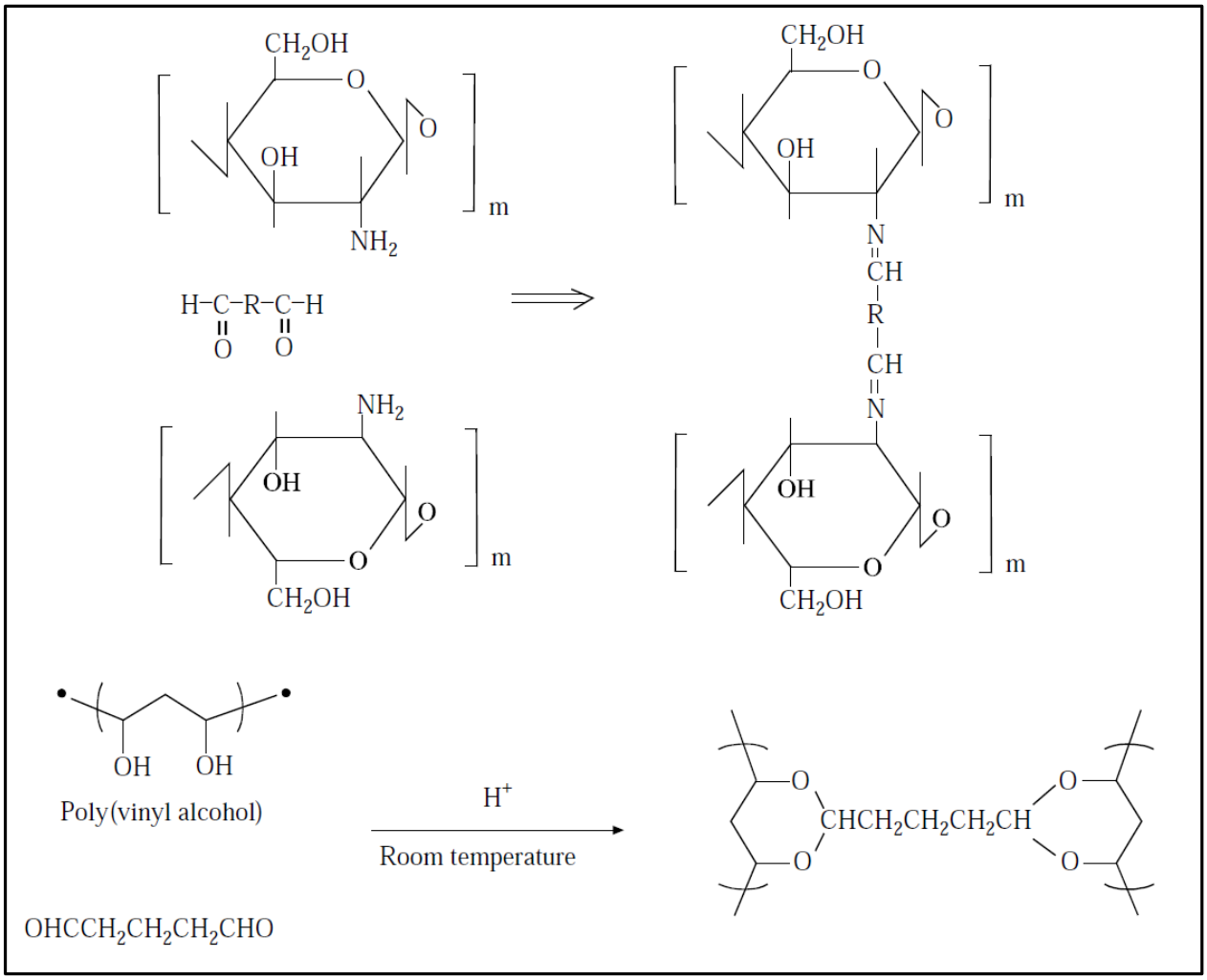

Fig 1.3: Mechanism showing cross-linking of chitosan-PVA

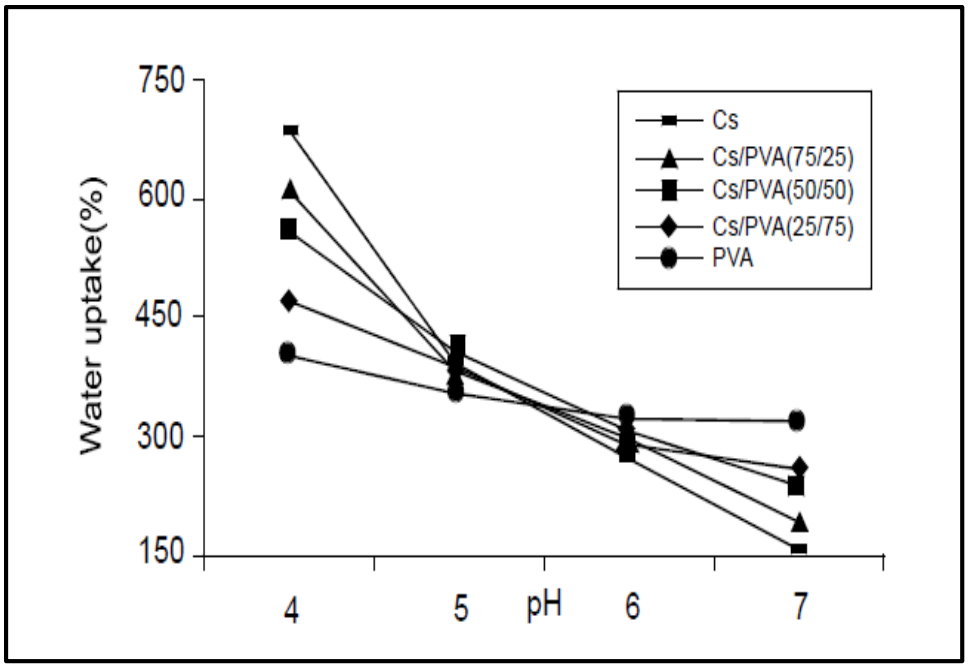

Fig 1.4: Effect of pH and water uptake on the composite film of chitosan, PVA and chitosan/PVA in different ratio

1.7 Chitosan based antimicrobial films for food packaging

Antimicrobial packaging is a packaging organization that is capable to kill or inhibit decay and pathogenic microorganisms that might be contaminating the food. Antimicrobial packaging can lengthen the food shelf-life therefore improving the quality of the food. Chitosan is quite a promising biodegradable polymer that can be used for food packaging. Furthermore chitosan possess massive potential as a packaging antimicrobial due to its biodegradability, antimicrobial activity and also non-toxicity resulting in widespread use over a varied range of applications. The functional features of chitosan films are upgraded when chitosan films are combined with some other film- forming materials. Lastly has been ascertained that chitosan films have very good antimicrobial activity to prolong food shelf life.

Dutaa et al. made chitosan–silver oxide nanocomposite film using the solution casting method [33]. It was shown that the nanosilver comprising chitosan film had an exceptional antibacterial performance. Characteristically the nanocomposite film can be used for wrapping foods that are

highly vulnerable to microbial growth or that are directly used in surface coating mainly on perishable fruits and vegetables to improve microbial safety and prolong food shelf life.

In another study, Li et al. made glucomannan-chitosan-nisin ternary blend film. This research was done to enhance antimicrobial efficacy of eatable film centered on konjac glucomannan by combining chitosan and nisin [34]. Also the antimicrobial efficacy was evaluated against four types of food pathogenic bacteria specifically Escherichia coli, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus. Antimicrobial activity experiments of edible films were conducted using the agar diffusion technique. Film cuts were put on Mueller Hinton agar plates that were earlier seeded with 0.1 ml of inoculums which was containing indicator microorganisms in the region of 105-106 CFU/ml. The widths of inhibitory zones neighboring film discs along with the contact region of edible films with agar superficial were reported with substantial results. The antimicrobial result of chitosan or KC_2 combining nisin were much superior than those of konjac glucomannan combining nisin at each analogous concentration and showed substantial difference ($p<0.05$) nevertheless, there was no major difference on the antimicrobial outcome between chitosan and KC_2 incorporating nisin. Integrating chitosan into konjac glucomannan film (KC_2) consequently improved not only physical features but also antimicrobial activity. The ground work for the preparation of antimicrobial chitosan film that are cross-linked by a naturally occurring aglycone geniposidic acid is a matter that has been reported somewhere else. In this research, a relative study was carried out amongst chitosan film with no cross-linking (fresh) and the glutaraldehyde-cross-linked chitosan film and aglycone geniposidic acid-cross-linked chitosan film. A volume of 50 μL bacterial broth which contains E. coli or S. aureus was scattered onto the film and then cultured. The chitosan film which was fresh and glutaraldehyde-cross-linked chitosan films were also used as the control of the experiment. It was suggested that the contact amongst the polycationic chitosan and that of the negatively charged surface in the bacteria may change the permeability in the bacterial wall and result to leakage of intracellular electrolytes and proteins. These results proposed that cross-linking of chitosan films ensured that their antibacterial capability was not altered. This might have resulted to the circumstance that the cross-linking amounts of glutaraldehyde and aGSA cross-linked chitosan films incorporated in this part of the research were relatively as low-slung as 8%, with the concentration of cross-linking agent in the range of 0.8 mM. The aGSA-cross-linked chitosan film comprised a relatively lesser water vapor permeability, a lesser cytotoxicity,

and a slower rate of degradation than that of the glutaraldehyde-cross-linked film. Lastly, it was established that the aGSA-cross-linked chitosan film can be a favorable material that can be used as an edible film in food packaging. In a different study, Mathew et al. made ferulic acid integrated starch-chitosan blend films to prolong the shelf-life of food [35]. Integration of ferulic acid was seen as an improvement to the barrier features and the tensile strength in the starch-chitosan blend films and meaningfully improve the lipid peroxide inhibition size. The surface pictures took from scanning electron microscope discovered smooth structural films for both the control and the ferulic acid integrated films which indicate excellent compatibility between the components and the plasticizer. On the other hand, the blend film exhibited assured discontinuous zone and small pores whereas the control films were more solid as a result of the networking followed by the ferulic acid. The surface properties of the films were also confirmed by AFM topographic images.

In another study, Pranoto et al. [36] prepared chitosan films by integrating garlic oil, potassium sorbate and nisin to improve antimicrobial activity of the chitosan film. The antimicrobial activity was tried against food pathogenic bacteria specifically Escherichia coli, Listeria monocytogenes, Salmonella typhimurium, Bacillus cereus and Staphylococcus aureus. Antimicrobial examination was conducted using the agar diffusion technique. The agar diffusion technique is a technique commonly used to scrutinize antimicrobial activity concerning the diffusion of the compound which is tested through a water-containing agar plate. Integrating antimicrobial agents into chitosan eatable films thus enhances antimicrobial efficacy of chitosan as the diffused antimicrobial activity add to non-migrated antimicrobial effectiveness of chitosan. It was established that garlic oil integrated into chitosan film resulted in an increase in antimicrobial efficacy, and had slight influence on mechanical and physical features of chitosan films. Generally the integration of garlic oil into the chitosan film has the required characteristic of performing as a physical as well as an antimicrobial barrier to food contamination.

Li et al. [37] also studied on the antimicrobial activity of O-carboxymethylated chitosan / cellulose blended film from a solution containing LiCl/N,N-dimethylacetamide. The antimicrobial activity of the blended films against E.coli was assessed by using the optical density technique. In this method if the optical density (OD) in the medium was found to be smaller it meant that the antimicrobial activity of the film was high. Conferring to this study,

both the blend films show substantial antibacterial activity. Equated to pure medium and the cellulose film, optical densities of blended films are lower. It was established that the antimicrobial activity of blended films may improve if the O-CMCh contamination is elevated. The study discovered that both blended films unveil adequate antibacterial activity against E. coli even if the O-CMCh concentration was merely 2 wt%.

In a different study, Eraricar Salleh et al. [38] suggested the antimicrobial starch-based film integrated with chitosan and lauric acid to prolong food shelf-life. In this research, integrating lauric acid and chitosan into starch based film exhibited noticeable effects concerning inhibition of B. subtilis and E.coli signifying that the film ensured synergistic antimicrobial effect when lauric acid and chitosan were mixed. The solution which contained starch and chitosan at altered mixing ratio of (w/w) 8:2 and 9:1 were the best effective mixing ratio which comprised of greater inhibition on both E.coli and B. subtilis than any other solution in agar plate or liquid culture test. The tensile characteristics of antimicrobial starch-based film were improved by the incorporation of chitosan. The antimicrobial starch-based films can also be used to lengthen food shelf-life.

Thus, the incorporation of chitosan into various polymer matrices to obtain materials with active internal layer for food packaging and/or coating applications constitutes the main theme of the present work. This approach also encompasses the modifications needed to alter the hydrophobic character of the biocide formulations based on chitosan. Conclusively, it can be safely stated that chitosan may prove to be a potential candidate in adopting novel bioactive packaging technologies in order to improve the quality and safety of perishable foods.

1.8 Objectives

- To procure raw materials such as chitosan, PVA, acetic acid, glutaraldehyde
- To prepare chitosan and PVA in optimized weight ratio
- To make the composite film of chitosan and PVA in different weight ratios by using the casting method
- To characterize the binary composite film by use of FT-IR, XRD, TGA, SEM
- To characterize the mechanical properties of the binary film using the universal tensile testing machine

- To check the antimicrobial and anti-bacteria activity using the gram negative and gram positive bacteria
- To check on the toxicity, biocompatibility and biodegradability of the film

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CHAPTER 2

EXPERIMENTAL WORK

2.1 Materials used

Tryptone bacteriological with product code 02075 and batch number 09032 was purchased from Avarice Company Pvt. Ltd GB Nagar India, yeast extract powder bacteriological with product number 28605 and lot number 2076 7217-7 was purchased from Thermo Fisher Scientific India Pvt. Ltd Powai Mumbai, agar agar powder with product code 02008 and batch number 080707 and was purchased from Avarice Laboratories India and sodium chloride (NaCl) with molecular mass of 58.44 and product code of 02750 an batch number of 100304 was purchased from Avarice Laboratories GB Nagar India and was also ISO certified. Acetic acid glacial with molecular weight 106.17 was purchased from Speckpure Chemicals India, polyvinyl alcohol (hot) with product number 030573 was purchased from Central Drug House Laboratory New Delhi together with glutaraldehyde ($C_5H_8O_2$) 25% for synthesis with molecular weight 100.12 and the pure chitosan ($C_6H_{11}NO_4$) n with molecular weight of 193400 was purchased from Sisco Research Laboratories in Mumbai. Ethanol absolute for analysis was purchased from Merck Chemicals in India.

2.2 Film preparation

The polymer films were prepared by solvent casting method. Chitosan solution was prepared by dissolving 3g of chitosan in 15mls of 1% aqueous acetic acid solution at room temperature with stirring. Stirring was done until all the chitosan had homogenously dissolved in the acetic acid.

Fig 2.1: Preparation of chitosan solution

3g of PVA was dissolved in 15ml of distilled hot water by use of a magnetic stirrer and also heating at a temperature of about 60ºC. The PVA solution was allowed to cool so that it won't form scales when mixed with chitosan in its hot state. This cooled solution was then mixed with the chitosan solution and these two were homogeneously mixed so as to get a uniform solution.

Fig 2.2: Preparation of PVA solution

A cross-linking agent (glutaraldehyde) was then incorporated (2-3 drops) so as to avoid phase separation of the films and this blended solution was mixed thoroughly by use of a magnetic stirrer for about 5min. The samples were then allowed to air dry for 24h and their properties were tested.

The following images show how the films formed in the petri plates over 24h.

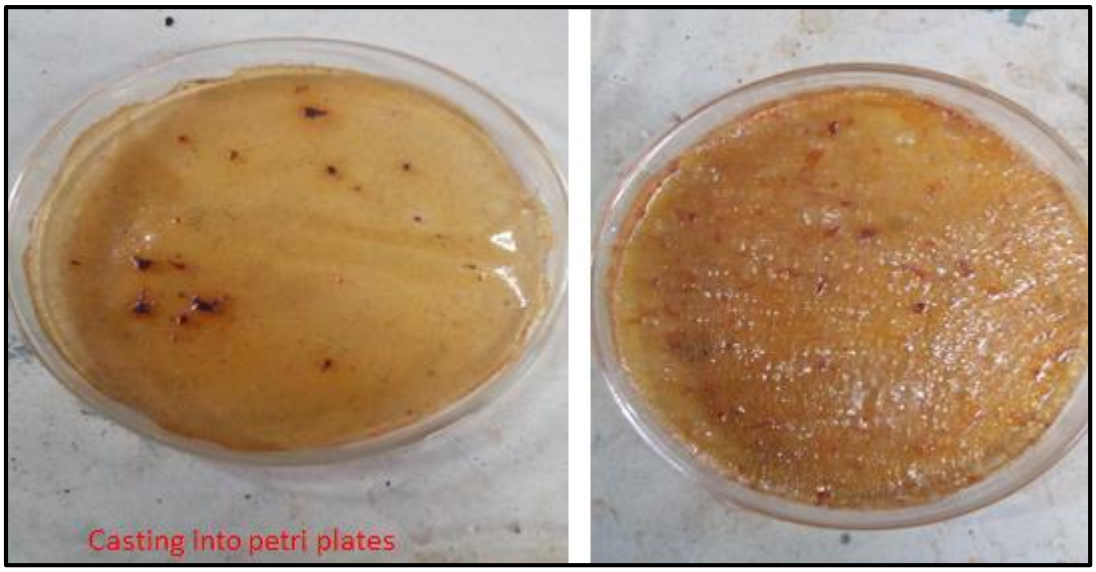

Fig 2.3: Casting method

In the casting method the solution containing chitosan/PVA was poured slowly making sure that no air bubbles are present ensuring that the solution is even spread over the petri plate so as to have a film with uniform properties when carrying out tests. The solution was to be put in petri plates that had a polystyrene sheet underneath to make sure that when the film solidify after 24hrs it would be easy to remove and won't stick to the walls of the petri plate.

2.3 Antimicrobial activity test

Microbiological culture, or microbial culture, is a method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions [1]. A suitable environment was made and then the bacteria were cultured and the films were spread over the solidified luria broth media. 1.25g of yeast extract, 2.5g of sodium chloride, and 2.5g of tryptone were dissolved in 250ml of water. They were mixed properly by stirring the pH of the solution was to be maintained at 7.4 and from the resulting mixture 3.75g of agar agar powder was added and the mixture was stirred well. The stirred mixture was sterilized by autoclaving at 15 psi pressure (121°C) for 15 min. The solution was allowed to cool and then poured under laminar air flow into petri plates. A solid media was observed after 24h and cultures of bacteria were spread on the solid media by use of an inoculation loop. Small pieces of the samples were cut and put on top of the solid media and daily results were noted. The samples were kept in an incubator at a temperature of 37°C for one week to see any observable changes.

Fig 2.4: Lb media solution under laminar flow

Laminar flow hoods work by protecting the working environment from dust and airborne contaminants by preserving a constant, unidirectional flow comprising of HEPA-filtered air over the working area. This flow can be blowing in different directions that is horizontal or parallel to the working surface or even vertically onto the work surface. This kind of hood provides protection to the culture that is it's mainly for product protection. Every equipment present and the hands of the user were disinfected with ethanol solution so as to avoid any source of contamination.

Fig 2.5: Streaking the bacteria on the solid using innoculation loop

When streaking the specimen the metal transfer loop is first sterilized by holding the wire in a red hot flame by use of a Bunsen burner. After it is passed through a red hot flame it is the allowed to cool making sure the wire is being hold still. The lb media is opened just enough to streak it with the wire and it should not be opened for a lengthy time. The bacteria is then taken using the sterilized innoculation loop then spread over the lb media in a zig zag fashion making sure that the area that has been streaked before is not streaked twice.

Fig 2.6: Placing binary film on solid media

After streaking was complete small pieces of the blended film of chitosan/PVA with different weight ratios were then placed on the nutrient broth and their bio-activity observed over 14days. The petri plates were then closed tightly and then put in the incubator at a temperature of about 37^{o}C as the bacterial growth is mostly favored by warm and dark environment.

2.4 Behaviour of a tomato in the chitosan/PVA solution

Small pieces of a tomato were cut and put into solution containing chitosan/pva and the behavior of the tomato was observed over 30days and see whether the tomato will go under bacterial attack when left under room temperature.

2.5 Packaging the tomato into the chitosan/PVA films

The prepared films were used to wrap a tomato to check whether they can be used as food packing material under different conditions. Two test were done in which the first one involved wrapping the tomato at room temperature to see if the a packaging film or tomato will undergo any form of changes at room temperature. In the second test the tomato was wrapped in the film and put in the refrigerator and note any observable changes to the packaging film.

2.6 CHARACTERIZATION TECHNIQUES

2.6.1 Fourier Transform Infrared (FT-IR) Spectroscopy

Infrared spectroscopy has been a pillar method for materials investigation. An infrared spectrum characterizes a fingerprint of a sample through absorption peaks [1] which match to the frequencies of vibrations among the bonds of the atoms building up the material. For the reason that each different material is an exceptional combination of atoms and no two compounds yield the exact identical infrared spectrum. Hence, infrared spectroscopy can end in a positive identification that is qualitative analysis of every different type of material. Furthermore, the magnitude of the peaks in the spectrum show a direct indication of the quantity of material present. With present software algorithms, infrared is an outstanding tool for quantitative analysis therefore it can be labelled as a method for studying the molecular orientation and ordering. Infrared (IR) spectroscopy identifies the vibration appearances of any chemical functional group in a given sample. When an IR radiation is permitted through a sample, more or less of the infrared radiation is captivated by the sample while some is passed through and the other transmitted. The resulting spectrum signifies the molecular absorption and transmission, generating molecular fingerprint of the sample. There are two kinds of fundamental vibrations for molecules: stretching, in which the distance between two atoms increases or decreases, but the atoms remain in the same bond axis, and bending or deformation, in which the position of the atom changes relative to the original bond axis. The various stretching and bending vibrations of a bond occur at certain quantized frequencies. When infrared light of that same frequency is incident on the molecule, energy is absorbed and the amplitude of that vibration is increased [1, 2]. When the molecule reverts from the excited state to the original ground state, the absorbed energy is released as heat. So after interaction of IR light with the material, chemical bonds will be stretched, contracts and bends. As a consequence, a chemical functional group will likely to adsorb IR in a particular wavenumber range irrespective of the structure of the whole molecule. For instance, the C=O stretching of a carbonyl group will come at around 1700cm-^{1} in a range of molecules. Therefore, the correlation of the bond and wavenumber position with the chemical structure is used to identify a functional group in a sample.

A beam splitter splits laser light going into an input port into which the transmitted beam and a reflected beam are perpendicular to each other. Right at the end of every beam's path, a mirror redirects the light towards the beam splitter. When these two beams' paths have just the same

length, beams' electric fields oscillate in a phase when the light goes back to the beam splitter. Also the beams recombine to yield a beam that leaves the beam splitter beside the output port. If these two paths vary in length by half wavelength, they are considered as out of phase. So therefore, they are said to interfere destructively at the beam splitter thus no light leaves from the output port. The strength of light exiting the output port fluctuates from maximum to zero and the relative distance mirrors at the end deviates by a quarter of a wavelength which is about 2.5 x 10-7 meters for a characteristic laser light. Exactly measuring this light intensity permits experimenters to identify even the smaller relative displacements of mirrors. Also any passing gravitational wave ought to compress space time in what is seen as one direction and stretching it in the perpendicular direction [1, 2].

A Simple Spectrometer Layout

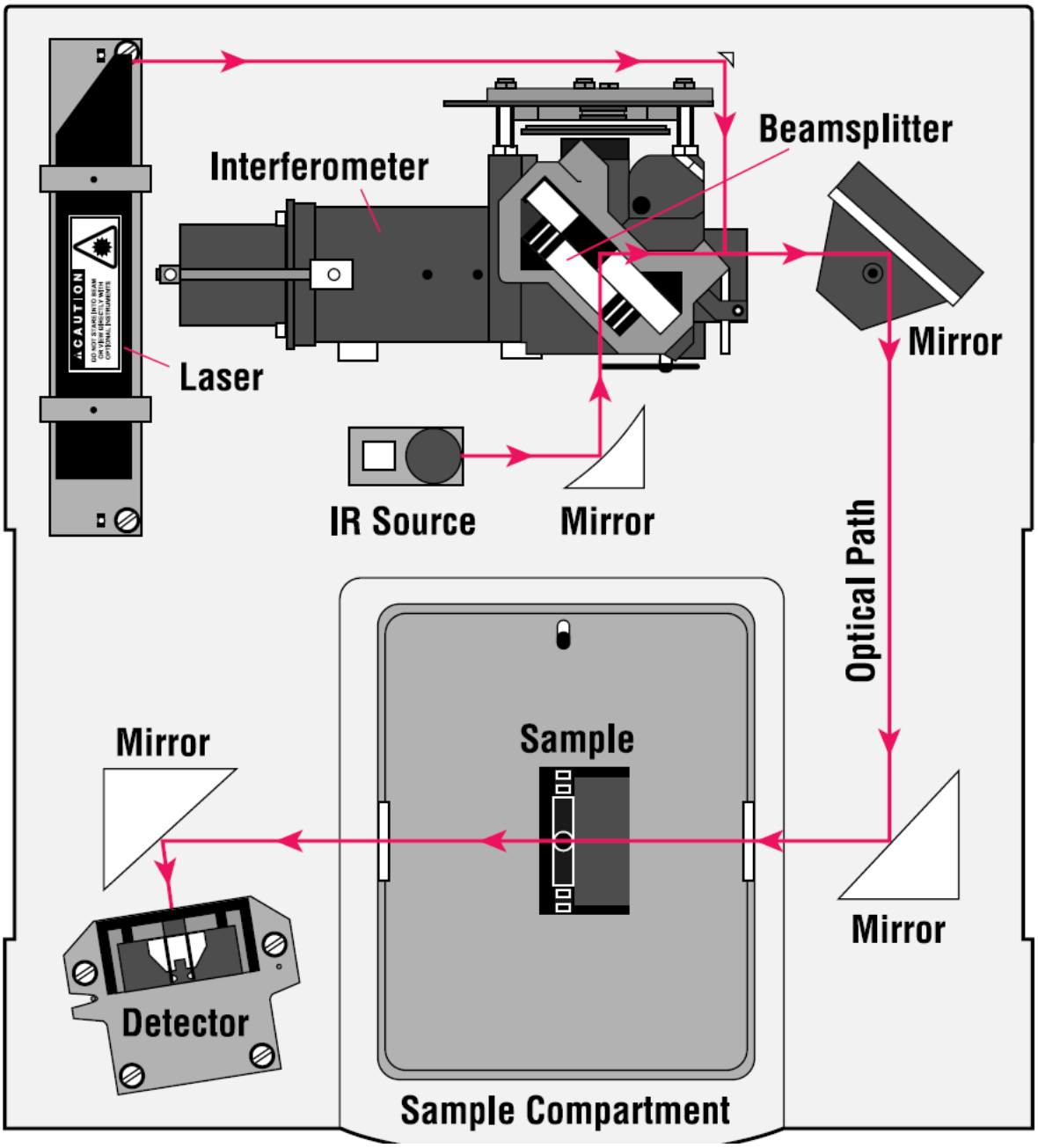

Fig 2.7: Schematic representation of FT-IR

2.6.2 Mechanical Testing

Tensile properties

The percentage elongation at break and also the tensile strength of the films is determined using a Universal testing machine. The initial grip distance is put at 50 mm and the rate of grip separation is 5mm/minute. The specimens were 5mm wide and almost 10mm. The thickness of each specimen was measured with a venier calipers [3].

The utmost common testing machine that is used in tensile testing is the universal testing machine. This type of machine has got two crossheads that are positioned in such a way that one is adjusted for length of the specimen/sample and the other is focused on applying tension to the test specimen. Thus there are two types which are:

- Hydraulic powered and
- Electromagnetically powered machines.

The machine must also possess the proper abilities for the test specimen to be tested. There are four key parameters:

- Force
- Capacity
- Speed, and
- Precision and accuracy

Force capacity mainly looks at the point that the machine need be able to generate sufficient force to cause fracture the specimen/sample. The machine must also be capable to exert the force rapidly or gradually enough to properly simulate the real application. Lastly, the machine must be capable of accurately and precisely measuring the gauge length and forces that are applied for example, a large machine that is intended to measure long elongations may perhaps not function on a brittle material that involves short elongations preceding to fracturing.

Test specimen alignment in the testing machine is key, because when the specimen is misaligned, at an angle or maybe offset to just one side, the machine will apply a bending force on the sample/specimen. This is particularly bad for brittle materials, as it will affectedly skew

the end results. This condition can be reduced by using either spherical seats or maybe U-joints between grips and also the test machine. When the initial area on the stress–strain curve is found to be curved and not linear, it shows the specimen was misaligned in the testing machine.

Also the strain measurements are most usually measured with an extensometer, however strain gauges are also normally used on small test sample or when Poisson's ratio is measured. Fresher test machines have the digital time, force and also elongation measurement systems that consist of electronic sensors that are connected to an information collection device and software to help manipulate and output the information. Nonetheless, analog machines endure to meet and surpass ASTM, NIST, and also ASM metal tensile testing accuracy prerequisites continuing to be used today.

Process

The test process comprises putting the test specimen/sample [3] in the testing machine and then slowly extending it until fracture is observed. In the course of this method, the elongation of the gauge area is then recorded against the force applied. The information is worked so that geometry of the test sample is not particular. The engineering strain, ε, is then calculated from the elongation measurement using the following equation:

$$\varepsilon = \frac{\Delta L}{L_0} = \frac{L - L_0}{L_0}$$

Where

ΔL: Change in gauge length

$L_{0:}$ Initial gauge length

L: Final length.

The force measurement is then used to calculate the engineering stress (σ) by means of the following equation:

$$\sigma = \frac{F_n}{A}$$ a

Where

F: Tensile force and A: Nominal cross-section of the sample

The machine prepares these calculations when the force increases, so that the information points can be put into a stress–strain curve.

2.6.3 Scanning Electron Microscopy

The surface Morphology of the film samples is determined using a HITACHI microscope under a high voltage of around 15-20kV.

A standard scanning electron microscope functions at a high vacuum [4]. The simple principle is that beam of electrons are produced by an appropriate source, characteristically a tungsten filament or some field emission gun. This electron beam is usually accelerated by means of a high voltage for example 20 kV and is passed through an arrangement of apertures and also electromagnetic lenses to yield a very thin beam of electrons and then the beam start scanning the surface of the sample by use of scan coils. Electrons are produced from the specimen by the use of the scanning beam and then collected by a properly-positioned detector [5]. The operator of the microscope will be viewing the image on a screen. Visualize a spot scanning through the screen from left to right. When at the end of this screen, it should drop down a line that scans across again, the method being repetitive down to the foot of the screen. The fundamental to how this scanning electron microscope works is that beam scanning the specimen surface should be precisely synchronized with the spot that is in the screen which the operator is watching.

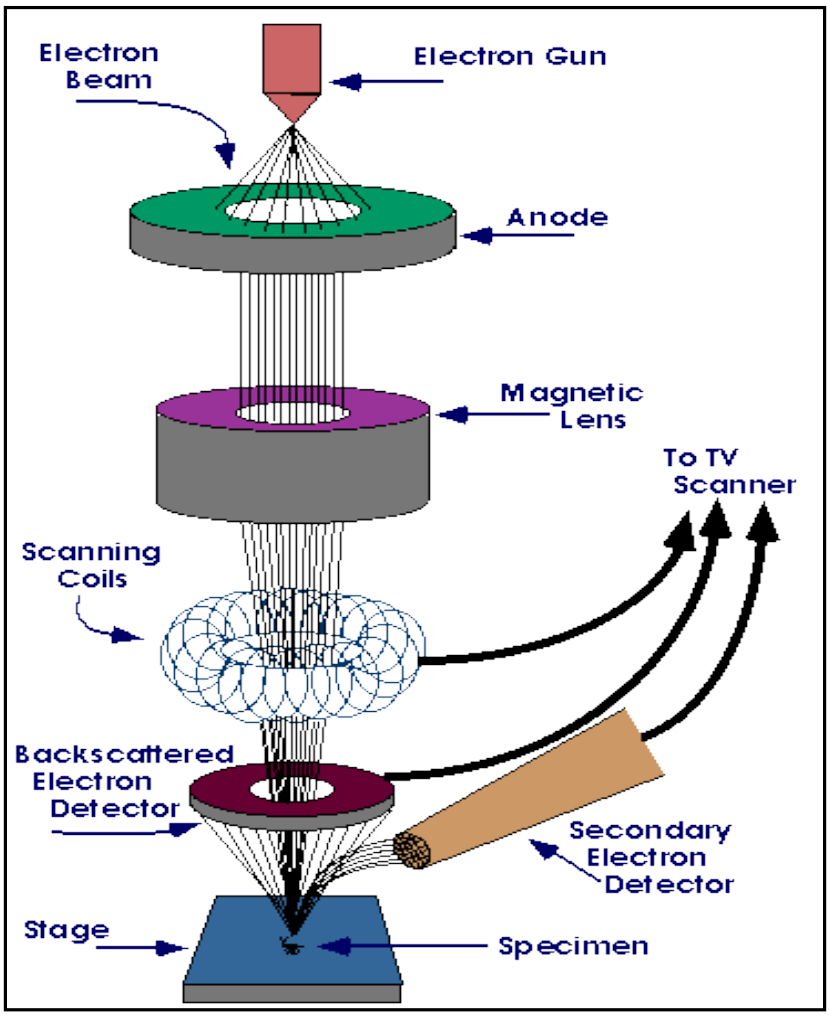

Fig 2.8: Schematic representation of scanning electron microscopy

An electron detector regulates the brightness on the screen as the detector identifies more electrons from a specific feature, the brightness of the screen is increased. Once there are less electrons, the spot which is on the screen becomes darker. Nowadays, the screen is normally a digital monitor, not of glass but the principle remains the same [5]. The enlargement of the image is known as the proportion of the screen's size to the scanned area on the specimen. When the screen is about 300 mm across with a scanned area of specimen as 3 mm across, the magnification should be x100. To get to a much higher magnification, the operator has to scans a slightly smaller area and if the scanned area is found to be 0.3 mm across, the magnification should be x 1000. There are different sorts of electron image and the two most known ones are the backscattered electron image (bei) and the secondary electron image (sei). The secondary

electron image is used mostly to image fracture surfaces and also gives a very high resolution image. The backscattered electron image is used characteristically to image a polished area and the brightness of the backscattered electron image is also reliant on the atomic number of a given specimen. For example, lead will seem brighter than iron and also calcium oxide will seem brighter than calcium carbonate. The backscattered electron image is, in principle, an atomic number map of any specimen surface. All the SEM images are found in black and white, even though they may have some made-up colours applied for aesthetic reasons.

Fig 2.9: Image of SEM

2.6.4 Atomic Force Microscopy

The atomic force microscope (AFM) is a well-known high-resolution type of scanning microscope with a proven resolution that is around a fraction of a nanometer which is far more than 1000 times superior to the optical diffraction limit. SPMs are intended to measure properties, such as height with, magnetism, friction with a probe [6]. To obtain an image, the SPM scans the probe on a small area of the specimen/sample, measuring the local property concurrently. The atomic force microscope textures the surface of the sample with such a selective touch which are able to sense even the individual atoms that are present on a crystal surface such as gold. The AFM performs this by raster-scanning a very small tip back and forward over the sample surface. This tip is on the cantilever end which repels when the tip come across features on the sample surface. This repulsion is sensed by an optical lever or otherwise the red line: a laser beam that reflects off the cantilever end onto a segmented photodiode then magnifies small cantilever deflections into very large changes in the intensity of the laser light upon the two photodiode segments. Using this principle the AFM produces a topographic map showing the sample surface.

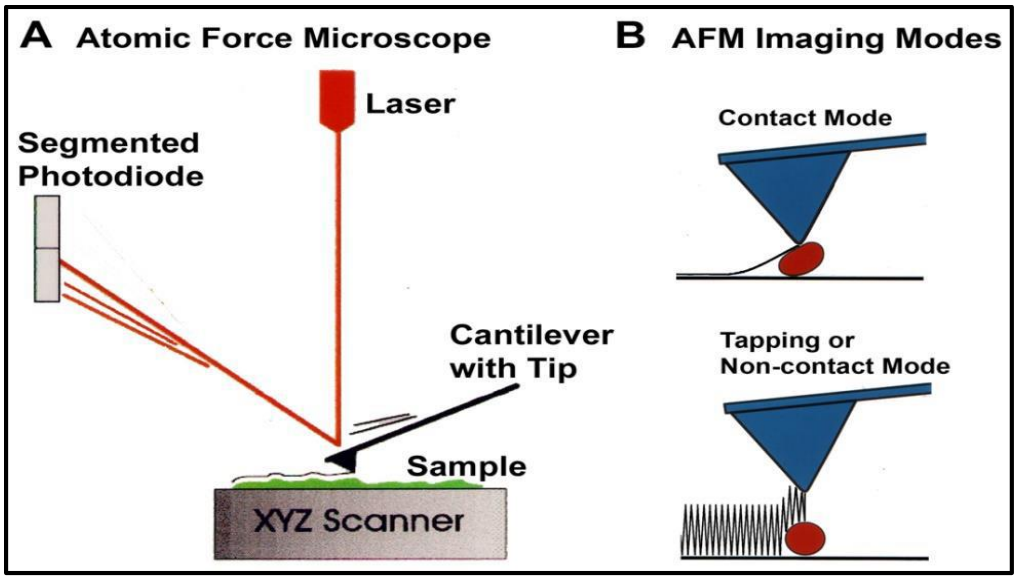

Fig 2.10: (a) Working principle of AFM and (b) AFM imaging modes

2.6.5 X-ray diffraction

X-ray diffraction (XRD) is operated for identification of polymorphism mainly in crystalline [7] organic materials. The stability of these organic materials is a vital issue in the production and performance of imaging devices and materials. A well-known problem that happens in organic materials is that of polymorphism. Polymorphism is the capability of singular molecular species to be able to crystallize in beyond one crystal structure. The multiple crystal structures are as a result of diverse packing configurations of molecules in a crystal. Pseudo-polymorphism is known as an irregular of polymorphism, where a solvent molecule is a portion of the lattice leading in a crystal structure change. By means of appreciating the existence or nonexistence of polymorphism in organics, XRD is regularly used as the technique of choice for detecting these crystal phase modifications. Hence the importance to know that if a material is found to be amorphous, XRD may give minimal data associated to polymorphism. X-ray diffraction is an influential technique for studying nanomaterials thus it can be seen as a primary tool for examining the structure of nano-materials.

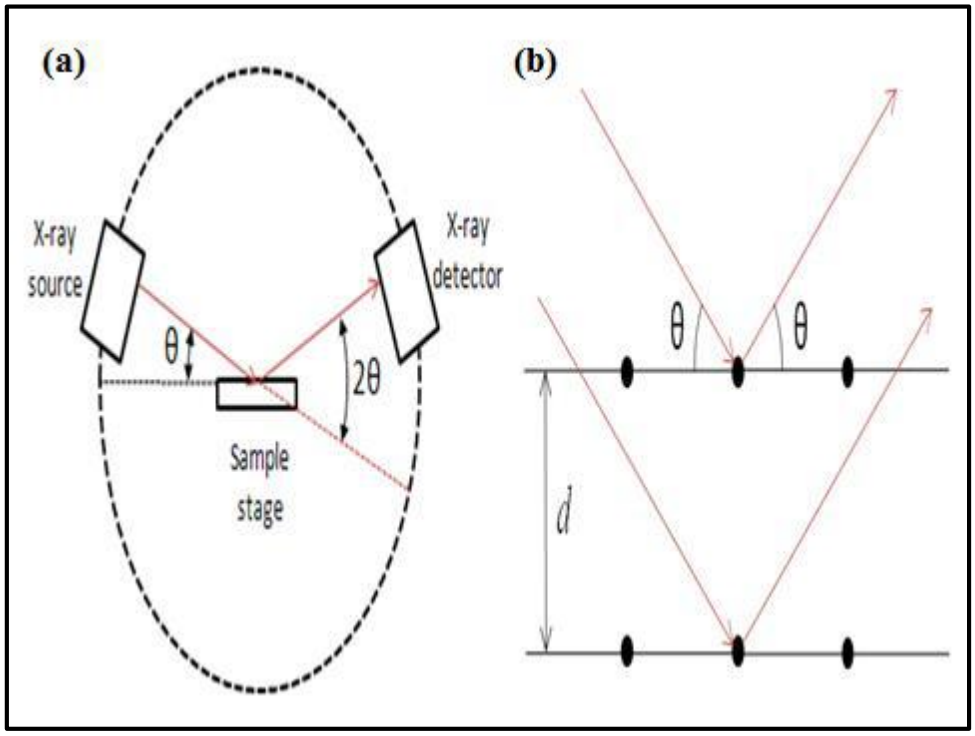

Fig 2.11: (a) Schematic representation of X-ray diffractometer and (b) Bragg‘s law

The lattice planes in the modest crystal above are separated only by a distance d. Now applying The Bragg's law links the wavelength (λ) of the reflected X-ray, the spacing between the atomic planes (d) and the angle of diffraction (Θ) as follows:

$2d \sin\Theta = n \lambda$ …… (2.1)

The angle stuck between the transmitted beams and diffracted beams will all the time be equal to 2Θ. This angle can be attained readily in experimental circumstances and the outcomes of X-ray diffraction are thus given in terms of 2Θ. However it is very essential to recall that the angle used in Bragg's equation must relate to the angle that is between the diffracting plane and incident radiation, that is Θ. In the first order diffraction the value of n=1 and also knowing Θ and λ, it is possible to calculate the interplanar spacing value of d for a specific plane. The first stage of X-ray diffraction pattern includes the indexing of XRD peaks. This indexing means allocating the exact Miller indices for every peak of the diffraction pattern. Also there are three key techniques for indexing a diffraction pattern [7, 8], which are as follows

- matching the measured pattern of XRD with that of the standard data base that is (JCPDS-cards)
- analytical methods
- graphical methods.

The intensity of diffraction signal is generally plotted against the diffraction angle of 2Θ [°], however d [nm] or 1/d [nm-1] can as well be used. The most known wavelength used in XRD is 1.54 Å (Cu Kα). The line broadening may be a measure of the typical size of the crystallites by use of the Scherrer formula.

$D_v = K \lambda / \beta \cos\Theta$ …… (2.2)

Where

D_v: average particle size

λ: wave length of the radiation

β: full width at half maximum of the reflection peak

K: Scherrer constant

The Scherrer constant in the above formula justify for the nature of the particle and is usually taken to have the value of 0.9. The size found from the Scherrer formula produces the outward or average particle size of a given material. Powders of materials are mostly aggregates of the smaller particles and therefore comprise of a dispersal of particle size.

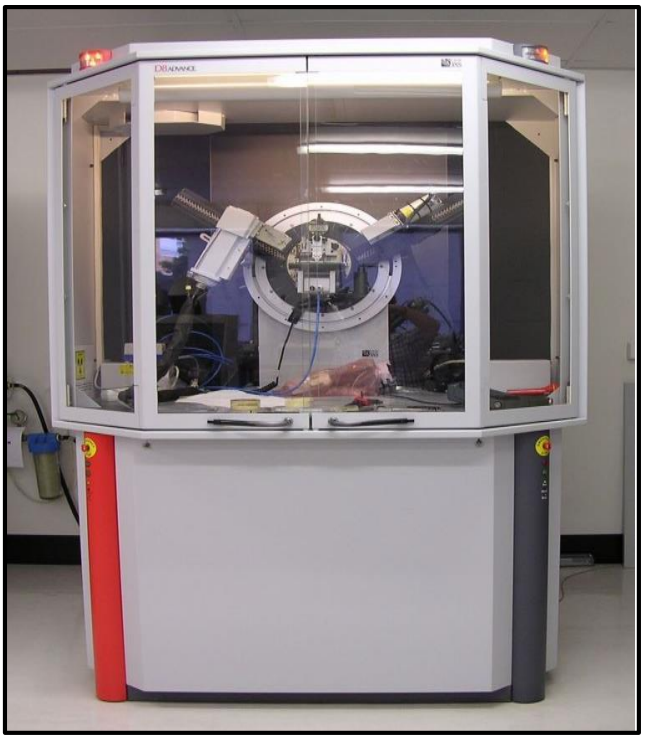

Fig 2.12: X-ray diffraction machine

2.6.6 Swelling analysis

The swelling analysis of the dried blend films is carried out in distilled water. The film samples were first conditioned at 50^{o}C for 24h in an oven, and the conditioned samples were weighed on a digital balance with a precision of 0.0001 gm. The specimen were then immersed in distilled water under room temperature for at least 10h. The water that was on the film surface was removed using tissue paper and the weight of the film was weighed [9].

The swelling degree was obtained using the following formula:

Degree of swelling (%) = [(W2-W1)/ W1] x 100

Whereby W1: weight of dried sample

W2: weight of swelled sample

2.6.7 Thermogravimetric analysis

Thermogravimetric analysis is an analytical method that is used to determine the thermal stability [10] of a material and also its portion of volatile components by observing the weight change that occurs after the specimen is progressively heated. The weight of the sample is checked continuously as the temperature is increased at a constant rate or in a series of steps [11]. The compositions of the given polymer or elastomer will volatiles or decompose at various temperatures this results in having a serious of weight losses that can be measured quantitatively. The apparatus comprises of an analytical balance that will be supporting a platinum crucible which will be placed in an electric furnace where there will be thermocouple to accurately measure the temperature. This measurement is usually carried out in an inert atmosphere containing either Helium or Argon whereby the weight loss is noted as a function of temperature increase. Occasionally this measurement is done in a lean oxygen atmosphere that contains between 1-5% oxygen in nitrogen or helium enable to slow down oxidation.

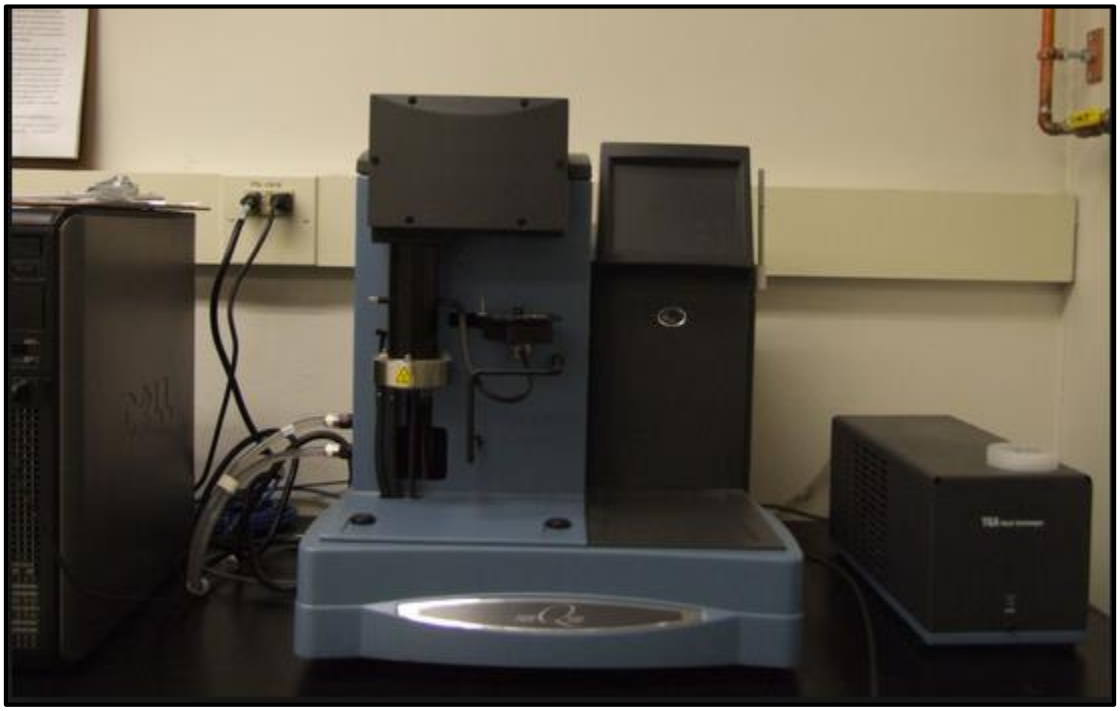

Fig 2.13: Thermogravimetric analyzing machine

TGA is mainly employed in researches and testing to investigate the characteristics of materials like polymers thus obtaining their degradation temperatures [10,11], content of moisture absorbed, level of both organic and inorganic component present in the material, solvent residues and also decomposition point of explosives. It can also be used in determining the corrosion

kinetics in very high temperature oxidation. In most situations, TGA analysis is carried out in an oxidative atmosphere with a linear temperature ramp. Maximum temperature is selected in such a way that the specimen weight will be stable at the completion of the experiment, indicating that all the chemical reactions are completed thus all of the available carbon is burnt.

A technique known as the hi-res TGA is often employed to achieve a greater accuracy in areas were the derivative curve show peaks. In this technique an increase in temperature slows as the weight loss of the samples increases. This is usually done to ensure that the precise temperature at which a peak happens can be more accurately recognized. Concurrent TGA-DTA/DSC measures heat flow and weight loss of the material as a function of the given temperature in a controlled atmosphere. Concurrent measurements of these two material properties does not only improve productivity but also simplifies the interpretation of the results.

2.7 REFERENCES

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CHAPTER 3

3. RESULTS AND DISCUSSION

3.1 The resulting films in terms of their compositions

The films were casted into the petri plates using different blend ratios of chitosan and PVA to obtain films which had different properties.

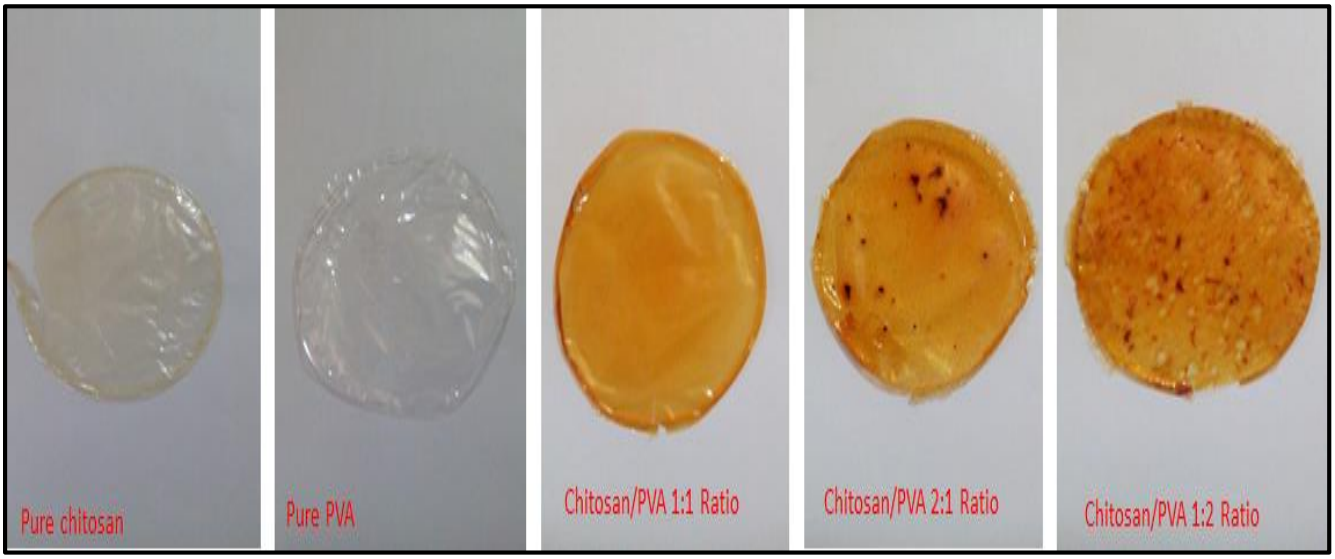


Fig 3.1: Blend films in terms of their weight compositions

Pure chitosan is a brittle film which shows some signs agglomeration whereas pure PVA film is a clear film which has no signs of and is very flexible showing very high elongation. From the above images it can be seen that as the ratio between the weight compositions of chitosan and agglomeration PVA drift away from 1:1 composition the film ceases to be smooth. A high concentration of chitosan resulted in a more brittle film which shows some form of agglomeration as can be seen by the chitosan/PVA 2:1 ratio and a high concentration of PVA resulted in having a film that was very flexible like a rubber with high elongation properties and this was observed in the film containing chitosan/PVA 1:2 ratio. The best film properties were observed in the 1:1 ratio whereby a smooth film that had good flexibility and elongation properties was observed. This film did not show any signs of agglomeration and its transparency was also excellent.

3.2.Structural characterization

3.2.1. X-ray diffraction

This technique was used to investigate the crystal lattice arrangements and also to determine the sample degree of crystallinity the x-ray patterns of the pure chitosan and that of the blended chitosan/PVA as shown below.

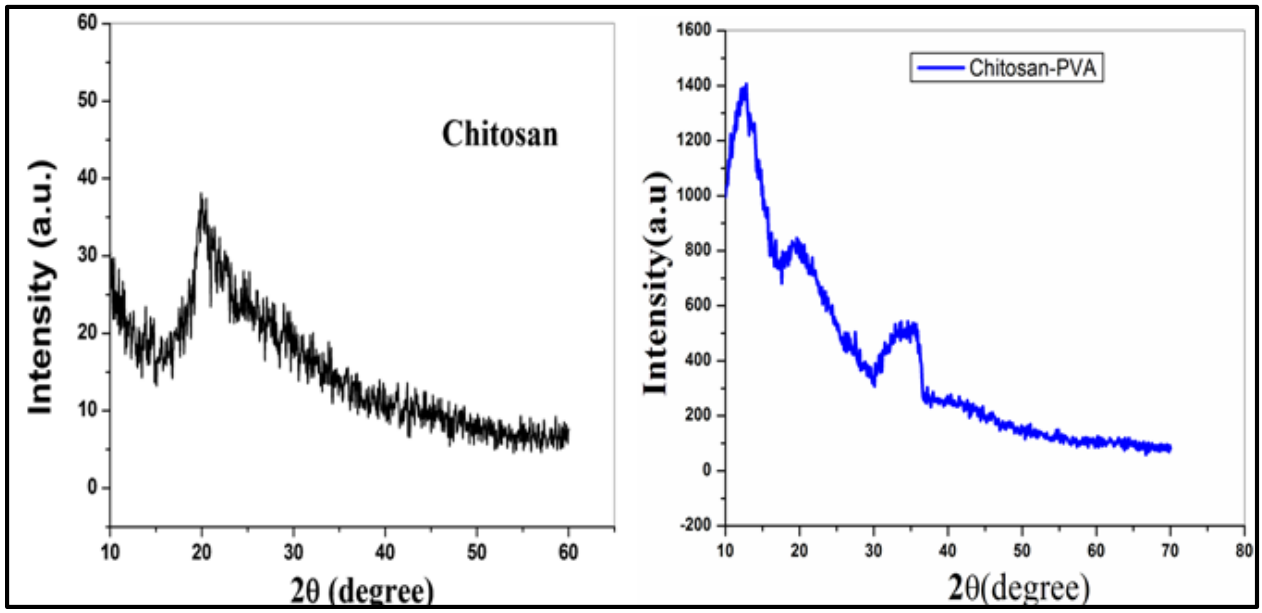


Fig 3.2: XRD of pure chitosan and chitosan/PVA

The diffraction peak of pure chitosan was found to be at 10º and 20º of 2ϴ. The highest peak of pure chitosan was noted at 40counts at 2ϴ =20 which was far much less than the peak obtained from the blended films. From this information it could be seen that pure chitosan is semi crystalline in structure. Blending the films improved the crystallinity of these bioactive chitosan films as the diffractions peaks are very higher than in pure chitosan. The chitosan/PVA diffraction peaks shows a very high peak intensity of about 1400 counts at 2ϴ =14.5. Thus from this data it can be seen that the film will become more crystalline once the amount of PVA increases [1].

3.2.2 Fourier Transmission Infrared Spectroscopy

This technique was used to analyze or determine the functional groups that are present in the pure chitosan by checking the absorption peaks which corresponds to the frequency of vibration among the bonds of the atoms building up the material.

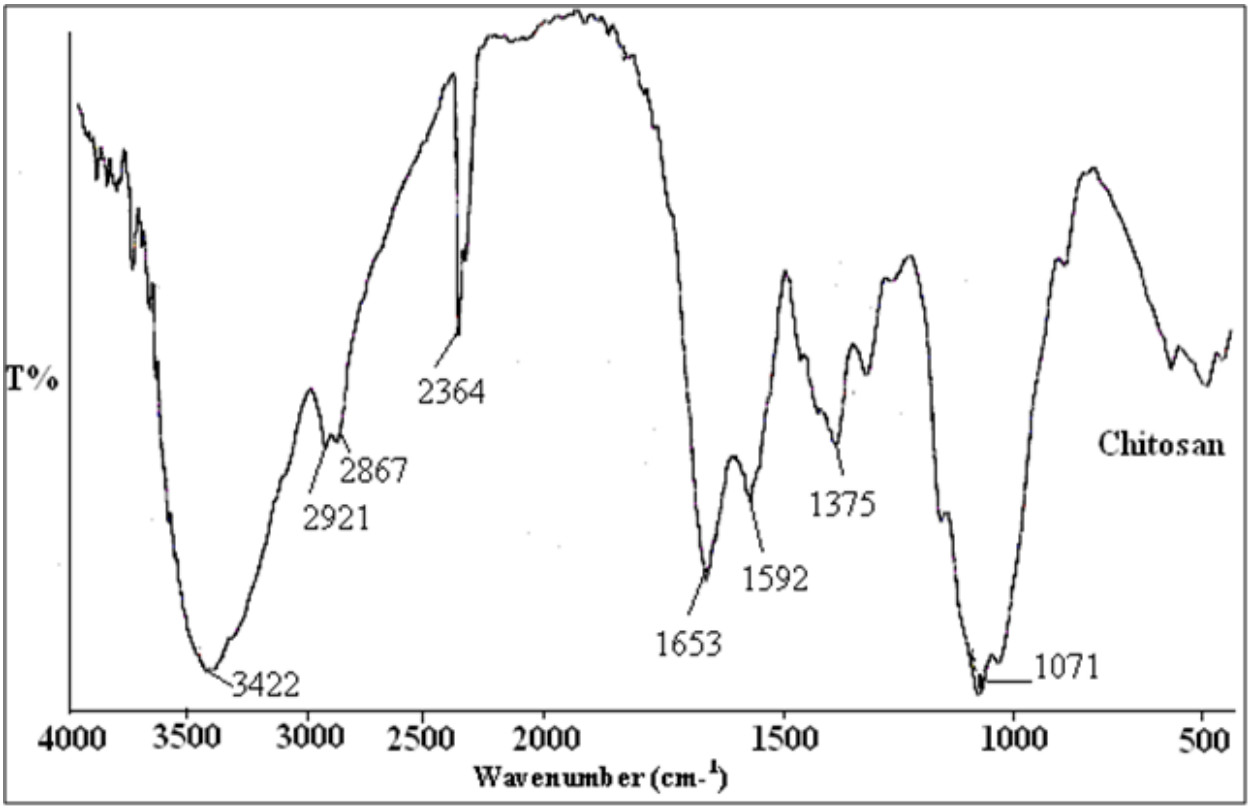


Fig 3.3: FTIR of pure chitosan

Pure chitosan spectrum shows peaks around 893 and 1156 cm^{-1} corresponding to saccharide structure. There were several peaks clustering in the range from 1510 to 1570 cm^{-1}, but there were still very strong absorption peaks at 1660 and 1320 cm^{1}, which are characteristic of chitosan. The sharp peaks at 1383 and 1424 cm^{-1} were given to the CH_3 [2] symmetrical deformation manner. The broad peak observed at 1040 and 1090 cm^{-1} shows the C-O stretching vibration in the chitosan. A different broad peak at 3450 cm^{-1} is a result of the amine N-H symmetrical vibration, and is mostly used with 1650 cm^{-1} for quantitative investigation of the deacetylation of chitosan. Peaks at 2810 and 2900 cm^{-1} are the typical C-H stretch vibrations [3-5].

FTIR spectroscopy was used to evaluate the polymer chemical groups (chitosan and PVA) and studying the formation of mainly cross-linked networks [6, 7] from the blends of glutaraldehyde. The above diagram shows the FTIR of the chitosan/PVA blend film. The IR spectra of the Chitosan/PVA blended films are different from that of the chitosan due to the ionization of primary amino functional groups.

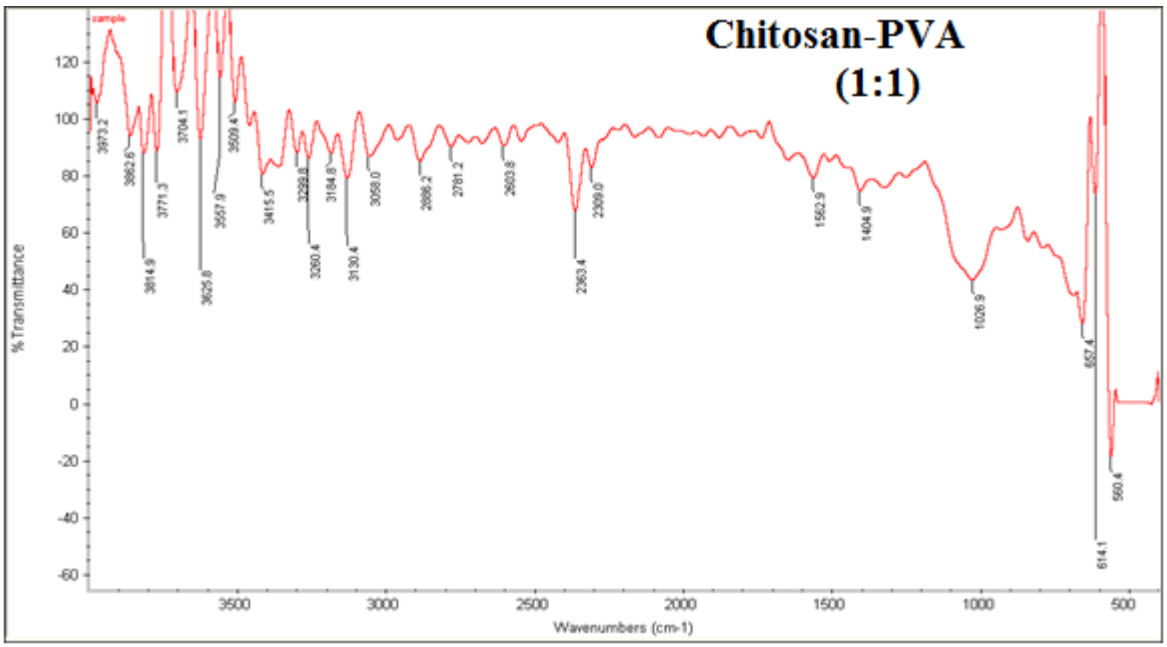


Fig 3.4: FTIR of chitosan/PVA

There are basically two distinct peaks at 1408 and 1550 - 1560 cm^{-1}. Formation of the 1550 - 1560 cm^{-1} peak is a symmetric deformation of the NH_3 resultant from ionization of primary amino functional groups in the acidic medium however the peak at 1408 cm^{-1} shows the existence of carboxylic acid. The peaks which are observed at 1700 - 1725 cm^{-1} are typical of the carboxylic acid. In recent study, the existence of carboxylic dimmer was a result of the acetic acid which was used to dissolve the chitosan. Also the peak at 1210 - 1300 cm^{-1} is due to the C=H vibration.

The FTIR spectrum of pure PVA from literature shows absorption peaks where all main peaks associated to hydroxyl and acetate groups are detected. More precisely, the broad band detected between 3550 and 3200 cm^{-1} is connected with the stretching of the O-H group from the intermolecular and also intra molecular hydrogen bonds. Furthermore the vibrational band

detected between 2840 and 3000 cm^{-1} refers to the stretching of the C=H group from alkyl groups and also the peaks between 1760 and 1740 cm^{-1} are caused by the stretching of the C=O and C-O from the acetate group residual from PVA that is saponification reaction of the polyvinyl acetate.

3.2 Antibacterial activity test

The control experiment (a) was used to check whether any unknown hazardous bacteria would have affected the test results. The nutrient broth media was tightly covered to avoid any misleading result. The petri dish was placed under incubation for about 10 days as it needed a warm, dark place in order for the bacteria to grow without any disturbances. The temperature was kept at about 37^{o}C. As expected the culture grew as shown in (b) and a smell signifying the growth of bacteria [1, 8] started to develop. Water droplets were not supposed to hinder the bacteria growth thus these petri dishes were placed upside down. The film placed on the lb media (c) did not show any visible changes until after day 10 when signs of an inhibition zone appearing could be seen (d).

Fig 3.5: Control experiment and the film on luria broth media

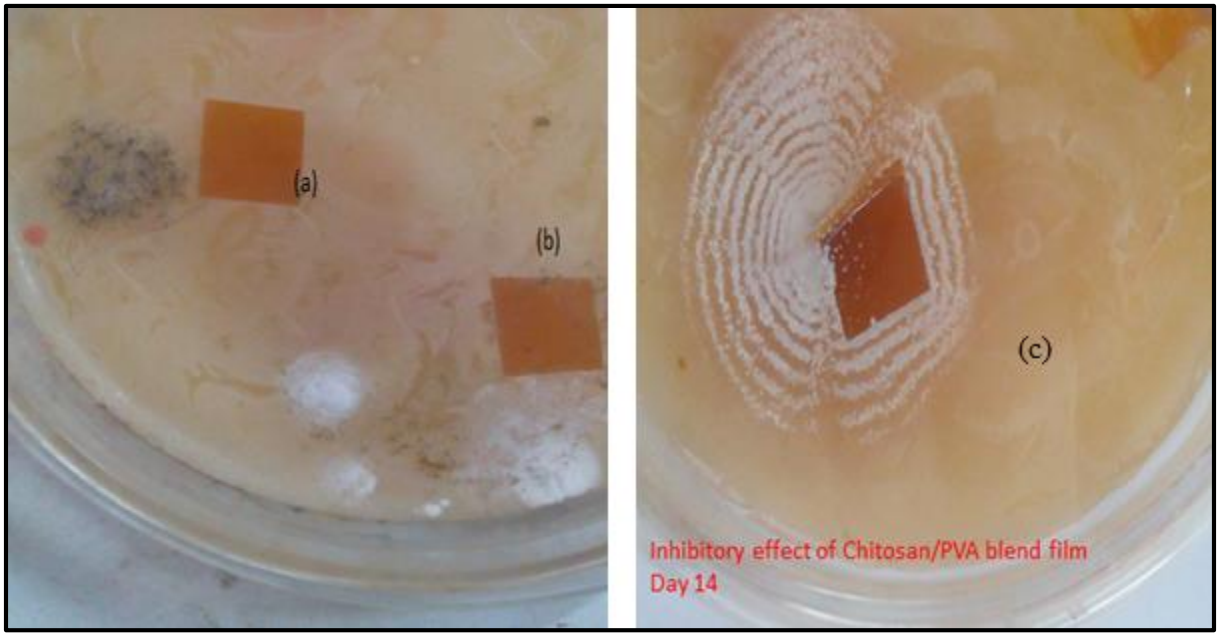


Fig 3.6: Chitosan/PVA at (a) 1:2 ratio and (b) 1:1 ratio and (c) the inhibitory effect of the chitosan/PVA blend film

This bio-active chitosan based film works as an antibacterial agent whereby a circle forms round the area where the chitosan/PVA film was placed and this area is the kill zone because no bacteria growth is seen around this area. The effectiveness of the blended films that is chitosan/PVA could be evaluated by means of the size or diameter of the kill/inhibition zone [1, 8] around the bio-active film. The larger the inhibition zone the more effective is the bioactive film thus for the film containing the 1:1 ratio it had a larger inhibiton zone diameter of 25mm as compared to that with the one that had the ratio of 1:2 chitosan/PVA. This mainly as a result if the fact that inbition is mainly caused by chitosan and was masked by the presence of more PVA unlike in the one were the ratio was 1:1.

From these results it can be seen that this bioactive film has an inhibitory effect against E coli bacteria thus an inhibition zone is formed which prevents or delay the attack of bacteria on the blended film. From the studies the film contains some antimicrobial properties which mainly results from chitosan and due to that the film is able to improve food safety and increase shelf life of semi processed food.

3.4 The tomato test

Due to high water potential in the tomato water moved from the region of high water potential to a region of lower potential thus shrinkage of the tomato occurred. This showed that the tomato survived attack from bacterial or fungi infection [1] when left in the PVA/chitosan solution at room temperature and thus the film can act as excellent barrier to extend shelf life of the packaged food due to the inhibitory effect of the bioactive chitosan/PVA solution caused by presence of chitosan.

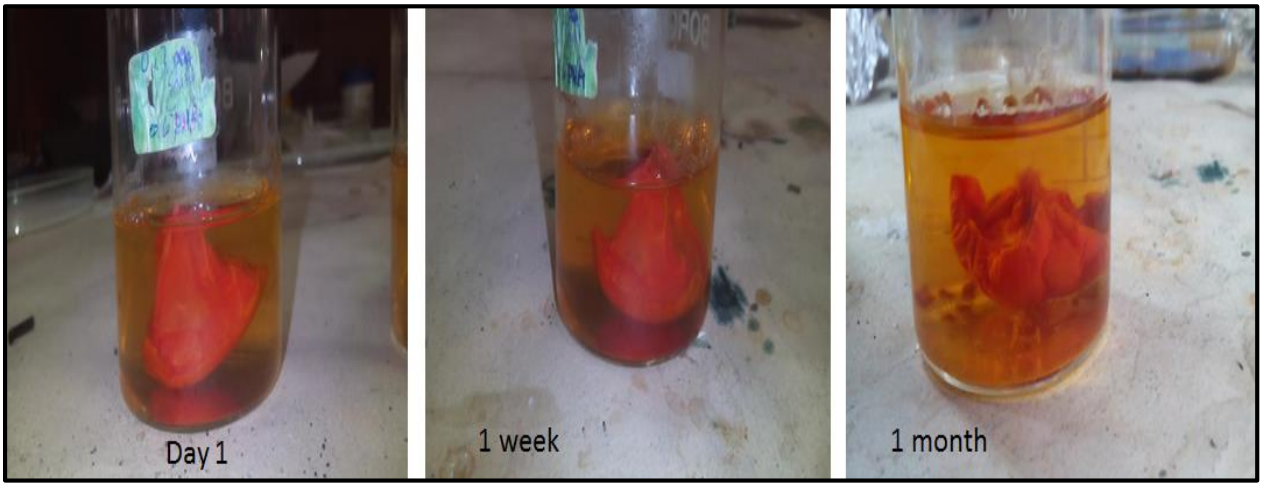


Fig 3.7: Effect of chitosan/PVA solution on a tomato

Fig 3.8: Packaged tomato at room temperature

The tomato was packaged and left at room temperature and showed it can go for weeks without going under any degradation of biological attack. This bioactive chitosan/PVA film can be used to extend the food shelf life.

In the second test the film was put in temperatures below $0^{o}C$ and no observable changes to the film or tomato were noticed.

Fig 3.9: Packaged tomato in the refrigerator

3.5 Thermogravimetric analysis

The Thermogravimetric analysis was used to determine the thermal stability and also the amount of volatiles that is present in the films by observing the weight loss as the heat is gradually increased.

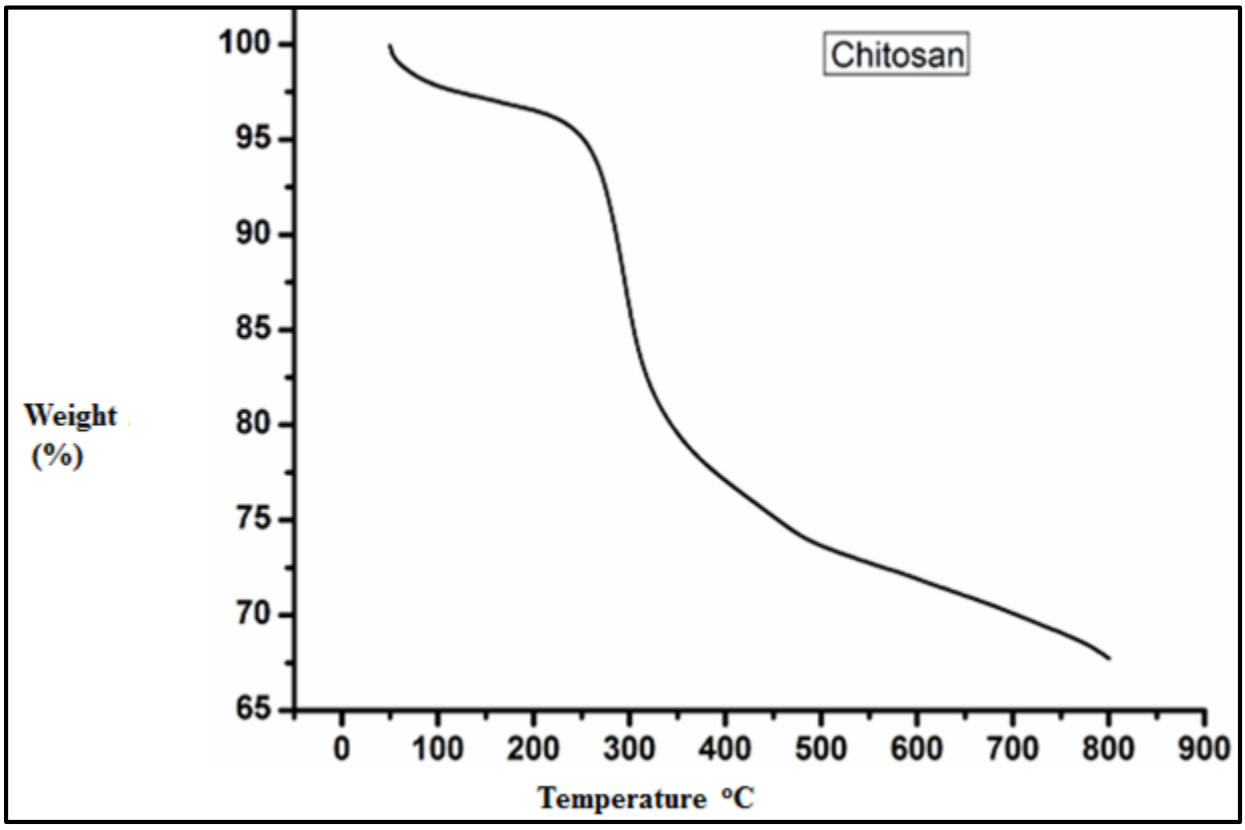


Fig 3.10: TGA thermogram of pure chitosan

The behavior of the chitosan film can be described as a two stage weight loss phenomenon because the thermograph of chitosan shows two major weight losses [1, 8]. The first transition occurs at about 50-180^{o}C and shows 7% weight loss which was caused by the moisture vaporization of the loosely bound water inside the film. The second weight loss which occurred at about 280-450^{o}C was a result of the degradation of the chitosan film. As the temperature increases the bond are strained until the lattice structure of the chitosan is weakened and results in the polymer backbone being raptured hence degradation due to the excessive heat. Similar behavior of the chitosan film was observed from the literature.

In this TG curve it can be seen that there are two main major weight losses. The first one at 50-250^{o}C was mainly due to the moisture vaporization of the loosely bound water molecules [1, 8-10].

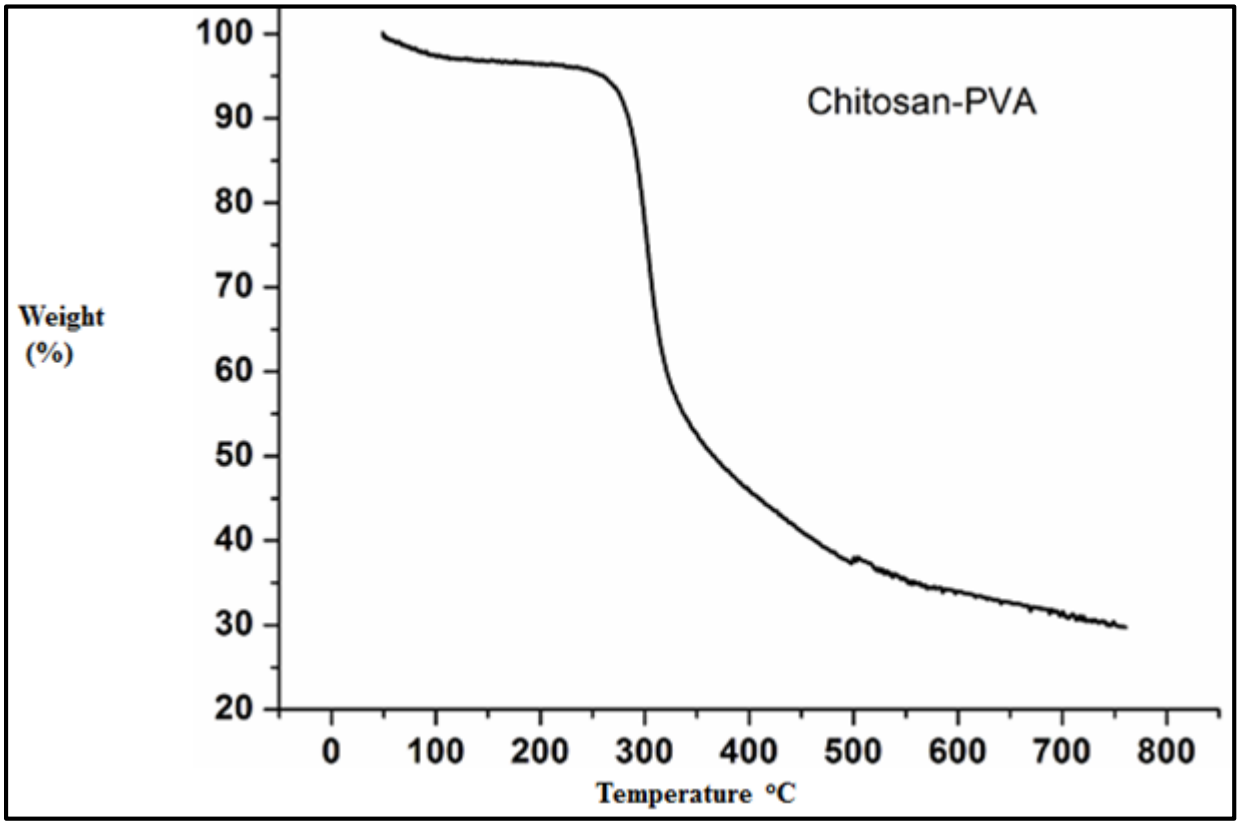
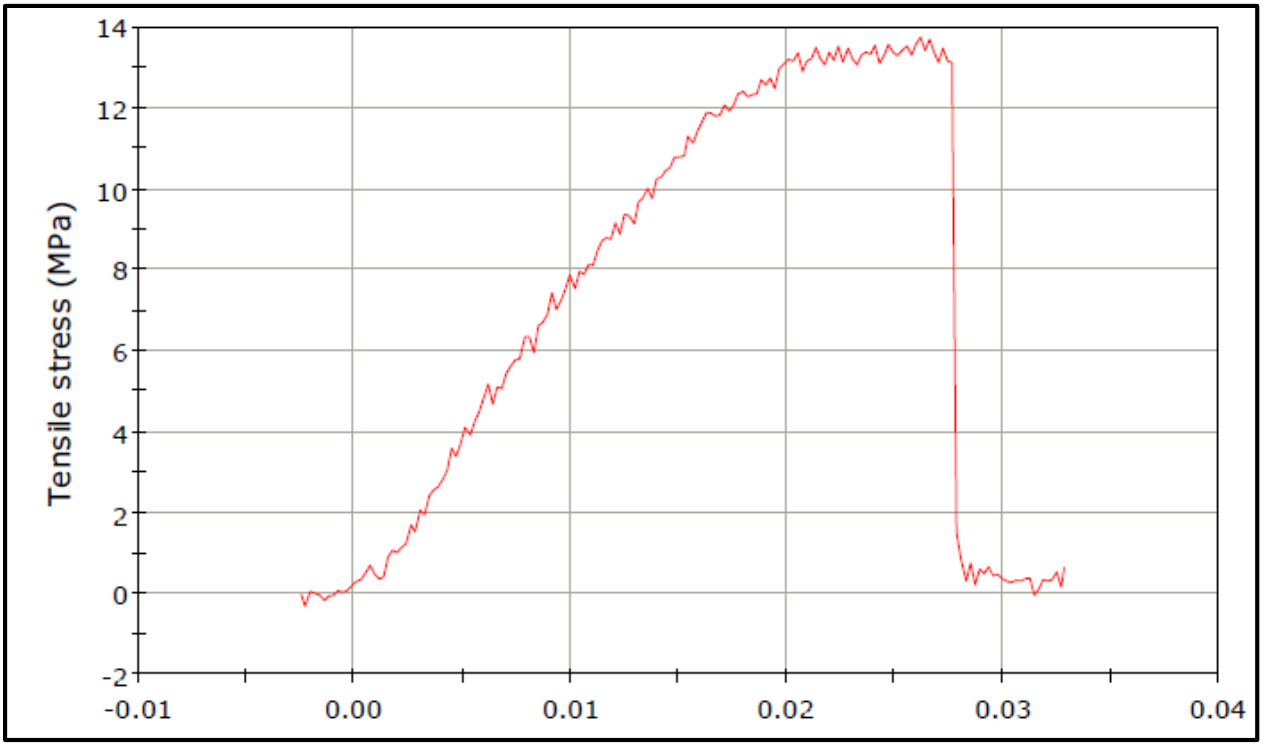


Fig 3.11: TGA thermogram of chitosan/PVA

In this case there was a lot of water content unlike in the chitosan due to the presence of PVA which increase the water uptake of the film and the second one at 300-500^{o}C was mainly a result of the thermal degradation of the chitosan/PVA film as the strong intermolecular hydrogen bonding existing between the two components functional groups that is the amino group in the chitosan and the hydroxyl group present in the PVA is broken down by the excessive temperature. As the temperature increases the bonds are strained until the lattice structure of the chitosan/PVA is weakened and results in the polymer backbone being raptured hence degradation due to the excessive heat.

3.6 Mechanical Properties

This technique used the stress strain relationship to determine the strength of the films and the elongation at break. Chitosan tend to have a high modulus of elasticity owing to its high glass transition and also its crystallinity. The chitosan film had a tensile stress at maximum load of 0.6663MPa and an extension at break of 1.3971mm. The modulus was found to be 766.11MPa.



	Maximum Load (kN)	Tensile extension at Maximum Load (mm)	Tensile stress at Maximum Load (MPa)	Length (mm)
1	0.01152	1.03802	0.66625	39.59000

	Thickness (mm)	Width (mm)	Load at Tensile Strength (N)	Area (mm^2)
1	0.07000	12.00000	11.52153	0.84000

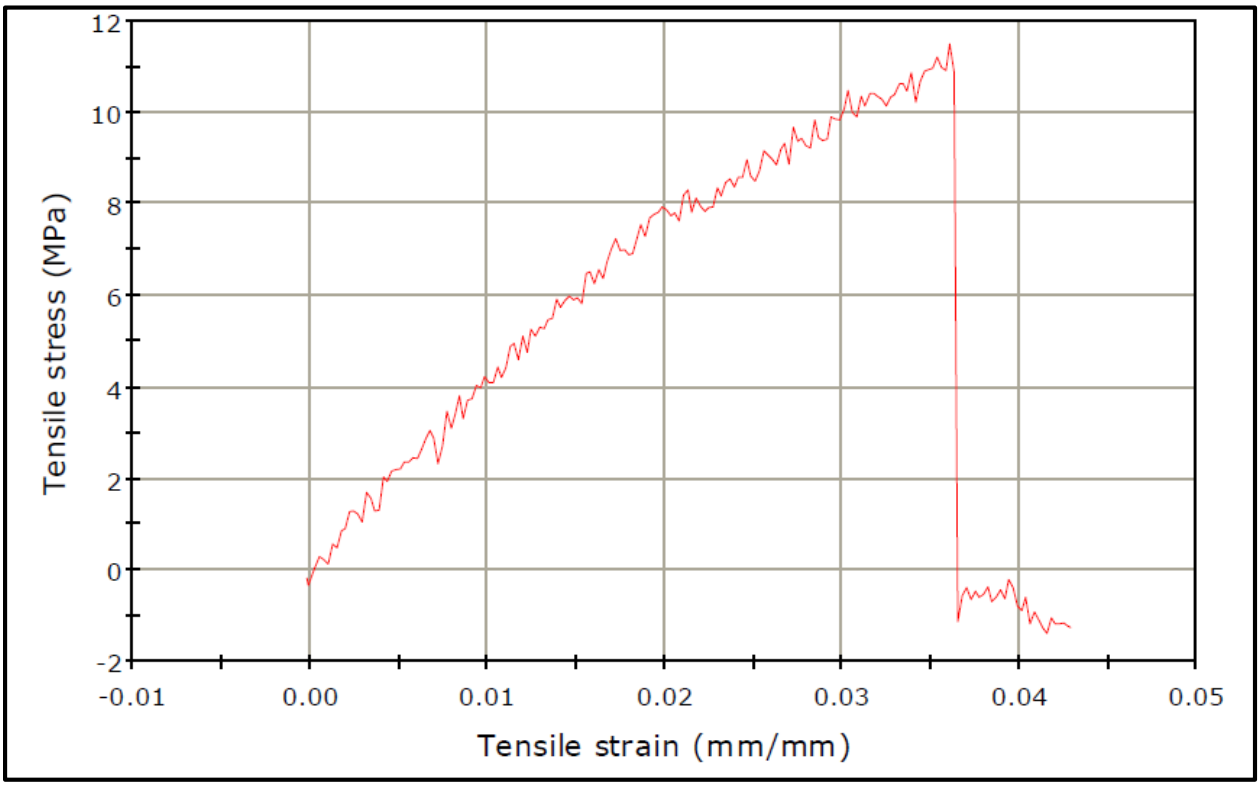
	Tensile stress at Tensile Strength (MPa)	Tensile strain at Tensile Strength (mm/mm)	Extension at Break (Standard) (mm)	Tensile strain at Break (Standard) (mm/mm)
1	13.71611	0.02622	1.39706	0.03288

	Maximum Extension (mm)	Load at Maximum Extension (N)	Modulus (Automatic) (MPa)	Extension at Tensile Strength (mm)
1	1.39706	0.55965	766.11222	1.13325

	Modulus (Automatic) (MPa)	Final area (cm^2)
1	766.11222	0.03600

Fig 3.12: Tensile strength of pure chitosan

The tensile strength of pure PVA was found to be more than that of pure chitosan because the film had a higher elongation at break therefore hence its flexibility was high. The tensile strength at maximum load was found to be 1.2264MPa and its extension at break was 1.5043mm. The modulus was 429.6448MPa.



	Maximum Load (kN)	Tensile extension at Maximum Load (mm)	Tensile stress at Maximum Load (MPa)	Length (mm)
1	0.00895	1.26330	1.22639	35.00000

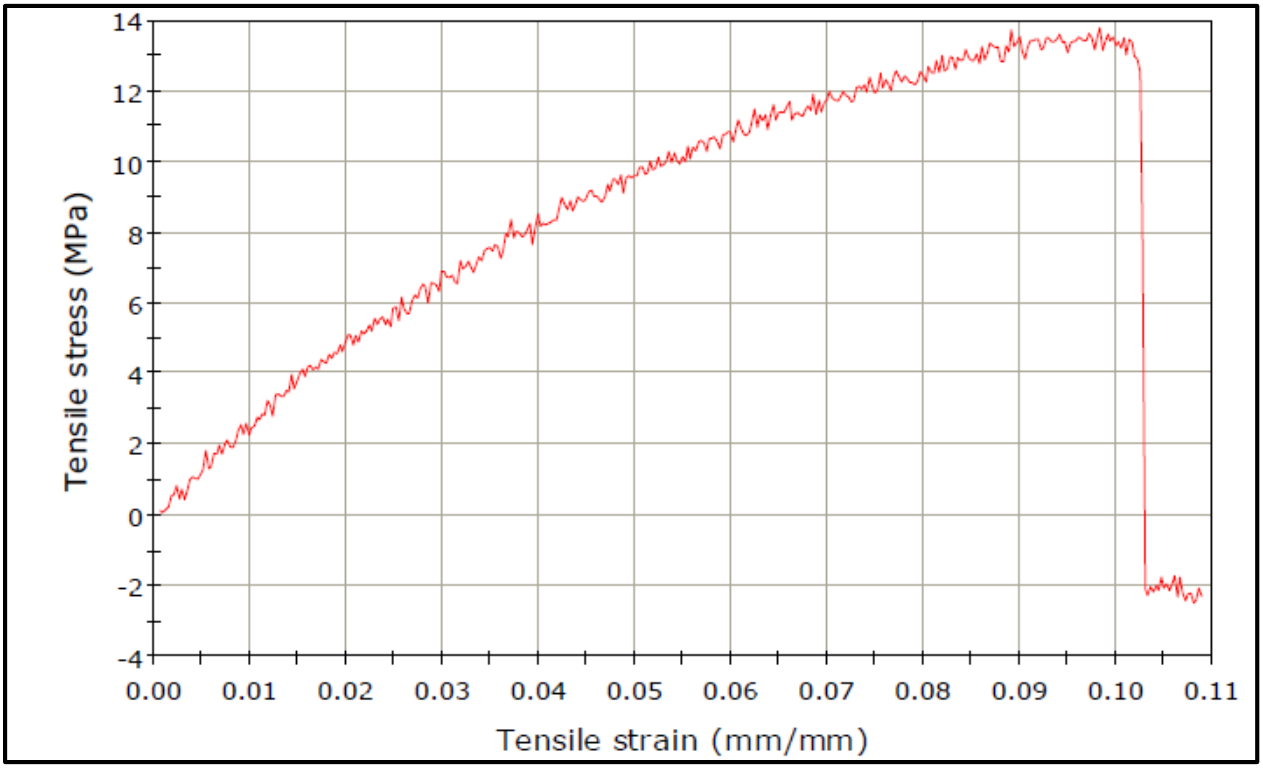
	Thickness (mm)	Width (mm)	Area (mm^2)	Extension at Break (Standard) (mm)
1	0.06000	13.00000	0.78000	1.49969

	Tensile strain at Break (Standard) (mm/mm)	Maximum Extension (mm)	Load at Maximum Extension (N)	Modulus (Automatic) (MPa)
1	0.04275	1.50453	0.95658	429.64479

	Modulus (Automatic) (MPa)	Final area (cm^2)
1	429.64479	0.03600

Fig 3.13: Tensile strength of pure PVA

The tensile strength of the chitosan/PVA in the 1:1 ratio was found to be higher than that of both pure chitosan and pure PVA. Due to intermolecular interactions as a result of blending the tensile strength was improved. The tensile stress at maximum load was found to be 2.2458MPa and the extension at break was 3.2428mm. The modulus was 251.4806MPa.



	Maximum Load (kN)	Tensile extension at Maximum Load (mm)	Tensile stress at Maximum Load (MPa)	Length (mm)
1	0.01252	2.95030	2.24581	30.00000

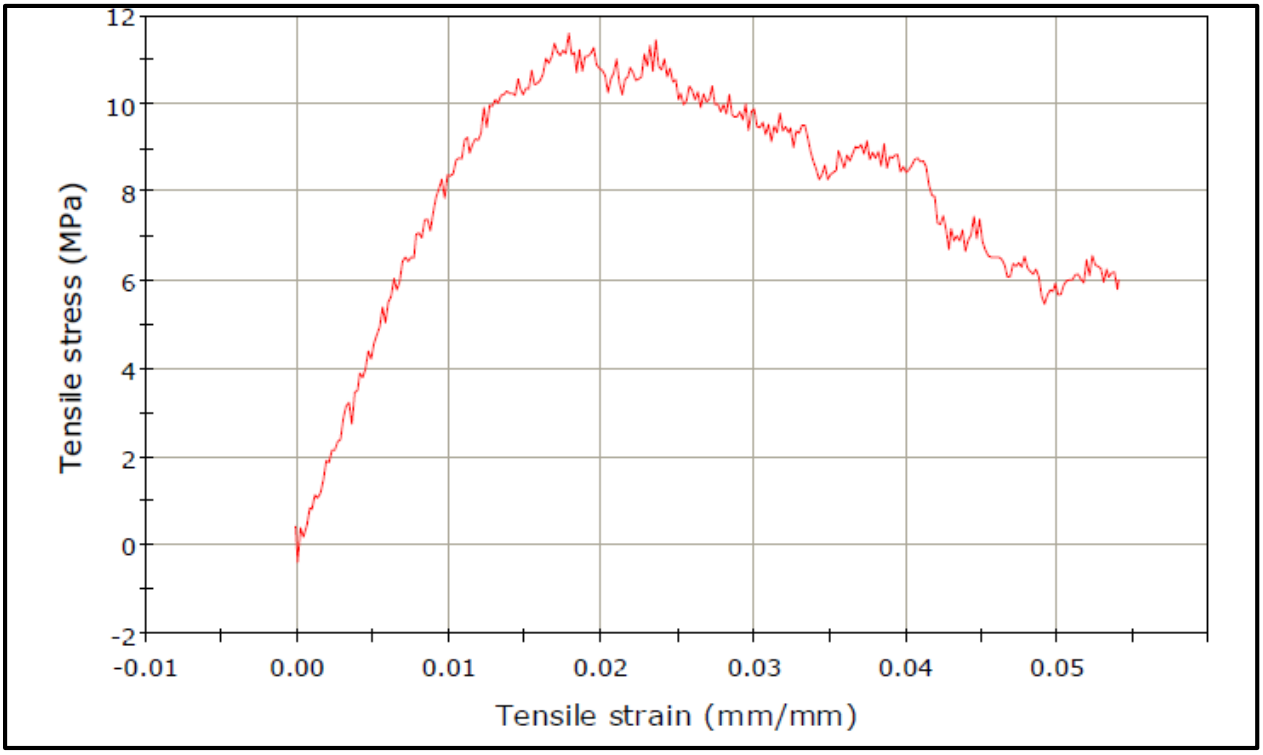
	Thickness (mm)	Width (mm)	Area (mm^2)	Extension at Break (Standard) (mm)
1	0.07000	13.00000	0.91000	3.24281

	Tensile strain at Break (Standard) (mm/mm)	Maximum Extension (mm)	Load at Maximum Extension (N)	Modulus (Automatic) (MPa)
1	0.10894	3.24281	2.04369	251.48056

	Modulus (Automatic) (MPa)	Final area (cm^2)
1	251.48056	0.03600

Fig 3.14: Tensile strength of chitosan/PVA 1:1 Ratio

The tensile strength of the chitosan/PVA in the 1:2 ratio was higher than all other samples due to an increase in the concentration of PVA. The tensile strength was found to be 6.0241MPa and the elongation at break was 2.4367mm.the modulus of this film was 904.6625MPa.



	Maximum Load (kN)	Tensile extension at Maximum Load (mm)	Tensile stress at Maximum Load (MPa)	Length (mm)
1	0.00973	0.80323	6.02414	45.00000

	Thickness (mm)	Width (mm)	Load at Tensile Strength (N)	Area (mm^2)
1	0.07000	12.00000	9.73044	0.84000

	Tensile stress at Tensile Strength (MPa)	Tensile strain at Tensile Strength (mm/mm)	Extension at Break (Standard) (mm)	Tensile strain at Break (Standard) (mm/mm)
1	11.58386	0.01785	2.43669	0.05404

	Maximum Extension (mm)	Load at Maximum Extension (N)	Modulus (Automatic) (MPa)	Extension at Tensile Strength (mm)
1	2.43669	5.06028	904.66254	0.80831

	Modulus (Automatic) (MPa)	Final area (cm^2)
1	904.66254	0.03600

Fig 3.15: Tensile strength of chitosan/PVA 1:2 Ratio

The tensile testing offers a sign of the strength and also elasticity [11] of the films, which can be reflected by the strength and also strain-at-break. Blending improved tensile strength of chitosan/ PVA blend significantly. These results indicate that blend films have higher tensile strength than pure chitosan and PVA films. Blending leads to an intermolecular interaction between two polymers and this improves mechanical strength of the blends. Due to possibility of interaction between -OH and -NH2 groups in these two polymers, blending improves mechanical properties of the films.

As it can be seen the tensile strength of sample increases with increase in PVA concentration the sample has more elongation-at-break, so with increasing the PVA content in the blend, the flexibility of the films were increased. Cross-linking with glutaraldehyde improves tensile strength and decreases tensile strain of the blend films. By increasing glutaraldehyde concentration, the films become more rigid and show less flexibility. It was found that the cross-linking improves mechanical properties of chitosan as compared to PVA. The effect of glutaraldehyde to improve tensile strength increases by increasing chitosan content in the blend films.

3.7 Water Uptake and swelling analysis

The blended films seems to have a high water uptake due to the presence of PVA. An increase in PVA resulted in an increase in water uptake. This was mainly caused by an increase in the number of hydrophilic groups (-OH) in the blends. Cross-linking with glutaraldehyde decreases water uptake in all samples as cross-linking reactions occur thus all the available hydroxyl and amino groups in the blended film are consumed thus no hydrogen bonding will occur.

Another parameter which was investigated was the effect of pH on water uptake. The water uptake increased when pH decreased and this was more predominantly on samples that contain more chitosan and this was decreased by increasing the concentration of the cross-linking agent. Due to the presence of the amino group in chitosan which could be protonized in acidic medium pH has more effect to chitosan than PVA. This could be clarified by the fact that in acidic medium the amino groups of chitosan ($-NH_2$) are protonized ($-NH^+_3$) so that the hydrogen bonds between chitosan and PVA are inhibited, therefore the network has more potential for hydrogen bonding with surrounding water. The other reason is that chitosan molecules in the acidic

condition are being uncoiled and form rods, which, might be another parameter to enhance hydrogen bonding with water.

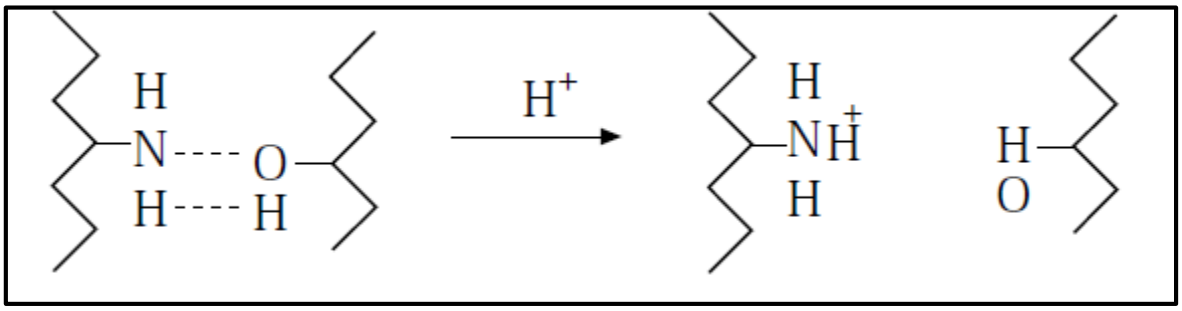


Fig 3.16: Effect of pH and water uptake on the blend film

Form the results the blended films had much higher degree of swelling compared with that of pure chitosan film, indicating that they have got higher hydrophilicity. The blended films showed a degree of swelling ranging from about 1050% to 2200% while that of pure chitosan was 90% and that of pure PVA was 680% which shows that the swelling behavior is mainly influenced by the amount of PVA present in the blend film. This is mainly caused by the fact that PVA being a water-soluble polymer and blending it with chitosan the PVA have a tendency to increase the water uptake as a result of the increase of hydrophilic groups that is (-OH)groups in the blend film. Furthermore the PVA chains being physically entangled with the chains of chitosan it leads to hydrogel network formation [12]. Thus blending chitosan with PVA results in an increased in the uptake of water as the concentration of PVA increases. [13, 14]

3.8 Morphology analysis

3.8.1 Scanning electron microscope

The surface morphology of the pure chitosan film was observed using SEM to determine the porosity and particle size that is in the chitosan films.

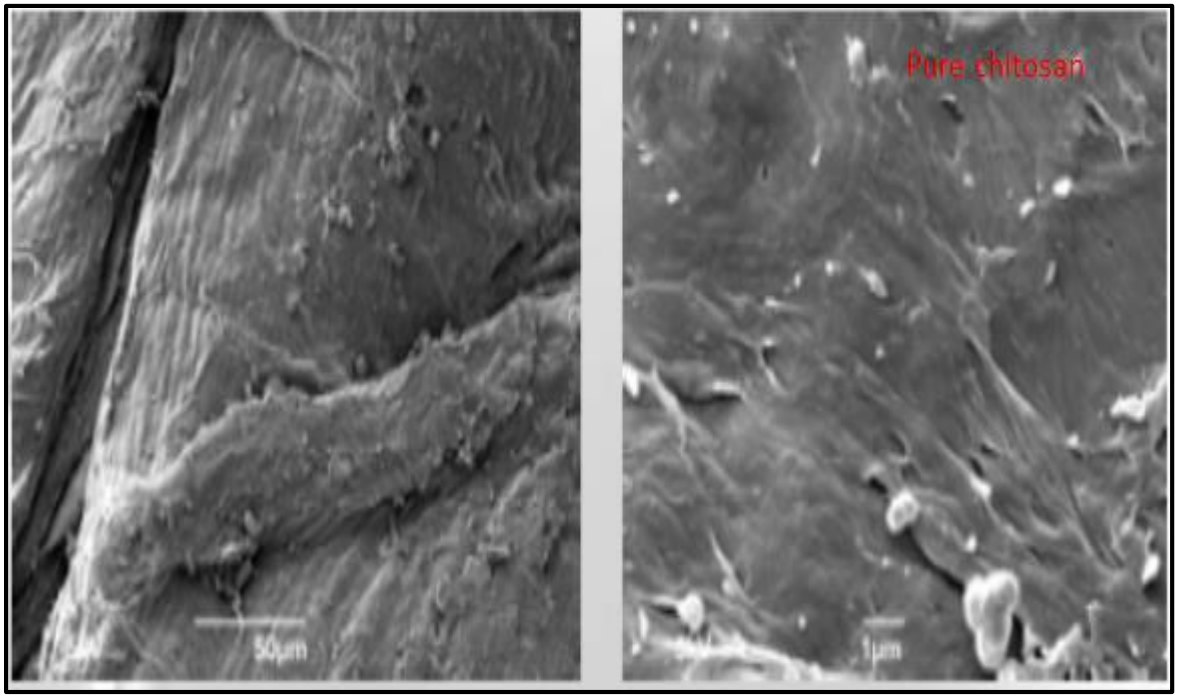


Fig 3.17: SEM micrograph of pure chitosan

The scanning electron images for the pure chitosan film discovered that the film is seen as nonporous and also the texture is plain lacking any pores. Pure chitosan film has a surface structure that is smooth and uneven. There were very small pores present at the surface of this film. From the images under higher magnification, the surface of the chitosan film shows a randomly dispersed microstructure space resembled a crack. This structure proposed the fact that the chitosan film particles could have failed to crystallize.

The films were then viewed at low resolution to check if the film appear to be homogenous or heterogeneous.

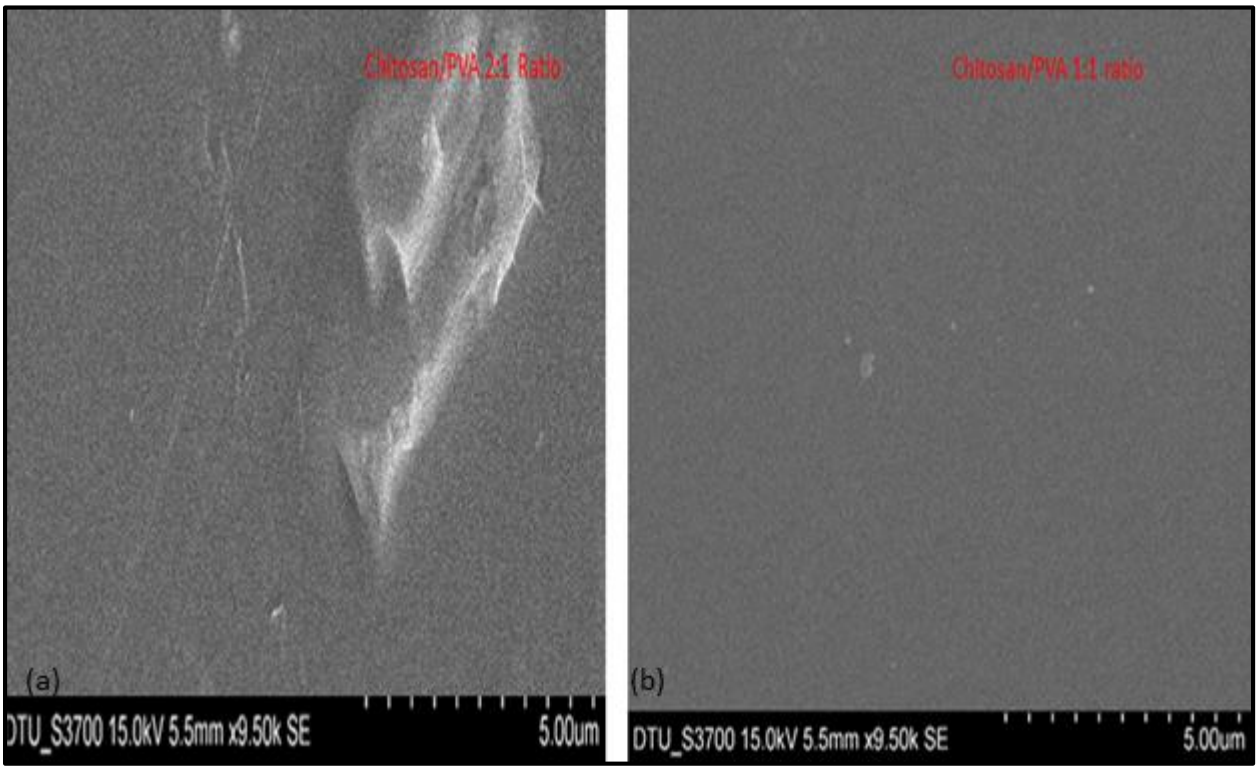


Fig 3.18: SEM micrograph of (a) chitosan/PVA 1:1 ratio and (b) 2:1 ratio at low resolution

The images above shows the surface area of chitosan-PVA by use of SEM and it can be seen that the surface is rough and heterogeneous which may have resulted from the reorientation of the polar functional groups. In the film containing more chitosan no obvious agglomeration is seen thus showing no signs of phase separation when a cross-linking agent is incorporated. No pores are seen on this film surface [15].

When the films were viewed at high resolution it was observed that the films that contained more chitosan exhibited particles that looked like air bubbles.

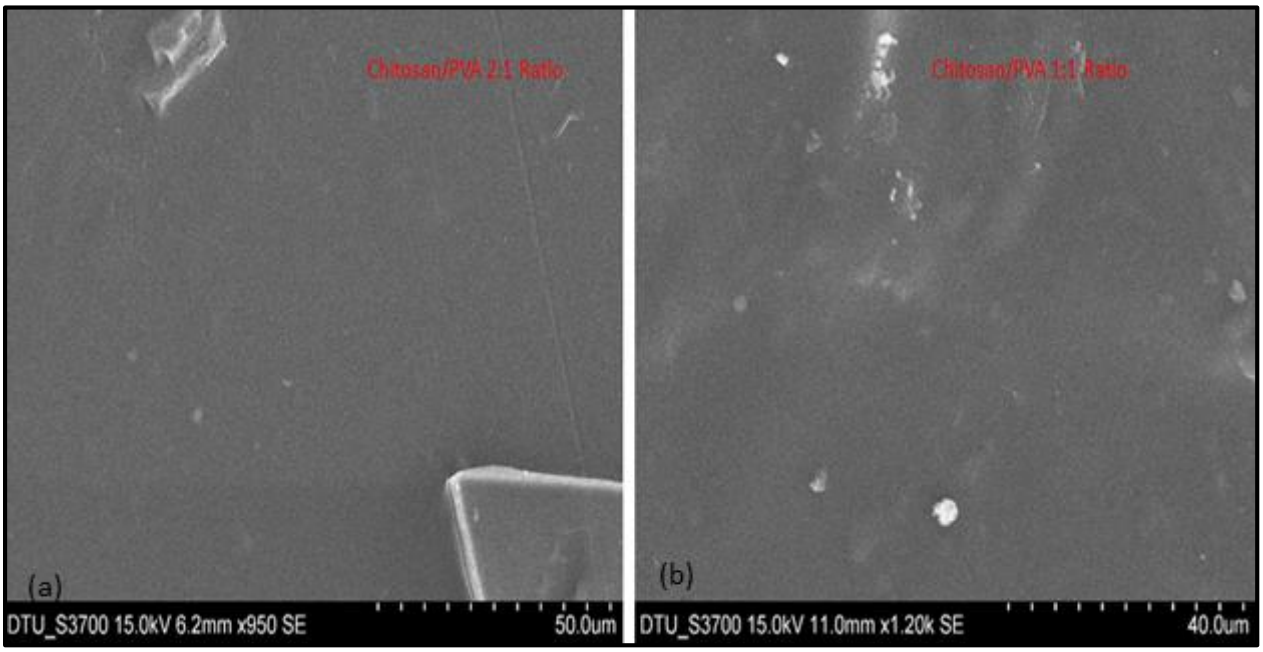


Fig 3.19: SEM micrograph of (a) chitosan/PVA 2:1 ratio and (b) 1:1 ratio at higher solution

However, in case of a film containing more PVA the agglomeration of various sizes of chitosan particles which haphazardly dispersed inside the chitosan-PVA matrix were observed. The mixture of the two is not a homogeneous mixture and thus air bubbles like surfaces are observed on the film surface.

3.8.2 Atomic Force Microscope

This technique was used to observe the surface roughness of the binary film.

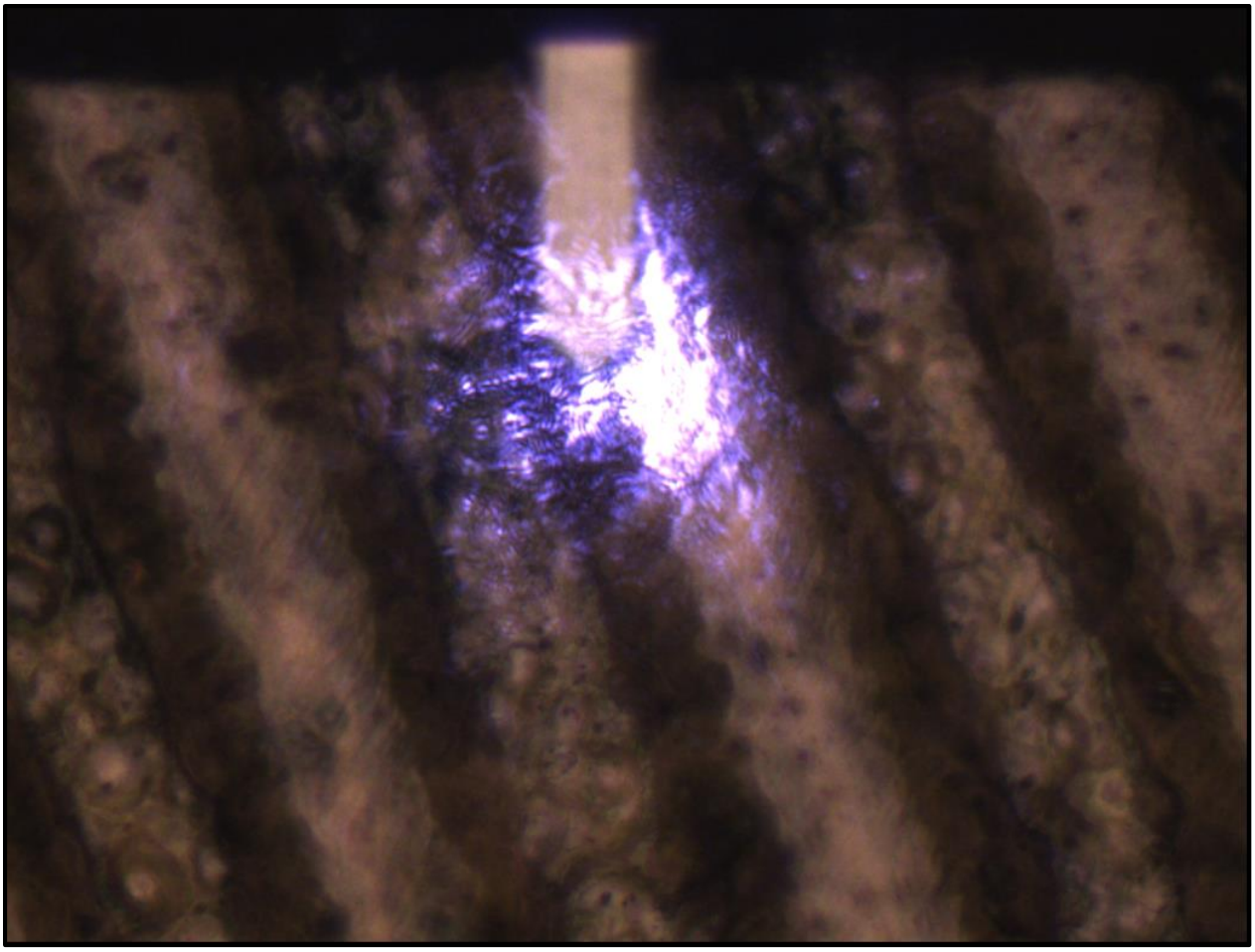


Fig 3.20: AFM image of chitosan/PVA blend film

The results from the atomic force microscope show that the surface roughness of the film could not be determined due to the unevenness of the surface. A homogeneous surface is required to get valid results.

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CHAPTER 4

CONCLUSION AND FUTURE PROSPECTS

Materials with film forming capacity and having antimicrobial properties will improve food safety and shelf life. It is an effective method for inhibition of certain bacteria in food as shown by their larger inhibition zone diameter of 25mm. These films can be used to replace antibacterial sprays which contains active substance that can be neutralized or diffuse into the food staff.

In this study the blending of PVA and chitosan improves tensile strength and flexibility of blended films. Cross-linking with glutaraldehyde improves tensile strength and decreases elongation of blends. Cross-linking effect of glutaraldehyde increases by increasing the chitosan content. As the bulk and surface hydrophilicity properties of biomaterials are very important parameter for bio application, we studied the effect of component content, cross-linking and pH on water uptake, contact angle and surface tension of different blended samples. With increasing PVA content in the blends, water uptake increases. Cross-linking of the blends with glutaraldehyde decreases water uptake. The water uptake increases when pH decreased. The effect of pH on increasing the rate of water uptake, is more important for samples with more chitosan and the effect of pH on water uptake decreases when glutaraldehyde concentration is raised. It seems likely that pH has more effect to increase water uptake for chitosan in comparison with PVA. Water uptake in PVA-chitosan blend films can be controlled by variation of their contents, cross-linking agent and the pH of solution. PVA Films showed highest surface tension and with its increases in the blends, surface tension (γ) and polar part (γp), were increased. Cross-linking with glutaraldehyde increases water contact angle. It seems likely that, the blended films are homogeneous on both sides. Blending the PVA with chitosan improves the tensile strength, antimicrobial activity, bulk and surface hydrophilicity and also flexibility of the blended films. The thermal stability was determined using the TGA and a weight loss was observed at a temperature of 50-250^{o}C as the volatiles had been driven off. As heating progressed there was another weight loss at 350-500^{o}C which was a result of degradation as the crystal lattice structure had been broken down. The blended films showed a degree of swelling ranging from about 1050% to 2200% while that of pure chitosan was 90% and that of pure PVA was 680% which shows that the swelling behavior is mainly influenced by the amount of PVA

present in the blend film. Blending improved tensile strength of chitosan/ PVA blend significantly. These results indicate that blend films have higher tensile strength than pure chitosan and PVA films. The tensile strength of the chitosan/PVA in the 1:1 ratio was found to be higher than that of both pure chitosan and pure PVA. Due to intermolecular interactions as a result of blending the tensile strength was improved. The tensile stress at maximum load was found to be 2.2458MPa and the extension at break was 3.2428mm. Blending the films improved the crystallinity of these bioactive chitosan films as the diffractions peaks are very higher than in pure chitosan. The chitosan/PVA diffraction peaks shows a very high peak intensity of about 1400 counts at 2Θ =14.5. Thus from this data it can be seen that the film will become more crystalline once the amount of PVA increases. The FTIR revealed basically two distinct peaks at 1408 and 1550 - 1560 cm^{-1}. Formation of the 1550 - 1560 cm^{-1} peak is a symmetric deformation of the NH_3 resultant from ionization of primary amino functional groups in the acidic medium however the peak at 1408 cm^{-1} shows the existence of carboxylic acid. The film that proved to have the best properties was that which had chitosan/PVA in the 1:1 ratio as it showed excellent antimicrobial activity, tensile strength, thermal stability as well as swelling behavior.

Thus the incorporation of chitosan into various polymer matrices to obtain materials with active internal layer for food packaging and/or coating applications constitutes the main theme of the present work. This approach also encompasses the modifications needed to alter the hydrophobic character of the biocide formulations based on chitosan. Conclusively, it can be safely stated that chitosan may prove to be a potential candidate in adopting novel bioactive packaging technologies in order to improve the quality and safety of perishable foods.

FUTURE PROSPECTS

- To incorporate polymeric additives such as antioxidants, colorings, plasticizers
- To put a p type and n type dopant and see how the film works as a conducting polymer can be used in electrochemical applications such as biosensors, sensor catalytic activity, energy storage, etc
- Bio-compatibility, biodegradability and toxicity test
- To check on the miscibility of the binary films using DSC