Green Synthesis of Silver Nanoparticles and Ag/TOPAS Nanocomposites for Packaging Applications

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Dedication

I dedicate this work to my grand dad, who has always been a source of inspiration to me.

Acknowledgement

I am privileged to express my deepest gratitude to my mentor Prof. Dr. Zakir Hussain for his support, suggestions and guidance, without which completing the research work would be an impossible task for me.

I greatly acknowledge all faculty members, lab engineers, lab technical staff and non-teaching staff for their support.

I also acknowledge all my fellow students and friends for their help at various stages during this research work.

Hareem Zainab

Abstract:

The time honored Silver is known for its anti microbial properties. An augmentation of the antimicrobial, electrical, optical and chemical properties of silver takes place at nano scale. This calls for a lucrative market of silver nanoparticles in medicine, anti bacterial coatings, paints, textiles and packaging industry. Since the use of plant extract provides an innocuous route for synthesis of nanoparticles, a study concerning the synthesis of silver nanoparticles using plant extract has been presented in the paper. The biogenic route for synthesis of nanoparticles was preferred because of its comprehensibility and environmental friendly essence. The biogenically synthesized nanoparticles were further used in another environmental friendly application i.e. formation of biodegradable food packaging material. Predominantly plastics are used for packaging, which linger in the environment even after the end of their shelf life; this poses a great threat to the soundness of the planet. In this research, nanocomposite films of TOPAS and biogenically synthesized silver nanoparticles are formed and characterized using disparate characterization techniques including SEM, XRD, TGA, FTIR, DSC and UV-vis spectroscopy. Physical tests like mechanical testing, antimicrobial testing, and Water vapor permeability test have also been executed/ performed to probe into the properties of nanocomposite films.

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List of Abbreviations:

PDA: potato dextrose agar

SEM : Scanning Electron Microscope

XRD: X-ray Diffraction

FTIR: Fourier Transform Infrared Spectroscopy

DSC : Differential Scanning Calorimetry

Chapter 1

1. Introduction:

Packaging is of utmost importance when it comes to green approach. Packaging materials when disposed off, can cause lots of environmental issues. Some of the common packaging materials used include; plastics which are light, strong and cost effective to manufacture, alloys like aluminum are used for canned goods, brick cartons as tetra packs are used for basic food stuff like milk etc. Cardboard packaging is used mostly for materials that are already packed and need to be stored or transported. Last but not the least is glass packaging used for storing drinks etc. Of all these packaging materials some are easily recyclable like glass, card board etc , some are difficult to recycle like brick cartons while others like plastics are not recyclable at all. In fact they form a major component of landfills and degrade to produce more contamination. An ideal packaging material must show following properties [1]:

1.1 Properties of Ideal Packaging material:

- Mechanical
- Thermal
- Environmental friendly
- Antimicrobial
- Moisture resistant

In order to meet these requirements polymers are most widely studied. Some of the most common polymers used for packaging include PP (polypropylene), PVP (polyvinylchloride), PS (Polystyrene) etc. The ever exceeding demand for attaining the best never ends and thus the fuel for research is never exhausted out.

Although plastic packaging is cheap, durable and light in weight, there are still drawbacks that come with these advantages like pollution. Classical polymers have the tendency to stay in the environment even after their shelf life because they do not degrade easily. The transition of plastics packaging to biodegradable packaging is mandatory to restore the health of the world. Biodegradable polymers are derived from organic resources and are biodegradable; these polymers have many benefits like [1]:

- **Easy Decomposition:**

These polymers do not need take much time to decompose; by replacing plastics with biodegradable polymers the contamination in landfills due to plastics can be reduced. When polymers face conditions like weather, aging and pressure due to burying they undergo thermal, chemical and mechanical changes as a result of which the structure of the polymer changes. Mechanical degradation occurs due to forces like tensile, shear and compression. Sensitivity to other stimuli like light, heat and chemicals can also enhance the mechanical degradation process. In biodegradation the polymers are degraded by microorganisms. Microorganisms grow on the surface of the material due to bio deterioration.

- **Renewability**

Biodegradable plastics are made from renewable resources which include trees, plants and decomposable organic matter.

- **Environment friendly:**

Biodegradable polymers do not need oil for their manufacturing also no green house gas and carbon emissions occur during synthesis of biodegradable polymers. In case of synthesis of non biodegradable polymers all the above mentioned problems occur.

- **Nontoxic:**

During the breakdown of plastics harmful byproducts are emitted while biodegradable polymers breakdown into byproducts that easily get absorbed in the earth. Biopolymers are an excellent alternative but the mechanical properties of these polymers need to be enhanced by adding some filler materials e.g. nano particles or by blending them with some other polymer.

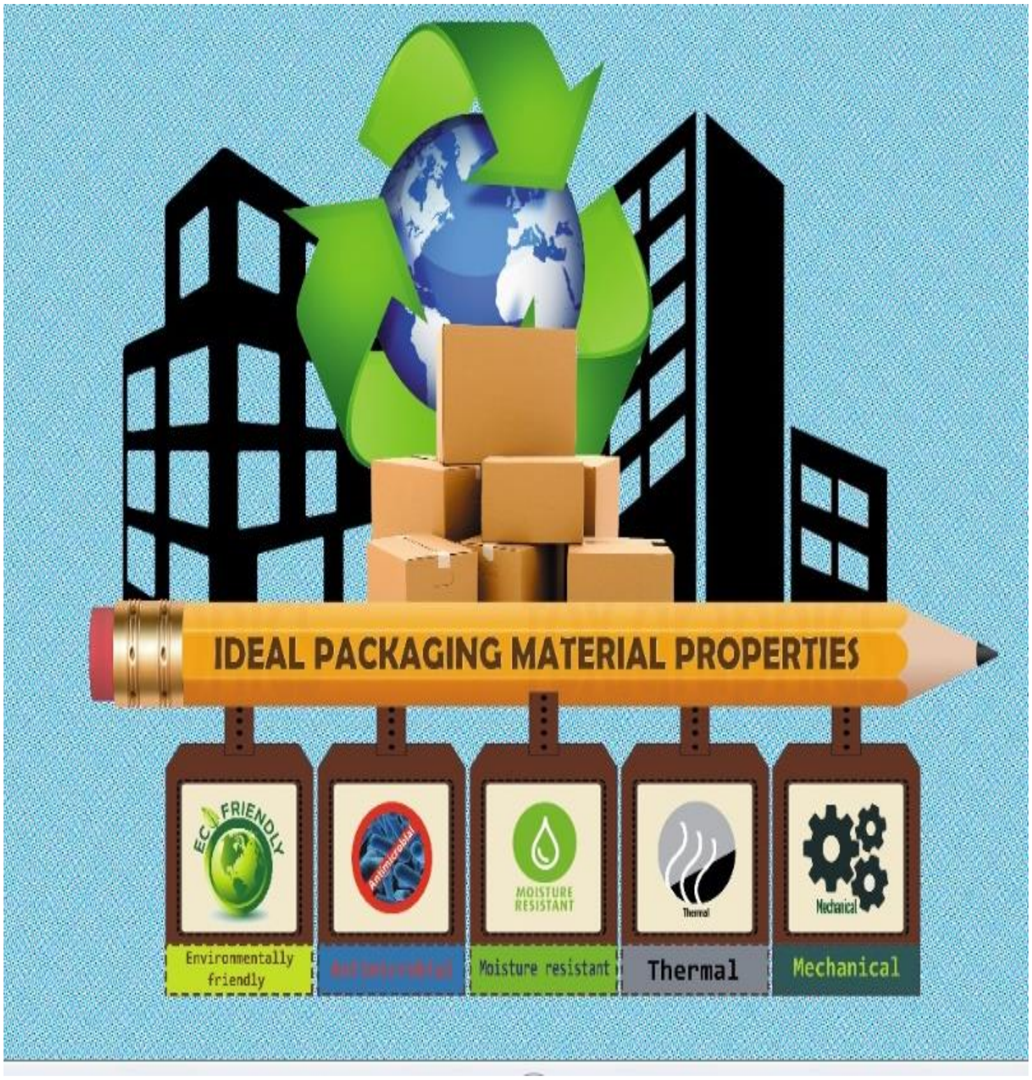


Fig. 1: Ideal packaging material

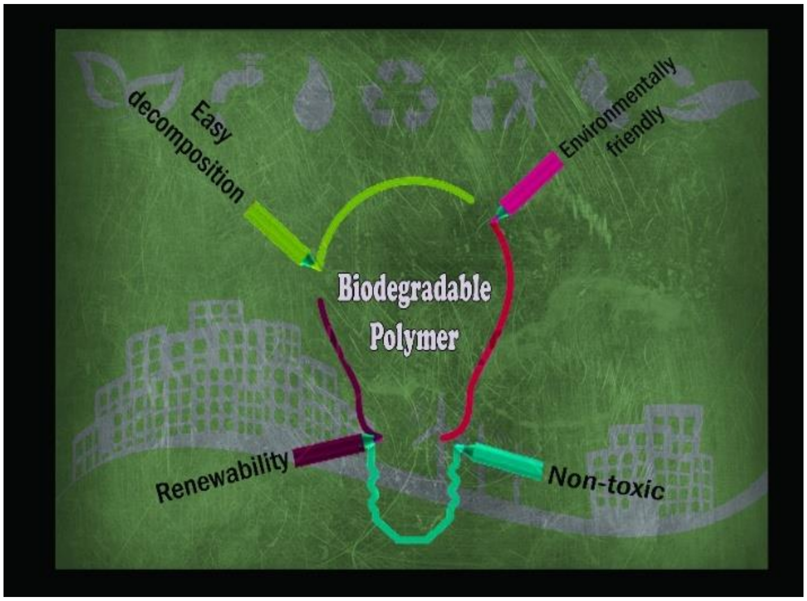


Fig.2: Biocompatible polymer

1.2 Nanocomposites:

When polymers are reinforced by nanosized organic or in organic phase the thermal, mechanical and barrier properties of the polymer change. The reinforcing phase is called nanofiller and the material thus formed by the incorporation of filler is called nanocomposite. By the addition of nanoparticles different desirable properties can be achieved [2]. When polymer matrices are filled with nanomaterials they exhibit remarkable mechanical, thermal, electrical and optical properties.

1.3 Nanofiller synthesis by green route:

Different nanoparticles can be synthesized via different approaches like chemical, physical and green synthesis. Green synthesis is gaining more and more attention due to its cost effective, environmental friendly and simple approach [3].

1.3.1 Green Synthesis:

With the cumulative demand of nanomaterials in different areas of consumer products the demand for environmental friendly synthesis methods for these materials is also elevating. Green synthesis is not only eco friendly but is cost effective and simple as well when compared to chemical and physical synthesis approaches. Chemical approaches require the use of reducing agents like sodium borohydride, heat treatments and harmful byproducts [3]. Anastas and Warner highlighted some of the principles of green chemistry as [4]:

- Prevent waste
- Atom economy
- Less hazardous chemical synthesis

In order to achieve the above mentioned goals due consideration is taken in the choice of solvent (mostly water is used), reducing agent (nontoxic) and stabilizing agent (eco friendly). Biological systems like algae, fungi, yeast, plants and bacteria can work at ambient conditions to produce nano materials.

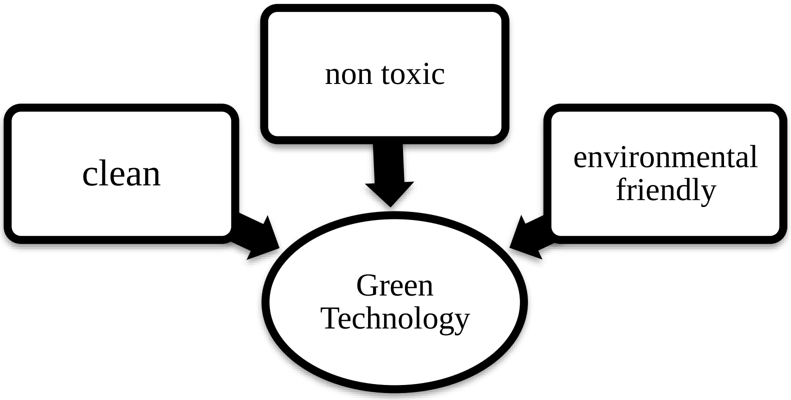


Fig.3: Importance of Green Synthesis

Silver was the first noble metal synthesized using silver resistant bacterial strains. Bacteria are cultured in high concentrations of silver nitrate solution. The bacteria accumulate silver on their cell wall in the form of nano particles, but with this type of synthesis method the synthesis rate is slow and precise control over the shape and size of the nano particles cannot be achieved [4].

In case of Fungi the silver ions in the solution get adsorbed on the surface of the fungal cell, the ions are then reduced by the enzyme present in the fungal cell wall and the nano particles begin to form.

Plants are another agent used in green synthesis. Plants are safer then bacteria and fungi as they do not pose any harm to human health and are easily available. Plants contain flavanoids, vitamins, alkanoids, amino acids etc which act as reducing and capping agent in the synthesis of nano particles. The metal ions in metal salt solution are reduced by the reducing agents present in the plant extract. Nucleation of ions results in nano particle formation. The plant extract also provides the capping agents to stabilize the nanoparticles. Nanoparticles of different shapes and sizes can be obtained usingplants’extract[4].

Chapter 2

Literature Review:

2.1 Silver

Nanotechnology is a miraculous technology that can provide us with materials at nanoscale. At nanoscale the due to small size physical and chemical properties of nanomaterials differ significantly from their bulk counterpart [5]. The materials with enhanced properties at nanoscale can be used for wide range of potential applications in medicine, space technology, sports goods and packaging [6–7]. When it comes to nanomaterials the mentioning of silver nanoparticles cannot be ignored. At nanoscale silver exhibits remarkable physical, chemical and biological properties that are not present in silver at macro scale [8].

At nanoscale silver shows [9]:

- Catalytic behavior
- Anti fungal properties
- Antibacterial properties
- Enhanced optical behavior

Due to these properties silver nanoparticles can be used in microelectronics, bio-imaging and anti microbial applications [10]. As an anti microbial agent silver nanoparticles can be used in a wide range of consumer goods like in medicines, in food as a packaging material and in textiles as a disinfectant [11].

2.2 Properties and Applications of Silver Nanoparticles:

2.2.1 Anti Bacterial Properties:

There are three possible ways in which silver nanoparticles can destroy the bacteria;

a) Silver nanoparticles would release silver ions that would disrupts ATP production and DNA replication
b) Silver ions could generate ROS that would affect DNA and membrane proteins
c) Silver nanoparticles would directly attack the cell membrane.

Due to their anti bacterial property silver can be used in food packaging materials, in soaps, bandages, antibacterial coatings and in wound healing gels [12].

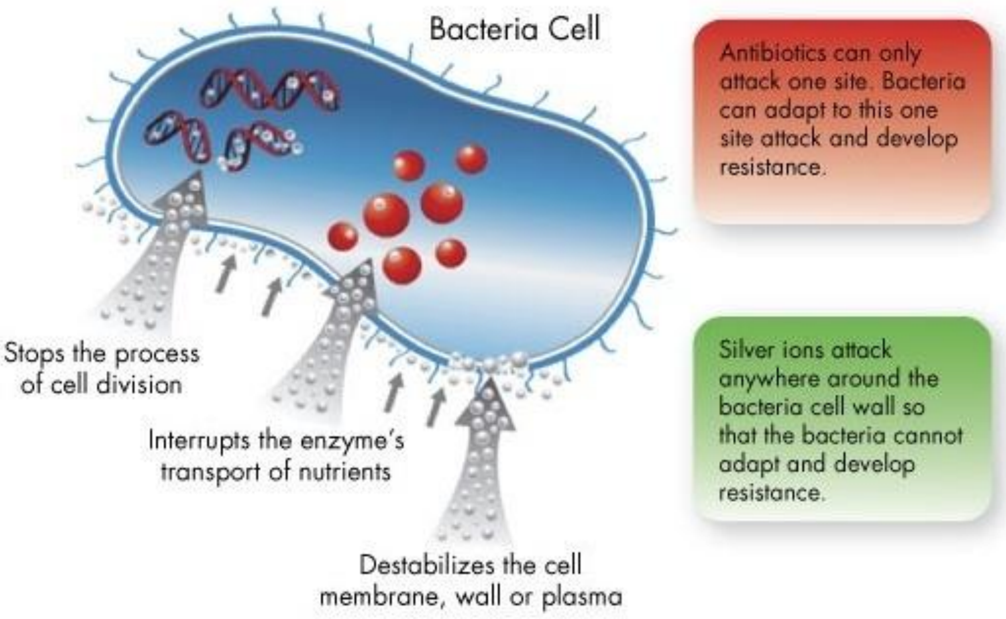


Fig.4: Nano silver killing bacteria [12]

2.2.2 Anti Fungal Properties:

Anti fungal activity of silver nanoparticles is due to their penetration into the fungal cell membrane. This would inhibit the normal growth and budding of the funji as the cell membrane is destroyed.

Anti fungal properties of silver nano particles can find their use packaging, bandages and in medicine for the treatment of fungal infections [13].

2.2.3 Anti Viral Properties:

A lot of infectious diseases like HIV, HBV, influenza etc are emerging due to viruses. Silver nanoparticles have shown antiviral activity against some viruses like HIV-I, monkeypox, influenza and MNV-I virus. Silver nanoparticles get attached to the viral proteins which prevents the binding as well as replication of viral particles in the cultured cells.

Silver is being used in biosensing devices like aptamers to detect ad kill viruses [14].

2.2.4 Anti Cancer Properties:

Silver nanoparticles exhibit anti cancer behavior. Tumor cells due to their leaky vasculature and permeable membrane absorb silver nanoparticles easily than healthy cells. Upon entering the tumor silver nanoparticles generate reactive oxygen species that can kill cancer cells.

Silver nanoparticles are used in cancer therapy application like hyperthermia. In this technique the nanoparticles are introduced in to the tumor cells. The nanoparticles are inside tumor are then ablated using and external stimuli like light, magnetic field, radio waves and ultrasounds. This results in the production of heat inside the tumor which kills the cancer cells [15].

Due to these properties silver nanoparticles are used in:

2.2.5 Drinking Water Disinfection:

Porous ceramic materials and membranes decorated with silver nanoparticles can be used to filter out bacteria and some viruses from drinking water [17].Airborne bacteria, viruses and fungi are cause of many diseases. Silver can be used to eliminate airborne pathogens when used in air filters e.g activated carbon filters decorated with silver nanoparticles indicated the inhibition of *Bacillus subtilis* and *E. coli* bacteria. In the same way polymer air filters incorporating silver nanoparticles are also used [16].

2.2.6 Surface Disinfection:

For surface disinfection silver nanoparticles embedded paints are used. Those surfaces which are coated with paint of silver nanoparticles show marvelous antimicrobial properties by killing pathogens [18].

2.2.7 Wound Healing Properties:

Silver is used in antimicrobial gel formulations for topical use. These gels can heal burn wounds without leaving a scar behind [18].

2.2.8 Packaging Applications:

Silver nanoparticles are used in packaging applications to impart anti microbial properties to the material. This helps in preserving the food for a longer period of time [18].

2.2.9 Clothes Containing Nanosilver for Clinical Use:

Clothing of health care workers can get contaminated by bacteria. Some of these bacteria can survive over 90 days. Such contamination is harmful for the health of the wearer and the patients. Silver impregnated clothing can help in reducing the growth and spreading of bacteria thus preventing from major health issues [19].

2.3 Synthesis Methods of Silver Nanoparticles

Silver nanoparticles can be synthesized via chemical, physical and biological routes.

2.3.1 Chemical Synthesis:

Chemical reduction method is the most common way of producing silver nanoparticles. In this method a silver salt ($AgNO_3$) is dissolved in water with a reducing agent. Some of the common reducing agents are NaBH4, sodium tri citrate and hydrazine. Reducing agent reduces the silver salt into silver ions that nucleate further to form nanoparticles. By controlling temperature, PH, solvent and the type of reducing agent size of nano particles can be controlled. Some of the other methods used are sol gel method, polyol method, chemical vapor deposition and co precipitation [18].

2.3.2 Physical Synthesis:

In physical synthesis methods the nano particles are synthesized using physical forces like heat and pressure. Some of the physical synthesis methods are:

- Ball milling
- Evaporation
- Sputtering
- Gas condensation
- Molecular beam epitaxy
- Themolysis
- Laser ablation
- Pulse laser deposition

Physical methods allow for high throughput but the processes are expensive.

2.3.3 Biological Synthesis:

Physical synthesis methods are expensive and chemical synthesis methods produce harmful by products. Reducing and capping agents used in chemical reduction methods are not eco friendly in order to avoid this issue green or biogenic synthesis approaches are used. In green synthesis as the name indicates biological organism like bacteria, fungi and plants are used to produce nanoparticles. Capping and reducing agents are replaced by the molecules provided by these living organisms [18].

Plants are most widely used in biogenic synthesis of nanoparticles as they are easy to handle and safe to use. Plants contain reducing agents like alcohols, phenols, flavanoids, vitamins, carboxylic acid [18].

Metal salt is introduced into the solution containing plant extract. The plant extract acts as the reducing and capping agent and reduces the metal salt into nanoparticles and also stabilizes them. The nano particles are recovered by centrifugation. Water is mostly as the solvent however other organic solvents like ethanol, methanol and ethyl acetate are also used. Following are some of the plants that are used successfully to synthesize silver nanoparticles;

Some of the plants used for green synthesis include Alternanthera dentate, Acorus calamus, Boerhaavia diffusa etc [18].

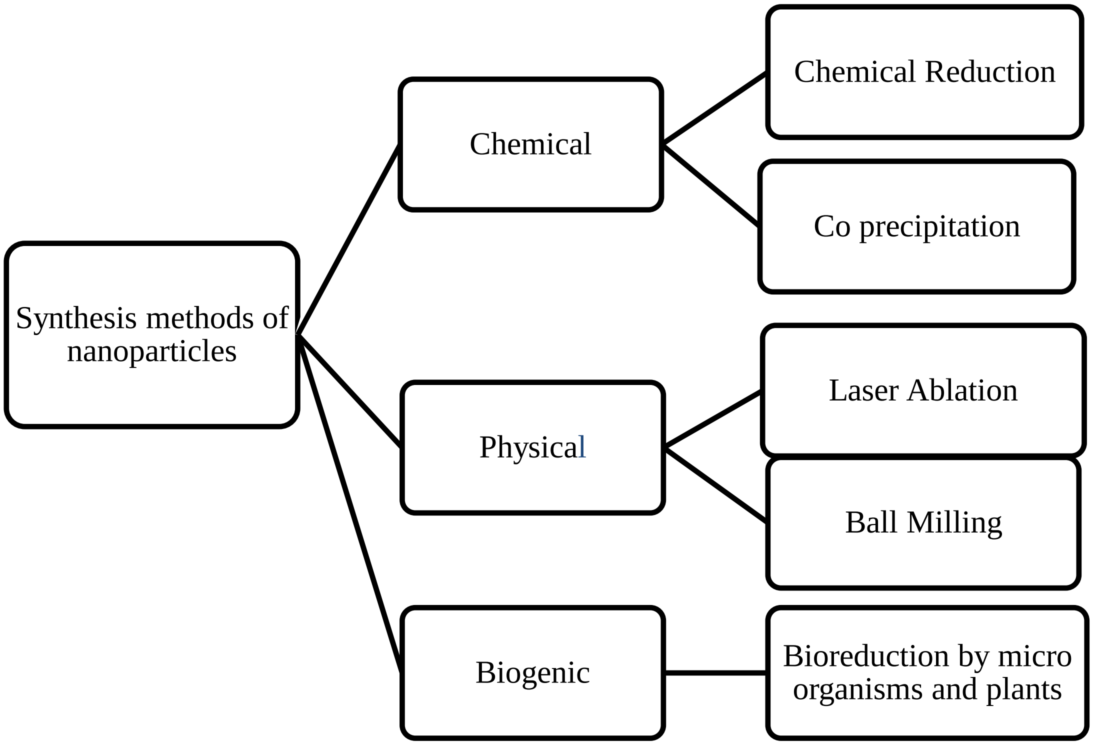


Fig.5: Synthesis methods of Silver nanoparticles

2.4 Importance of Silver in Packaging

There is an ever increasing requirement for increased shelf life of canned foods and even fresh food. In order to increase the shelf life of edibles and to prevent food borne diseases the need of antimicrobial food packaging arises. Different packaging materials are developed and one of them is organic–inorganic packaging, in this type of packaging polymer matrix is embedded metal nanoparticles. Metal nanoparticles not only enhance the anti microbial properties but also improve mechanical properties. As silver nano particles show antimicrobial, anti-fungi, anti-yeasts and anti-viral activities so they can be combined with both non-degradable and edible polymers for active food packaging. Nanotechnology is used in two types of packaging [20]:

a) Improved Packaging

Nanomaterials when combined with the polymer matrix improve the gas barrier properties of the matrix such as polymer/clay Nanocomposites.

b) Active Packaging

The nanoparticles react directly with the food and the environment and thus protect the food in a better way.

2.5 Packaging Materials

A polymer is a long-chained molecule build up from small repeating units called monomers that are covalently bonded together to form long chains. Many polymers occur in nature like proteins, cellulose, and silk. Polymers can also be synthesized like polystyrene (PS), polyethylene (PE), polypropylene (PP), and polyamides, etc., they are called synthetic polymers. Plastics are derives from synthetic polymers.

Plastics are derived from petroleum resources which are depleting. Landfill space for dumping of plastics is also reducing. Plastics persist in the environment and get ingested by marine animals and birds.

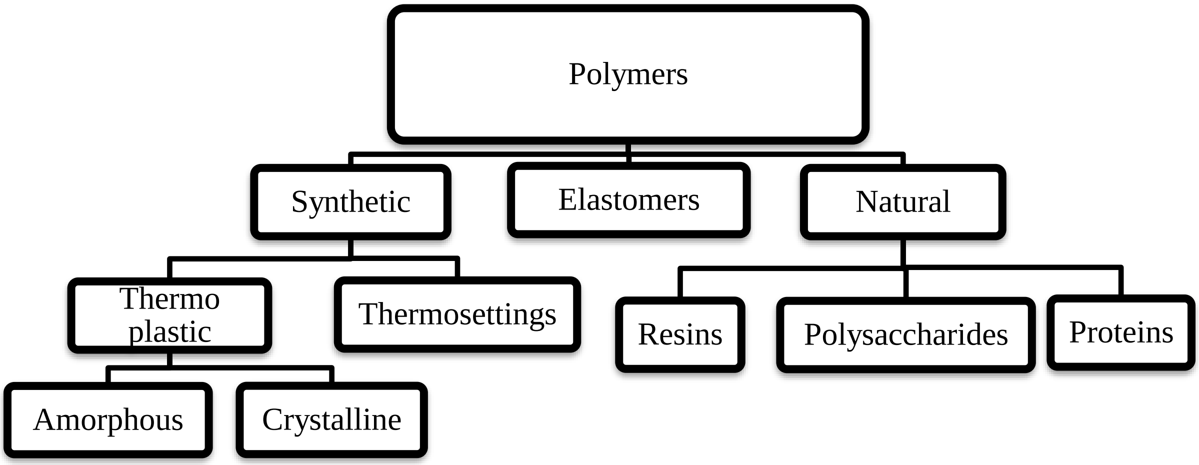


Fig.2: Types of Packaging materials

2.6 Biodegradable Polymers:

Biodegradable polymeric films are an alternative option in food packaging because they can be obtained at low costs from renewable sources and pose no threat to the environmental. As a result of these benefits, biodegradable polymers are attracting much attention as alternatives to non-degradable polymers which are currently in use in food packaging. Some of the commonly used are biodegradable polymers are polysaccharides such as cellulose, pullulan, agarose, starch and chitosan. Cellulose binds electropositive transition metal atoms by electrostatic interactions. Consequently silver ions are adsorbed during immersion in silver nitrate and the porous structure of cellulose favors the synthesis and stabilizations of AgNPs. Absorbent pads are realized in this polymer material which is widely used in fresh food modern packaging strategies [20].

2.6.1 Pullulan:

It consists of maltotriose units forming an edible polysaccharide polymer. Pullulan occurs in the form of white powder which is water soluble and forms colorless and tasteless films. The films are and resistant to oil and show low permeability to oxygen [21-23]. Apart from being biodegradable and biocompatible, pullulan also inhibits fungal growth in food, so it can be regarded as one of the good packaging materials [21-23, 31].

2.6.2 Agarose

Agarose is a polysaccharide based polymer that is mostly obtained from red algae. As the structure shows it is made up of the repeating unit of agarobiose, a disaccharide formed by D-galactose and 3, 6-anhydro-L-galactopyranose. Agar–agar is a derived from agarose. When dissolved in water it forms a transparent gel which is non-toxic, biodegradable and non-immunogenic because of all these characteristics agar–agar is used to make coatings for “ready to eat” food [24].

2.6.3 Sodium Alginate

Sodium alginate is sodium salts of alginic acid (sodium alginate). It is a biodegradable and biocompatible polymer. The polymer is often used in the form of blends with other polymers and Nanocomposite films to show good mechanical properties, flexibility, tear resistance and oil resistance [25]. The chemical formula for sodium alginate is:

2.6.4 Starch

Starch is made up of two polymers that consist of D-glucose repeating units, amylose and amylopectin [32]. Studies are underway to use starch as a substitute for plastics as starch shows poor mechanical properties and high moisture sensitivity. Starch based nanocomposide films incorporating silver nanoparticles show improved mechanical and gas barrier properties along with anti microbial properties [26].

2.7 Synthetic Polymers:

Synthetic polymers are manmade polymers derived from renewable or non renewable resources; some of common types of synthetic organic polymers are [27]:

- Low-density polyethylene (LDPE)
- High-density polyethylene (HDPE)
- Polycaprolactone (*PCL*)
- Polystyrene (PS)
- Nylon, nylon 6, nylon 6,6
- Polyethylene terephthalate (PET)
- Polyvinyl Alcohol (PVOH)

2.7.1 TOPAS

Chemical Structure of TOPAS:

Topas is an amorphous cyclic olifin copolymer formed by the polymerization of norbornene and ethylene. It is a good replacement for plastics [30, 40].

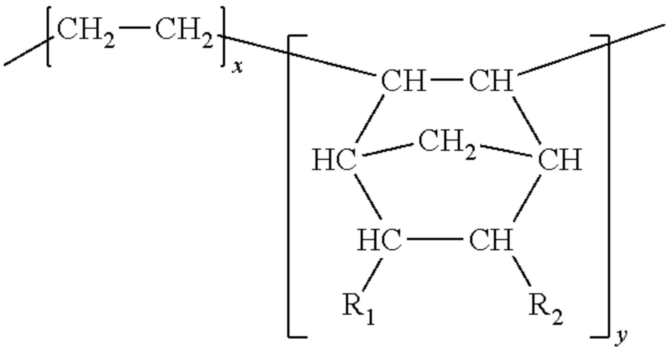


Fig.7: Chemical structure of TOPAS [30]

2.7.2 Properties and Applications of TOPAS:

Cyclic olefin copolymers are a new class of polymeric materials which exhibit unique properties which can be tuned by changing the chemical structure of the copolymer. Some of the properties are:

- High Glass Transition Temperature
- High transparency
- Extremely low water absorption
- Water vapor barrier properties
- Good Heat Resistance
- Good mechanical properties like high rigidity, strength and hardness
- Excellent Film Forming Ability
- Biocompatibility
- Good Chemical Resistance
- Electrical insulating properties
- Low Double Refraction

Topas (COC) resins can find their application in digital cameras, electrical insulation; medical products etc. These materials have shown potential to be used in primary packaging of pharmaceuticals, medical devices and diagnostic disposables [30]. There are five grades of TOPAS 8007, 5013, 6013, 6015 and 6017.

2.7.2.1 Applications of TOPAS in Medicine:

TOPAS show greater resistance to shattering, high barrier properties and transparency which are prerequisites for pharmaceutical packaging materials. Along with these properties TOPAS is also biocompatible with high transparency which makes it ideal for pre fillable packaging and vials. Due to increased barrier properties the TOPAS, the shelf life of packed pharmaceuticals can be increased [30-31].

2.7.2.2 Applications of TOPAS in Optics:

Due to the combination of properties like heat resistance, high transparency, low specific gravity and low water absorption, COCs can be used in lenses for mobile telephones, digital camera LEDs and printers. Along with these applications COCs can be used in other optical applications [30-31].

2.7.2.3 Applications of TOPAS in Films:

Transparent and metalized films of COCs can be prepared by extrusion and metal plating. The films can be used in the back light and image light areas of LCDs such as diffusion film, protective film, and retardation film and as an anti-glare polarizing film for high resolution LCDs [30-31].

2.8 State of the Art:

TOPAS is a new material and not much research has been done so far. Some of the papers reported include the nanocomposite of TOPAS with CNTs, exfoliated graphite and silica nanoparticles. Topas /SiO_2 nanocomposites were synthesized using solution blending method. TGA, DSC and Water Vapor Permeability tests were done to study the thermal and gas barrier properties of nanocomposite films. Nanocomposite films showed enhanced properties as compared to pure Topas film [40].

Hybrid composite of Topas and Hydroxyaptite nanoparticles was synthesized using solution casting method. The hybrid system was studied for bone grafting applications [42].

Nanocomposite films of Topas and multiwalled CNTs were synthesized by solution precipitation method. Thermal, electrical and mechanical properties of the nanocomposite films were studied. DSC results showed no appreciable difference between the Tg values if pure Topas and that of nanocomposite films. Degradation temperature of nanocomposite films was 40°C higher than pure Topas. Similarly electrical properties also showed an increase with the addition of CNTs [30].

2.9 Nanocomposites

Nanocomposites are materials that are formed by the incorporation of nanosized particles into the matrix of a polymer. By the addition of nanoparticles is pronounced improvement in properties like mechanical strength, toughness and electrical and thermal conductivity. The addition of nanoparticles remarkably improves the properties normally the amount of nanomaterial added is between 0.5 and 10% by weigh [28-29].

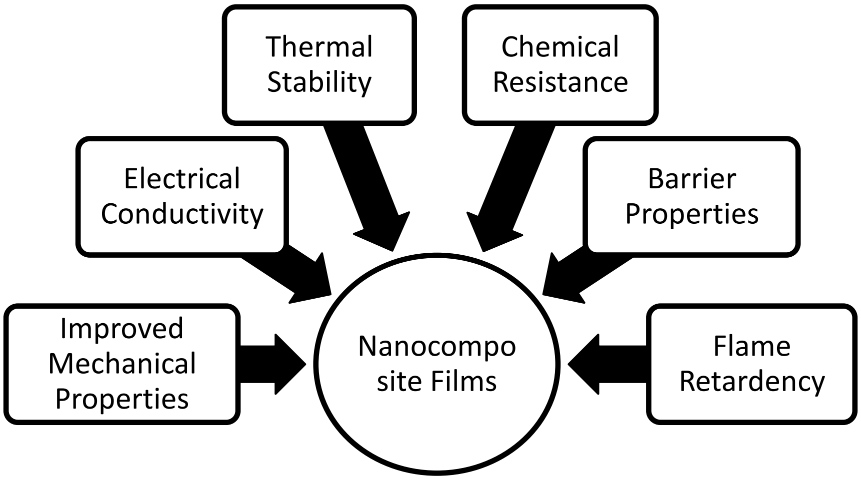


Fig.8: Nanocomposite films show enhanced properties

Nanoparticles due to their small size have an extremely high surface to volume ratio which dramatically changes their properties when compared with their bulk counterparts. Small size enables the nanoparticles to interact with the matrix or bulk material in a much effective manner. The result is that the composite show improved properties with respect to their individual components. Nanocomposites are currently being used in many fields and new applications are being continuously developed. Applications for nanocomposites include [28-29]:

- Thin-film capacitors for computer chips
- Solid polymer electrolytes for batteries.
- Automotive engine parts and fuel tanks
- Impellers and blades

- Oxygen and gas barriers
- Food packaging

2.9.1 Nanocomposite of TOPAS and Silver

In order to meet the need for better packaging materials researchers search for new materials. By looking at the mechanical properties of COC, as reported by the manufacturers, it appears quiet attractive for packaging applications [30]. Incorporating silver has affected the mechanical properties, water barrier properties and thermal properties of TOPAS.

Chapter 3

3 Experimental

3.1 Materials

Following table give information regarding the materials used in the experimental part.

Table 2 Description of materials used for experimental portion

S No	Material	Manufacturer and specifications
1	Topas (COC)	TICONA GmbH, Germany
2	Chloroform	Sigma Aldrich Co, USA (Lab grade)
4	Silver nitrate	Sigma Aldrich Co, USA (Lab grade)

3.2 Procedure:

3.2.1Green Synthesis of Silver Nanoparticles:

3.2.1.1 Selection of Plant:

In order to select the plant I randomly picked up different families of kingdom planate. Out of those families 10 plants were selected that were not used for the synthesis of silver nanoparticles. The selected plants were sorted out on the basis of novelty and nine were left. The selected plants were further studied on the basis of phytochemical screening and those were selected who have already been studied on the basis of their phytochemicals. So out of nine plants only six were left behind. Out of those six plants three were selected on the basis of availability. Experiments were conducted to find out which plant would best suit for synthesis of nanoparticles. One of the experiments failed and two became successful leaving behind two plants for synthesis of nanoparticles. Again one plant was selected out of the two plants on the grounds of novelty.

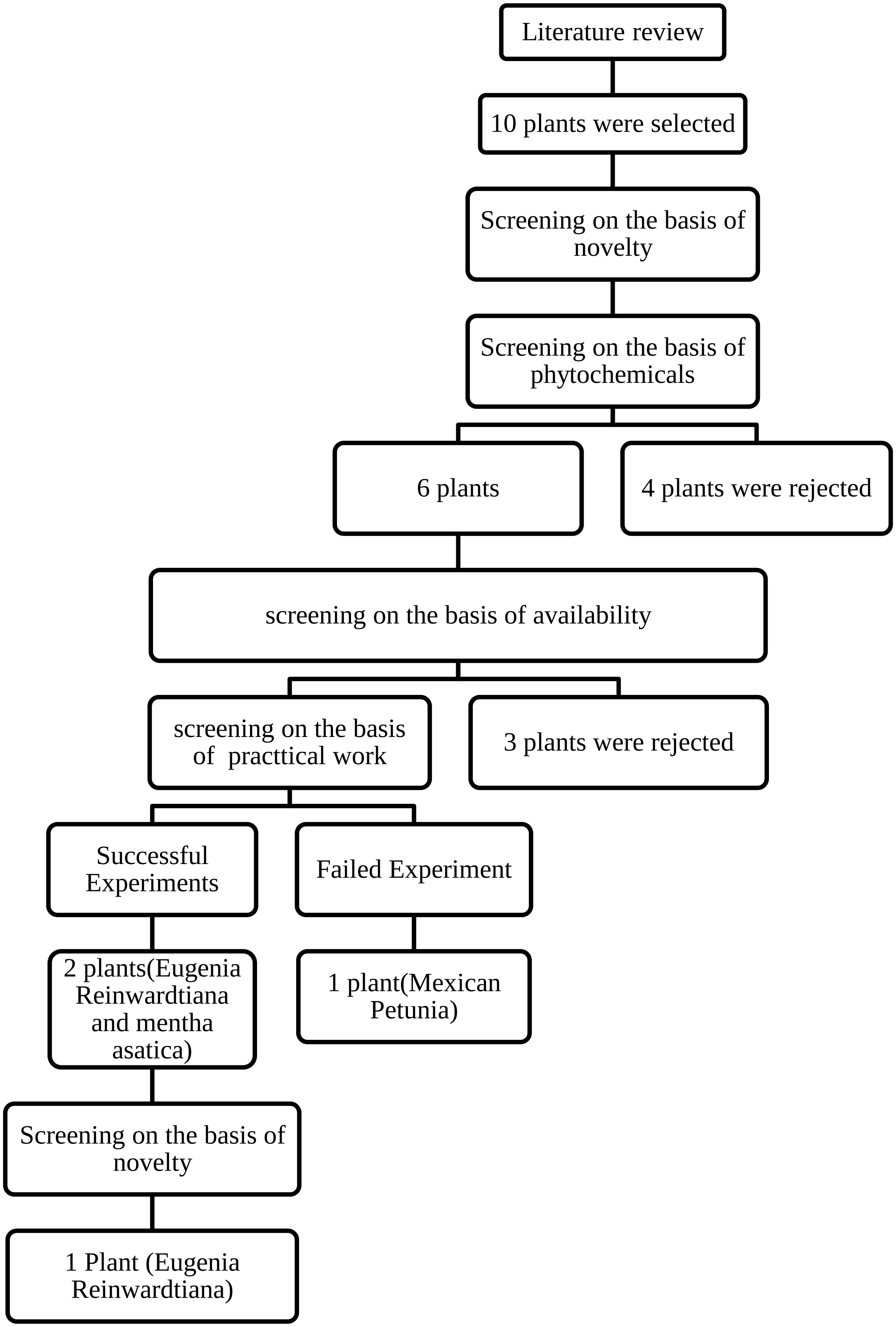


Figure.9: Selection of Plant

3.2.1.2. Drying of leaves

There are different methods for drying of leaves:

Hot Air drying

Main components of a hot air dryer are a heating section, a blower and temperature sensor. Sample is placed in a chamber where heating and drying is done. Weight of the sample is measured after regular intervals and drying is done till the mass of the sample becomes constant.

Sun drying

In sun drying samples are directly placed under sun light till dried.

Shade drying

In this type of drying extreme conditions are avoided and samples are dried at ambient conditions.

Microwave drying

Microwave drying is another type of drying that can be done by precisely modifying domestic microwaves.

Fluidized bed drying

Fluidized bed dryer is used in this type of drying. 50g of fresh leaves are used for each cycle of drying at a temperature of 50˚C to 80˚C and drying is done in 30 minutes.

Out of all these techniques shade drying was most suitable for us in terms of cost, availability and quality of the final product. Hot air drying, fluidized bed drying, microwave drying all use extreme conditions which might result in damaging the phytochemicals in the leaves. Although sun drying requires no extreme conditions, yet exposure to direct sunlight can result in loss of chlorophyll and other useful constituents of the leaves.

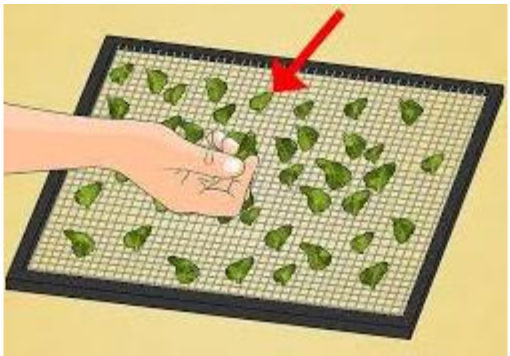


Fig.10: shade drying of leaves

Preparation of Leaves' Extract:

Fresh leaves of cedar bay cherry plant were washed and left for drying under ambient conditions for 20 days. After 20 days the leaves were finely grinded and stored in an airtight glass container.

For the preparation of extract, 1 gram of grinded leaves was boiled in 150ml distilled water. The leaves were boiled till the volume of water reached 100ml mark of the volumetric flask. The extract was allowed to cool at room temperature and then filtered using what man filter paper.

Preparation of Silver Nanoparticles:

In order to synthesize the nanoparticles, 20ml of above mentioned leaves' extract was mixed in 80ml of distilled water. Into this 100ml solution 1mM (0.166g) of silver nitrate was added. Immediately after mixing, the mixture was transparent. The mixture was kept at 80°C for heating for 2 hours.

After first 15 minutes the color of the solution turned from transparent to reddish. Upon further heating the solution became brownish black after 30 minutes. The color changes indicated the formation of silver nanoparticles. After that no further color change was observed till the end of the reaction.

After two hours the solution was allowed to cool and then centrifuged at 12000rpm for two hours with five washings. The supernatant was discarded and the particles were dried at 80 °C for two hours to obtain dried nanoparticles.

Synthesis of silver nanoparticles:

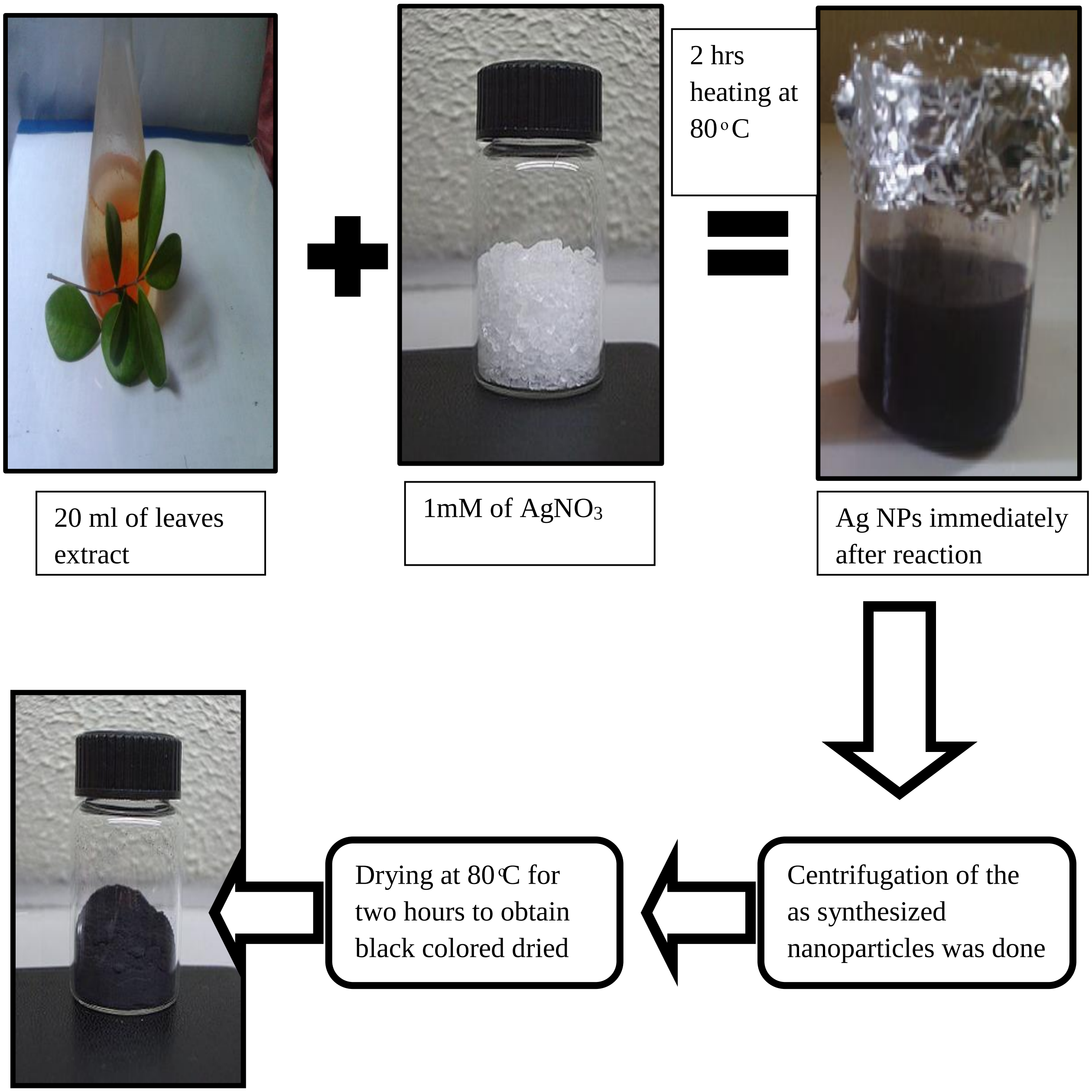


Fig.11: Green synthesis of silver nanoparticles

3.2.2 Preparation of TOPAS/ Ag Nanocomposite:

Solution casting method was used to prepare the Nanocomposite films of Topas and silver nanoparticles .The weight percentage of Ag nanoparticles in Topas is mentioned in table below

Table 3: Ag concentration in Topas matrix

S No	Wt.% of Topas	Wt.% of Ag Nanoparticles
1	100(1 g)	0% Ag (0g)
2	99(1 g)	1% Ag (0.01g)
3	95(1 g)	5% Ag (0.05 g)
4	90(1 g)	10% Ag (0.1 g)

For preparation of these nanocomposite films, at first 1g of TOPAS was dissolved in 20ml of chloroform and the solution was allowed to stir for 5 hours. Silver nanoparticles were sonicated for one hour in 10ml of chloroform to make them finely dispersed. The two solutions were mixed and probe sonicated for 1 hour after which the solution was again stirred for 24 hours to obtain a homogenized dispersion of nanoparticles.

In order to get uniform dispersion of silver nanoparticles prob sonication was done. In prob sonication ultrasound probe is directly immersed into the sample providing much greater energy to the suspension as compared to bath or cup sonicaton method. In sonication aggregated nanoparticles are broken down into finely dispersed ones by acoustic cavitation which results in the formation of microscopic bubbles that collapse producing shock waves which fracture the agglomerates of nanoparticles. After sonication the nanocomposite films were stirred overnight and then solution casted. There are different ways for film formation:

Film Deposition:

There are many film deposition methods like dip coating, doctor blade, capillary immersion force, and solution casting method.

Dip Coating;

Dip coating is a simple and inexpensive technique. In this type of film deposition method a substrate is simple immersed in the solution of polymer and is drawn out with uniform speed. This results in the deposition of film over the substrate.

Knife Edge Coating:

Substrate is first covered with the liquid to be deposit and is then moved towards a sharp knife with deposits a uniform layer over the substrate by removing excess liquid. This process involves many parameters and is quiet complex.

Spin Coating:

In spin coating the dissolved polymer is deposited over the rotating substrate in the spin coater. The thickness of the film deposited depends up the angular velocity of the substrate. Spin coating results in the deposition of thin films with uniform thickness.

Solution Casting:

The film forming material is dissolved in a solvent and is then poured out in a petri dish. The solvent is evaporated by drying and the polymer in the form of a film is left behind which is peeled off for further use.

I have used solution casting method because of its simplicity. The solution of nanocomposite film was allowed to air dry for 24 hours after which it was subjected to vacuum drying for 24 hours. The film obtained after vacuum drying were carefully packed for characterization.

Formation of Nanocomposite Films:

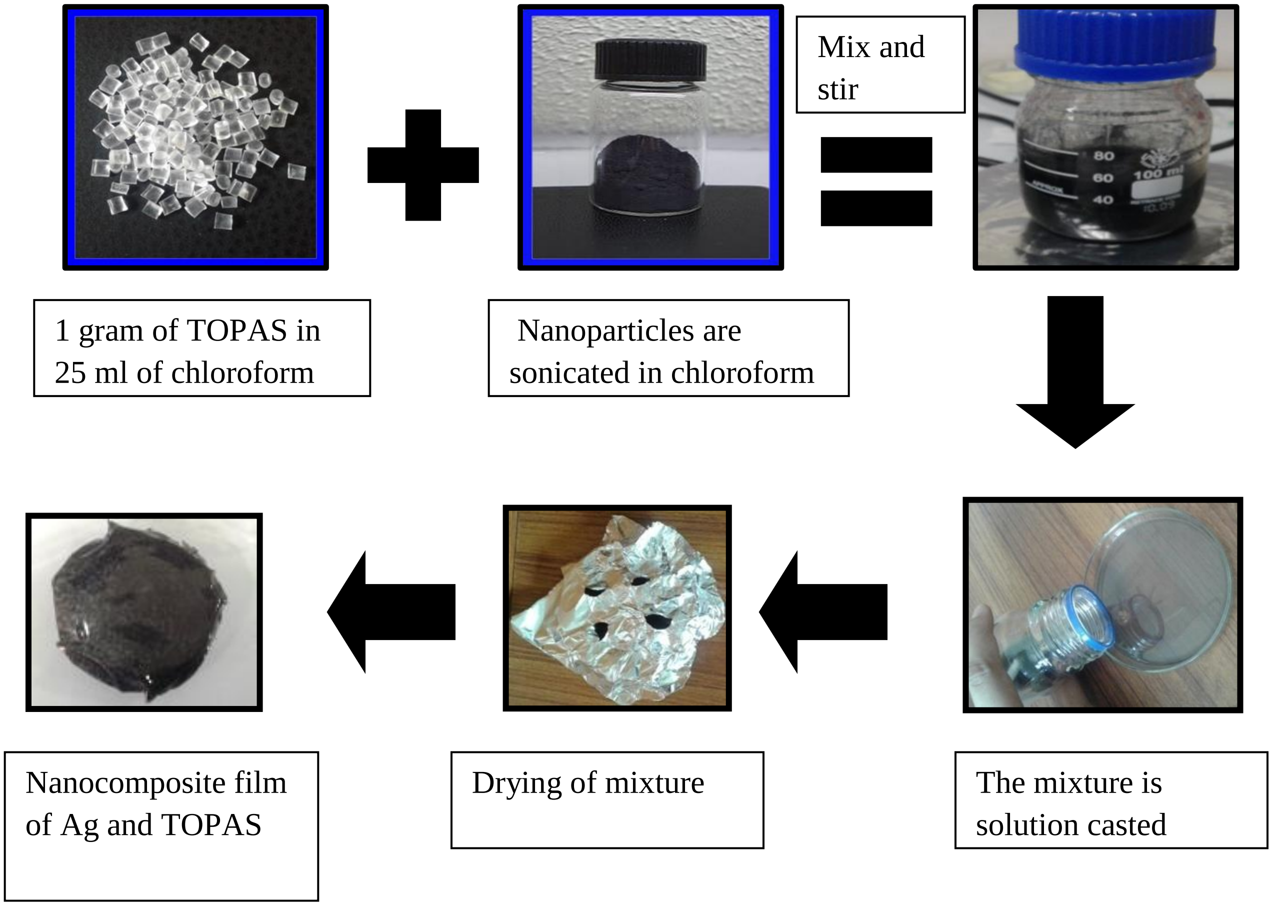


Fig. 12: Formation of nanocomposite films

3.3 Characterization of Samples:

3.3.1 Scanning Electron Microscope

Morphological analysis of Topas/Ag nanocomposite films was done using JEOL JSM-6490A Analytic scanning electron microscope. Small square pieces of films were cut and sputtered to form a layer of gold (250A approximately) by using a JOEL JFC-1500 Ion sputtering device. Samples were mounted on aluminum stubs for further examination.

3.3.2 Fourier Transform Infrared spectroscopy

FTIR (ATR-FTIR JASCO 7890) analysis was carried out for chemical analysis and to identify different functional groups present in nanocomposites films. FTIR was used in the range of 400-4000cm-^{1} with a spectral resolution of about 4cm^{-1}.

3.3.3 UV-Vis Spectroscopy

UV-Vis spectroscopy (PerkinElmer, LAMBDA 35 UV/Vis spectrophotometer) was carried out to indentify the absorption maxima for Ag nanoparticles.

3.3.4 Differential Scanning Calorimeter

In order to investigate the thermal properties of specimen, DSC was used. The samples were heated to 250°C/min, at a scanning rate of 10° C/min in order to generate a graph, from that plot of heat flow vs. temperature; we recorded glass transition temperature of nanocomposite films.

3.3.5 X- ray Diffraction

For crystallite size determination and phase identification of specimens, XRD was used. X-ray diffraction patterns of the samples were obtained by using Xpert’s PRO PAN Analytical X-ray diffractometer. The machine uses Ni-filtered Cu K alpha.

3.3.6 Tensile Testing

TRAPEZIUM-X Universal Testing machine (AG- 20KNXD Plus) by Shimadzu Corporation was used for tensile testing. The force was applied using cross head speed of about 5mm/ minute. Hand cutter was used to cut the films of 10x 90 mm^2 (WIDTH X LENGTH) having gauge length 20mm. The tensile test was performed on 3 samples of each concentration, at a strain rate of 5mm/min.

3.3.7 Water Vapor Permeability

Water vapor permeability test was performed to determine the barrier properties of nanocomposite films. The nanocomposite films were wrapped over the neck of reagent bottle which was filled with 10 ml of distilled water. The films were wrapped, using Teflon tape, in a manner to make the reagent bottle as much air tight as possible. The bottles were weighed and then placed in the oven for 24 hours at 40 °C. After which they were weighed again. The decrease in their weight was recorded for calculations.

3.3.8 Antibacterial study

Escherichia Coli and Staphylococcus Aureus were used to investigate their responses toward TOPAS/Ag nanocomposite films. For antibacterial study of TOPAS/Ag nanocomposite films, “Anti bacterial parallel streak AATCC 147(modified) method” was used. Samples were cut into round shape of 2cm diameter. For the preparation of bacterial cultures, 1ml of given bacteria was added into 9ml of freshly prepared broth and placed it in shaking incubator at 37°C for 24 hrs. The OD of these bacteria was measured using spectrophotometer at wavelength of 600nm.Agar was prepared by dissolving 1.4 gram of nutrient agar into 50ml of distilled water. In each Petri dish 25ml of agar solution was poured. The plates were then incubated overnight at 37˚C to check any type of microbial growth. After this 50µl of bacterial broth was poured and spread into each petri dish and then samples were placed. Petri dishes were sealed and placed into incubator for 24 hours. Antibacterial activity of films was visually assessed.

3.3.8 Antifungal Study

For anti fungal study of nanocomposite films, standard "ASTM G 21-96" method was used. Solution of potato dextrose agar (PDA) was formed by dissolving it in water. The PDA solution was poured into petri dish and the perti dish was then incubated overnight at 27℃. One fungal strains of Aspergillum Flavus and was used to study anti-fungal resistance. Sample of each concentration were cut in to round shape of 1.5 cm diameter and placed in perti dishes. After 15 days the results were studied and images were taken.

Chapter 4

4. Results and Discussion:

4.1 Surface Morphology of Silver Nanoparticles and Ag /TOPAS Nanocomposites:

In order to prepare samples for SEM dry powder of Ag nanoparticles was mixed in 20 ml of distilled water. The mixture was sonicated for one hour to break the agglomerates and to get a fine dispersion of silver nanoparticles. Out of well dispersed solution of silver nanoparticles one drop was taken out and poured on a glass slide with the help of a dropper. The sample was dried using a lamp as a heating source. After mounting the slides on aluminum stubs sputtering was done after which the analysis started. SEM images show that the nanoparticles are successfully synthesized by green approach. The nanoparticles are spherical in shape and are well dispersed. The size of the particles varies from 16nm to 40nm.

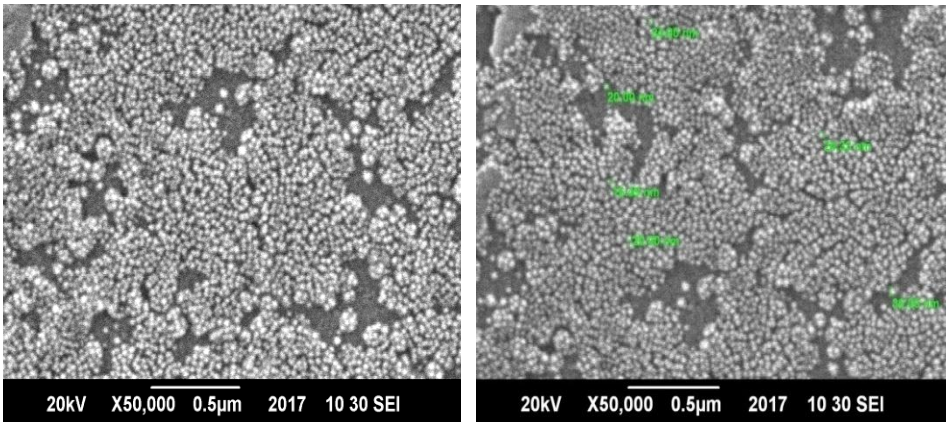


Figure 13 (a) Figure 13 (b)

Figure 13: SEM images of Silver Nanoparticles

4.2 UV-Vis Spectroscopy of Nanoparticles:

Following is the UV spectra of silver nanoparticles.1mg of silver nanoparticles were dissolved in 20 ml of distilled water and sonicated to get a fine dispersion.5 ml out of that dispersion was taken in a cuvett to get the absorption spectra.

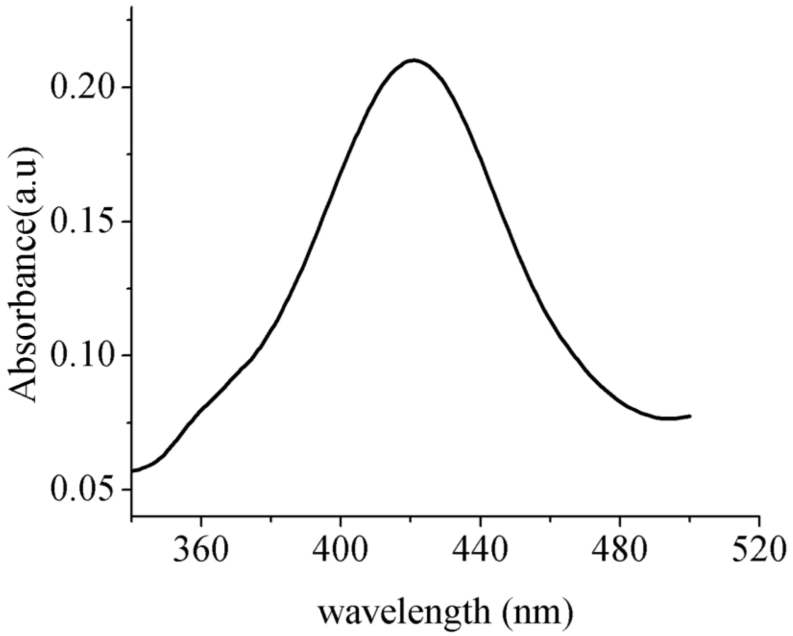


Figure 14: UV Visible Spectra of Silver Nanoparticles

Silver nanoparticles have free electrons that give rise to surface Plasmon resonance upon irradiation with light having frequency in resonance with the electronic vibrations. The UV spectra show that nanoparticles of silver are successfully synthesized. The peak at 420nm corresponds to the formation of particles with average size around 20nm. The Plasmon band is quiet broad with the tail region in the longer wavelengths. The reason for which is that the non-uniform size distribution for silver nanoparticles. Small sized nanoparticles absorb in the small wavelength region where as particles with large size show absorbance in the longer wavelength region [32-33].

4.3 FTIR Spectra of Leaves' Extract and Silver Nanoparticles:

The FTIR spectrum was used to identify the active components' functional groups present in the plant extract. Following is the FTIR spectra of leaves extract and nanoparticles.

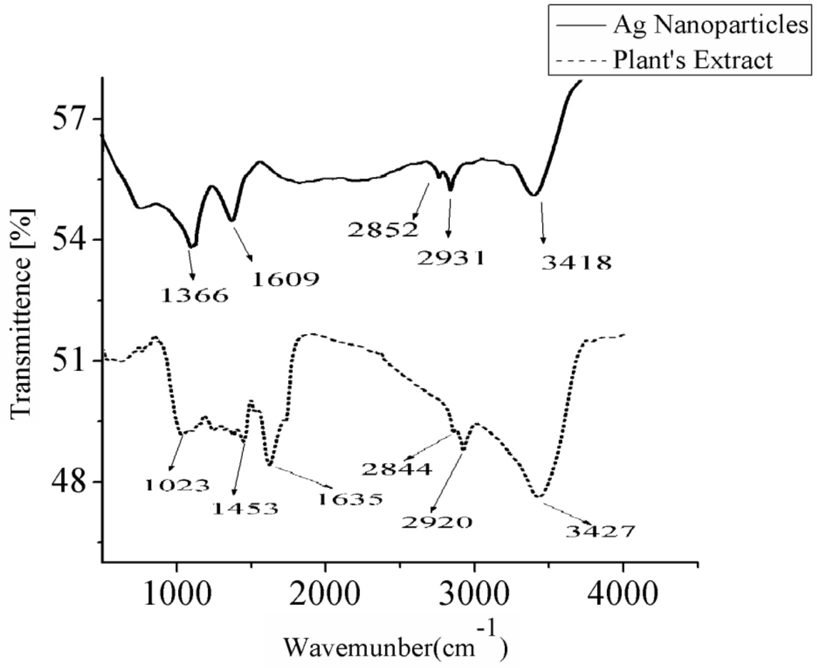


Figure 15: FTIR of leaves' extract and Ag nanoparticles

The FTIR spectra show the peaks at 1023cm^{-1}, 1453cm^{-1}, 1635cm^{-1}, 2844, 2920cm^{1} and 3427cm^{1} ·Eugenia Reinwardtiana contains anthocianines and phenols [34]. A band of peaks is observed from 1023cm^{-1} to 1453cm^{-1} which represents the vibrations of C=C bond present in anthocyanines and phenols [35]. At 1635cm^{-1} and a characteristic peak at 3427 cm^{-1}again show the presence of polyphenols such as gallic acid, tannic acid compounds [36]. The peaks at 2844cm^{-1} and 2920cm^{-1} are due to asymmetrical and symmetrical stretching vibrations of C–H groups such as CH_2 and CH_3 of reducing sugars present in the plant [36-37].

All the absorption peaks present in the FTIR spectrum of *Eugenia reinwardtiana* extract could also be observed in the FTIR spectrum of green synthesized phytocapped silver nanoparticles with some variation in their positions. This shows that the phytoconstituents of the plants are involved in the synthesis and capping of silver nanoparticles.

4.4 X- ray Diffraction of Silver Nanoparticles:

The biosynthesized silver nanoparticles using E. Reinwardtiana leaf extract were further studied by XRD. Following is the graph for XRD

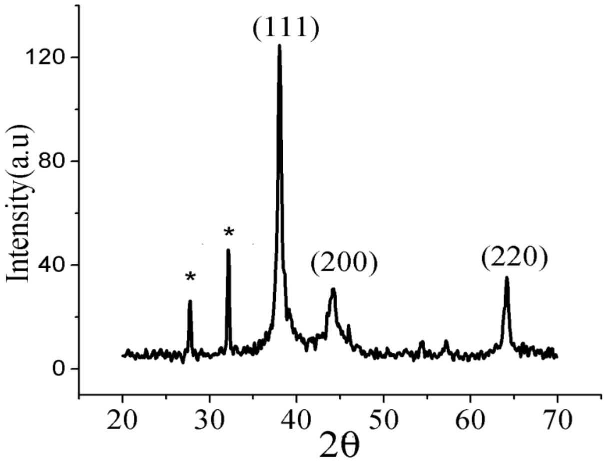


Figure 16: XRD of silver nanoparticles

The four distinct diffraction peaks with 2 Ɵ values of 38.02, 44.02 and 64.73 are due to the presence of (1 1 1), (2 0 0) and (2 2 0) FCC planes [38]. The XRD thus confirms that the nanoparticles are FCC and crystalline. Unassigned peak (marked with star) also appeared which suggests the crystallization of bio-organic phase on the surface of the silver nanoparticles [39]. The average crystallite size was found to be 11.6nm calculated using Debye Scherer formula.

Table 3: Calculation of lattice parameters from XRD

	[°2Th]	d-Spacing[Å]	Lattice constant [Å]	Crystallite size (nm)
1	38.2	2.35	4	16.8
2	44.1	2.04	4	7.8
3	64.1	1.45	4.1	10.4

4.5 Morphology of Nanocomposite Films:

Scanning electron microscope was used to analyse the surface morphology of TOPAS/Ag Nanocomposite system. Small square pieces of films were cut and sputtered to form a layer of gold (250A approximately) by using a JOEL JFC-1500 Ion sputtering device. Samples were mounted on aluminum stubs for further examination.

Following are the SEM images of nanocomposite films:

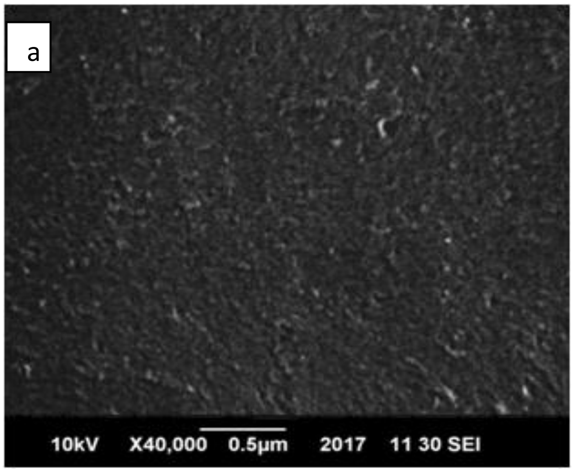


Fig. 17(a): SEM image of pure TOPAS

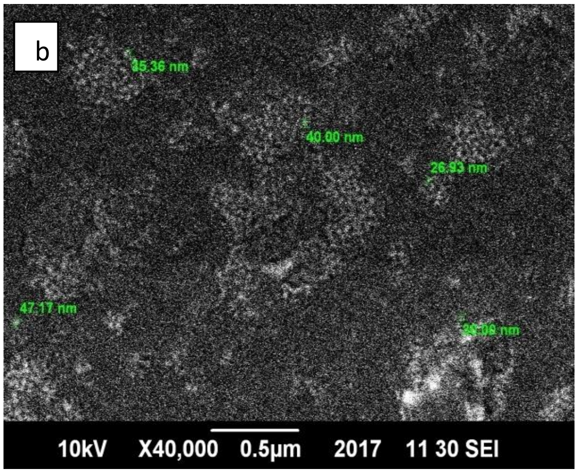
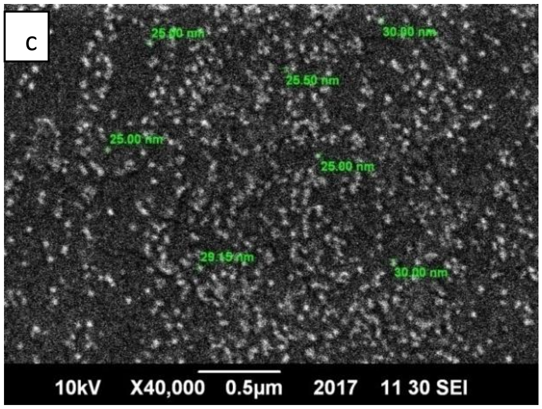


Fig. 17(b): SEM image of 1% Ag+ TOPAS

From SEM images it can be clearly observed that varying concentration of Ag nanoparticles in topas impacts the morphology of the system. In this system Ag is dispersed phase while TOPAS is matrix. Figure 14(a) shows the surface of pure TOPAS film. The image indicates that film of pure TOPAS has a smooth and crackles surface. This is the same as the SEM images of the surfaces of many other pure polymers' films. Figure 14(b) shows the SEM image of TOPAS film containing 0.01g of Ag nanoparticles, as this constitutes only 1wt% of nanoparticles in polymer matrix so in the SEM images we can see small patches of blank polymers that are not reinforced with the filler material. In 1% Ag in TOPAS film, few nanoparticles can be observed.

Following are the SEM images of nanocomposite films of Topas with 5 wt% Ag and 10 Wt % Ag.



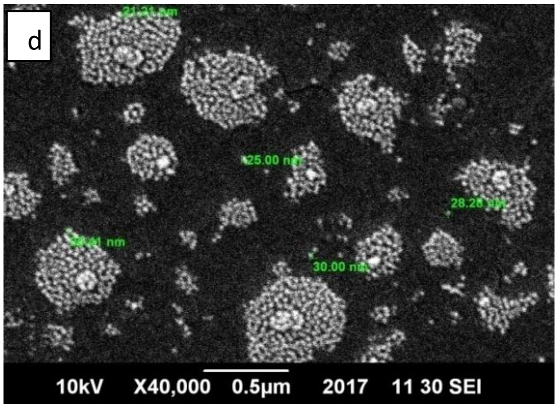


Fig.17(c): SEM image of 5% Ag +TOPAS Fig. 17 (d):SEM image of 10% Ag+ TOPAS

The film with 5% Ag shows increased amount of Ag nanoparticles. The Ag nanoparticles are well dispersed and are distributed throughout the surface of the films. The size distribution of nanoparticles as seen in the image indicates that there is no aggregation of particles. By further increasing the concentration to 10%, the SEM image of the film shows the aggregation of nanoparticles. As the concentration of nanoparticles increases the interactions between the filler have increased as compared to the interactions between the matrix and the nanoparticles formed aggregates. By comparing the above two figures we can see that in 10% film the nanoparticles are not well dispersed and are aggregated.

At lower concentration (≤ 5 wt%), the AgNPs are dispersed uniformly in the TOPAS matrix without any visible aggregation (Fig. 14b and 14c). However, at higher concentration of 10 wt%, the AgNPs are agglomerated in the TOPAS matrix (Fig. 14d) as evident from x-ray diffraction graph also (Fig. 13). The size of AgNPs did not change in the nanocomposites, however, the dispersion is influenced with the increase in the concentration of the AgNPs from 1 wt% to 10 wt% which would eventually affect the properties of the TOPAS/AgNPs system as discussed further.

4.6 XRD of Nanocomposite Films:

In order to further investigate nanocomposites XRD was done. XRD plots of pure TOPAS as well as nanocomposite films are shown in fig. 19.

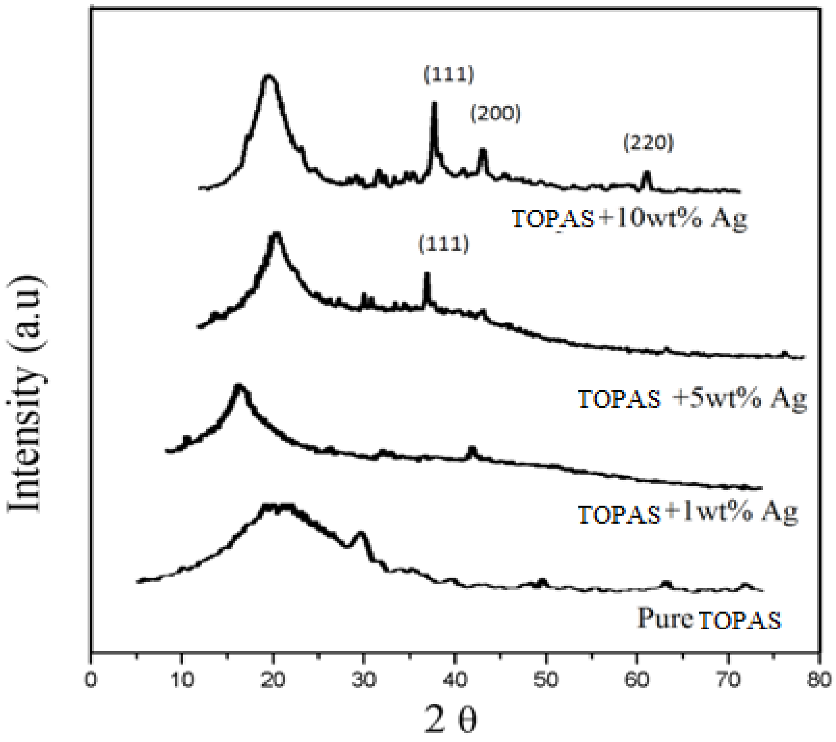


Fig. 18: XRD of Nanocomposite films

Peaks of silver can be observed in nanocomposite films with 5wt% and 10wt% silver content. Pure Topas and the nanocomposite film with 1 wt % of silver showed no peak of silver. The reason could be very low concentration of silver in 1 wt % film to be detected by XRD. SEM image of 1% nanocomposite film shows that the amount of nanoparticles is very small and the particles are not distributed throughout the film. The peaks of silver begin to appear at higher concentrations i.e. we can see the peaks of silver for 5% and 10% nanocomposite films. The SEM images also indicate the greater concentration of nanoparticles in these films. The graph show sharp crystalline peaks for silver nanoparticles as well as a hump which indicates the semi crystalline nature of

TOPAS. The XRD shows that silver nanoparticles are successfully incorporated in the polymer matrix.

The pure TOPAS exhibits a broad hump indicating the amorphous nature of the copolymer. However, a small peak adjacent to the broad hump of neat TOPAS appears at $2\theta = 33^o$ inferring the localized ordered arrangement of the rigid chain structure of norbornene present in the main chain of TOPAS. Recently, such localized ordering of the rigid chain structures like phenyl and/or nitrile groups in acrylonitrile butadience styrene has also been observed [40]. For TOPAS/Ag NPs nanocomposites, the diffraction peaks of Ag NPs pertaining to the (111), (200) and (220) crystal planes are clearly discerniblealong with the broad hump of TOPAS indicating that the Ag NPs are embedded in the TOPAS matrix. Although, at lower concentration of Ag NPs up to 1 wt%, the diffraction peaks of AgNPs are barely visible inferring well dispersion of Ag NPs in the TOPAS matrix. While at higher concentration of AgNPs≥ 5 wt%, the peak intensity of Ag NPs is enhanced inferring the stacking and/or agglomeration of Ag NPs in the TOPAS matrix.

4.7 DSC of Nanocomposite Films:

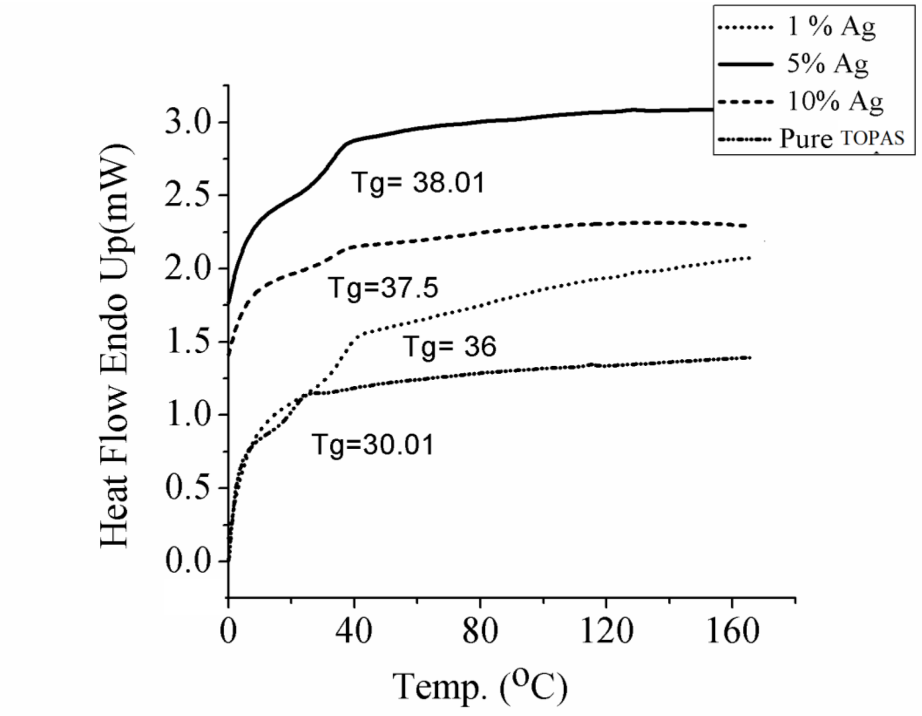


Fig. 19: DSC of Nanocomposite Films

At glass transition temperature (T_g) the polymer becomes brittle and hard from a ductile material. Glass transition temperature is the temperature below which the chains of polymer matrix don't have enough energy to move or to rearrange themselves, it the point when material can be labeled as glassy or fragile. When such a material is heated its energy as well as volumes is increased. When temperature is increased till T_g the chains become more mobile and the polymer becomes plastic or rubbery.

Crystalline polymers do not show glass transition temperature; while amorphous polymers do not have a melting temperature; they only have a glass transition temperature. There are polymers that semi crystalline which means they have both amorphous and crystalline nature. Such polymers have both a glass transition temperature and a melting temperature. The glass transition temperature is lower than the melting temperature. Amorphous polymers consist of entangled segments or polymer chains. These polymer chains require some activation energy in order to move freely. Below glass transition temperature they do not move as they not have enough energy to do so.

By the addition of filler the T_g values of our samples show at first an increase with the increase in nanoparticles concentration in the nanocomposite. When nanoparticles are added in polymer matrix, they restrain the movement of polymer chains, but addition of Ag above 5% causes a decrease in the value of T_g because by increasing the amount the particles further, agglomeration occurred that affected the internal structure of the polymer resulting in the decrease in glass transition temperature. Although there is not much variation in the T_g values among nanocomposite films, the T_g of overall system showed an increase by the addition of nanoparticles [41].

4.8 FTIR Spectroscopy of Nanocomposite Films:

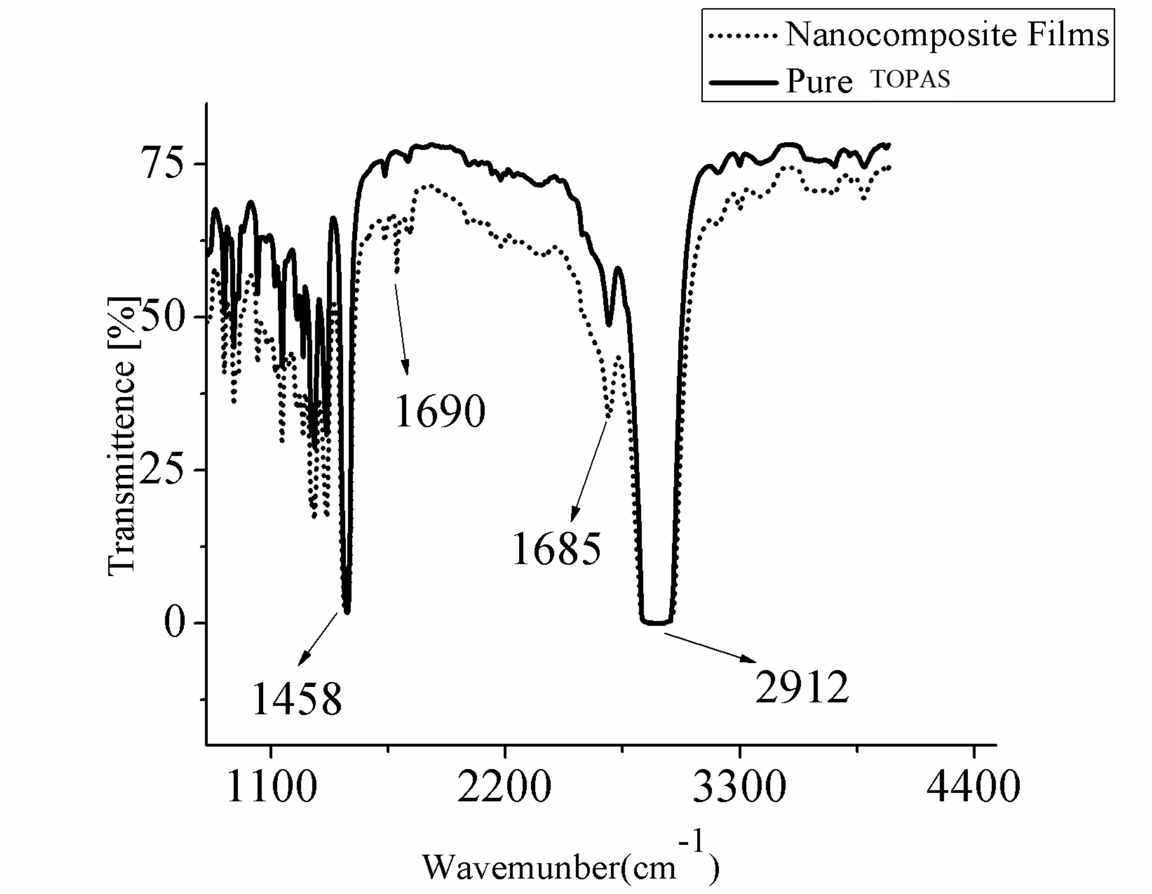


Fig.20: FTIR spectroscopy of Nanocomposite Films

FTIR of nanocomposite films shows peaks at 1458cm^{-1}, 1690cm^{-1}, 2685cm^{-1} and 2912cm^{-1}. The peak at 1458cm-^{1} corresponds to the C-H vibrations of methylene groups (= C-H_2) present in the structure of Topas, those at 2685 and 2912 (due to methyene groups=CH- and methyl groups-CH_3) represents C-H stretching [40]. There is also a band between 800 and 1300 which represents C-C stretching of aromatic rings in TOPAS. The peak at 1690cm-^{1} is due to phenols on the silver nanoparticles [37].

4.9 Water Vapor Permeability of Nanocomposite Films

In water vapor permeability test we measure the passage of water vapors through the polymer film. If water vapor transmission rate is higher through a film this means the film has poor water barrier properties which results in shorter shelf life of the packed products. In general permeability test is used to measure the barrier properties of plastic packaging materials.

If a material has no defects in its structure, the basic mechanism for the flow of water vapor through it would be activated diffusion. For activated diffusion the water vapors first dissolve in the polymer film at the higher concentration side and then disuses through the film, the diffusion occurs due to concentration gradient as a result of difference in concentration of vapors on both sides of the film. After diffusion the water vapors evaporates from the other side of the film

Some of the factors affecting the diffusion of the permeant molecule are its size, shape and polarity along with this the crystallinity, degree of cross-linking and segmental motion of the chains of polymer matrix also count. Gas molecules have difficulty in permeation through the polymer crystallites, as they are insoluble into the material. In case of semicrystalline polymers vapors permeate through the amorphous regions only. In short there are three steps involved in transmission of water vapors through polymer films.

- Adsorption of molecules on the surface of the polymer matrix
- Diffusion through the membrane due to concentration gradient and
- Desorption at the other end

When some filler is added in the polymer matrix water vapor transmission rate decreases, as now the spaces between chains of amorphous polymer are being occupied. This results in a decrease in the amount of free space available to the water vapors to travel through the membrane. As the addition of filler blocks the paths for vapors to travel across the membrane, the vapors have to change their path and move through longer paths in order to reach the other end. The results for water vapor permeability test are presented below in the form of bar graph.

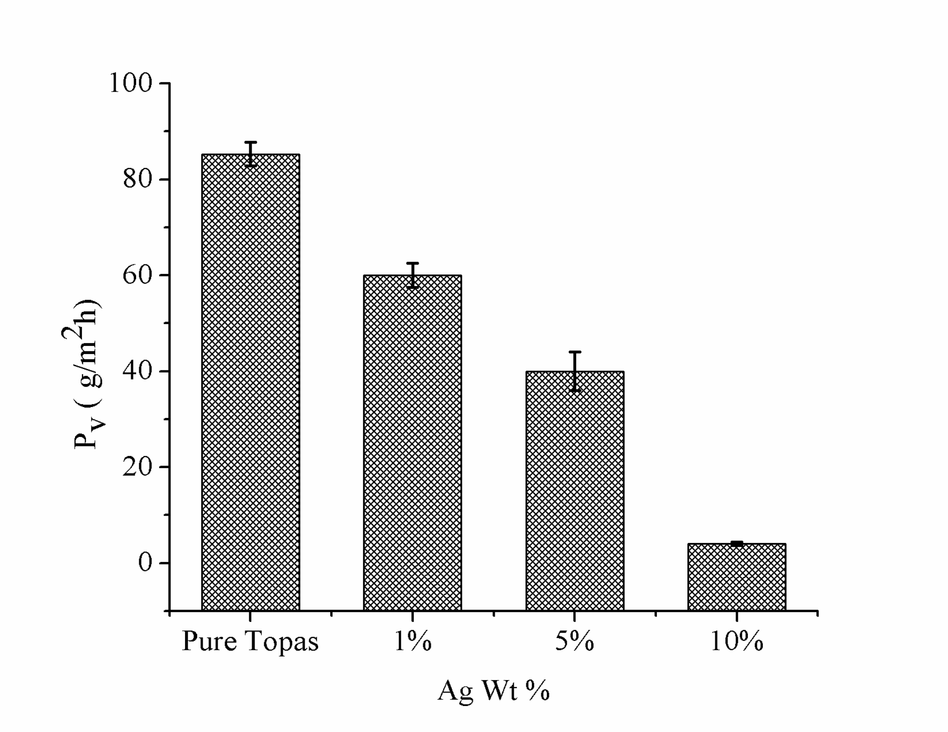


Fig. 21: Water Vapor Permeability of Nanocomposite Films

The results of the permeability test show that water permeability of theTOPAS/ Ag nanocomposites at 40 °C is dependent on the silver content. The water transmission rate for pure TOPAS was 85.3 (g/m^2 h). The water transmission rates of the nanocomposites decreased with increasing nanoparticle content to 10 wt. %. All of the nanocomposites exhibited less water transmission rates when compared to that that of pure TOPAS. By increasing the amount of nanoparticles the path way for the water vapor to diffuse through becomes tortuous and the vapors have to pass through a long distance in order move across the polymer matrix [41-42].

4.10 Mechanical Testing of Nanocomposite Films

A polymer is a network of a large number of chains. When nano particles are added the polymer chains adsorb on the surface of the particles which increase the degree of cross linking of polymer chains. The presence of filler reduces the motion of the polymer chains to the extent that the chains closed to the filler become bound to it. The samples were tested to evaluate the tensile strength and elastic modulus and to plot their stress-strain curves.

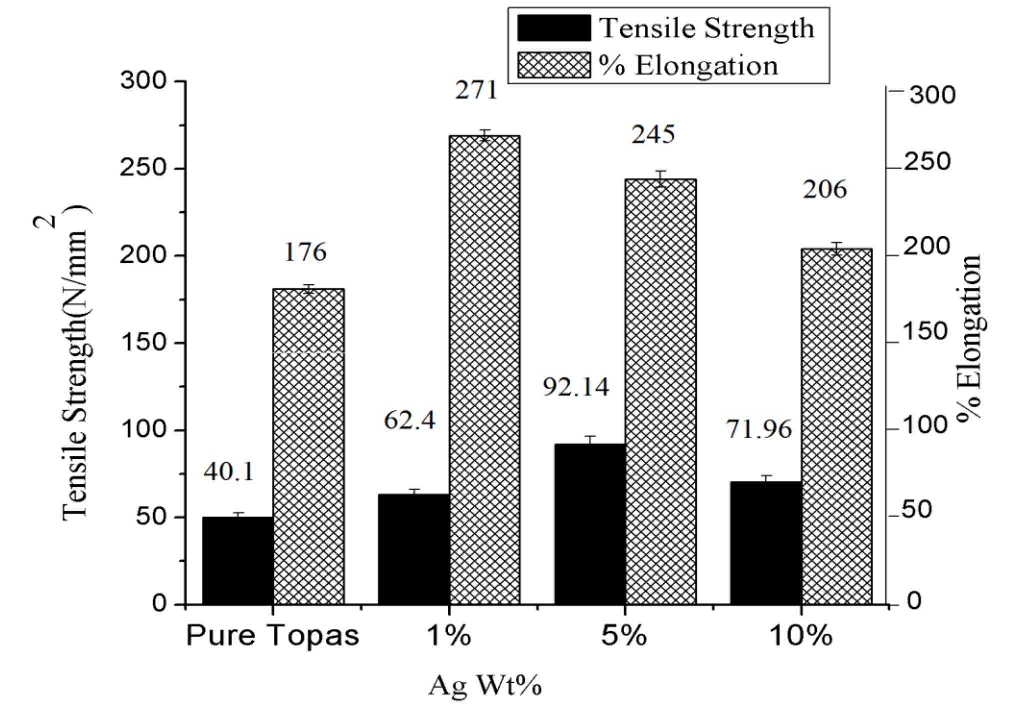


Fig. 22: Tensile Testing of Nanocomposite Films

We can explain these results on the basis of SEM results. With increasing Ag nanoparticles concentration, the tensile strength has increased. This increase is up to 5% and after this concentration, the values of tensile stress are decreasing. The reason for first increase and then decrease is good dispersion of nanoparticles at lower concentration and the dispersed nanoparticles increase the stiffness as well as tensile strength of the material because the polymer chains are anchored in the vicinity of the Ag nanoparticles. After 5 %, most of the nanoparticles starts making agglomerates and do not help in increasing the strength of nanocomposite [43]. At 10 % concentration, aggregation of nanoparticles has occurred and in aggregated nanoparticles the effective interfacial area, which contributes in the chain constriction, between the nanoparticles and the polymer matrix has decreased.

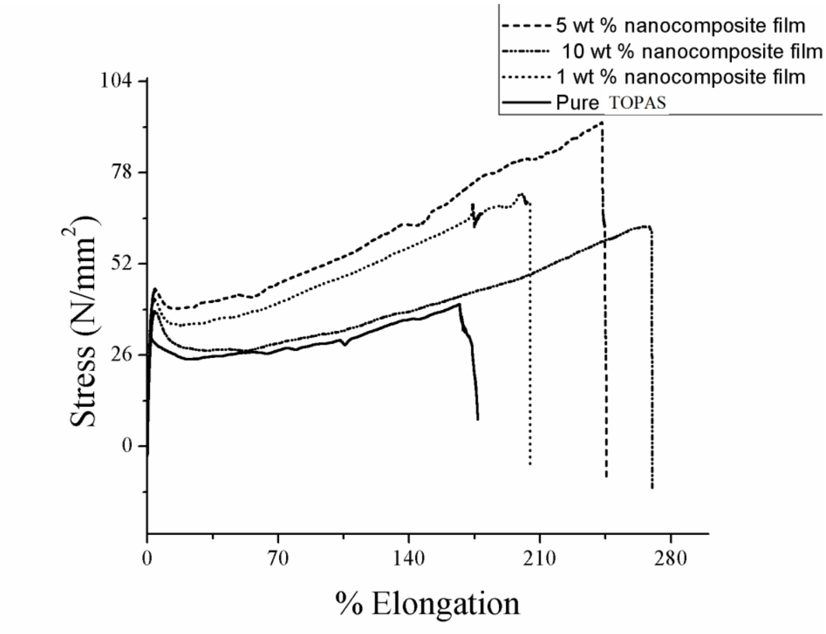


Fig.23: Force- extension graph of films

We can conclude from these results that 5 % nanoparticles concentration in TOPAS is good and useful for the application. Elongation at break of nanocomposite films is greater than pure Topas due to the load transfer at interface. In nanocomposite films the presence of nanoparticles evenly distribute the applied load throughout the polymer matrix, this buys more time for fracture to occur. Among the films we can see that 1 % nanocomposite film has shown maximum value of elongation at break then come 5 % and 10 % respectively. This has happened due to increase in the rigidity because of the restriction of polymer chain movement by the increase in the amount of filler content.

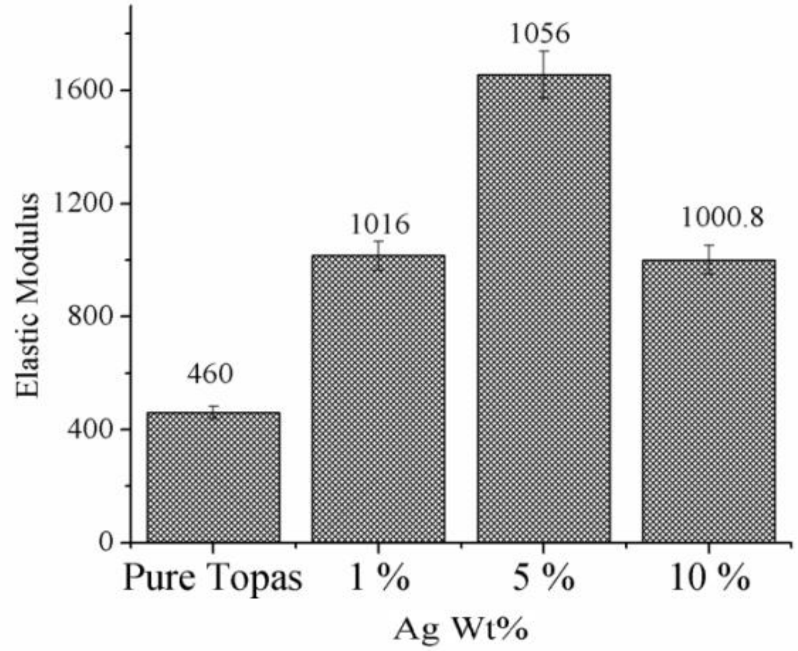


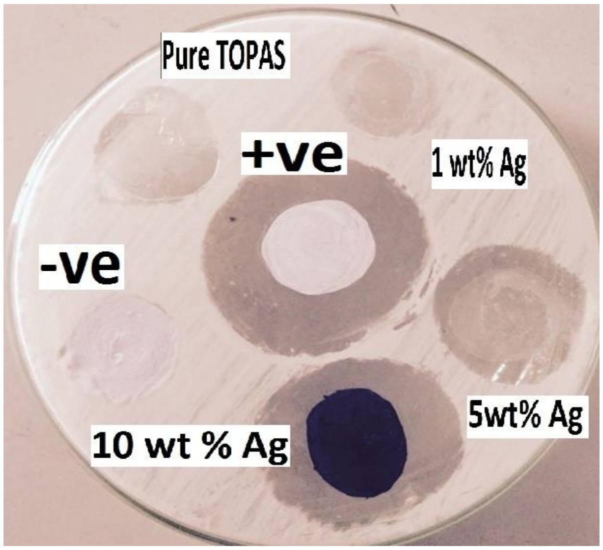
Fig. 24: Elastic modulus of samples

By the incorporation of AgNPs in TOPAS, not only the tensile strength and modulus but elongation at break is also improved (Fig. 20). The tensile strength of TOPAS (Fig. 19) is increased ~ 130 % from 40.1 to 92.14 MPa by the addition of 5wt % of AgNPs. At higher concentration of AgNPs up to 10 wt%, the tensile strength of TOPAS is reduced to 72 MPa which is ~ 80 % higher than pure TOPAS. Similarly, the modulus of TOPAS/AgNPs (5wt %) nanocomposite (Fig. 4b) is enhanced ~ 130 % from 460 to 1056 MPa and at higher concentration up to 10 wt% of AgNPs, the modulus of TOPAS is reduced to 1000 MPa that is 117 % higher than pure TOPAS.Such relative reduction in the tensile strength and modulus of TOPAS at higher concentration of AgNPs is due to the possible agglomeration of the nanoparticles in the matrix. Interestingly, at lower concentrations (≤ 5 wt%) elongation at breakof TOPAS/AgNPs nanocomposites (Fig. 20) is also increased from 176 to 271 % which is then reduced to 206 % by increasing the AgNPs concentration to 10 wt %. Notably, TOPAS exhibited the best set of mechanical properties at lower concentrations (≤ 5 wt%), thus making TOPAS more stiff, strong, ductile and tough.

Both strengthening as well as toughening mechanisms are occurring in TOPAS/Ag NPs nanocomposites systems. Usually polymer nanocomposites exhibited the increase in stiffness and strength at the loss of ductility and toughness [44-45]. However, few reports also indicate the increase in toughness along with the increase in stiffness and strength [46-47].The strengthening mechanism is based on the increase in the tensile strength and modulus of TOPAS/Ag NPs nanocomposites. At lower concentrations (≤ 5 wt%), the Ag NPs are well dispersed, resulting in the enhancement of the tensile strength and modulus of TOPAS matrix as the load can effectively be transferred from the polymer chains to the nanoparticles. However, at higher concentrations (10wt%), Ag NPs are stacked together to form the agglomerates, thereby reducing the tensile strength and modulus of TOPAS as agglomeration provides the stress concentration sites to weaken the matrix and the toughening mechanism is induced due to the retardation or delaying of crack growth within the system. Possibly, by dissipating the energy through successful transmittance of the load across TOPAS/Ag NPs interfaces delayed the process of the crack growth as this would avoid the generation of stress concentration sites in the system and thus improved the overall toughness of the nanocomposite system along with the increase in strength and stiffness.

4.11 Antibacterial Test for Nanocomposite Films:

For antibacterial study of Topas/Ag nanocomposite films, “Anti bacterial parallel streak AATCC 147(modified) method” was used. Bacteria were grown on nutrient agar poured in petri dishes. The test was 24 hours long and results were recorded in the form of images. Following are the pictures showing the antibacterial activity of nano composite films.



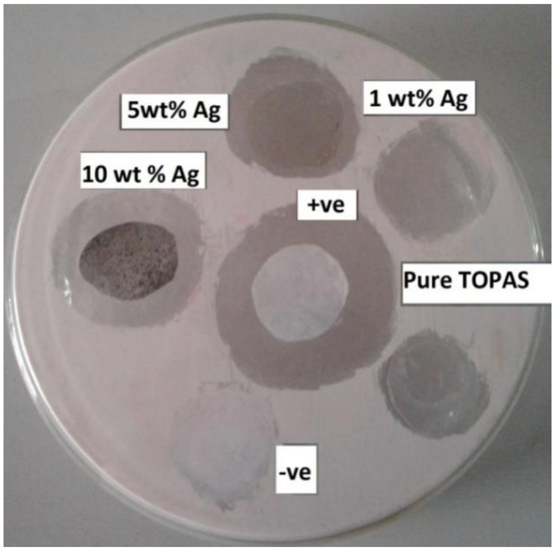


Fig. 25(a):Anti bacterial Test against *E. coli* Fig. 25(b):Anti bacterial test against *S. aureus*

By looking at the pictures it can be observed that there is no bacterial growth above and under the nanocomposite films. Bacterial growth could be seen on the negative controls that were prepared by dipping filter paper in distilled water. Positive controls for both of the bacteria gave zone of inhibition for bacterial growth (Fig. 21). Positive controls were made by dipping filter paper in a solution of Augmentin.

Incorporation of silver nanoparticles is an effective strategy for antimicrobial packaging materials. By adding antimicrobial agents into packed foods, the growth of microorganisms can be prevented, but preservatives can cause health related issues. If packaging itself is made antimicrobial the growth of food borne pathogens can be prevented and the shelf life of food will increase [51]. Another advantage is the simplicity of the production as now the addition of preservatives is eliminated. By direct addition of antimicrobials in to the food, their activity would reduce with the passage of time because of leaching into the food, and reaction with other components of the food. Antimicrobial packaging is a better option as it results in controlled release of the antimicrobial agent into the food, which allows for inhibition of microorganisms, and also provides residual activity over time, during the transport and storage of food during distribution .There are different mechanisms through which silver nanoparticles can destroy bacteria. The nanoparticles may interact with the bacterial cell wall and affect its permeability and respiration processes by attaching to the respiratory chain proteins and transport proteins [48-49]. Silver nanoparticles can form holes and pits in the cell membrane that result in the leakage of cytoplasm. The nanoparticles can also penetrate the cell membrane to destroy sulfur and phosphorous containing compounds like DNA. DNA also loses its replication ability upon interaction with silver ions. Ag^+ also destroys nucleic acid and damage respiratory chain enzymes. SNPs generate reactive oxygen species like hydrogen peroxide that can produce oxidative stress and attack membrane lipids leading to the breakdown of membrane and mitochondria and can also cause damage to DNA leading bacterial cell death [48]. In short there are different mechanisms of anti microbial activity of nanoparticles like Ag nanoparticles destroy petidolycan cell wall, denature ribosomes and bind to DNA.

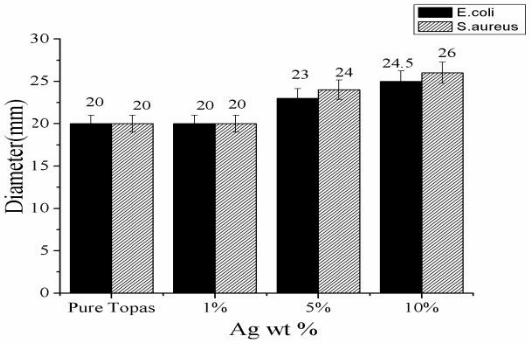


Fig. 23: Anti Bacterial Activity of Silver Nanoparticles

4.12 Anti Fungal Test for Nanocomposite Films:

For anti fungal study of nanocomposite films, standard “ASTM G 21-96” method was used. A solution of potato dextrose agar was poured into petri dish and the perti dish was then incubated overnight at 27 ° C. A fungal strain named Aspergillum Flavus was grown by streaking method on the incubated petri dish. Sample of each concentration were cut in to round shape of 1.5 cm diameter and placed in perti dishes. After 15 days the results were studied and images were taken.

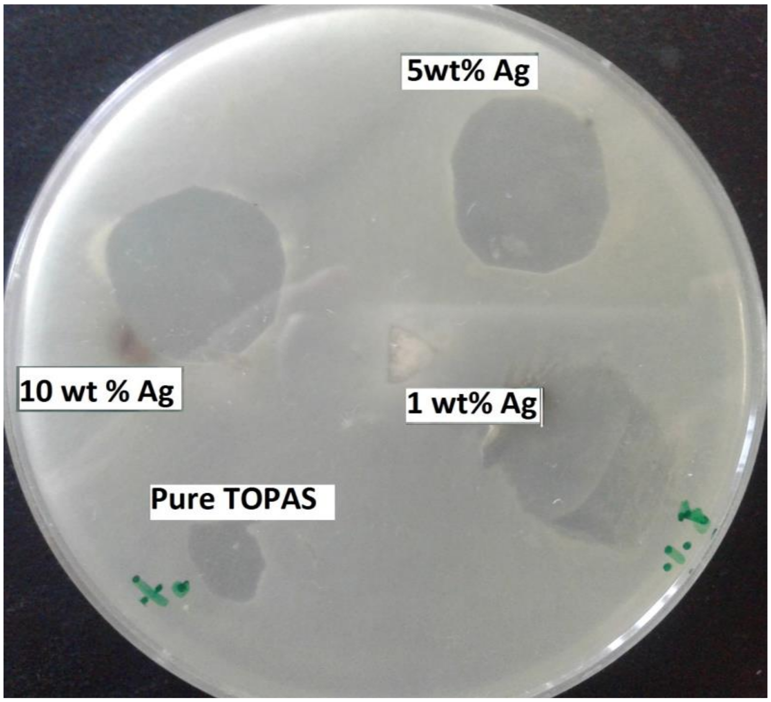


Fig. 24: Anti fungal test against *Aspergillus Flavus*

Anti fungal activity test is important for packaging materials. Packaging materials intend to prevent the food present in the packaging from temperature, moisture, dust and microbes. The migration of substances from the environment and the packaging material into the packed food material will cause its spoilage. The packaging material should be anti fungal in order to prevent the passage of fungal spores through it. The spores can damage the packed items. Apart from damaging the food, fungi can also grow on the surface of the packaging and can cause damage to it. If packaging material packaging material is anti microbial, it will inhibit the growth of food borne pathogens [51]. Pure TOPAS film did not show antifungal activity whereas nanocomposite films showed anti fungal activity. The reason for anti fungal activity is that the silver nanoparticles damage the cell wall and destroy mitochondria, ribosome and chromatin. The nanoparticles can also diffuse through the cell wall into cell and then cause cell death. Silver nanoparticles form Ag+ which binds to thiol groups of the proteins resulting in the denaturing of proteins that again results in bacterial demise [48-49].

Nanocomposite films are polymers embedded metal nanoparticles. Biocidal activity of these films is through ion release. Ions from the nanocomposite films are released through the corrosion of nanoparticles present in the bulk of the polymer when water molecules from the bacteria medium diffuse and get in contact with the particle’s surface in the nanocomposite films [50]. Following is the mechanism for the antimicrobial behavior of Nanocomposites of polymer and metal [50]:

(1) Bacteria is adhere to the polymer surface which trigger the diffusion of water through the polymer matrix

(2) As water with dissolved oxygen reaches metal nanoparticles, metal ions are realized

(3) Metal ions damage the bacterial membrane;

(4) Metal ions can also diffuse into the bacterial cell.

Chapter 5

Conclusion

In this study it has been observed that silver nanoparticles can successfully be synthesized using leaves extract of cedar bay cherry plant. SEM, XRD, UV and FTIR, all confirmed the successful formation of silver nanoparticles. The as synthesized silver nanoparticles were used for the formation of Ag/TOPAS nanocomposite films. Nancomposites of Ag and TOPAS showed remarkable properties. By the incorporation of silver nanoparticles mechanical properties of Topas were enhanced, tensile strength of nanocomposites became almost double the value of pure TOPAS, while elongation at break also increased remarkably as compared to pure TOPAS. WVP of nanocomposites was less than pure TOPAS. All concentrations of nanocomposite films showed antibacterial and antifungal behavior. At higher concentration of silver nanoparticles the films formed zone of inhibition against bacterial growth. SEM, XRD and FTIR spectra indicated successful incorporation of silver nanoparticles in polymer matrix. DSC showed that glass transition temperature of nanocomposite films was higher than pure Topas film.

Cytotoxicity and degradation profile of nanocomposite films is not known. In order for industrial application in packaging, cytotoxity and degradation profile of these nanocomposite films should be known.

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