# IMPREGNATION AND CHARACTERIZATION OF PECTIN-PVA BLEND FILM WITH SILVER NANOPARTICLES FOR FOOD PACKAGING

**A PROJECT REPORT**

***Submitted by***


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**(An Autonomous Institution Affiliated to Anna University, Chennai)**


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## BONAFIDE CERTIFICATE

Certified that this project report **"IMPREGNATION AND CHARACTERIZATION OF PECTIN-PVA BLEND FILM WITH SILVER NANOPARTICLES FOR FOOD PACKAGING"** is the bonafide work of "Deepika V, Jothi Sailaja C A C, Santhanalakshmi K, Sowmiya Devi S**"** who carried out the project work under my supervision.

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## ABSTRACT

In this research paper, semi-synthetic films have been prepared to check its compatability to be used for food packaging. Pectin, PVA, Pectin-PVA blend and silver nanoparticles incorporated pectin – PVA films at three different concentrations were prepared, using glycerol as the plasticizer and calcium chloride as the binding agent which were made into film by solvent casting method. The incorporation of silver nanoparticles in the film was confirmed using Fourier Transform Infra Red spectroscopy (FTIR), X-Ray Diffraction analysis (XRD). The mechanical properties of the film were characterized using Tensile strength, Elongation factor, Young's modulus. Antibacterial activity was checked against *Klebsiella Pneumonia*.



Keywords: Pectin, PVA, Blend film, Silver nanoparticles, Food packaging.

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| SYMBOL | ABBREVIATION |
|---|---|
| % | percentage |
| °C | Degree Centigrade |
| µl | Microlitre |
| Ag | Silver |
| $AgNO_3$ | Silver Nitrate |
| AgNps | Silver nanoparticles |
| C 30B | Cloisite 30B |
| cm | centimeter |
| $cm^{-1}$ | per centimeter |
| Conc. | Concentration |
| DSC | Differential Scanning Calorimetry |
| EPS | Extracellular Polymeric Substances |
| FTIR | Fourier Tansfrom Infra Red spectroscopy |
| IMTECH | Institute of Microbial TECHnology |

| | |
|---|---|
| ml | Millilitre |
| mM | Millimolar |
| MTCC | Microbial Type Culture Collection and genebank |
| nm | Nanometer |
| NMR | Nuclear Magnetic Resonance |
| OEO | Oregano Essential Oil |
| PLA | Poly Vinyl Alcohol |
| PVAc | PolyVinylAcetate |
| PVDF | Poly Vinylidene Fluoride |
| SDFCL | S D Fine Chemicals Limited |
| SEM | Scanning Electron Microscopy |
| SPR | Surface Plasmon Resonance |
| TEM | Transmission Electron Microscopy |
| $T_g$ | Glass transition temperature |
| TGA | Thermo Gravimetric Analysis |
| UV-Vis spec | Ultra Violet Visible spectrophotometer |
| WVP | Water Vapor Permeability |
| XRD | X-Ray Diffraction analysis |

# CHAPTER 1

# INTRODUCTION

## 1.1 FILMS IN FOOD PACKAGING

Food packaging has become a significant concern of food technology for the preservation and protection of various food stuffs from microbial spoilage and oxidative damage. Plastic industry is one of the most rapidly expanding industry in the past several decades. This plastic packaging is used annually worldwide which is put to onetime use and discarded. The treatment of waste plastics pose serious threat to environment. The industry is now facing multiple ecological issues for handling the plastic raw materials and their finished products. The increased environmental consciousness has led to the focus on an alternative packaging made of natural biopolymer or its blend with a synthetic polymer. A polymer is a macromolecule that is formed by the process of polymerization when many monomers are linked together in a single unit. These polymers either occur naturally such as cellulose, starch, chitosan, pectin or may be synthetic such as Polyethylene, Polyvinyl chloride, Polystyrene, Polyvinyl alcohol, Polypropylene and many more. Irrespective of being natural or synthetic, polymers are used to synthesize thin films by a variety of methods like solution casting, extrusion, solution deposition and many others.

In recent years, consumer's demand for microbiologically safer foods, greater convenience, smaller packages, longer shelf life is on a rise in the market. This demand has created an impact for production of films that proves to be reliable against microbial spoilage with enhanced antibacterial activity. A blend film with enhanced antibacterial activity helps in effective preservation of food from pathogens. Incorporation of silver nanoparticles into the film plays a significant contribution for the antibacterial activity. Further, the biosynthesis of silver nanoparticles using a plant extract adds great value to the antibacterial activity. However, the concern for longer shelf life with reliable mechanical and barrier properties has lead to the production of a blend film with a natural polymer and a synthetic polymer.

### 1.1.1 PECTIN – A NATURAL POLYMER

Pectin is a structural polysaccharide which is widely found in land plants. It is an anionic polysaccharide composed of β-1,4- linked D-galacturonic acid residues. The major traditional and commercial sources of pectin are citrus peel, apple pomace, sugar beet, sunflower, etc. Citrus peel and apple pomace are usually waste materials obtained from other industries like fruit processing, food and juice industries. This proves pectin to be one of the cheapest source available for the synthesis of a blend film(Fishman and coffin, 1998).

On the other side, pectin is widely used in manufacturing of medicines. Further, it is used to prevent colon and prostate cancer. These medicinal values of pectin makes it more reliable for the synthesis of a novel blend film which can be potentially used in food packaging.

### 1.1.2 POLY VINYL ALCOHOL (PVA) - A SYNTHETIC POLYMER

Poly Vinyl Alcohol (PVA) is a unique polymer that doesn't result polymerization but by the alcoholysis of PolyVinylAcetate (PVAc) in alcohol using any alkaline catalyst. The resulting PVA is soluble in water but insoluble in most organic solvents. PVA are widely used in plastic films with water-resistance capacity, contact lenses, wound dressing, artificial heart lining and drug delivery. PVA is proved to be edible and non-toxic (Cortez-Mazatan*et al.*, 2011), having compatability with the body system (Kenawy*et al*., 2014). Thus, addition of PVA to the pectin will cause no harm and also could enhance the mechanical, thermal properties of the resulting film with increase in its shelf-life. In addition, glass transition temperature of the film decreases by addition of PVA and again with the addition of glycerol to the film. This has an added advantage of increasing the tensile strength and elasticity (Fishman and Coffin, 1998).

### 1.1.3 NANOPARTICLES

Nanotechnology has gained advantage in the recent years. It has a wide range of applications in the fields of nanomedicine, drug delivery, imaging (Panneerselvam *et al*., 2011). With the use of nanotechnology, nanoparticles can be synthesized in the controlled range of 1 to100 nm (Ravi *et al*., 2013). The present work focuses on synthesis of silver nanoparticles.

Currently, the use of silver nanoparticle has been increased because of their unique properties which include electromagnetic, optical and catalytic activity (El-Nour *et al*., 2010). It can be synthesized by physical, chemical and biological method. Physical methods are time consuming process and chemical methods cause environmental toxicity as it involves chemical agents (Jain *et al*., 2009). In biological method, nanoparticles can be synthesized rapidly when compared to physical and chemical approach and it is also eco-friendly. The synthesized nanoparticles has a potent application in medical, food and cosmetic sector (Song and Kim, 2009). In biological method, the nanoparticles can be synthesized by micro organisms and plant extract. On comparision, the nanoparticles synthesized by plant extracts are more stable and efficient than micro organism based method(Ismail *et al*., 2014)

### 1.1.4 PLANT SOURCE

*Andrographis paniculata* (Siriyanangai) belongs to the family of Acanthaceae. This medicinal plant is widely present in India and Sri Lanka (Mishra *et al*., 2009). It is often referred as "The King of Bitter" (Aniel Kumar *et al*., 2010) because of the presence of an active compound called Kelmegh. The plant is primarily used for the treatment of snake bite (Ravi *el al*., 2013). In addition, the plant is known for its antibacterial, anti inflammatory, anti viral, anti tumour and many more (Aniel Kumar *el* al., 2010) . The plant contains bioactive compounds like diterpenoids, flavonoids and polyphenols which shows multiple biological actions (Rajasekar *et al*., 2013). The plant extract has been

chosen because they are economical, cost effective and eco-friendly (Kotkadi *et al*., 2014).

### 1.1.5 SYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticles can be synthesized by the following methods

(a) Sunlight: The prepared sample should be kept in sunlight for 1hour to synthesize silver nanoparticles (Sulaiman *et al*., 2013).
(b) Room temperature: For the synthesis of Silver nanoparticles, the sample should be kept at room temperature for 5hours (Jain *et al*., 2009)
(c) Shaker: The sample should be kept in Shaker at 150 rpm in dark for the synthesis of silver nanoparticles (Kulkarni *et al*., 2011).

In the present work, Silver nanoparticles were synthesized by treating plant extract of *Andrographis paniculata* with silver nitrate and the sample was kept in sunlight as it is a natural source and for rapid synthesis of silver nanoparticles (Sulaiman *et al*., 2013)

### 1.1.6 CHARACTERIZATION OF SILVER NANOPARTICLES:

The presence of nanoparticles were initially confirmed by visual color change from yellowish green to brown (Panneerselvam *et al*., 2011). Further the formed nanoparticles were characterized by UV-Visible Spectrophotometer, TEM, SEM, XRD, FTIR (Song and Kim, 2009).

### 1.1.7ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES:

The antibacterial activity of silver ions and silver nanoparticles was previously reported. The mechanism is that silver ions interact with thiol groups present in the protein molecules that are present inside and outside of the cell membrane. This in turn inhibits the replication capacity of DNA of the microbes (Vimala *et al*., 2010)

The synthesized silver nanoparticles showed good antibacterial effects against various pathogens. Silver nanoparticles were incorporated in the media at various concentration and their inhibitory effect against pathogens has been studied (Kim *et al*., 2006)

## 1.2 MOTIVATION

The use of plastics for packaging purposes pose serious threat to environment which increased the demand for an alternative packaging material. The present work is focused on preparation of a novel pectin – PVA blend film which helps to meet consumer's demand for microbiologically safer foods, greater convenience, longer shelf life. The blend film with the incorporation of biosynthesized silver nanoparticles helps in effective food packaging against microbial spoilage.

## 1.3 PROBLEM STATEMENT

Pectin, a natural biopolymer has been blended with several other natural or synthetic polymers for the commercial packaging purposes. However, free pectin itself possess poor moisture barrier properties. So, this study focuses on enhancing the barrier and mechanical properties of the pectin by blending it with a synthetic polymer. Further, the incorporation of silver nanoparticles into the blend film enhances the antimicrobial activity of the film thus in favoring effective protection of food stuff against microbial spoilage.

## 1.4 OBJECTIVES

- Preparation of film using pectin, PVA as the base materials .
- Incorporation of silver nanoparticles into the blend film.
- Characterization of film by Fourier Transform Infra Red Spectroscopy (FTIR), X-Ray Diffraction studies (XRD), Thermo Gravimetric Analysis (TGA) , Differential Scanning Calorimetry (DSC) and determining their tensile strength.

# CHAPTER 2

# REVIEW OF LITERATURE

## 2.1 FILMS IN FOOD PACKAGING

A review of recent developments on Biopolymer Films and Coatings in packaging applications was reported by Vartiainen *et al.,* (2014). Biobased plastics and biopolymers were widely found to be studied in recent research. For a sustainable economy, biomass derived raw materials can be used for high volume applications like food packaging. The barrier properties of packaging proves to be a crucial factor which is to be of great concern. Special emphasis was given on barrier properties. Moisture resistance was also studied to analyse the excessive water vapor transmission through packaging which may dilute the quality of foods. A variety of biopolymers like starch, chitosan, pectin, xylan, lignin, cellulose etc and their unique properties have been discussed. In addition to single layer biopolymer films, the biopolymers can also be integrated into multilayer structures, thin film coatings like sol-gel and Atomic Laser Deposition (ALD).

Sebti *et al.*, (2007), has studied several properties of chitosan films, Hydroxy Propyl Methyl Cellulose films (HPMC), HPMC incorporated with nisin, HPMC incorporated with fat and HPMC cross-linked chitosan films. Nisin at the concentration of 250μg $ml^{-1}$ and

chitosan at 1% concentration were more efficient for total inhibition of microorganisms like *Aspergillusniger* and *Kocuriarhizophila.* Tensile strength and ultimate elongation measurements for chitosan and HPMC initial films were found to be elastic and flexible. Less elasticity was induced by High thermal treatments and additive incorporation. Film water barrier property was very limited by incorporation of fat. The evaluated characterisation of the film makes it more reliable for food packaging application.

Pelissari *et al.,* (2009), has attempted to synthesize starch-chitosan films incorporated with oregano oil. By disc inhibition zone method,the antimicrobial properties of starch-chitosan-OEO films was determined against *Bacillus cereus, Escherichia coli, Salmonella enteritidi s*and *Staphylococcus aureus.* This incorporated OEO in the film effectively inhibited the above four test microoganisms and also improved the barrier properties of the films. The water vapor permeability (WVP) of the film was determined. Further, the techniques like Fourier transform infrared spectra (FTIR) and Thermogravimetric analysis (TGA) were used to determine interaction of starch-chitosan-OEO and thermal stability respectively. The thermal stability of the film was not affected by the addition of chitosan and OEO which was determined by TGA. The improved antimicrobial activity of the film due to incorporation of oregano oil helps the food to be preserved from microbial spoilage.

## 2.2 PECTIN- A NATURAL POLYMER

Pectin based formulations for biomedical applications was reviewed by Mishra *et al*., (2012), which elucidates the widespread application of pectin. Pectin itself is an excellent biodegradable and biocompatible in nature. The various commercial sources of pectin are apple pomace, citrus peel, sunflower, etc. It can be widely used as gelling agent and stabilizer in food industries since it is consider to be a high value functional food ingredient. It can also be widely used for targeted drug delivery and other biomedical applications. A wide variety of pectin based formulations such as polymer hydrogels, films, tablets, scaffolds and many more have become one of the significant subject of recent research.

Pectin and fish skin gelatin or soybean flour protein was used for the preparation of composite films and this was studied by Liu *et al*., (2007). Protein which was included in the films promoted molecular interactions that resulted in a homogeneous structure. The composite were found to possess increased stiffness and strength. Pectin films which are protein-free can be treated with glutaraldehyde or methanol to improve Young's modulus and tensile strength. Further, the films were found to be biodegradable with moderate mechanical properties and low water vapor transmission rate. This implies that the film can be potentially used for food packaging applications or drug industries.

The mechanical, microstructural and solubility properties of pectin/polyvinyl alcohol blends was studied by Fishman and Coffin, (1997). Pectin and PVA were found to be miscible in all proportions.

Pectin control exhibited no thermal transitions and PVA control exhibited a glass transition temperature ($T_g$) between 0°C and 50°C. Lower storage moduli and more flexibility was exhibited by a mixture of 49% pectin, 21% PVA and 30% glycerol. Pectin/PVA films showed increased ductility when glycerol was added. Solubility studies revealed that pectin is readily soluble compared to PVA with even less concentration. Pectin- like properties were found to be exhibited by the films when pectin is added to PVA films and vice versa.

Pervaporation dehydration of isopropanol and water using PVA-Pectin blend membranes in which phosphomolybdic acid can be embedded was studied by Rao, (2014). Pervaporation test was carried out on phosphomolybdic acid loaded PVA-pectin blend membranes crosslinked with glutarladehyde. The characterisation of these blend membranes was done with various studies like Fourier Transform Infra Red spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Thermal gravimetric Analysis (TGA) and Differential Scanning Colorimetry (DSC).The flux and selectivity increased when the amount of phosphomolybdic acid in PVA-Pectin matrix was increased. Further, this selectivity was found to be high in a composite membrane than a plain blend membrane.

A ternary film made from chitosan/Polyvinyl alcohol/Pectin was prepared and studied by Tripathi *et al.,* (2009).The film was prepared by solution casting method and characterised by Fourier Transform Infra Red spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD). The IR spectra peak

depicted the intermolecular ionic salt bonds between amino groups of chitosan and carboxyl group of pectin. XRD analysis proved the ternary film to be crystalline. The surface morphology and weight losses of the film were observed from SEM and TGA respectively. Further, the antimicrobial activity of the film against pathogenic bacteria was also investigated. This characterisation of the ternary film collectively helps in providing a suitable material for food packaging applications.

## 2.3 PVA – A SYNTHETIC POLYMER

Santos *et al.,* (2014) studied the use of PVDF (Poly Vinylidene Fluoride) as the basal membrane, nanofibrous membrane of PVA (Poly Vinyl Alcohol), PVA-Chitosan, PVA- Extracellular Polymeric Substances (EPS) were prepared separately. In order to compare their efficiencies in water filtration, properties of the prepared films were compared for making a thin film which will be suitable for water filtration.The morphology of the prepared film was characterized using Energy-Dispersive X-ray diffraction, Atomic Force Microscopy, Scanning Electron Microscopy. On comparing the films, there were better mechanical and tensile properties were found for PVA/EPS films than PVA, PVA/Chitosan films.

Chitosan – PVA blend films have been made in the ratio of (1:1, 2:1, 3:1). Silver nitrate was added to chitosan solution and observed for the color change from yellow to red. Then PVA of 1% was added to it with 2% glutaraldehyde. The presence of silver nanoparticles in the resulting film was confirmed by the Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction studies (XRD) and UV-Vis

spectroscopy. Swelling capacity of the film increase with the increase in chitosan concentration. The intense peak for the formation of silver nanoparticles was found to be at 421nm which was reported by Vimala *et al.,* (2011).

Chitosan, PVA ratio amount were taken in various compositions to form films. These films were tested for its mechanical strength by with or without the addition of glutaraldehyde. Cloisite 30B was used as the drug carrier since it can carry hydrophobic molecules easily inside the body. Curcumin was dissolved in ethanol which is then added to the chitosan, PVA, cloisite 30B and was made into film by solvent casting method. Various kinetic equations were framed for the drug release kinetics mechanism.SEM analysis showed that 1%, 2.5% of C 30B were homogenously distributed. Swelling amount was found from the formed equation which indicated the total amount of drug carried by the film. Tensile strength of the film was found to be high for the blend film. Drug release was found to be fast for highest loading in the matrix that was studied by Parida *et al.,* (2011).

PVA mixed with sodium alginate aqueous solution was made in the form of hydrogel. This was loaded with ampicillin drug. The blend was freeze-thawed for making crosslinking stronger. By the addition of Sodium alginate and ampicillin, the gelation frequency gets decreased and swelling capacity increases. The porosity of the membrane, weight loss increases but the amount of drug being released decreases with the increase in sodium alginate content which was reported by Kamoun *et al.,* (2015).

## 2.4 SYNTHESIS OF NANOPARTICLES

A novel method for biosynthesis of gold nanoparticles using aqueous extract of grape leaf and seed was reported by Ismail *et al.*, (2014). The synthesized gold nanoparticles were by UV-Visible spectra, TEM analysis and XRD analysis. UV-Vis spectra of gold nanoparticles which is formed using grape leaf extract by varying concentration of extract (0.02-0.1%) with constant concentration of Chloroauric acid and varying concentration of Chloroauric acid (0.025-0.25mM) with constant extract concentration, along with effect of time on gold nanoparticle formation was reported. UV-Vis spectra of gold nanoparticles which is formed by aqueous extract of grape seed was also shown. XRD analysis of gold nanoparticles synthesized from leaf and seed extract showed the size of 18-25nm and 10-17nm respectively. TEM images of gold nanoparticles synthesized by grape leaf and seed extract and also the effect of pH was shown. Photoluminescence effect (fluorescence spectra) of gold nanoparticle was studied. Further FTIR and TGA of gold nanoparticles were reported.

Kumar *et al*., (2011) has attempted to synthesize Gold nanoparticles from green techniques. The plant extracts were prepared from *Terminalia chebula* which acted as reducing and stabilizing agent for the synthesis of gold nanoparticles. Then the aqueous extract was mixed with Chloroauric acid and kept in shaker. The formation of gold nanoparticles can be visually confirmed by color change from Yellow to Pinkish red. The presence of nanoparticles were further confirmed by UV-Visible Spectrophotometer with the formation of peak at 535nm. Further the formed nanoparticles were characterized by Fourier

Transformed Infrared Spectroscopy, X-Ray Diffraction analysis, Transmission Electron Microscopy.

In this study, Kumar *et al*., (2012) synthesized Silver nanoparticles from extract of *Terminalia chebula***.** The aqueous extract was prepared and treated with silver sulphate. The sample was kept in room temperature for the synthesis of silver nanoparticles. This plant contains high amount of polyphenolic contents which were responsible for the formation of silver nanoparticles. The formed nanoparticles were initially confirmed by visual color change from Yellow to Reddish brown. Then it was further confirmed by UV-Visible Spectrophotometer with the formation of peak at 452nm. The formed nanoparticles were further characterized by Fourier Transformed Infrared Spectroscopy, X-Ray Diffraction analysis, Transmission Electron Microscopy, Atomic Force Microscope.

Synthesis and characterization of silver nanoparticle from the ethanol extract of *Andrographis paniculata* and its cytotoxicity effect was studied by Ravi *et al.,* (2013). Using the ethanolic extract of *Andrographis paniculata* the silver nanoparticles were produced with the help of Silver Nitrate ($AgNO_3$) which was indicated by UV-Visible spectra and also from their Surface Plasmon Resonance spectra and their absorbance value. The aggregated nature of silver nanoparticles were viewed by their TEM image which also showed the size of the spherical nanoparticle was between 11 to 22 nm. The biomolecules that are responsible for both the reduction of silver ions and also for the capping of bio-reduced silver nanoparticles were identified by FTIR spectrum. The antibacterial activity of the synthesized nanoparticles were revealed

by their zone of inhibition for various concentration. The cytotoxic effect of synthesized silver nanoparticles was performed against sheep bone marrow cells.

Green synthesis of silver nanoparticle is gaining more importance nowadays. Because they utilizes cost effective non-hazardous reducing and stabilizing agent such as plant extracts for the synthesis of silver nanoparticles. Three medicinal plants viz *Musa balbisiana, Azadirachta indica, Ocimumtenui florum* were used as the reducing agent and silver nitrate ($AgNO_3$) as the source of silver was used for synthesizing silver nanoparticles. The UV-Vis analysis showed the absorption maxima in the range of 425-475nm which was indirectly by the Surface Plasmon Resonance. The FTIR analysis showed functional group responsible for the capping and stabilization of silver nanoparticles. The SEM image of AgNPs were in the shape of spherical, triangular and cuboidal for banana, neem and tulsi extracts respectively. TEM images of the AgNPs were at the size of 200nm. The EDS profile has the strong silver peak in all, along with oxygen and carbon peak which might be due to the biomolecule at the surface of AgNPs that are capping and stabilizing them. Antibacterial activity against *E.coli* and *Bacillus* showed maximum zone of inhibition for the AgNPs from banana when compared to those of neem and tulsi. Further toxicity and oxidative stress studies were enquired by Banerjee *et al*., (2014).

Song and Kim, (2009) has synthesized silver nanoparticles from five different leaf extracts of plants which include Pine, Persimmon, Magnolia, Ginkgo and Plantanus. Silver nanoparticles were

synthesized by treating plant extract with silver nitrate solution which acts as a reducing agent. From the above five leaf extracts, magnolian leaf broth showed maximum synthesis of silver nanoparticles. The formed silver nanoparticles were characterized using UV-visible spectrometer, TEM, SEM. The size of nanoparticles ranged from 15 to 500nm. It depends upon reaction temperature, concentration of extract and silver nitrate solution. Thus Ag nanoparticles had been synthesized and widely used in various fields.

Rodriguez-Leon *et al*., (2013) has attempted to synthesize silver nanoparticles from roots of *R.hymenosepalus*. Ag nanoparticles were synthesized using plant extract and silver nitrate solution at room temperature. Plant extract acted as a good reducing agent. Five different concentrations of silver nitrate had been taken in order to optimize the synthesis of Ag nanoparticles. The synthesized silver nanoparticles were characterized using UV-visible spectrophotometer, TEM, NMR. The size of nanoparticles ranged from 2 to 40nm. Thus synthesized nanoparticles were used for further studies.

## 2.5 ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

Kim *et al*., (2006) has attempted to synthesize silver nanoparticles in order to know its effect against microorganisms. The synthesized silver nanoparticles were stable and it was characterized using TEM. Muller Hinton media was used for the growth of *Escherichia coli* and *Staphylococcus aureus*. Antibacterial activity of silver nanoparticles were investigated against these microorganisms by disk diffusion

methods. Ag nanoparticles were incorporated at various concentrations. These nanoparticles acted on the bacterial cell wall and inhibited their growth. It was revealed that silver nanoparticles had shown more inhibitory effect on *Escherichia coli* than *Staphylococcus aureus.* Thus silver nanoparticles synthesized using reduction method has shown promising effect against microorganisms.

In this study, Rajasekar *et al*., (2013) proposed that plants acts as a good source for synthesizing of silver nanoparticles. Here a medicinal plant, *Andrographis paniculata* has been chosen for synthesizing of Ag nanoparticles as it is enriched with bioactive compounds. Ag nanoparticles was produced by green synthesis method. The synthesized silver nanoparticles were characterized using SEM, TEM, FTIR. The bactericidal activity of silver nanoparticles were investigated against *P.aeroginosa, S.aureus* and *E.coli* by Agar-well diffusion method. It was reported that the size of the nanoparticles had a great impact on inhibitory effect. The size of Ag nanoparticles less than 10nm interact with the cell membrane and damage DNA which ultimately leads to the death of microbes. The zone of inhibition for the bacterial strains clearly indicated the inhibitory potential of Ag nanoparticles. Thus the biosynthesized Ag nanoparticles have bactericidal effect against pathogens.

### *2.6 Andrographis paniculata*

Aniel Kumar *et* al., (2010) studied the antibacterial activity of extracts of *Andrographis paniculata* which is a potent medicinal plant. Bacterial cultures like *E.coli, Bacillus subtilis, Proteus vulgaris, Klebsiella*

*pneumonia and Staphyloccous aureus* were adapted for study. Agar well diffusion method was followed with the well diameter of 6mm and tetracycline was used as the control. Aqueous and methanol extracts of various plant parts like leaf, root, stem and whole plant were prepared and 50µl of each was loaded in order to report their antibacterial activity. It was showed that methanol extract of various parts of plant showed significant activity against both gram positive and gram negative organisms. It was also further suggested that the contain potential antibacterial compounds and so it can also used in pharmaceutically.

*Andrographis paniculata* belongs to the family of Acanthaceae has been widely cultivated in all over India. Ravi *et al*., (2013) has reported that the plant extract has been widely used in medical applications. Traditionly, it has been used in Siddha and Ayurvedic system of medicine. The plant extract is used in the treatment of some infectious diseases. It also exhibits good anti-fungal and anti-typhoid activities, anti malarial, anti snake venom. The plant is also used in the treatment of leprosy, fever, cough, inflammation, skin diseases.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 MATERIALS

Pectin was purchased from Himedia Laboratories Private Limited, Mumbai, India. PVA of 98% - 100% hydrolysis with the molecular weight of 60,000 – 1,25,000 was purchased from Himedia Laboratories Private Limited, Mumbai, India. Calcium chloride of molecular weight 110.99 was bought from S-D Fine Chemicals Limited (SDFCL), Mumbai. Glycerol was also bought from SDFCL, Mumbai which has the molecular weight of 92.09. Silver nitrate was bought from Himedia laboratories Private Limited, Mumbai, India. Leaves of *Andrographis paniculata* were collected on summer season from Tiruppur district of Tamilnadu.

### 3.1.1 INSTRUMENTS USED

UV-spectrophotometer of model no UV-1800 from Shimadzu was used.

Magnetic stirrer Tarson Digital Spinot MCO2

Weighing balance - Uni Bloc shimadzu model AUW220D

Hitachi high speed refrigerated centrifuge CR22GIII was used for centrifugation.

Culture of *Klebsiella pneumoniae* MTCC 3384 was obtained from IMTEC, Chandigarh

Tensile strength, Elongation property, Young's modulus were checked

using the Universal Testing Machine with ASTM D-638M-93 standard, Dak system Inc.

## 3.2 METHODS

### 3.2.1 PLANT EXTRACT PREPARATION

Fresh leaves of *Andrographis paniculata* were collected on summer season from Tiruppur district, Tamil Nadu. Leaves were shade dried for a week and ground to fine powder. The aqueous extract of plant was prepared by mixing Leaf powder (5g) with 100ml of deionized water and maintained in water bath at 100°C for 20 minutes. The prepared solution was filtered to get clear extract. The extract was then stored at cold condition for future use (Panneerselvam *et al*., 2011)

### 3.2.2 SYNTHESIS OF SILVER NANOPARTICLES

In the present study, Silver nanoparticles were synthesized by adding leaf extract as reducing agent to the silver nitrate solution and kept in sunlight in order to fasten the reaction. The presence of silver nanoparticles were visually confirmed by the color change from green to reddish brown. Further it was confirmed by UV-visible spectrophotometer analysis. The effect of extract on formation of Silver nanoparticle was revealed by adding varying concentration of extract (0.025-0.175%) to the constant concentration of silver nitrate (1mM).

The effect of silver nitrate on producing nanoparticle was performed by adding constant concentration of extract (0.125%) to varying concentration of silver nitrate (0.4-0.6mM). Simultaneously the optimum time for the formation of maximum amount of silver nanoparticle formation was also studied. Silver nanoparticle solution was synthesized in bulk as per the optimized concentration of extract and silver nitrate, which is then centrifuged in order to obtain the silver nanoparticle powder (Ismail *et al.*, 2014). The synthesized nanoparticles was further characterized by Fourier Transform Infra-Red Spectroscopy and X-Ray Diffraction analysis.

### 3.2.3 ANTIBACTERIAL ACTIVITY

Agar well diffusion method was used to carry out the antibacterial assay of silver nanoparticles. Fresh bacterial culture was obtained from MTCC 3384. About 0.1 ml of the obtained culture was spread on the agar (Muller Hinton) plate. With the help of a sterile cork borer, a well of 6mm diameter was punched off into agar medium. 50µl of each sample of silver nanoparticles with different concentrations 20,40,60,80 (µg/ml) was loaded into each well. The plates were incubated at 37◦C for 24hrs. Zone of inhibition clearly depicts the antibacterial activity of the silver nanoparticles. (Bonev *et al.*, 2008)

### 3.2.4 FILMS PREPARATION

Pectin control film was prepared by taking 0.88g of pectin in 20ml of distilled water which was mixed using magnetic stirrer instrument maintained at 50°C. Glycerol of 0.88ml was added to the solution to

increase plasticity of the film. Calcium chloride of 0.02g was also mixed with the film which was then poured in petriplates by gel casting method. Similarly, PVA control film was also made by taking 0.88g of PVA, 0.88ml of glycerol, 0.02g of calcium chloride in 20ml of distilled water by maintaining at 90°C. Pectin-PVA blend film was made by taking 1:1 ratio (Sekharnath *et al*., 2014) of pectin-PVA (0.44g & 0.44g) with 0.88ml of glycerol, 0.02g of calcium chloride in 20 ml of distilled water. Initially PVA was melted at 90°C and then pectin was added at 50°C to prevent denaturation of pectin at high temperatures.

### 3.2.5 IMPREGNATION OF PECTIN-PVA FILMS WITH SILVER NANOPARTICLES

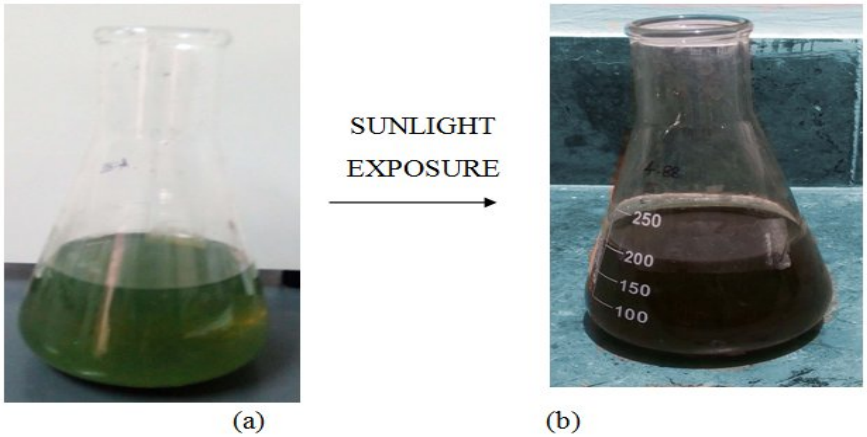
Silver nanoparticles added pectin-PVA film was made by taking 0.88mg of silver nanoparticles with 0.44g of pectin, 0.44g of PVA, 0.88ml of glycerol and 0.02g of calcium chloride in 20ml of distilled water. pectin-PVA-AgNp blend films were made in increasing concentrations by the increase in silver nanoparticles concentration alone as 0.89mg, 0.9mg respectively. These nanoparticles incorporated films were ultrasonicated for the homogenous distribution of silver nanoparticles before casting in petriplate. All the films were maintained in the incubator at 40°C.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 SYNTHESIS OF SILVER NANOPARTICLES

The silver nanoparticles were synthesized using silver nitrate as the source of silver ion and the aqueous extract of *Andrographis paniculata* as the reducing agent. After the addition of extract to the silver nitrate solution it was exposed to sunlight which act as the catalyzing agent. Within few minutes of exposure to sunlight the color of the solution turns to reddish brown from green color. The color change shows the reduction of silver nitrate to silver ions ($Ag^{+}$) by utilizing plant extract as the reducing agent (Ravi *et al*., 2013). Fig.4.1 is the pictorial representation of the color change from green to reddish brown.




(a) (b)

Fig.4.1 (a) 5% (w/v) Plant extract of *Andrographis paniculata* mixed with silver nitrate solution

(b)Silver nanoparticle solution.

## 4.2 UV-VISIBLE SPECTROPHOTOMETRY

The silver nanoparticles formed by the mixing of extract to silver nitrate solution was confirmed by the UV-Visible spectrophotometric analysis. In the UV-Vis spectra, the pronounced peak was formed in the range of 400-475nm for all the nanoparticle solution obtained. Initially by visual observation, the formation of nanoparticle was indicated by the color change from the green to reddish brown which was due to the Surface Plasmon Resonance (SRP) that occurs by excitation of nanoparticles on account of their strong absorption of visible light (Leon *et al.,* 2013).

Fig.4.2 illustrates the spectra of the extract optimization. On increasing the concentration of the extract from 0.025%-0.125% the absorption value increases and peaks were also obtained in the range of 400-450nm. Upon increasing the concentration of the extract, the absorption was decreased and the peaks were also distorted which is due to the presence of high amount of plant compounds incapable for capping and stabilizing the formed nanoparticles. Fig 4.3 illustrates the effect of $AgNO_3$ concentration on the synthesizing nanoparticles. Maximum peak (422nm) and the absorption(2.67) were obtained at the concentration of 0.55mM of $AgNO_3$ when compared to the concentration of $AgNO_3$ for the range of 0.4-0.6mM. Thus the optimum concentration of $AgNO_3$ for synthesizing maximum silver nanoparticle was found to be 0.55mM. Simultaneously the effect of time was monitored as 80 min for obtaining the good amount of nanoparticles which was shown in the Fig4.4 The nanoparticle started to synthesize within 10 min. Beyond 80min, the nanoparticle started to form aggregates that lead to distortion of the peak.

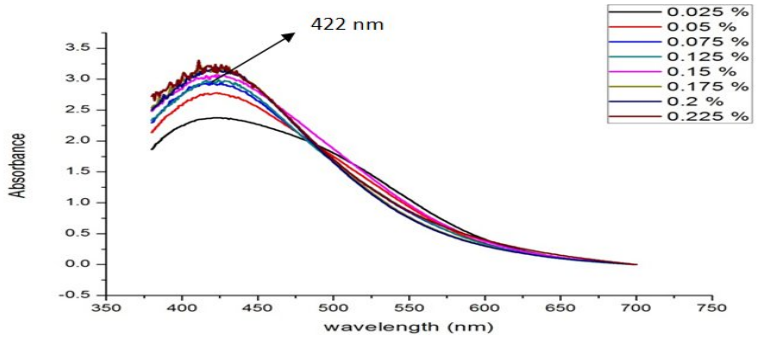



Fig 4.2 Effect of extract concentration on synthesis of silver nanoparticles

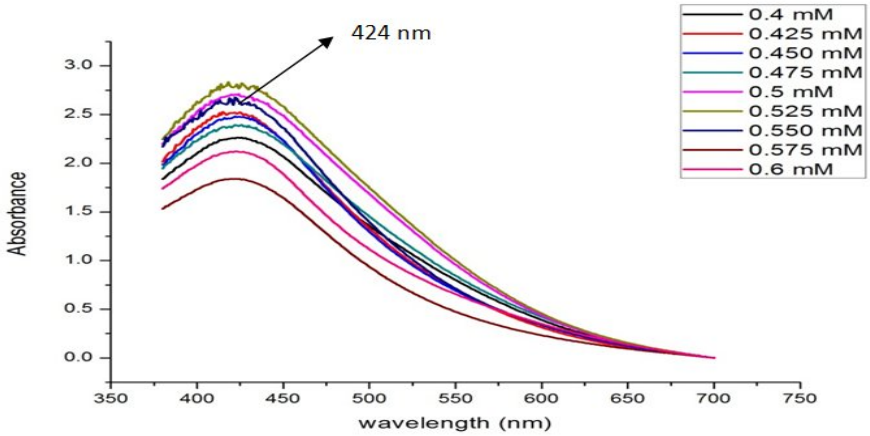



Fig 4.3 Effect of silver nitrate on synthesis of silver nanoparticles

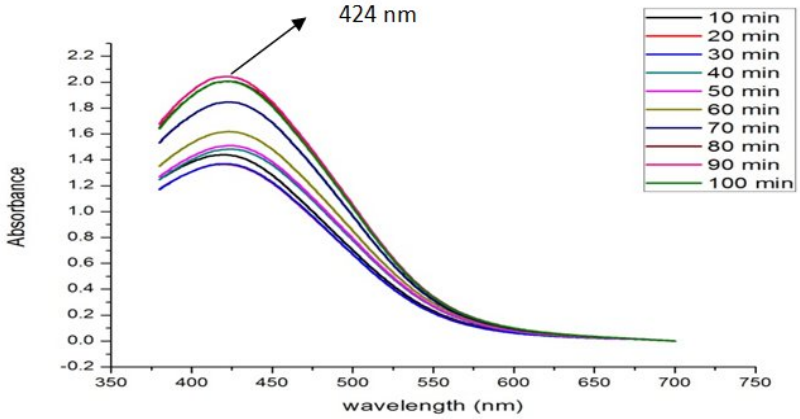



Fig.4.4 Optimization of time for the synthesis of silver nanoparticles

## 4.3 FTIR ANALYSIS OF PLANT EXTRACT AND SILVER NANOPARTICLES

FTIR analysis clearly shows which functional group is responsible for reduction of the $Ag^{+}$ ions and capping of bio-reduced silver nanoparticles (Ravi *et al*., 2013). Fig 4.5 depicts the FTIR analysis of plant extract and the nanoparticles synthesized. The range of the spectrum measures is in between 500-4000$cm^{-1}$. The IR spectrum of leaf extract has the vibrational stretching peaks at 3232.70, 3109.25, 1681.93, 1211.30 $cm^{-1}$ which corresponds to hydroxyl group OH stretch, aromatic CH stretch, secondary amine NH bend and tertiary amine CN stretch respectively. In case of nanoparticle spectrum vibrational stretching peaks were at 752.23, 1064.71, 1990.54, 2962.66 $cm^{-1}$.The hydroxyl group peak showed broad peak change in nanoparticle spectrum which might be assumed that hydroxyl group was responsible for stabilizing and also capping the synthesized nanoparticles.

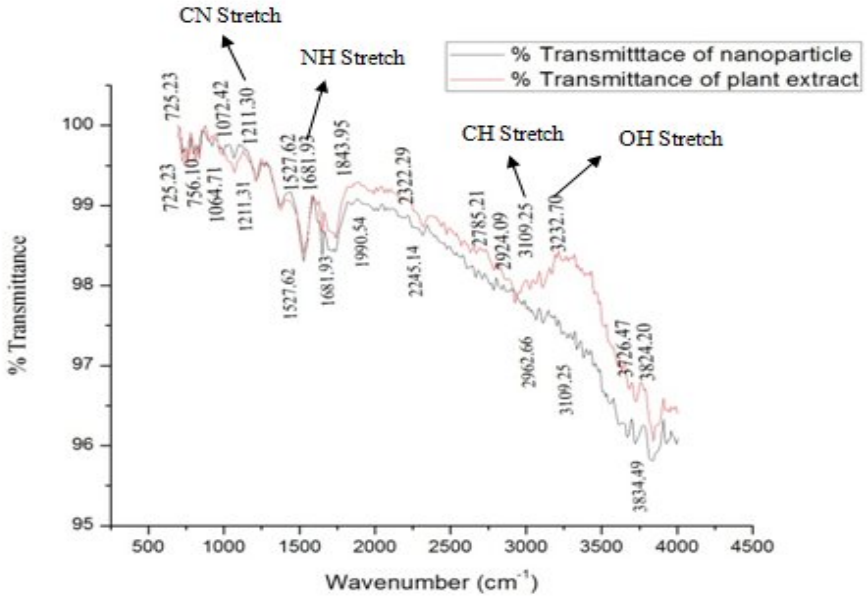



Fig 4.5 FTIR analysis of plant extract and silver nanoparticles

## 4.4 XRD ANALYSIS OF SYNTHESIZED SILVER NANOPARTICLES

XRD analysis was done for the silver nanoparticle solution which was obtained by centrifuging the silver nanoparticle solution at 10,000rpm.The analysis is done for the 2 theta values in the range of 30 to 100. The analysis showed the intense peak at four regions which corresponds the 2 theta values at 46.305, 54.825, 64.355 and 77.775 shown in Fig 4.6. By using Debye-Scherrer's equation and the 2 theta value further the size of the nanoparticle was determined as 357.9 nm . More and more broadening of the peak indicates the formation of nanoparticles.

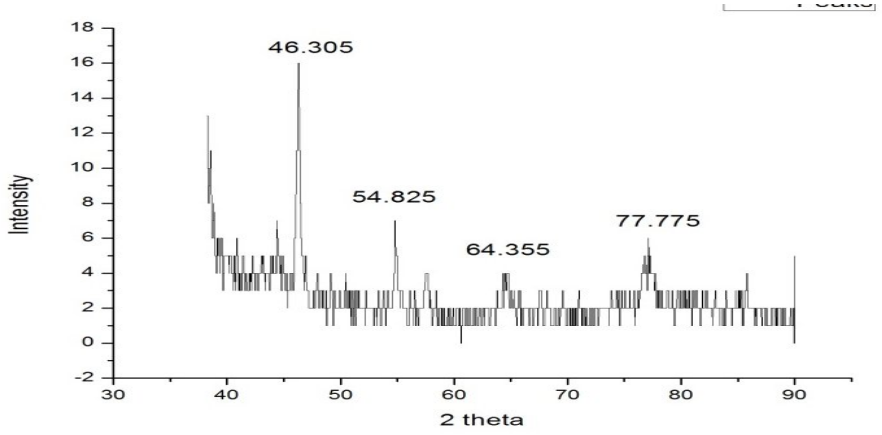



Fig.4.6 XRD analysis of synthesized nanoparticles

## 4.5 ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

The antibacterial activity of silver nanoparticles with different concentrations 20, 40, 60, 80 (µg/ml) was determined against a gram negative bacteria *Klebsiella pneumoniae* (MTCC 3384) using Agar well diffusion method. The zone of inhibition was observed to be 12mm,

13mm, 15mm, 18mm (Fig 4.7) for the concentrations 20, 40, 60, 80 (µg/ml) respectively which depicts the effective antibacterial activity of the silver nanoparticles.

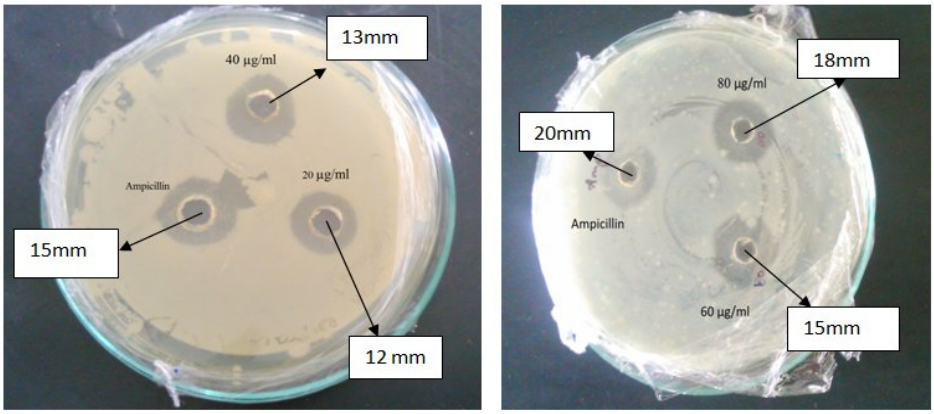



Fig 4.7 Antibacterial activity of various concentrations of silver nanoparticles against *Klebsiella pneumoniae*

## 4.6 PREPARED FILMS

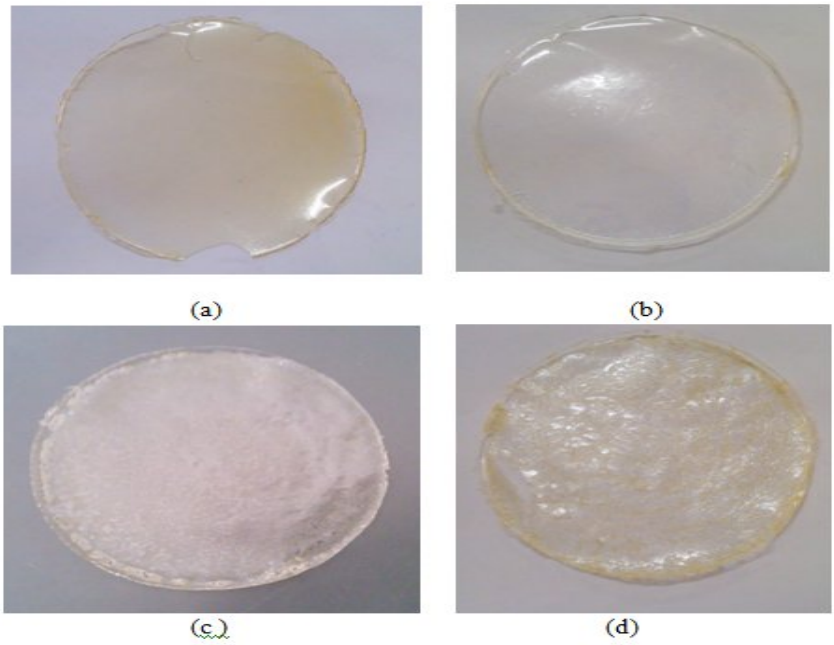



Fig. 4.8 (a) Pectin control film, (b) PVA control film, (c) Pectin-PVA blend film, (d) AgNp incorporated Pectin-PVA blend film.

Pectin, PVA, Pectin-PVA films were used as the control films. The pectin control film appeared yellowish in color. PVA films were transparent. Pectin-PVA blend films were slightly yellowish. The silver nanoparticles were added to the blend film by ultrasonication which enabled the uniform distribution of nanoparticles which resulted in the alternation of film color to light brown. Fig. 4.7 shows the Pectin, PVA, Pectin-PVA control films, Silver nanoparticles incorporated blend films in various concentrations.

## 4.7 TENSILE STRENGTH, THICKNESS, ELONGATION AND YOUNG'S MODULUS OF FILMS

All the films were cut into 90*20 mm for testing the tensile strength and other related parameters like percentage elongation, Young's modulus, thickness. The thickness of the films were found to be 0.020, 0.029,0.34, 0.29, 0.34, 0.37 cm for Pectin control, PVA control, Pectin-PVA blend, Pectin-PVA-AgNp(0.88 mg), Pectin-PVA-AgNp(0.89 mg), Pectin-PVA-AgNp(0.90 mg) respectively. From Table 4.1, it was found Tensile strength, %Elongation, Young's modulus.

Table 4.1 Mechanical properties of various films

| Film | C.S.A (Sq.cm) | Peak load (kg) | Tensile strength (kg/sq.cm) | Elongation @break % | Young's modulus 5% (kg/sq.cm) |
|---|---|---|---|---|---|
| Pectin control | 0.0400 | 1.9344 | 48.3590 | 32.8742 | 9.8344 |
| PVA control | 0.0580 | 4.1811 | 72.0874 | 440.5244 | 2.4303 |
| Pectin- | 0.0680 | 0.5363 | 7.8861 | 50.2988 | 1.7398 |

| | | | | | |
|---|---|---|---|---|---|
| PVA control | | | | | |
| Pectin-PVA-AgNp (0.88 mg) | 0.0580 | 0.6214 | 10.7142 | 214.8488 | 2.1006 |
| Pectin-PVA-AgNp (0.89 mg) | 0.0740 | 0.4701 | 6.2536 | 79.7326 | 0.2313 |
| Pectin-PVA-AgNp (0.90 mg) | 0.0680 | 0.1751 | 2.5746 | 18.5494 | 1.0157 |

The tensile strength of the Pectin, PVA films were found to be highest. Blend film of Pectin-PVA and Pectin-PVA-AgNp incorporated films has less tensile strength than the control films. But, 0.88 mg AgNp incorporated film has more tensile strength when comparing with Pectin-PVA blend film. Upon its inferred that increase in the conc.of nanoparticles further decreases the tensile strength.

## 4.8 FTIR ANALYSIS OF FILMS

The pectin control film has shown absorption peaks at 3317.56 $cm^{-1}$ and 2931.80 $cm^{-1}$ relating to N-H stretch and C-H stretch. The corresponding functional groups related to the absorption peak was observed to be amines, amides and alkynes. In the fig.4.6 absorption peak for the PVA control film was observed at 1635.64 $cm^{-1}$ and 1589.34 $cm^{-1}$ relating to N-H stretch and C-C stretch. All the above characteristics of the peaks where also observed in the Pectin-PVA blend film with a slight shift of peak at 1604.77 $cm^{-1}$ relating to the N-H bend. The silver nanoparticles incorporated blend film has shown a peak shift at 3039.81 $cm^{-1}$ and 2924.09 $cm^{-1}$ respectively. The shift in the peak clearly depicts that the incorporated silver nanoparticles are bounded to the alkene and alkane

groups of both pectin and PVA respectively. The incorporation of a high concentration of silver nanoparticles into the blend film has shown peak variations at 3294.42 $cm^{-1}$ and 3271.27 $cm^{-1}$ when compared with a film composed of silver nanoparticles at low concentration.

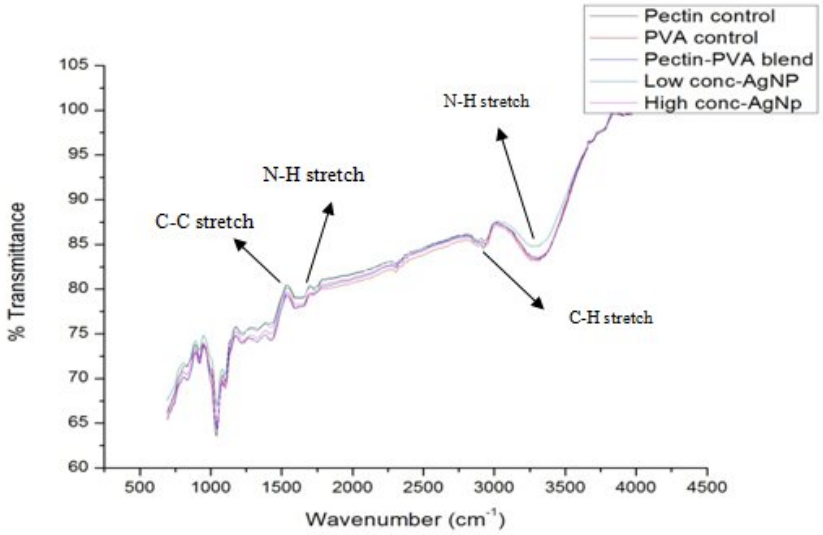



Fig.4.9. FTIR analysis of Pectin, PVA, Pectin-PVA, Pectin-PVA-Ag (low concentration), Pectin-PVA-Ag (high concentration) films.

# CHAPTER 5

# CONCLUSION

Silver nanoparticles were synthesized using the aqueous extract of *Andrographis paniculata* as the reducing agent and optimum condition for the synthesis of silver nanoparticles was identified. Pectin which is a natural polymer was blend with PVA which is a synthetic polymer to obtain a film. The Pectin-PVA blend film which was found to have enhanced antibacterial activity due to the incorporation of biosynthesized silver nanoparticles. The film was characterised by various studies such as Fourier Transform Infra Red spectroscopy (FTIR), X Ray Diffraction(XRD), UV-Visible Spectroscopy, Tensile strength, Elongation property, Young's modulus. Further, the antibacterial activity of the film clearly depicted that the prepared blend blend film can be potentially used for food packaging.

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