Biodegradable antimicrobial polymeric nano-composite films for packaging

2.1 Introduction

Packaging is used to protect food materials by isolating them from external influences [1]. It is an essential and integral part of food processing and preservation [2]. A good packaging material must be safe to the consumer and convenient to produce and dispose of in an eco-friendly manner. Several technologies have been used till date to manufacture good packaging materials [3]. Plastics are widely used for packaging purposes. The unique functionality of the conventional plastics, their low cost and weight are the major reasons for their extensive application [4]. But, the discarded plastic packaging and containers have raised issues of environmental pollution due to their non-biodegradable nature. The biodegradable takeaway food containers and plastic bags are being used these days as a substitute, but these degrade completely only when subjected to a harsh thermal treatment above 50 °C [5].

Recent research reports point to an alarming condition of the presence of micro-plastics and nano-particles of plastics (5µm to 1nm) in the natural river and marine ecosystems generated by the weathering of macro-plastics [6]. The micro-plastics can easily enter the human food chain from aqueous ecosystem either through fish, seafood, marine biota, plastic food wrappings, and beverages packed in plastic bottles. It has been reported that micro-plastics can pass across the gastrointestinal tract and may accumulate in the other vital organs such as liver, brain and other tissues of the aquatic species. It can also affect the organisms' central nervous and reproductive systems [7,8]. The presence of micro-plastics in aquatic animals have been ascertained through

laboratory feeding studies using non-commercial fish species [6,8,9]. Some researchers have also pointed out that the micro-plastics have already entered into the food chain, and the day is not far away when it can be found in human body [8]. It is already detected in the stool of individuals who largely depend on seafood [6]. More research is still needed to assess the potential danger of micro-plastics to human beings.

In view of the above, biodegradable packaging materials as a replacement for plastic packaging is the need of the day. The biodegradable materials which have been tested so far normally suffer from the limitations of hydrophilicity and crystallinity, which negatively influence their wide application [10]. Several additives have been used to improve these and other properties. Zhang et al. used glycerol, polyethylene glycol, and sorbitol as plasticisers to enhance the flexibility, elasticity, water vapour barrier, moisture resistance, mechanical strength and optical properties of the biodegradable packaging materials [11]. Bio-based packaging materials derived from renewable resources offer several environmental benefits such as biodegradability and nontoxicity. Some eco-friendly, active packaging materials and bio-plastics having excellent mechanical properties have already been used and reported to enhance the shelf-life of the packaged food items [1].

2.1.1 Bio-plastics

The mounting plastic waste and spread of micro-plastics have attracted the attention of policymakers and researchers towards development of eco-friendly bio-based polymers as alternative packaging materials. Bio-plastics degrade naturally under appropriate aerobic conditions of moisture and temperature without producing any toxic residue. Depending on the type and origin of the polymer, some of the bio-polymers

degrade in a few weeks, whereas synthetic polymers take years [12]. In countries where the primary method of waste management is landfill, the use of biodegradable polymer packaging materials is the most promising. The bio-plastics derived from natural polymers such as cellulose, starch, chitosan, agar, and proteins such as casein are being considered as viable alternatives [13]. These may be utilized as eco-friendly food packaging material in increasingly large amount in coming years.

2.1.2 Food packaging

Increasing consumers demand for healthy and safe food items free from synthetic chemicals and preservatives has become one of the prime concerns for food manufacturers and food businesses. Role of packaging has become very important as it provides a medium for decreasing post-processing contamination and assures food safety during marketing. By definition, the primary function of food packaging is to separate the food items from the surrounding environment, preventing or minimizing exposure to factors responsible for spoilage including microorganisms, humidity, oxygen and temperature to retain or extend the quality of nutrition along with the shelf-life of perishable food products. Moreover, packaging makes an important contribution to the consumer convenience, communication with consumers, marketability and acceptability of the packed products [14].

2.1.3 Foodborne pathogens

One of the major hindrances in assuring global food security is the microbial contamination of food which leads to spoilage and food wastage and the growth of pathogenic microorganisms [15]. Pathogenic microorganisms, such as *Toxoplasma gondii*, *Campylobacter* species, *Salmonella* species, *Staphylococcus aureus*, *Yersinia*

enterocolitica, Escherichia coli, and Listeria monocytogenes grow primarily on food surface and lead to food spoilage and deterioration [13]. These microorganisms are one of the critical reasons for illnesses, hospitalization, and even deaths of humans. Foodborne diseases cost billions of dollars annually for immediate medical care. Nearly one-fourth of this expenditure is due to foodborne illness linked with the use of contaminated processed, canned and fresh products. In the food market, normally, the proliferation of pathogens occurs on the packaging and external surface of foods as these are the most exposed surfaces. As per the data of the Center for Disease Control and Prevention (CDCP) of USA, bacterially contaminated food causes over 3000 deaths and 48 million illnesses in the United States alone [16].

Traditional methods of food preservation, such as drying, salting, heating, freezing, and fermentation are used to extend the shelf-life of food products but are not sufficient to inhibit the growth of pathogenic microorganisms that may endanger consumers' health, hence effective packaging becomes vital [15]. Improvements and advancements in the effectiveness of packaging materials and techniques can help to prevent foodborne pathogens and reduce wastage.

2.1.4 Antimicrobial packaging

In recent years antimicrobial packaging has attracted the attention of the food industry and consumers for packaging minimally processed food. It comprises techniques such as antimicrobial coatings, modified atmosphere packaging or active packaging films/materials containing natural antimicrobials, nanomaterials, nano-composites etc. as antimicrobial agents to control growth and reduce activities of microbes [14]. In antimicrobial coatings, edible natural antimicrobial agents are sprayed

on the food to reduce moisture loss during storage, spoilage and growth of pathogenic microorganism on the surface of food products, the rate of rancidity causing lipid oxidation and brown colouration, and loss of volatile flavour [15]. Usually, the coatings lack the practical functionality due to rapid diffusion of antimicrobial agents and reactions with the food to affect the taste of food negatively [14]. Antimicrobial agents carrying polymeric films are more efficient in interacting actively with the surface in contact to prevent foodborne microbial growth and slow the diffusion of antimicrobial agents, thereby ensuring the effective presence of adequate antimicrobials on the packed food surface [13]. The primary goals of a bio-polymeric antimicrobial packaging system are; assurance of food safety, maintenance of food quality and the product shelf-life enhancement in an eco-friendly way.

2.1.4.1 Chitosan (CH) as antimicrobial packaging

Chitosan (CH) has emerged as a promising bio-polymeric material for antimicrobial packaging in the food industry. CH is a polysaccharide of N-acetyl D-glucosamine and D-glucosamine units, mainly obtained by the partial deacetylation of chitin. CH is the soluble form of chitin and has been extensively used in various industrial applications including biodegradable packaging and food preservation [14,17,18]. CH possess several desired characteristics such as, biodegradability, non-toxicity, biocompatibility, film-forming ability, economical as well as abundant natural availability to be a bio-polymer of choice [16]. CH is primarily a by-product of crustacean fish and seafood processing and handling industries. CH possess the ability to easily form bio-composites with numerous natural and synthetic antimicrobials materials, antioxidants, and nanomaterials having the potential of shelf-life extension of fruits, vegetables, meat and fish products [14].

Poor barrier moisture and mechanical properties and shorter shelf-life compared to conventional food packaging materials are the major limitations of natural bio-based materials. The incorporation of different nanomaterials in the matrix of bio-based polymers can improve the quality of packaging materials by altering their properties. Incorporation of nanomaterials in the film provide a viable solution for enhancing the antimicrobial activity, therefore preventing foodborne pathogens [16]. Bio-nanocomposite films with biodegradable and antimicrobial properties have the potential to solve the issues of both plastic waste and food products spoilage [2]. Sometimes metallic nanoparticles are used in nano-composites to enhance the desired characteristics.

2.1.4.2 Silver nanoparticles (AgNPs) as antimicrobial material

Silver nanoparticles (AgNPs) are the most widely used nanoparticles due to the ease of their preparation in the pure form [19]. The AgNPs share more than 50% of the global market of nanomaterial-based consumer products [20] and have already been approved by the regulatory agencies for use in various applications requiring direct human contact [21,22]. Their unique antimicrobial, electronic, optical, and magnetic properties have resulted in their application in several diverse fields such as biomedical sensors [19,23], antimicrobial products [24], food packaging materials [25], fabrics, bandages [26], catalysis [27], optoelectronics and photonics [28] and pharmaceuticals [29]**.** *Figure* 2.1 depicts the share of AgNPs in various commercial products.

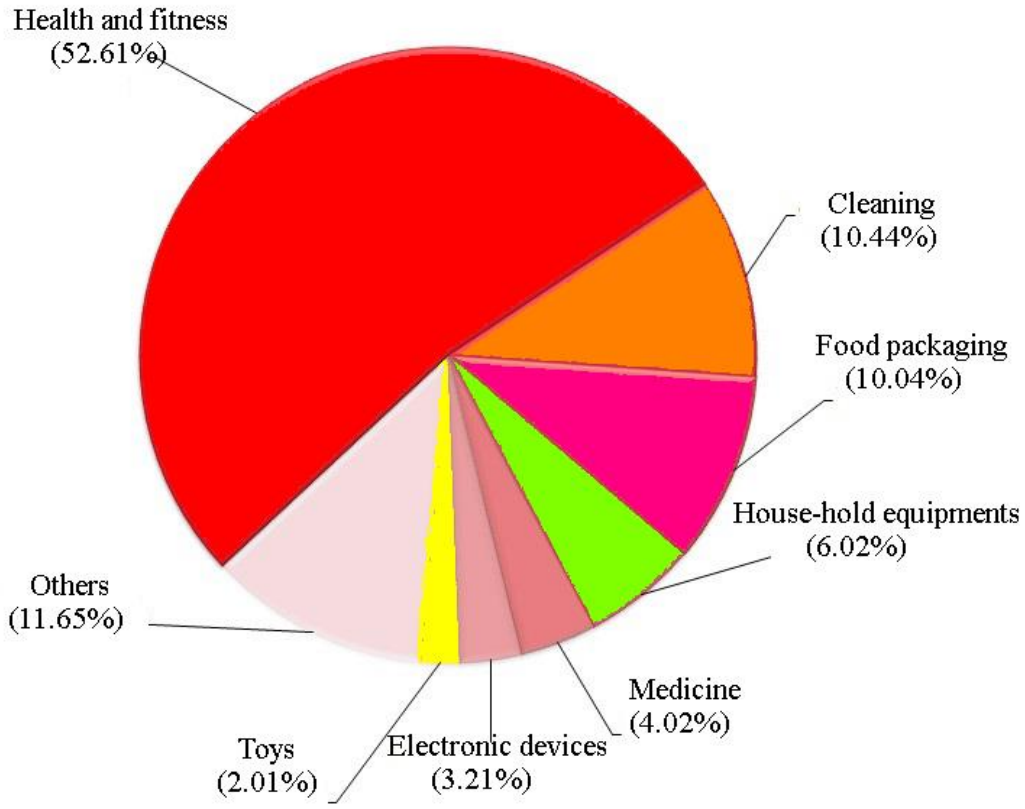

Figure 2.1: AgNPs utilization in commercial products [20]

The unique properties of AgNPs depend mainly on their shape and size. However, the variation in their size and size distribution is the most significant hurdle in the synthesis and application of AgNPs [30]. Thus, there is a need to explore new eco-friendly methods for AgNPs synthesis, having the possibility of large scale production of monodispersed AgNPs. Size regulation is crucial to utilize the antimicrobial activity of AgNPs in packaging films as the antimicrobial efficacy of AgNPs depends on the size [29]. The AgNPs as such or embedded within different polymeric materials have been used for developing antimicrobial films using different materials for applications such as antifouling membranes [31], packaging [13], wound dressing [32] etc. The current focus is on the development of active biocompatible polymer-AgNPs films with antimicrobial property to permit the diffusion of some active ingredient to the applied

site. Attempts have been made for developing active food packaging materials, which not only protect the food material from bacterial infection but also protect their flavor and nutritional value [33]. Antimicrobial nanofibrous composite films have also been used against foodborne pathogens. The green synthesised AgNPs have been used previously and are reported to be safe for antimicrobial packaging [34].

2.1.5 Electrospinning

The electrospinning technique is a simple technique for large scale production of non-woven fibers of nanoscale diameter with large surface area to volume ratio. Electrospinning assembly can be modified and arranged in different ways for combining materials in composites to obtain different morphological structures. Electrospun fibers play a significant role in biomedical applications like tissue engineering, drug release, wound dressing, and food packaging [35–37]. Nano-fibers produced via electrospinning provide ease of functionalization for different purposes and have excellent mechanical properties making them suitable for use in active packaging due to high surface to volume ratio.

2.1.6 Packaging of perishable meat products

Packaging of the food products like dairy and meat products which got easily contaminated is very challenging. Available reports indicate that 22-33% of the meat produced gets spoiled and wasted at various stages of processing and distribution. Low shelf-life and improper packaging of meat products is the primary reason for spoilage [38]. Growth in the demand for packaged meat products has led the researchers to search for the solutions to increasing the shelf-life of meat products.

In this study, the leaf extract of *Ocimum tenuiflorum* (Tulsi plant), a common household medicinal herb has been used for the biosynthesis of AgNPs. The plant is rich in essential oils, mostly eugenol and is known to possess medicinal properties [39]. The colloidal AgNPs suspension has been solution cast to develop polyvinyl alcohol (PVA)-based active antimicrobial polymeric film for packaging. To enhance the antimicrobial property CH has been selected as the natural polymer additive to design an electrospun biodegradable antimicrobial fibrous composite nano-layer as an active packaging material for meat. The CH possesses the characteristic antimicrobial property, and this property get synergistically enhanced by incorporating AgNPs within its fibrous nano-layers [40]. The CH-based packaging film is an eco-friendly value-added product that can be obtained from the processing of waste generated from the seafood processing industry [41]. The dispersion of active constituent over a limited surface area of conventional packaging films will reduce the effectiveness of the active film packaging. The electrospun fibrous nano-layers prepared and used in this work provide a very large surface area and high effectiveness due to its nano-scale diameter [42]. The PVA has been used to improve the electrospinning behaviour, elastic nature and enhance the strength. PVA is also biodegradable both under aerobic and anaerobic environmental conditions [43].

The antimicrobial property of the new biodegradable packaging film and nano-layers has been tested against Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Listeria monocytogens* (*L. monocytogenes*) bacteria. *L. monocytogene*sis a foodborne pathogenic bacterium which usually grows in meat and is responsible for highly fatal disease listeriosis. *E. coli*, on the other hand, is the most common pathogen found in the gastrointestinal tract of human and a typical foodborne pathogen.

2.2 Literature review

Traditionally packaging materials are derived from non-renewable fossil fuels. Internationally plastic production during 2015 climbed up to 322 M tons out of which a total of 49 M plastic tons was used for food packaging [12]. Around 30% of the plastic waste had filled lands [44]. Considering this, plastic recycling has become a motivating factor in decreasing the accumulation of environmental plastic waste and gradually replacing synthetic packaging materials with biopolymers [16].

Although with the increase in demand, extensive research efforts have been made with emphasis on enhancing the biodegradable nature of packaging films, their mechanical, thermal and physical features. But the reported information are still unsatisfactory and are often impractical to be used in industrial applications [2]. A summary of advantages and disadvantages of bio-based packaging is given in Table 2.1.

Table 2.1: The advantages and disadvantages of bio-based packaging [16]

Advantages of bio-based polymers	**Disadvantages of bio-based polymers**
Bio-based polymers can decrease carbon dioxide and greenhouse gas emission levels	May produce methane in landfills under anaerobic conditions
Complete degradation and decomposition occur by natural microbes in specific condition	Require the weather co-operation for degradation and herbicides and pesticides not taken in consideration
Energy efficient with least waste generation during the manufacturing cycle	Low durability of polymers matrices in the long run
Save petroleum consumption for other needs	Expensive
Could create new export industries & marketing platform	No proper safety guidelines for nano-composites

Non-chemical process, comparatively safe for the operators.	Requires moderate operator skill and engineering support. Bio-based polymers must follow a precise disposal procedure.

2.2.1 Biodegradation of plastic/polymer

Biodegradable plastics are often considered as a feasible solution to the plastic related ecological issues. Degradation which refers to polymer deterioration from abiotic reactions, like hydrolysis, oxidation, and photo-oxidation is a crucial step in the biodegradation [45,46]. Biodegradation occurs when the polymer fragments in the presence of appropriate enzymes in aerobic or anaerobic conditions that can ultimately convert the residuals into benign products, like carbon dioxide, methane, nitrogen, and water [46]. Plastics degradation in the environment takes place through different processes as shown in *Figure* 2.2. These include:

(1) **Biodeterioration:** Microorganisms form biofilms and deteriorate the substrate surface into smaller sizes, like micro-plastics, through biological, chemical and physical means.

(2) **Depolymerization:** Microorganisms release extracellular enzymes to depolymerize the substrate for further biodegradation.

(3) **Bioassimilation:** Small molecules are consumed as metabolites by cells whereas toxic chemicals may get adsorbed on these micro-plastics to bioaccumulate then result in the transmission of pathogens that may have attached unto the micro-plastics or ingestion of the toxic chemicals adsorbed on the micro-plastics [47].

(4) **Mineralization:** Degradable molecules are converted into benign products, such as carbon dioxide, nitrogen, methane, and water depending upon the temperature, relative humidity, oxygen levels, and population and diversity of microorganisms.

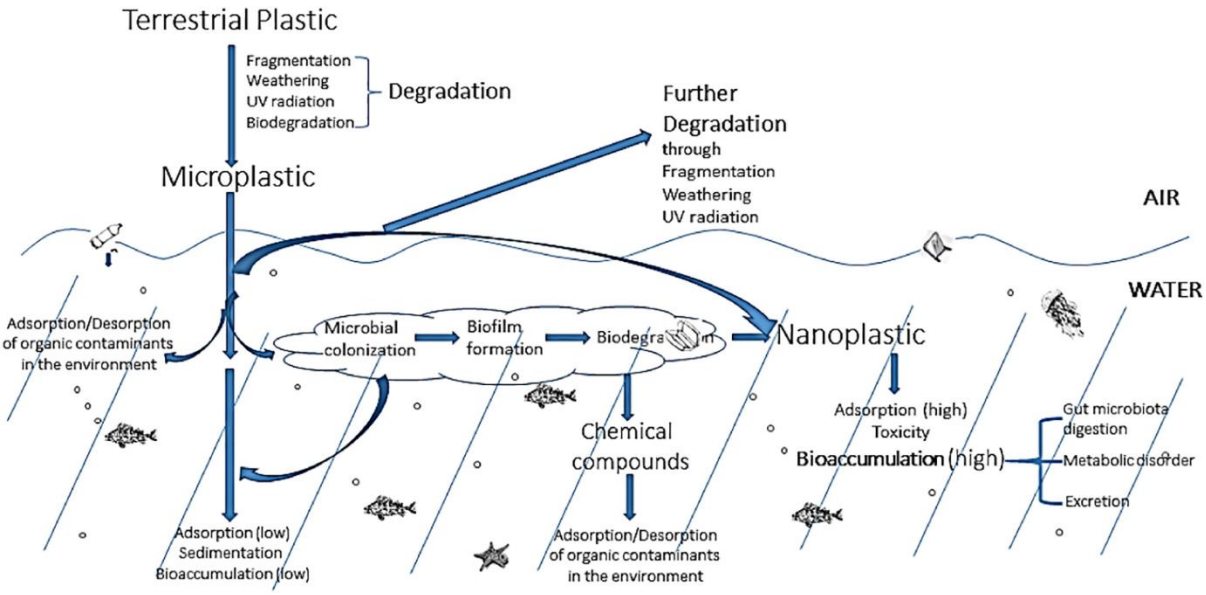

Figure 2.2: The fate of plastics in the aquatic environment [47]

2.2.2 Antimicrobial packaging

Antimicrobial packaging is an emerging technology proposed for shelf-life extension, ensures food safety and preserves quality of products. Many natural antimicrobial compounds have been evaluated as additives in film structures, synthetic polymers, and edibles [15]**.** Natural antimicrobials are mainly obtained from animals, plants, and microbes, hence these are natural, biocompatible, biodegradable and have low toxicity [14]. The most commonly used and commercially available natural antimicrobial packaging materials with their applications are listed in Table 2.2.

Table 2.2: Natural antimicrobial agents used in food packaging systems [15,48]

Category	Antimicrobials	Packaging materials*	Target microorganisms
Organic acids	a. Benzoic acid	LDPE	Total bacteria
	b. Sorbates	LDPE MC/chitosan Starch/glycerol	Yeast, molds
	c. Sorbic anhydride	PE	*Saccharomyces cerevisiae*, molds
Enzymes	a. Lysozyme, Nisin, EDTA	Zein	*E. coli, Lactobacillus plantarum*
	b. Immobilized lysozyme	PVOH, nylon, cellulose acetate	Lysozyme activity test
	c. Glucose oxidase	Fish	
Bacteriocins	a. Nisin	PE, HPMC	*Staphylococcus aureus*
	b. Lauric acid	Zein	Migration test
Fungicides	a. Benomyl	Ionomer	
	b. Imazalil	LDPE, PE	Molds
Polymers	a. Chitosan	Chitosan/paper	*E. coli*
	b. Chitosan, herb extract	LDPE	*E. coli, Lb. plantarum, Fusarium oxysporum, S. cerevisiae*
	c. UV/excimer laser irradiated nylon	Nylon	*Enterococcus faecalis, S. aureus*

Natural extract	a. Grapefruit seed extract	LDPE, nylon	Aerobes, coli-forms
	b. Clove extract	LDPE	*E. coli, Lb. plantarum, F. oxysporum, S. cerevisiae*
	c. Eugenol, cinnamaldehyde	Chitosan	Enterobacter, lactic acid bacteria
	d. Horseradish extract	Paper	*E. coli*
Essential oils	a. Clove essential oils	Films	*Aspergillus niger, Bacillus coagulans, Bacillus cereus*
Others	a. Silver zeolite	LDPE	*E. coli, S. aureus, S. cerevisiae, Salmonella typhimurium*
	b. Antibiotics	PE	*E. coli, S. aureus, S. cerevisiae, S. typhimurium*

** PE, polyethylene; HDPE, high-density polyethylene; LDPE, low-density polyethylene; HPMC, hydroxypropyl methylcellulose; MC, methylcellulose.*

2.2.2.1 Polymeric antimicrobial composites of CH and their blends

CH itself has inherent good antimicrobial activities, however, to enhance its antimicrobial properties, natural antimicrobials and antimicrobial nanostructures (metal and metal oxide nanoparticles) have been incorporated in the films [40]. It has been reported that CH, Zn^{+} and Ag^{+} complex demonstrated increased antimicrobial activity in comparison to CH alone [49]. When implied in the nanoscale Mn^{2+}, Zn^{2+}, Cu^{2+}, and Ag^{+} improved the antibacterial activity considerably [14]. The list of natural antimicrobial compounds used in the CH-based composites for antimicrobial food packaging by different researchers is given in Table 2.3.

Table 2.3: Natural antimicrobial compounds used in the chitosan-based composite for antimicrobial food packaging [14].

Natural antimicrobial	**Composite composition**	**Target microorganisms**	**Food item(s)**	**Ref.**
Agro-industrial residue (AR) extracts	AR - 0.84% and 1.90% (v/v) and CH - 2% (w/v)	Mesophilic and Psychrotrophic aerobic counts	Chicken product	[50]
Apple peel polyphenols (APP)	APP - 0.25 to 1.0% (w/w) and CH - 2% (w/ v)	*B. cereus, E. coli, S. typhimurium* and *S. aureus*	Food packaging	[51]
Apricot kernel essential oil (AKEO)	AKEO- 0–1% (w/v) and CH- 2% (w/v)	*B. subtilis, E. coli,* and fungi	Sliced bread	[52]
Bamboo vinegar (BV)	BV - 2% (v/v) CH - 2% (w/v)	Lactic acid bacteria and *P. aeruginosa*	Pork chops	[11]
Cinnamon essential oil(CEO)	CEO - 10% (w/w) CH - 1% (w/v)	*E. coli* and *S. aureus*	Food packaging	[53]
Custard apple leaves (CAL)	CAL - 2/10 (w/v) and CH- 2.5% (w/v)	*Colletotrichum gloeosporioides*	Papaya fruit	[54]
Lemon essential oil (LEO)	LEO - 3% (w/w) CH - 1.0% (w/v)	Fungus	Strawberry fruits	[55]
Lysozyme	Lysozyme in CH - 2.75% (w/v)	Mesophilic, Psychrotrophic, yeasts and molds	Cheese	[56]
Melissa officinalis essential oil (MOEO)	MOEO - 0.25 and 0.5% (w/v) CH -1.5% (w/v)	*E. coli*	Antimicrobial packaging film	[57]
Nisin (N)	N - 0.2% (w/v) CH - 1.5% (w/v)	*Lactobacillus species*	Mutton meat	[58]

	N - 500 and 1000 ppm CH - 1.4% (w/v)	*L. monocytogenes*	Cheese	[59]
Oregano essential oil (OEO)	OEO - 1.0% (v/v) Carboxymethylchitosan - 1.0% (w/v)	*L. monocytogenes*	Raw chicken meat fillets	[60]
Plum peel extract (PPE)	PPE - 25% (w/w) and CH - 2% (w/v)	*E. coli, S. aureus, and L. monocytogenes*	Food packaging	[61]
Spirulina extract (SE)	SE – 0 to 20% (w/v) and CH - 2% (w/v)	*E. coli, S. aureus, P. aeruginosa, L. monocytogenes, S. typhimurium, B. subtilis and B. cereus*	Food packaging	[62]
Thyme essential oil (TEO)	TEO - 1.5% (w/v) CH - 2% (w/v)	Yeasts and molds	Mushroom	[63]

Bio-nanocomposites are novel, eco-friendly materials that are non-toxic and biodegradable. However, they have poor antimicrobial activity, mechanical properties, and low water resistance. Therefore, nanomaterials are employed to improve their antimicrobial activity, thermal, mechanical, and gas barrier properties while retaining the biodegradable and non-toxic characteristics [2,16].

2.2.2.2 Utilization of nanomaterials as antimicrobial agents in packaging

Nano-materials (films, particles, rods and tubes) exhibit entirely different physico-chemical properties than the bulk form of parent material. This is primarily due to the large difference in the surface to volume ratio and difference in the nature of surface energy distribution that make them highly active [16]. The inorganic antimicrobial materials have higher thermal stability in comparison to the organic

materials. Metal oxide and metal nanoparticles both can bear the difficult processing situations [64]. Inorganic nanoparticles possess improved and novel biological characteristics because of their structure and size, less toxicity, specificity and selectivity in comparison to organic antibacterial agents [65]. Zinc oxide nanoparticles (ZnO-NPs) and titanium dioxide nanoparticles (TiO_2-NPs) have also demonstrated successful antimicrobial activity against pathogens commonly found in food by generating reactive oxygen species (ROS) and reducing membrane integrity. These nanoparticles are also biocompatible with blood cells so they can be used as a defense against food pathogens [66]. As this work is directly concerned with the use of nano-silver particles, the following pages present a relatively detailed account of properties and applications of nano-silver particles.

2.2.2.3 Silver nanoparticles (AgNPs)

Silver nanoparticles (Ag-NPs) display bactericidal characteristics against a broad variety of pathogenic microorganisms such as viruses, fungi, yeasts, and bacteria [67]. A greater volume to surface ratio of nanoparticle has resulted in increased exposure of the surface to microbes to act efficiently. Additionally, features such as phases, shape, and size play an important role in killing bacteria or their inactivation.

The AgNPs are the most extensively used nanomaterials in commercial products ranging from biomaterials, composites, ceramics, food packaging, healthcare, etc. [20,68]. Like other nanomaterials, two general approaches for the synthesis of AgNPs are the "top-to-bottom" and "bottom-to-up" (*Figure* 2.3). In the top-to-bottom approach, a suitable bulk material is broken down into tiny particles of the nanoscale by reducing the size using various techniques like grinding, milling, sputtering and thermal/laser

ablation, etc. On the other hand, in the bottom-to-up approach, nanoparticles are synthesized using biological and chemical methods through self-assembly of atoms to new nuclei which grow into particles of nanoscale dimensions [69]. The conventional techniques for AgNPs synthesis employ hazardous and toxic reagents which are expensive and cause pollution. Generally, the methods for the synthesis of AgNPs can be classified into three different categories: (1) physical methods, (2) chemical methods, and (3) biological or green synthesis methods (*Figure* 2.3). Out of these, the green synthesis route is an eco-friendly way of AgNPs biosynthesis. The green synthesis of AgNPs can be achieved by utilizing a natural biotic entity such as (i) microorganisms like actinomycetes, bacteria and fungi, etc.; (ii) plant-products and their extracts; and (iii) biological templates like DNA, membranes, and viruses [70]. Active biomolecules of biotic origin, such as amino acids, enzymes, proteins, flavonoids, and terpenoids from several plants extract act as reducing and stabilizing agents during the synthesis of AgNPs through green route. Despite several economic and ecological advantages of green synthesis, regulating the polydispersity of the nanoparticles is still the major challenge. To counter this problem, a scientifically controlled reaction condition like optimum pH, temperature, incubation period, irradiation, redox conditions and salt concentration need to be maintained [71].

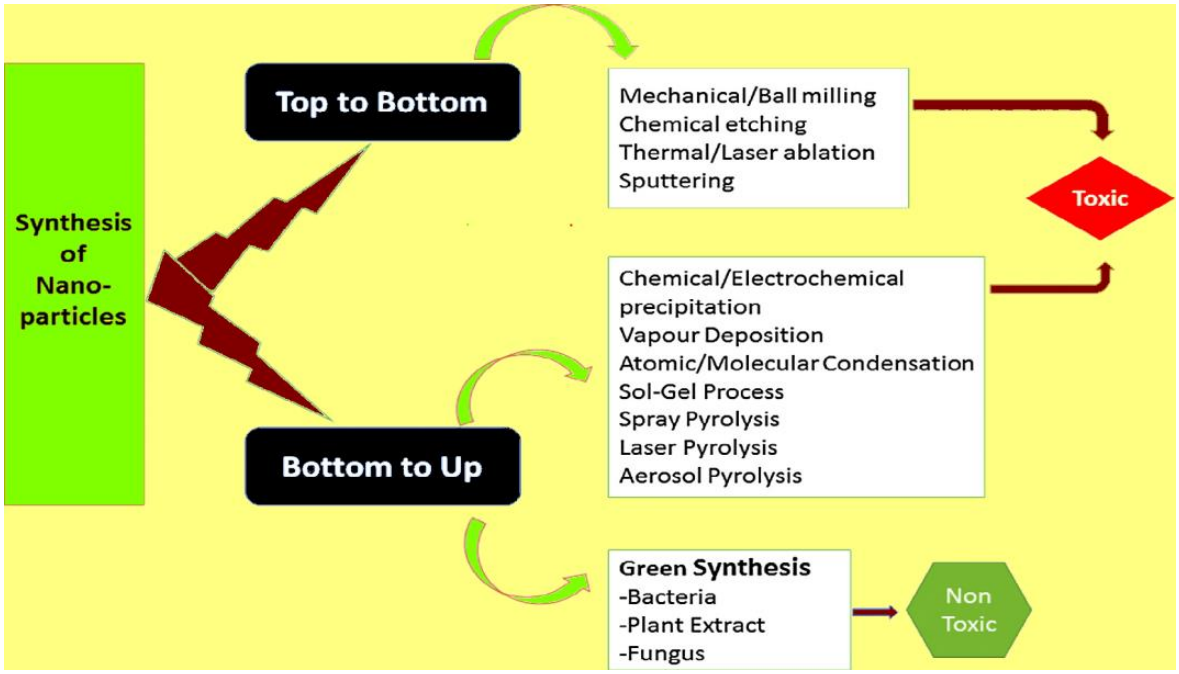

Figure 2.3: Different approaches for the synthesis of AgNPs [69]

The antimicrobial activity of AgNPs strongly depends upon the size, capping agent and environmental conditions (temperature, pH, ionic strength). Reported mechanisms behind the antimicrobial activity of AgNPs are: (i) generation of ROS [23]; (ii) bacterial protein denaturation by bond formation between silver ions and sulfhydryl groups interaction [72]; (iii) electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells causing severe damage to the bacterial membrane permeability leading to cell death [73]. AgNPs have already been reported to show antimicrobial activity against *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyogens*, *Vibrio cholera* etc. [74].

2.2.2.4 Antimicrobial bio-nanocomposite films

Kumar *et al.* (2020) demonstrated that incorporation of nanomaterials and phytochemicals into CH improve antimicrobial, mechanical and barrier properties of the fabricated films and coatings that lead to an improvement in food quality and shelf-life extension. Nanoparticles of silver, zinc oxides and titanium dioxide have been extensively used as the key candidates for the fabrication of CH nano-composites. CH matrices serve as carriers of active compounds such as nanomaterials, essential oil, fruit extracts, and other phytochemicals [14]. Table 2.4 presents a list of bio-nanocomposites with antimicrobial properties using different nanoparticles and nanofibers.

Table 2.4: Use of bio-nanocomposite films in antimicrobial food packaging applications [16]

Nanomaterials	**Composite materials**	**Enhanced properties**	**Inhibition of microorganisms**	**Reference**
AgNPs	PVA-Montmorillonite clay-Ginger extract	Antimicrobial activity, water resistivity, mechanical property, and light barrier ability.	*S. aureus, S. typhimurium*	[75]
AgNPs	Polyvinyl chloride	Antimicrobial activity	*B. subtilis, A. niger, F. solani*	[76]
Aluminum-doped ZnONPs	Polylactic acid	High transparency and UV blocking property, antibacterial activity	*E. coli*	[77]
CH-Gelatin NPs	CH/Gelatin - Moringa oil	Antimicrobial activity and maintain surface sensory quality and colour of cheese	*S. aureus, L. monocytogenes*	[78]
Graphene stacks	CH - cinnamaldehyde coating	Antifungal activity and increased mechanical properties	*Rhizopus stolonifer*	[79]
Lignin NPs	Cellulose - Polylactic acid	Antimicrobial activity, optical transparency and UV light blocking capability	*P. aeruginosa*	[80]
MgONPs	Polylactic acid	Bio-based, antimicrobial activity	*E. coli*	[81]

Nano-cellulose	CH/Polylactic acid - Rosin coating	Mechanical and antimicrobial property	*E. coli, B. subitilis*	[82]
Polylactic acid nanofibers	Cinnamon essential oil – β cyclodextrin complex	Antimicrobial activity and thermal stability of essential oil	*E. coli, S. aureus*	[83]
Silver-Copper NPs	Fish skin gelatin	Mechanical strength, thermal stability and antibacterial activity	*L. monocytogenes, S. thyphimurium*	[84]
TiO_2NPs	Polyvinyl alcohol-CH	Antimicrobial coating, mechanical, water vapor and gas barrier property.	*E. coli, S. aureus, S. enterica, L. monocytogenes*	[85]
ZnONPs	CH/Cellulose/Acetate /Phthalate	Barrier properties and thermal stability	*E. coli, S. aureus*	[86]
ZnONPs	PVA / Starch	Enhanced water barrier, mechanical and antimicrobial property	*S. typhimurium*	[87]

Meeting the demand and preferences of consumers, the innovative and novel packaging methods have given rise to bioactive, intelligent and active food packaging processes that are considered to be highly advanced [2]. These new technologies have made important contributions to the improvement of bioactivity, feasibility, safety, and quality of functional elements, as shown in *Figure* 2.4.

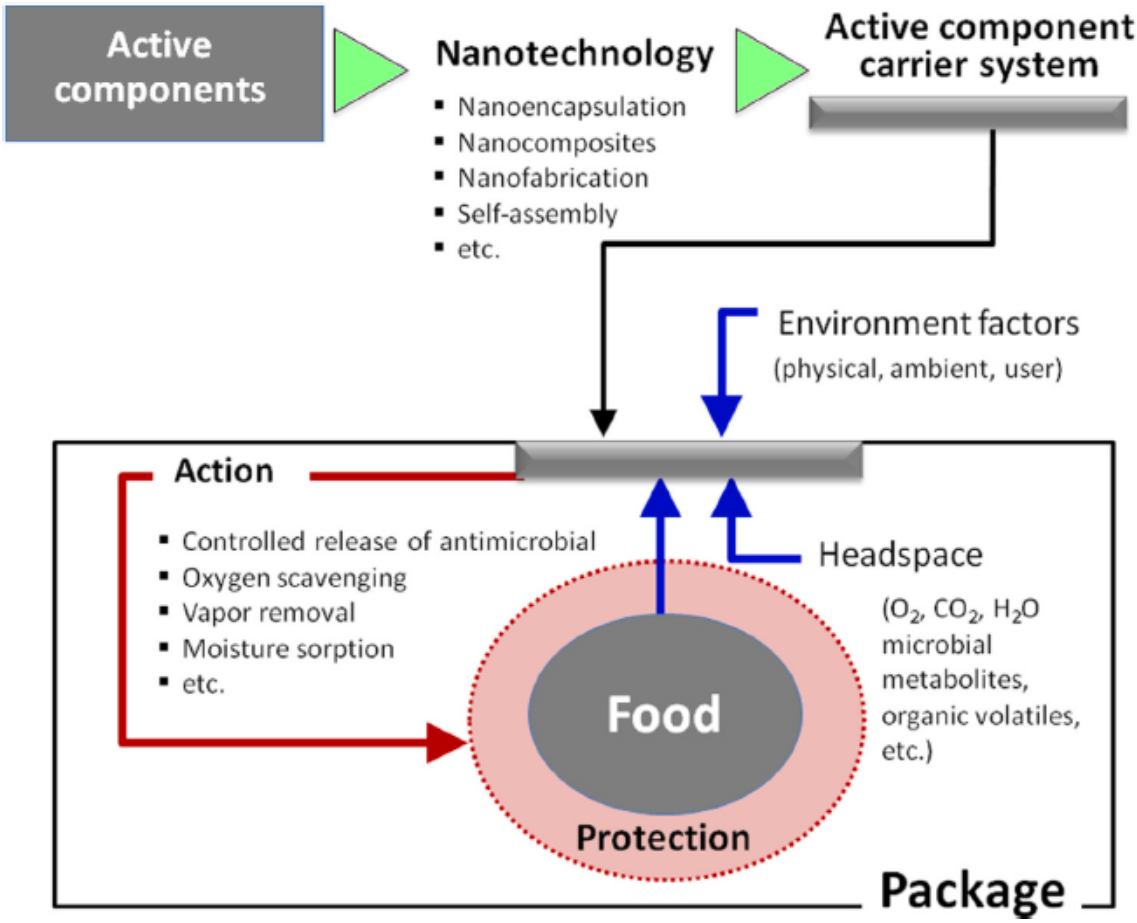

Figure 2.4: Schematic diagram displaying the active nano-composite packaging concept [88]

2.2.3 Nanofibrous packaging

Majority of antimicrobial active packaging materials reported so far involve the dispersion of the active agent in carriers with limited surface areas for controlled release

of active molecules, such as polymeric films and sheets. But in nanofibers by virtue of their submicron to the nano-scale diameter and very large surface area this limitation can be overcome. The commonly used methods for nanofiber production are listed in Table 2.5.

Table 2.5: Commonly used methods for nanofiber production [89]

Method	Description	Advantages	Disadvantages
Drawing	The fabrication of fiber is done by contacting a previously collected polymer solution droplet with a sharp tip and then drawing it as a liquid fiber, which is further solidified by solvent evaporation.	Simple process	Limited amount of product, discontinuous process
Electrospinning	Nanofibers are produced in the one-step method from a viscoelastic solution of a polymer or its melt under applied high voltage.	Unlimited length, simple and core-shell nanofibers, great diversity of polymers and solvents used. Scale-up possible.	High voltage, solvents required, numerous parameters affecting the process
Force spinning	Fibers are produced using a very highly concentrated polymer solution or melt under applied centrifugal force.	Free from high voltage, simple method, high production yield. Scale-up possible.	Thermal degradation of melts, fibers are usually much thicker than 1 μm in diameter

Phase separation	Firstly, a gel is formed by cooling the polymer solution to the gelation temperature. Afterwards, the gel is immersed in distilled water for solvent exchange, followed by removal from the distilled water, blotting with filter paper, and transfer to freeze-drying	Simple, no special equipment required	Numerous parameters, limited to a few polymers
Self-assembly	Amphiphilic molecules are used as basic building blocks that self-associate to produce nanofibers	Suitable for production of very thin nanofibers	Poor control over morphology and orientation of nanofibers
Template melt extrusion	The molten polymer is forced by an extruder screw through a mold or spinning die in the head of extrusion devices and then cooled to solidify. Anodic aluminium oxide membranes are used as a template.	No need for solvents, homogenous fiber diameter	Short length of fibers, time-consuming
Template synthesis	Nanofibers are formed within the numerous cylindrical pores of a nonporous membrane.	Aligned nanofibers, homogenous and easily controlled fiber	Complex method

2.2.3.1 Electrospinning as a method of choice in packaging

Electrospinning is a versatile method to prepare continuous fibrous polymeric nonwoven layers that exhibit excellent properties such as large specific surface area, high molecular orientation and porosity [90]. Electrospinning takes place at ambient conditions so the electrospun fibers are suitable for encapsulating thermally-labile active agents as compared to the fibers made by the conventional melt spinning process. No requirement of heat in electrospinning is a key advantage over other encapsulation methods to incorporate and preserve the efficacy of bioactive substances during fibrous

layer formation [90]. Considering the numerous advantages, electrospun nanofibers have been employed as a potential candidate for the fabrication of active food packaging materials (*Figure* 2.5).

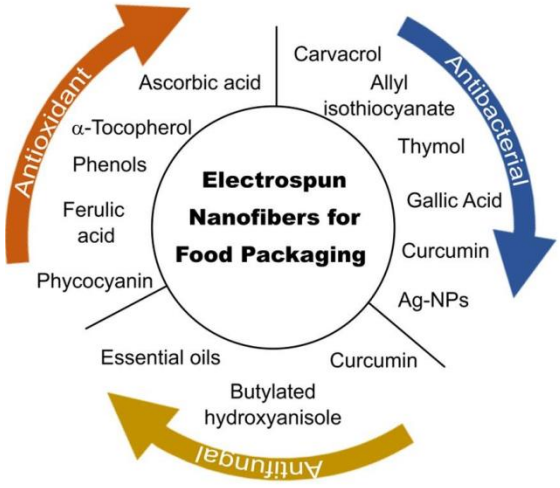

Figure 2.5: Packaging precursors for antimicrobial packaging in nanofibers

The biggest challenge in electrospinning is the choice of safe, effective and economical solvent. Therefore water or other non-toxic solvents with environment friendly property are chosen to prepare fibrous food packaging films. During the electrospinning, the solvent gets evaporated, but for safety non-toxic solvents are used [91]. Some natural polymers cannot be directly electrospun into nanofibers, so it is crucial to select a suitable polymer blend, which is also a problem. Some industries already have begun large-scale production of nanofiber membranes, for example Jiangxi Xiancai Nanofibers Technology Co., Ltd in China produces electrospun polyimide fibrous membranes, yarns and short nanofibers [90]. The process of electrospinning depends on a number of parameters (solution, processing, and ambient), and these parameters affect the fiber morphology. A summary of different parameters and their influence on fiber diameter is given in Table 2.6.

Table 2.6: Electrospinning parameters (solution, processing, and ambient) and their effect on fiber morphology [35,36,92]

Parameters	**Effect on fiber morphology**
Solution parameters	
Viscosity	Low-bead generation, high-increase in fiber diameter, disappearance of beads
Polymer concentration	Increase in fiber diameter with increase in concentration
Molecular weight of polymer	Reduction in the number of beads and droplets with increase in molecular weight
Conductivity	Decrease in fiber diameter with an increase in conductivity
Surface tension	No conclusive link with fiber morphology, high surface tension results in instability of jets
Processing parameters	
Applied voltage	Decrease in fiber diameter with increase in voltage
Distance between tip and collector	Generation of beads with too small and too large distance, minimum distance required for uniform fibers
Feed rate/Flow rate	Decrease in fiber diameter with decrease in flow rate, generation of beads with too high flow rate
Collector type	Influence structural morphology of electrospun fibers. A non-conductive collector creates a porous structure with circular pores on the fiber surfaces
Ambient parameters	
Humidity	High humidity results in circular pores on the fibers
Temperature	Increase in temperature results in decrease in fiber diameter

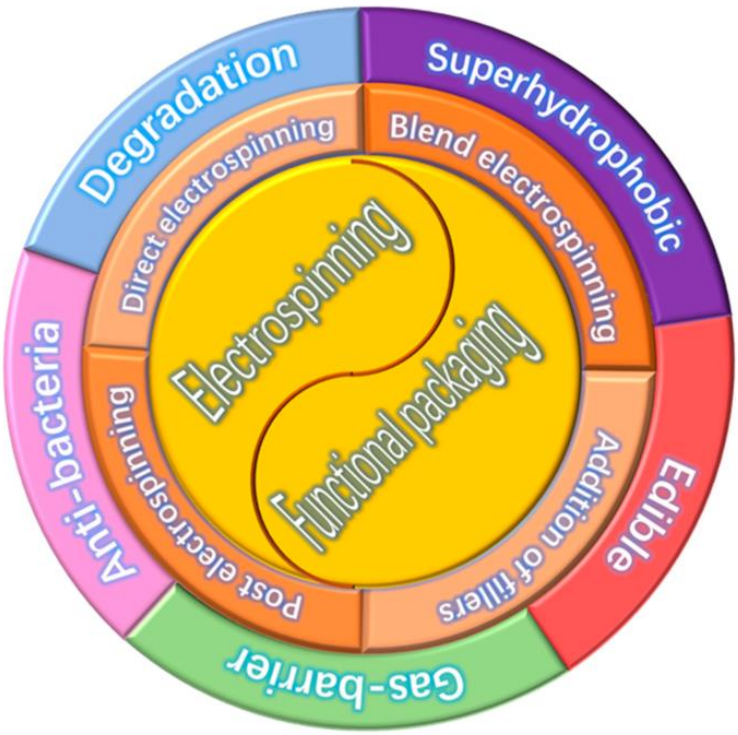

Figure 2.6: Functional electrospun and food packaging materials [90]

Functional packaging materials like moisture absorbing [93], antioxidant releasing [94], antimicrobial and flavor or odor absorbing [95] are gradually evolving, and their demand is increasing day by day. Electrospun fibers offer several additional advantages in comparison to films and sheets. Fibrous layers are more responsive to the surrounding atmospheric changes (e.g., relative humidity, acidity and temperature changes) and could satisfy the requirements of designers and consumers for packaging materials. Therefore, the development of functional packaging materials based on electrospinning technology has become a hot field in the food packaging sector. In order to maintain the quality of food and extend its shelf-life, food packaging materials also need to have some functionality like biodegradability, super hydrophobic nature, edible coating materials, antibacterial materials and high barrier property (*Figure* 2.6). These

properties can be easily realized through electrospun fibrous nanolayers just by selecting suitable polymer, solvent and modifying design and parameters of electrospinning [36].

2.2.4 Regulation for using nanomaterials in food packaging

Very little are known about the capabilities of nanomaterials as these are is still in their infancy stage. Due to lack of sufficient scientific evidence, it is impractical to make suggestions, regulations and legislation. Moreover, each nanomaterial is unique, and a specific case-by-case evaluation is needed [16].

World-wide the current legislation is extremely generic at the moment and requires extensive research and evaluation for specificity and risk assessment separately for each nanomaterial. Current FDA guidelines state that it does not consider all the products containing nanomaterials as dangerous [96]. The most detailed regulations on the use of nanomaterials for food applications has been implemented by the European Union to assist the industry in making safe and suitable choices of nanomaterials for their products [97]. The EU Commission Regulation (EC, 2011) reinforces the need to assess nanomaterials on case-by-case basis. Later in the regulations (EC, 2017, 2015, 2016) an amendment mentioned regarding the details of nanomaterials that have been permitted for use in plastic-based food packaging [16]. Some elements like aluminium oxide and TiO_2 are also allowed as food additives or contact materials, though not in the nanoform. Nano-coatings are not included by any European legislation [2].

Currently, even the term “nanoparticle” is defined differently by ISO (ISO/TS 80004-2, 2015), American Society for Testing and Materials ASTM (ASTM E2456-06, 2012) and IUPAC. It demonstrates that definitions and terms in the nanotechnology

field are still evolving. Some of the currently enforced ASTM standards for packaging include D5988-12 and D6691-09 for aerobic biodegradation of plastic materials in soil and natural marine environment, respectively. The ASTM standard D5526-12 holds good for anaerobic plastic biodegradation under accelerated landfill conditions [45].

2.3 Materials and methods

2.3.1 Materials

Medium molecular weight chitosan (CH) (viscosity- 200–800 cP, degree of deacetylation- 85%), polyvinyl alcohol (PVA) (Mol wt. 85000-124000), silver nitrate and glutaraldehyde were purchased from Sigma-Aldrich (Saint Louis, U.S.A.). Acetic acid and food-grade glycerol were purchased from Merck Life Science Pvt. Ltd., Mumbai, India. All these chemicals were used as received.

2.3.2 Collection & processing for preparation of *Ocimum tenuiflorum* leaf extract

Fresh green leaves of *Ocimum tenuiflorum* (*O. tenuiflorum*) were collected from the Botanical Garden of the Department of Botany, Banaras Hindu University, Varanasi, India. The leaves were washed thoroughly first with tap water to remove the externally adhered dust particles and then rinsed several times with double distilled water (DDW) prepared in the laboratory. Around 30 g of the filter-paper dried finely cut leaves of *O. tenuiflorum* were grinded using mortar and pestle then boiled in 100 ml of DDW for 20 min. The aqueous extract thus prepared was cooled and filtered through the Whatman filter paper no. 6. The filtrate was centrifuged twice at 10,000 rpm for 15 min, and the supernatant was collected and refrigerated at 4 ^{o}C for further use.

2.3.3 Green synthesis of AgNPs via plant extract and Chitosan (CH)

For the preparation of 10 mL of AgNPs suspension, 1 ml of silver nitrate ($AgNO_3$) solution (1 mM) and 1 mL of the previously obtained aqueous leaf extract were mixed with 8 mL DDW at room temperature (28 ± 2 ºC) in separate test-tubes and placed in sunlight and UV-light chamber to get the light of the desired wavelength range for 2 to 6 hours. The UV-light source was a laboratory UV lamp (Philips TUV 15W/G15 TB).

In CH mediated green synthesis method for preparing AgNPs, 6.0% (w/v) of CH in 2.0% (v/v) aqueous acetic acid acts both as reducing and stabilising agent for the AgNPs biosynthesis. 0.5 mL of 1 mM $AgNO_3$ solution was added to 100 mL of CH solution, and the mixture was kept under constant stirring at 95 °C for 6 h until the solution turned dark yellow marking the formation of AgNPs [98].

The AgNPs suspensions thus prepared were further analyzed using UV-Visible spectrophotometer for AgNPs synthesis and the dynamic light scattering (DLS) device (Particulate Systems NanoPlus-3, Micromeritics, Norcross, USA.) for particle size determination.

2.3.3.1 UV–Visible spectrophotometry

The change in the spectral behaviour of the synthesised AgNPs was monitored in the wavelength range of 300–900 nm using a UV–Visible Spectrophotometer (Model 2202, Systronics, Ahmedabad, India). The spectrum was observed for sample after 2 and 6 h of the exposure to the light.

The UV–Visible Spectrophotometer was also used to monitor the bacterial cell growth by measuring optical absorbance at 600 nm.

2.3.4 Electrospinning solution

2.3.4.1 Preparation of CH/PVA/AgNPs solution for electrospinning

The 6.0% (w/v) of CH solution with AgNPs prepared before and 12.0% (w/v) of PVA solutions in 2.0 % (v/v) aqueous acetic acid were mixed with constant stirring for 8 h. The PVA solution was heated to 70 °C with continuous stirring. Solutions having different proportions of PVA:CH (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 0:100) were prepared by mixing and properly homogenized. After complete homogenization, a clear transparent yellowish solution was obtained. This resultant solution was used for electrospinning to form the composite nano-layers as described below;

2.3.4.2 Preparation of fibrous composite nano-layers (FCNLs) through electrospinning

For electrospinning, 3 mL of above prepared solutions were taken in a 5 mL syringe. Electrospinning of pure PVA, CH and blended solutions was carried out using a horizontal electrospinning set up with a flat plate collector. The schematic diagram of electrospinning process and set-up is shown in Figure 2.7 and parameters are listed in Table 2.7. All the experiments were carried out at the ambient temperature of 23 ± 1 °C and relative humidity of 40-45%. The nanofibers collected on the collector plate were placed in glutaraldehyde fumes for 5 min and then left overnight to evaporate the solvent.

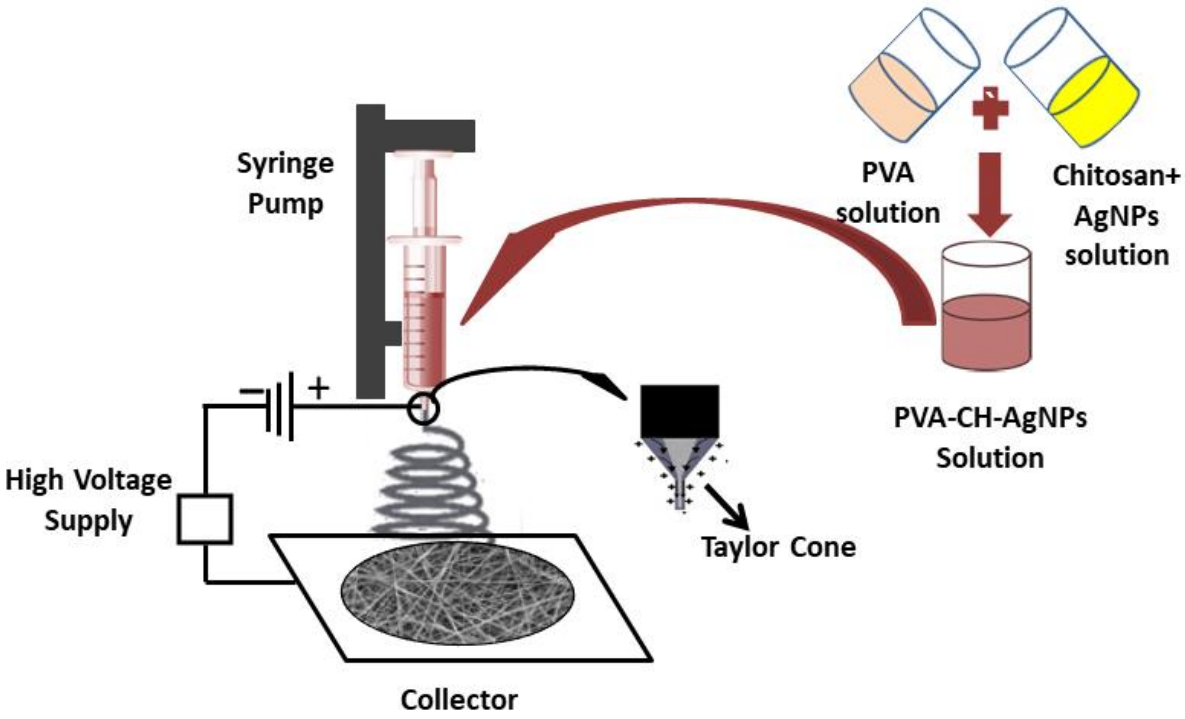

Figure 2.7: Schematic diagram of electrospinning process and set-up

Table 2.7: Electrospinning parameters used for preparing PVA-based nano-layers

Parameters	**Description**
Solvent system for Polymer (PVA)	2% aqueous acetic acid
Co-solvent for CH & AgNPs	2% aqueous acetic acid
Syringe	A 5 mL syringe fitted with a 22G blunt-end needle
Distance between needle and collector	12 cm
Applied DC voltage	17-25 kV (PVA 17 kV and CH or PVA/CH blend 25 kV)
Solution Flow rate	0.5 mL/h

2.3.5 Preparation of composite polymeric film

The solution casting method was employed for preparing AgNPs bearing polymeric films. 6% (w/v) PVA was dissolved in the 25 mL of the prepared colloidal suspension of AgNPs containing the plant extract. The mixture was then heated at 80˚C with continuous stirring until a homogenous solution was formed after the dissolution of PVA. The solution thus prepared was poured in a Petri plate and kept on flat surface at 45˚C for 24 h to get a clear transparent film of uniform thickness.

2.3.5.1 Diffusion of the active constituents

The diffusion of AgNPs from the polymeric film was spectrophotometrically analyzed by placing a piece of polymeric film of known area (0.5×0.5 cm^2) in a capped Eppendorf tube dipped in 1.5 ml DDW for 5, 10, 15, 20, 25, 30 min. After the desired period of incubation, the water sample was analyzed at 427 nm to estimate the release of AgNPs and 335 nm for the release of plant extract contents. All experiments were performed in triplicate.

2.3.6 Physico-chemical characteristics

2.3.6.1 Electrical conductivity

The conductivity of the electrospinning solution was measured using a digital conductivity meter (Model-304, Systronics, Ahmedabad, India).

2.3.6.2 Viscosity

Viscosities of pure and blended solutions were measured using a rotating viscometer (DV-III^{TM} Ultra Rheometer, AMETEK, Brookfield, USA.).

2.3.6.3 Thickness

The thickness of the fiber composite nano-layers (FCNLs) and polymeric film was measured using a digital micro-meter (Schut Geometrical Metrology, Groningen, Netherlands) up to the nearest 0.001 mm at six different locations. The values thus obtained were averaged.

2.3.6.4 Solubility

The solubility of the FCNLs and polymeric film in DDW was measured by immersing a small piece of known dimension (2cm x 2cm) in 50 mL of DDW in a Petri dish. The Petri dish was placed at ambient temperature (23 ± 1 °C) for 6 h. The samples were taken out and dried in the oven at 105 °C for 1h and weighed.

2.3.6.5 Scanning Electron Microscopy (SEM)

After 2 h of exposure of the mixture of aqueous leaf-extract and $AgNO_3$ solution to the light, the unbound moieties from AgNPs and the colloidal nanoparticle solution were separated by centrifugation at 15000 rpm for 15 min, and the supernatant was discarded. The pellets of AgNPs thus obtained were re-dispersed in DDW and repeatedly centrifuged thrice. The supernatant was discarded, and the washed pellets were dried in a hot air oven at 80 ºC for 10 h. The dried AgNPs thus obtained was used for SEM analysis.

The size and morphology of AgNPs and fiber diameter were analysed by using a scanning electron microscope (Quanta 200F, FEI, Tokyo**,** Japan) at an acceleration voltage of 10 kV for polymeric film and 20 kV and 4000X magnification for FCNLs.

ImageJ software was used to analyze the average size of the AgNPs and average diameter of the FCNLs.

2.3.6.6 Zeta (ζ) potential and particle size distribution

The Particulate Systems Nanoplus Particle Size/Zeta Potential analyser (Particulate Systems Nanoplus-3, Micromeritics, Norcross, USA.) was used to measure the zeta potential of the Tulsi plant extract and the synthesized colloidal AgNPs. The average particle size and size distribution was measured by the dynamic light scattering (DLS) method.

2.3.7 Water contact angle (WCA)

The WCA measurements were carried out to evaluate the hydrophilic or hydrophobic nature of the FCNLs and polymeric film surface. The WCA analysis in air was performed by the sessile drop test using a contact angle measuring drop shape analyzer (DSA25S, KRUSS, Hamburg, Germany). A drop of water (4 μL) was dropped on a piece of film (30 mm × 50 mm) placed over the movable stage leveled horizontally using an automatic micro-syringe and the static images for each surface were taken. Five replicates were taken and averaged per nano-layer to represent the mean water contact angle value of the FCNLs and polymeric film.

2.3.8 Fourier transforms infrared (FTIR) spectroscopy

The FTIR spectroscopic analysis was done to know the possible chemical interaction between various functional groups of active bio-molecules and polymer(s), the stability of the bio-molecules as well as the effect of electrospinning on the functional groups of constituents in nano-composites. Spectrum 100 spectrophotometer

(Perkin-Elmer, Shelton, USA) was used for scanning in the wavelength range of 4200-650 cm^{-1}. The FTIR spectra of the samples were obtained directly placing the sample in the sample holder at room temperature (~25 °C).

2.3.9 X-ray diffraction (XRD)

The XRD analysis was performed to study the degree of crystallinity of the nano-composites. The pattern for XRD was recorded in the 2θ range of 10-60° at a scan rate of 6° min^{-1} using the nickel filtered Cu K_α radiation source with a wavelength of 0.154 nm (40 kV, 15 mA) in the XRD unit (RigakuMiniflex 600 Desktop X-Ray Diffraction System, RIGAKU Corporation, Tokyo, Japan). The powder or thin sheet of nanofibrous samples were placed on the silica sample holder at room temperature (~25 °C) and were scanned.

2.3.10 Microbial analysis

2.3.10.1 Culture media and microorganisms

Tryptone soya broth (TSB), tryptic soya agar, nutrient agar, phosphate buffer saline (PBS), Luria Bertani (LB) broth, and LB agar were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. The standard bacterial strains of *Escherichia coli* (ATCC 25922) (Gram-negative) and *Listeria monocytogenes* (ATCC 7644) (Gram-positive) were obtained from the Institute of Medical Sciences, Banaras Hindu University, India used as the reference for the experimental purpose during the study. The *E.coli* was grown over the LB media, and *L. monocytogenes* on the tryptone soya media. The DDW rinsed and sterilized borosilicate glass-wares were used in these experiments.

2.3.10.2 *In vitro* antimicrobial activity

The antimicrobial activity of the nanofibers was tested against the standard bacterial strains of *E. coli* and *L. monocytogenes*. The agar disc diffusion assay was used to analyse the antimicrobial activity of AgNPs colloidal solution, plant extract, polymeric film, FCNLs [99]. 100µL of the overnight grown bacterial cultures ($1x10^7$ cells/mL) were spread over the LB agar and tryptone soya agar plates and incubated for 24 h at 37 ± 1 ºC, after placing the sterile pieces of composite films over the plates. 50 µL of the colloidal solution of AgNPs from test tube was added to the wells (~7 mm diameter) created by back of sterile micropipette tips and incubated for 24 hours at 37 ± 1 ºC. After incubation, bacterial growth and diameter of the inhibition zone were measured in millimeters (mm) after 24 h.

2.3.11 Antimicrobial application of FCNLs for meat packaging

The aseptically cut fresh meat pieces of goat were placed in a bacterial suspension of mixed culture of *E. coli* ($1x10^3$ CFU/mL), and *L. monocytogenes* ($1x10^3$ CFU/mL) for 30 s and then excess of the liquid was drained and dried for 5 min in a sterile environment using the protocol adopted by Arkoun et al. [18]. The dried meat was then packaged in the fibrous nano-layer. The normal plastic packaging was used for the comparative study. The packaged samples were stored at 4 °C and observed periodically. After one week of storage, the samples were unwrapped and placed in the 50 mL LB broth and shaken gently for 5 min. The meat piece was removed, and the broth was placed under observation for 24 h to monitor the bacterial growth. Microbial analysis was performed in triplicate, and average values were noted.

2.3.12 Raw Materials

2.3.12.1 Chitosan

Chitosan is a natural bio-polymer with intrinsic antimicrobial activity against various microorganisms [14]. Several mechanisms are proposed for antimicrobial activities, the most acceptable ones are ;

(i) Electrostatic interaction between negatively charged microbial cell membranes (carbonyl and phosphoryl groups of the phospholipid components) with positively charged amine groups in chitosan altering the membrane barrier properties leading to leakage of intracellular contents, and ultimately cell death [61].

(ii) Chitosan is selectively chelated with metals, inhibiting various metabolic enzymes of microbial cells by blocking their active centers, and reducing microbial growth [14,100].

(iii) Antimicrobial action of chitosan depends on molecular weight and degree of deacetylation.

High molecular weight chitosan can form an impervious polymeric layer on the surface of the microbial cell altering the cell permeability and ultimately blocking the entry path of nutrients into the cell. On the other hand, low molecular weight chitosan could penetrate the cytosol, and bind with DNA, resulting in interference with transcription and translation leading to cell death [100]

2.3.12.2 Polyvinyl alcohol

Polyvinyl alcohol (PVA) offers a wide range of advantages, because of its superior ability to form the film, completely synthetic water-soluble polymer, resistance to chemicals, semi-crystalline, good biodegradability and easy preparation [43]. PVA has significant physical characteristics because of its hydroxyl groups, which encourage the creation of hydrogen bonds [101]. PVA is used in numerous industries such as medicine and food, producing products such as food packaging materials, surgical threads, resins and lacquers. PVA is used as a coating material for different supplements of food and does not raise any concerns of safety [2]. For food package films, PVA has a wide range of significant features such as superior properties of forming a film, good stability and excellent hydrophilicity [43]. It is insoluble in organic solvents, less soluble in ethanol but soluble in water. PVA is appropriate to integrate with other polymers to enhance the barrier, thermal and physical properties because of its easy processability, good mechanical properties and polar character [75].

2.4 Results and Discussion

2.4.1 Analysis of UV–Visible spectra

The rapid change in the color of the leaf extract-$AgNO_3$ colloidal solution due to the reduction of $AgNO_3$ confirmed the formation of AgNPs. The color of the solution changed from light yellow to brownish-yellow and got intensified with time. The change in colour can be attributed to the reduction of Ag^+ to Ag^0 by various active bio-molecules present in the Tulsi extract in the presence of light [102]. The absorption

spectra (*Figure* 2.8) reveals that the peaks of biosynthesized AgNPs are observed within the range of 420-430 nm (*Figure* 2.8a) due to surface plasmon resonance, a characteristic phenomenon of AgNPs after 2 and 6 h of sunlight exposure. The observed results are in agreement with the findings of Prathna and co-workers who used lemon extract for the synthesis of AgNPs and reported that the single absorption peak observed in the wavelength range 400-420 nm corresponds to the spherical shape of formed AgNPs [103]. The stability of colloidal nanoparticles was monitored as the function of time up to which it nanoparticles do not aggregate. The UV light treated Tulsi leaf extract-$AgNO_3$ colloidal solution did not show any peak confirming that negligible AgNPs formed at wavelengths less than 380 nm (*Figure* 2.8b). The absorption spectra of Tulsi extract and $AgNO_3$ did not show any peak in the visible region (*Figure* 2.8c).

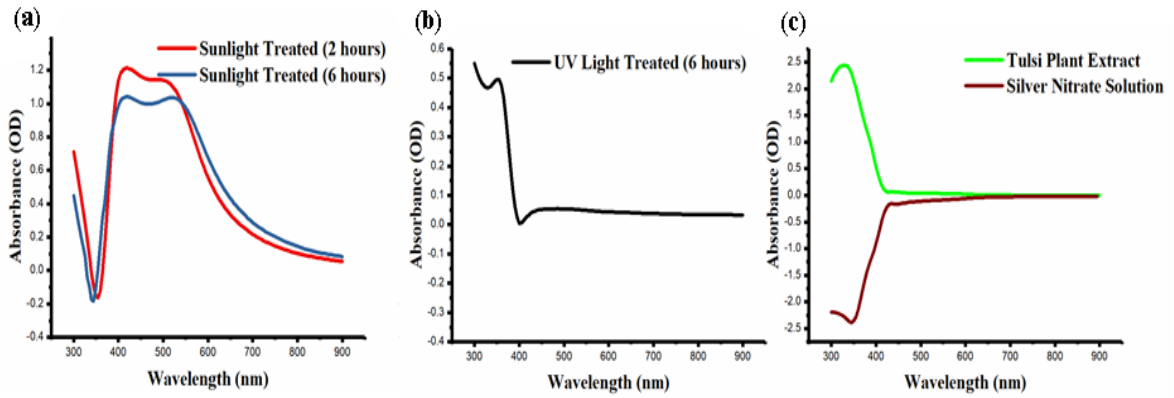

Figure 2.8: Absorption spectra of AgNPs under **(a)** Sunlight; **(b)** UV-light; **(c)** Raw materials

The UV-Visible spectra of CH-mediated synthesized AgNPs showed a clear peak at 427 nm (*Figure* 2.9a), which corresponds to the formation of AgNPs. The AgNPs formed from the $AgNO_3$ solution by the reducing and stabilizing action of CH. The SEM and DLS analysis results (*Figure* 2.9b & 2.9c) showed that the size of AgNPs was in the range of 70-130 nm. The SEM of the AgNPs, when analyzed by ImageJ software, showed the average size of the formed nanoparticle as 80 ± 11 nm, which was lower than that obtained from the DLS analysis (93.4 ± 26.0 nm). This difference in the values of average size can be attributed to the fact that the SEM image measurements show the actual diameter, but the DLS technique measures the hydrodynamic diameter, which is always greater than the actual diameter [104]. A thin dipole layer of the solvent is formed on the surface of AgNPs due to the adherence of solvent onto the surface of AgNPs. Thus the DLS analysis gives the diameter of the core molecule together with the solvent layer. Measurement of the hydrodynamic diameter is a vital parameter for understanding the effect of nanoparticles in biological systems and correlating particle size with the physiological responses of the body [105]. Further, the hydrodynamic diameter also affects the diffusion across the cell membrane.

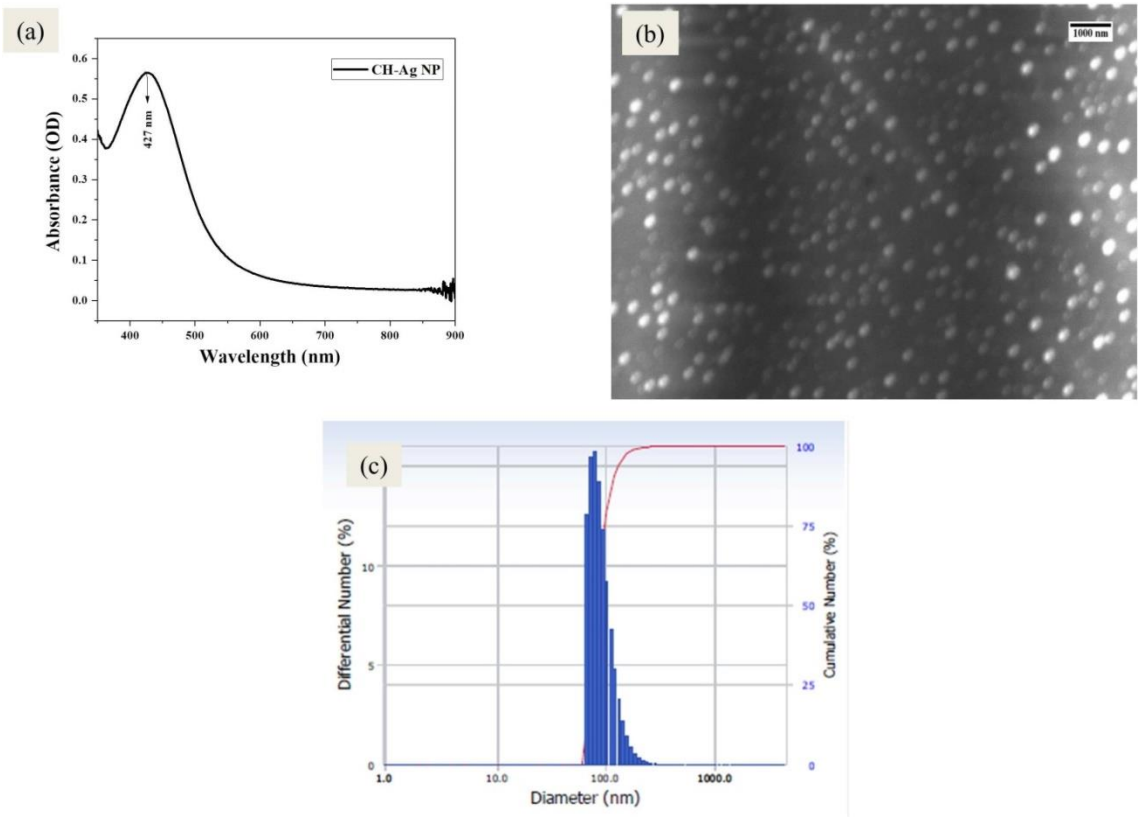

Figure 2.9: Chitosan-mediated green synthesized AgNPs; **(a)** UV-visible spectra; **(b)** SEM image of AgNPs; **(c)** Cumulative size distribution obtained through DLS method

2.4.2 Solution casting of polymeric film

A clear film of PVA containing AgNPs and Tulsi extract formed after 24 hours over the Petri plate kept in an incubator at 45 ºC (*Figure* 2.10). The average thickness of the formed film was found to be 0.15 ± 0.03 mm. The film obtained exhibited good strength.

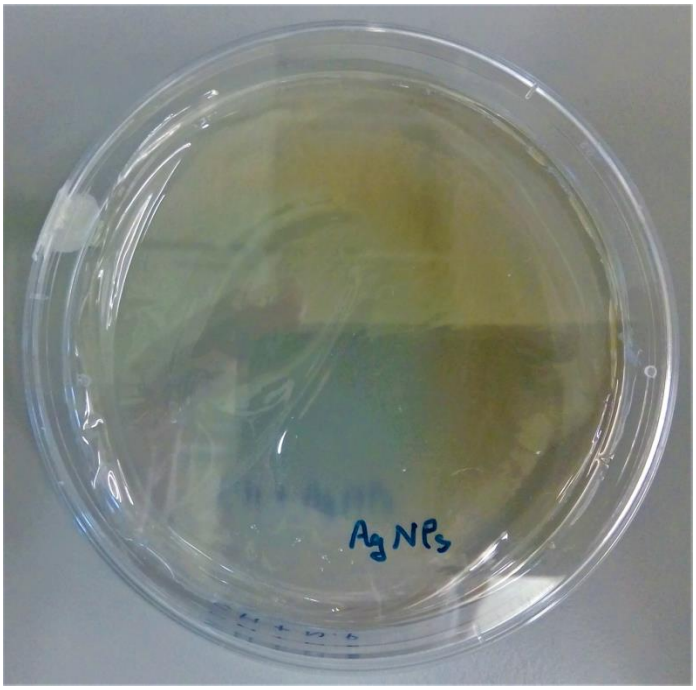

Figure 2.10: Morphology of antimicrobial active film

2.4.2.1 The release profile of active constituents

The diffusion of AgNPs and plant extract was monitored after 5, 10, 15, 20, 25, 30 minutes as spectrophotometric absorbance values at 427 and 335 nm, respectively. The corresponding increase in absorbance with time (*Figure* 2.11) represents the diffusion of AgNPs and plant extract in the surrounding microenvironment. Thus the prepared film can be considered as an active antimicrobial film, which releases the active constituents AgNPs and Tulsi extract simultaneously. The diffusion rate of active constituents is not constant. Initially, it is slow, but later on, it increases because the PVA film begins to swell and becomes porous to release the constituents actively.

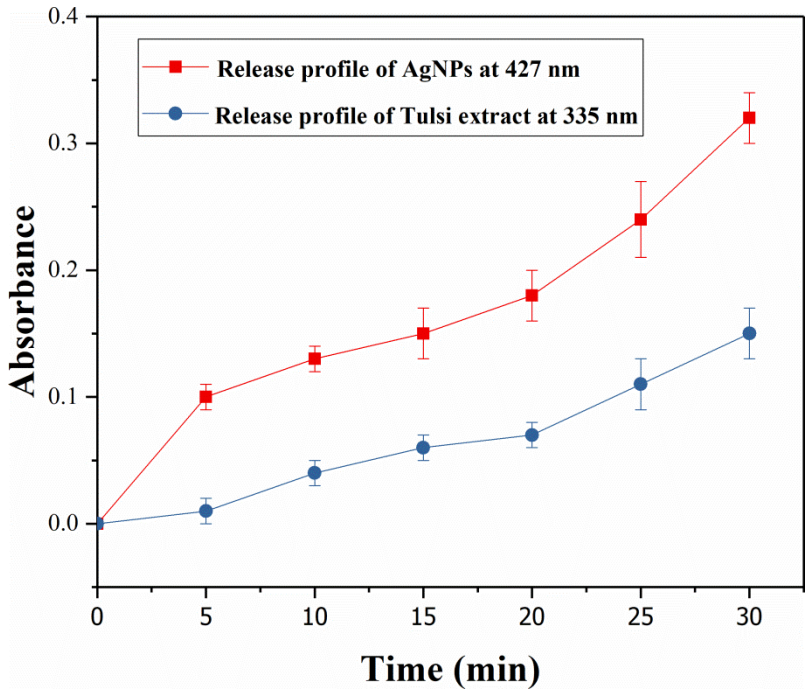

Figure 2.11: Release profile of AgNPs and *O. tenuiflorum* extract at 427 nm and 335 nm

2.5 Physico-chemical characteristics of electrospinning solution

The solutions were formed and further analyzed after complete homogenization for the parameters listed below:

2.5.1.1 Electrical conductivity

The electrical conductivities of the solutions are shown in Table 2.8**.** For CH, its value is higher (5.4 mS/cm) compared to that for PVA (2.8 mS/cm), but the fiber formation did not take place for CH alone. The blend solution 70:30 (PVA: CH) and (PVA: CH-AgNPs) have conductivities of 3.9 and 4.0 mS/cm, respectively. These can be molded into a fibrous form as seen in SEM images (*Figure* 2.12). The PVA can be easily electrospun due to its plasticizing nature [106]. The 6.0% solution of CH alone is difficult to electrospun. The 2.0% acetic acid has been selected to avoid any harmful effect, although it gets evaporated during electrospinning and drying [17,91,106]. The

pure CH is tough to electrospun, only fine spray of beads is formed, when electrospun (*Figure* 2.12f.).

2.5.1.2 Viscosity

The viscosity of the solution plays an important role in determining the electrospinnability, morphology, and diameter of nanofibers. The viscosity of pure PVA, CH and PVA:CH (70:30) blend and PVA:CH-AgNPs (70:30) blend solutions were found to be 7.10 Pa·s, 13.55 Pa·s, 8.20 Pa·s and 8.24 Pa·s, respectively (Table 2.8). Low viscous solutions do not form continuous fiber, and with very high solution viscosity, there is difficulty in the ejection of the jet from the needle tip. The higher viscosity of CH solution may be the reason why the CH fibers are difficult to form [107,108]. After blending with PVA, probably new hydrogen bonds between PVA-CH are formed, which increase its ability for electrospinning [106]. These are also supported by the change in FTIR peaks and XRD pattern of blended composite fibers (*Figure* 2.14 and 2.15).

Table 2.8: Properties of electrospinning solutions for making fibrous nano-layers

Sr. No.	Nano-layers	Composition of solution	Viscosity	Conductivity	Size of AgNPs
			(Pa·s)	(mS/cm)	(nm)
1.	P-100	PVA solution (A)	7.10	2.8	-
2.	CH-100	Chitosan solution (B)	13.55	5.4	-
3.	P70-CH30	A (70%) + B (30%)	8.20	3.9	-
4.	P70-CH30-Ag	A (70%) + B (30%) + AgNPs	8.24	4.0	93.4 ± 26.0

A- 12% aqueous polyvinyl alcohol (PVA) solution, B- 6% aqueous chitosan (CH) solution with 2% acetic acid, AgNPs- silver nanoparticles.

2.5.1.3 Thickness

The film formed by solution casting of CH was homogenous, transparent and light yellow in appearance (*Figure* 2.10) whereas the electrospun FCNLs was white and opaque. At the macroscopic scale, all the composite nano-layers were evenly smooth and had an average thickness of around 0.015 to 0.020 mm (Table 2.9). The CH film was slightly thicker (0.23 mm) as it was solution cast.

2.5.1.4 Solubility

The PVA is a water soluble polymer hence the water uptake capacity of the PVA nanofiber is maximum. The PVA film wholly dissolved in the DDW. CH is hydrophobic in nature. The solubility of composite nano-layers in water decreased as the proportion of CH increased and that of PVA decreased. The cross-linking of fibers due to the action of the glutaraldehyde reduces the solubility, hence the film made of PVA: CH (70:30) has low solubility (3.0-7.0%) and can be used as an active packaging material. The CH film is very weakly water-soluble (0.2-0.4%) due to its hydrophobic nature remained nearly intact on exposure to water. The PVA film containing plant extract-AgNPs swell and dissolved entirely in 8 h.

2.5.1.5 The SEM fingerprints

The SEM images confirmed that PVA easily forms smooth fibrous films with an average diameter of 466.84 ± 129.37 nm (*Figure* 2.12a, Table 2.9). Pure CH is extremely difficult to electrospun [42] and it formed only a spray of beads (*Figure* 2.12f). The PVA: CH concentration ratio of 70:30 resulted in the formation of fibers of average diameter 267.80 ± 148.64 nm and 196.02 ± 118.76 nm for P70-CH30 NL and

P70-CH30-Ag NL, respectively (Table 2.9, *Figure* 2.12b-c). As the proportion of CH increased, the formation of beads along with the fibers increased (*Figure* 2.12d-e), so the fibres made using PVA: CH ratio of 70:30 was used for further study. The SEM micro-photographs showed that the PVA solution formed fine fibers without any bead, but the tendency of fiber formation decreased as the concentration of CH increased (Table 2.9). The fiber diameter also decreased as the concentration of CH increased. Above 30% CH concentration, the fiber diameter decreased and the bead formation started. At 50% CH concentration, beads with few fibers formed but after this, only beads formed (*Figure* 2.12e-f). As the antimicrobial property is due to the CH, so the fiber with the maximum concentration of the CH will have greater antimicrobial activity. Thus the PVA: CH ratio of 70:30 is optimal for the formation of smooth and bead free fibrous film with maximum surface area and antimicrobial activity.

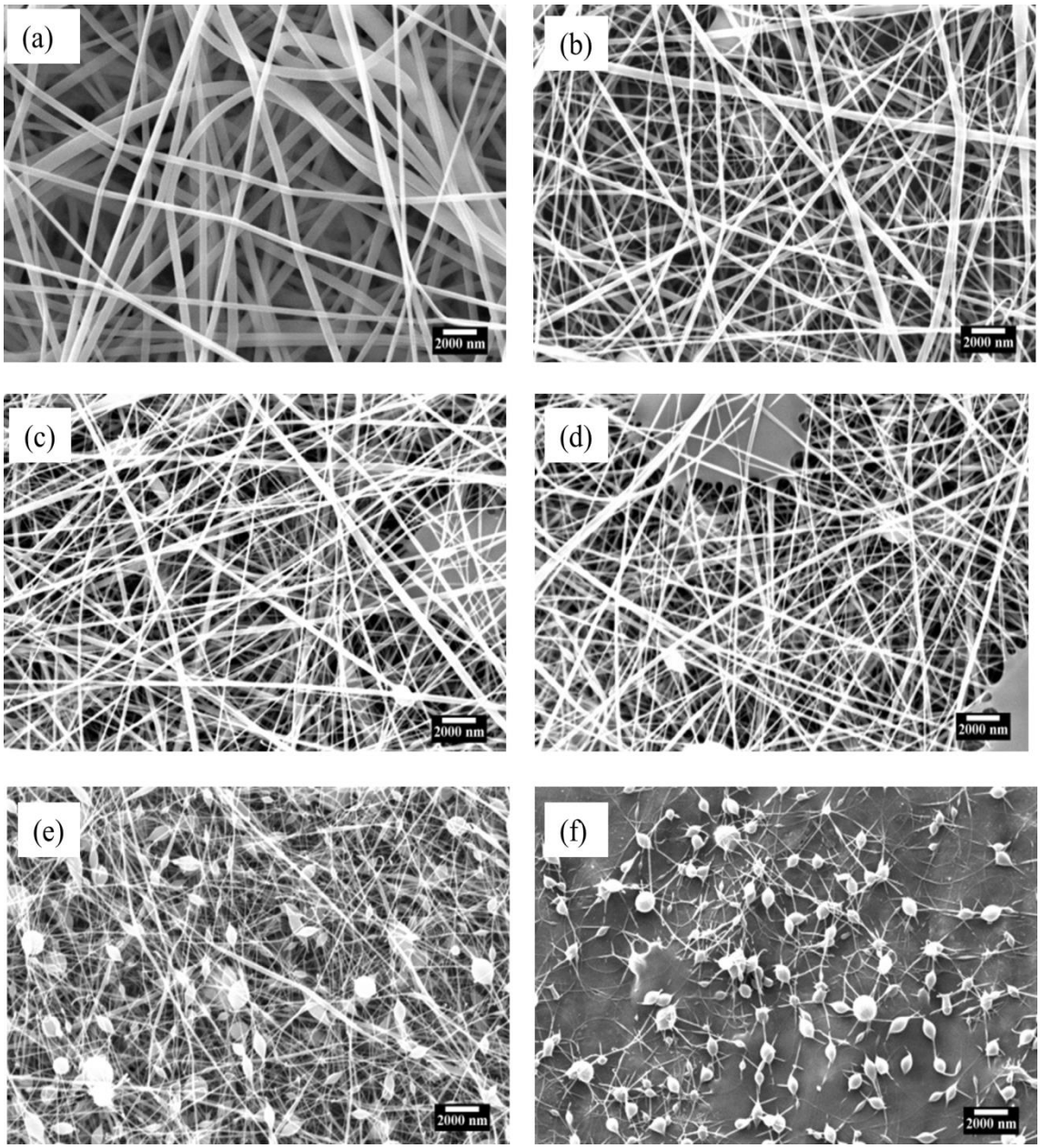

Figure 2.12: SEM photo-micrographs of electrospun composite nano-layers; **(a)** P-100 NL; **(b)** P70-CH30 NL; **(c)** P70-CH30-Ag NL; **(d)** P60-CH40 NL; **(e)** P50-CH50 NL; **(f)** CH-100NL

Table 2.9: Morphology, thickness, average fiber diameter and water contact angle of fibrous composite nano-layers

Sr. No.	Film/Nano-layer	Composition	Morphology	Thickness	Diameter of Nanofiber	Water Contact Angle
				(mm)	(nm)	(Degree)
1.	P-100 NL	A (100%)	Smooth Fiber	0.015	466.84 (±129.37)	73.12 (± 9.71)
2.	P70-CH30 NL	A (70%) + B (30%)	Fiber	0.018	267.80 (±148.64)	97.12 (± 9.28)
3.	P70-CH30-Ag NL	A (70%) + B (30%) + AgNPs	Fiber	0.020	196.02 (±118.76)	95.82 (± 10.27)
4.	P60-CH40 NL	A (60%) + B (40%)	Fiber + Bead	0.018	129.61 (±59.95)	88.72 (± 9.54)
5	P50-CH50 NL	A (50%) + B (50%)	Fiber + Bead	0.019	93.73 (±53.09)	81.26 (± 11.63)
6.	CH-100 NL	B (100%) + AgNPs	Beads	-	-	-
7.	CH-100 Film	B (100%)	Film	0.230	-	106.47 (± 9.98)
8.	Sunlight treated Polymeric Film	C (100%) + AgNPs	Film	0.150	-	70.37 (± 7.92)

A- 12% aqueous polyvinyl alcohol (PVA) solution, B- 6% aqueous chitosan (CH) solution with 2% acetic acid, , C- 6% aqueous plant extract polyvinyl alcohol (PVA) solution, NL- Nano-layer, AgNPs- silver nanoparticles.

2.5.1.6 Zeta (ζ) potential of plant extract-AgNPs

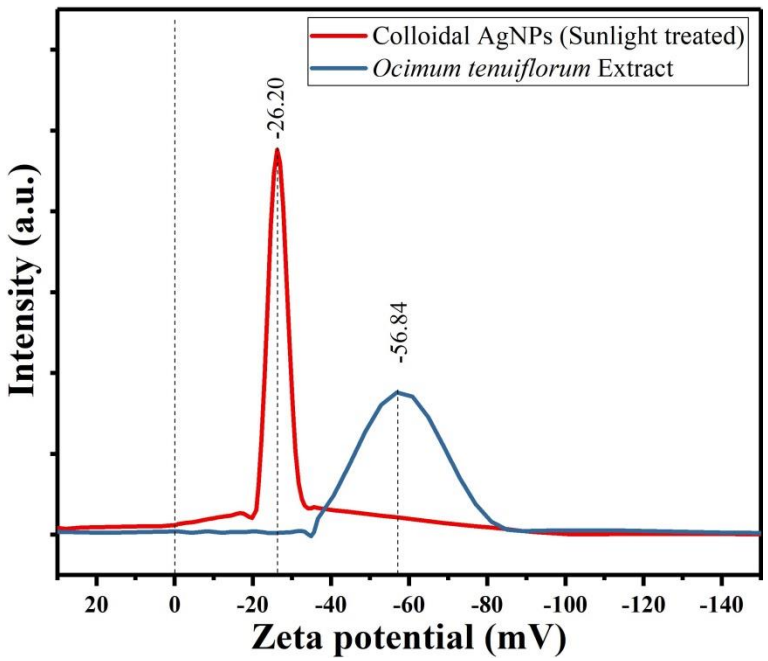

Figure 2.13: Zeta potential of *Ocimum tenuiflorum* extract and AgNPs colloidal solution

The zeta potential calculated using Smoluchowski equation based on electrophoretic mobility, $v = (\varepsilon E/\eta)\xi$ where v is the measured electrophoretic velocity, η is the viscosity of the solution, E is the electric field, ε is the electrical permittivity of the electrolytic solution and ξ is the zeta potential. The zeta potential measures the stability of the nanoparticles. It was determined as –26.20 mV for AgNPs colloidal solution and –56.84 mV for the plant extract (*Figure* 2.12). The high value of zeta potential, which provides stability to nanoparticles against agglomeration was probably due to the capping of AgNPs by the active biomolecules like eugenol (1-hydroxy-2-methoxy-4-allylbenzene) present in the Tulsi extract. Although the negative charge provided by the active molecules of Tulsi extract should have stabilized the size of AgNPs, however with time, an increase in the size of AgNPs has been observed. This

may be due to the ionic effect and agglomeration of some of AgNPs. A zeta potential of around ± 30 mV has been reported for the complete stability of the colloidal solution [109], but in the present case, it has been found as –26.20 mV for the colloidal solution, hence slow agglomeration is observed after some time.

2.5.2 Water contact angle (WCA)

The hydrophilic and hydrophobic nature of the FCNLs and polymeric film for its suitability as packaging material was evaluated by the sessile water drop contact angle test. The hydrophilic surfaces show a WCA < 90° while the hydrophobic surfaces show a WCA > 90° [110]. The results showed (Table 2.9) that the fibrous PVA surface is highly hydrophilic. The hydrophilic nature decreased as the concentration of the CH increased in the composite nano-layer. The contact angle of the solution cast CH film is the highest among the FCNLs. The fibre-forming blends of the PVA-CH (70:30) and PVA-CH-AgNPs nano-layer showed WCA of 97.12° ± 9.28° and 95.82° ± 10.27° as the hydrophobic nature of the FCNLs was enhanced by the CH. It showed the permissible hydrophilicity-hydrophobicity balance for use as a packaging film. For CH percentage more than 30%, a decrease in WCA (Table 2.9) was observed, it may be due to the beaded uneven surface of the FCNLs (*Figure* 2.12d-f). More the surface roughness (beaded structure) less is the WCA. On the basis of the WCA values, the order of hydrophilicity is; plant extract-AgNPs-PVA polymeric film > PVA fibrous nano-layer > PVA:CH blend composite nano-layer > CH film. The major disadvantage of hydrophilic polymers used for packaging is their poor water vapour and moisture barrier property and high manufacturing cost [1]. The CH blending is capable of removing these limitations.

2.5.3 Fourier Transform Infrared (FTIR) spectroscopy

The FTIR spectroscopic analysis of CH-100 film, electrospun nanofibrous PVA-100, PVA-CH (70:30) and PVA-CH-Ag (70:30) nano-layers were carried out to ascertain the presence of various functional groups and chemical interaction among the polymers. *Figure* 2.14 shows the FTIR spectra of the FCNLs. In the FTIR spectrum of CH film, the absorption peak at 1634 cm^{-1} corresponds to carbonyl stretching vibration in O=C–NHR groups and the absorption peak at 1533 cm^{-1} is due to N–H bending vibration in primary amine groups [106]. The broad absorption peaks of O–H have appeared at around 3200 cm^{-1}. A peak at 2873 cm^{-1} shows aliphatic C-H stretching vibration. The absorption peaks at 898, 1019 and 1254 cm^{-1} corresponds to the saccharides structure, and peak at 1403 cm^{-1} corresponds to stretching of N–H of amide III. The peak at 1065 cm^{-1} corresponds to the secondary hydroxyl group [111].

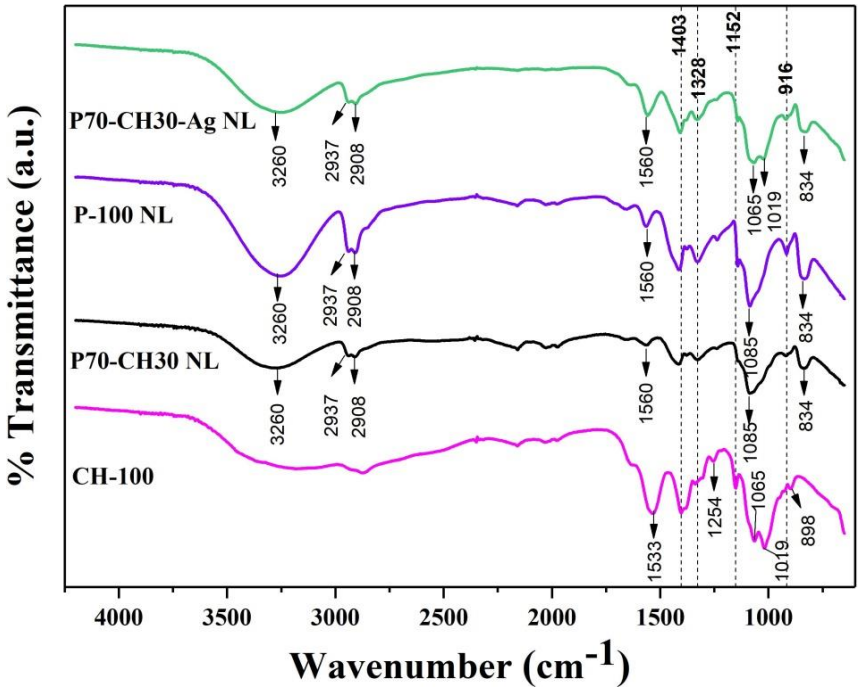

Figure 2.14: FTIR spectra of film and composite nano-layers; (a) CH-100 film; (b) P70-CH30 NL; (c) P-100 NL; (d) P70-CH30-Ag NL

The PVA nanofibrous film exhibits the stretching vibration peak due to its side hydroxyl groups at 3260 cm^{-1} associated with –OH stretch due to intermolecular and intramolecular hydrogen bonds. The C–H stretch from alkyl group corresponds to the peak at 2908 cm^{-1}.

In the electrospun FCNLs, the absorption peak due to amino group at 1560 cm^{-1} gets reduced in the composite films as compared to the fibrous PVA films due to the interaction between PVA and CH macromolecules. It may be due to the formation of hydrogen bonds between –OH groups in PVA and amino groups in CH. Therefore, the blending of PVA with CH improved its electrospinnability.

2.5.4 X-ray diffraction (XRD)

The CH films showed peaks at 2θ of 11.2° (100), 17.9° (101), and 22.3° (102) to mark the crystallinity. The pattern for the PVA fibers showed crystalline peaks at 2θ of 19.4° (101), 22.3° (200), 40.6° (111) [106,112]. In case of the composite P70-CH30 NL and P70-CH30-Ag NL two prominent peaks were observed at 2θ of 19.4°, 22.3°. Though there is a difference in the intensity of the peaks. In P70-CH30-Ag NL, the crystalline nature appears at 2θ of 38.0° and index to the (111) reflection plane (JCPDS number 04-0783, 1991). In the composite nanofibers, it has been observed that the peaks for CH at 11.2°, 17.9° and those for PVAs at 19.4°, 40.6° vanish (*Figure* 2.15). The possible reason may be the hydrogen bond interactions between CH and PVA macromolecules. These results also support the observations made through FTIR analysis (*Figure* 2.14).

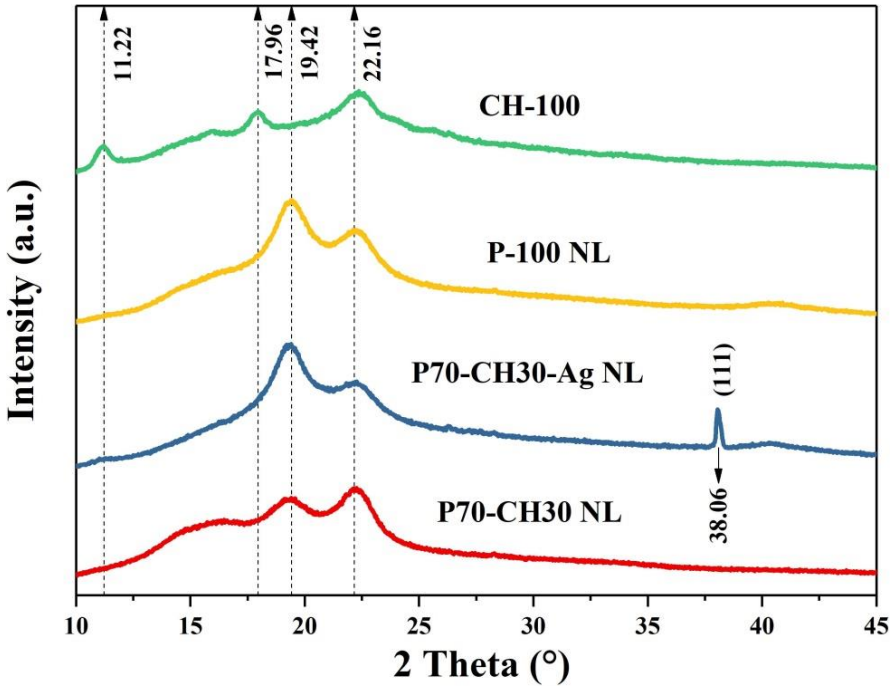

Figure 2.15: XRD pattern of film and composite nano-layers: (a) P70-CH30 NL; (b) P70-CH30-Ag NL; (c) P-100 NL; (d) CH-100 film

2.5.5 Microbiological analysis

2.5.5.1 *In vitro* antimicrobial activity of film and FCNLs

When tested for the antimicrobial activity by agar disc diffusion assay, the zone of inhibition was observed in some cases (Table 2.10). The P-100 NL did not exhibit any antimicrobial activity, but there was no bacterial growth over its surface. The P70-CH30 NL and P70-CH30-Ag NL showed remarkable antibacterial activity against the tested bacteria- *E. coli* and *L. monocytogenes*. The maximum zone of inhibition was observed for P70-CH30-Ag NL, possibly due to the synergistic antimicrobial activity of CH and AgNPs similar to the findings of Kumar et al. [40]. The antimicrobial action of CH molecule is believed to be due to the electrostatic interaction between the positively charged CH molecule and negatively charged outer bacterial cell membrane resulting in

a change in the membrane permeability leading to osmotic imbalance and hydrolysis of peptidoglycan wall of the bacteria [100].

The polymeric film, when tested for the antimicrobial property using the overnight grown bacterial culture, no growth over the film was observed, and a zone of inhibition all around the film was also observed indicating its antibacterial activity. The peripheral zone of inhibition is small due to the slow diffusion of active constituents from the film towards the solid media. The zone of inhibition shown in Table 2.10 indicates the antimicrobial property of the formed AgNPs against the pathogenic bacteria. Based on the results obtained (Table 2.10), it can be inferred that the UV treatment does not catalyze the AgNPs biosynthesis, hence mere plant extract and $AgNO_3$ solution showed limited antibacterial activity. A clear zone of inhibition occurs in sunlight treated mixture due to the presence of AgNPs. Some antimicrobial activity has been seen in case of $AgNO_3$ due to its inherent nature while Tulsi plant aqueous extract also showed a little antimicrobial activity as reported by Hanna et al.[39].

Table 2.10: Antimicrobial activity of fibrous composite nano-layers and polymeric films against Gram-positive and Gram-negative bacteria

Sr. No.	**Film/Nano-layer**	**Composition**	**Zone of Inhibition (mm)**	**Zone of Inhibition (mm)**
			E. coli	***L. monocytogenes***
1.	P-100 NL	A (100%)	00.0 ± 0.0	00.0 ± 0.0
2.	P70-CH30 NL	A (70%) + B (30%)	16.0 ± 0.6	15.0 ± 0.4
3.	P70-CH30-Ag NL	A (70%) + B (30%) + AgNPs	20.0 ± 0.7	21.0 ± 0.6
4.	UV light treated	C (100%) + AgNPs	3.0 ± 0.20	3.0 ± 0.20

	Film			
5.	Sunlight treated Film	C (100%) + AgNPs	15 ± 1.10	13 ± 1.20
6.	Silver Nitrate Solution	Aqueous solution	4.0 ± 0.20	4.0 ± 0.50
7.	Tulsi extract	Aqueous solution	1.9 ± 0.20	2.0 ± 0.30

A- 12% aqueous polyvinyl alcohol (PVA) solution, B- 6% aqueous chitosan (CH) solution with 2% acetic acid, C- 6% aqueous plant extract polyvinyl alcohol (PVA) solution, NL- Nano-layer, AgNPs- silver nanoparticles. n=5, zone of inhibition ± SD

2.5.6 Antimicrobial application of FCNLs for meat packaging

The meat packaged in conventional plastic and PVA nano-layers, when tested for microbial growth after a week, showed a rapid increase in the microbial count (*Figure* 2.16). This indicated that there were suitable conditions for microbial growth, and this may lead to the degradation of the packaged meat due to microbial action. In the case of the P70-CH30 NL and P70-CH30-Ag NL, initially there was no growth, but after a period of 16-17 h, the bacterial growth was observed. It may be due to the bacteria that reside in the deeper layers of meat which were not in close contact of the packaging layer. These bacteria must have escaped from the antimicrobial action of FCNLs. Compared to the bacterial growth observed in the case of convectional plastic and PVA nano-layers (P-100 NL), the observed bacterial contamination in P70-CH30-Ag NL nano-layer was negligible. This indicates that the composite P70-CH30 and P70-CH30-Ag FCNLs have effectively killed the initial bacterial contamination. The P70-CH30-Ag FCNLs exhibited maximum antimicrobial activity among the tested packaging materials. Further, when the meat was grinded in a grinder after seven days, the organoleptic quality (i.e. visual and smell) was much better in case of meat packaged within P70-CH30-Ag FCNLs (*Figure* 2.17).

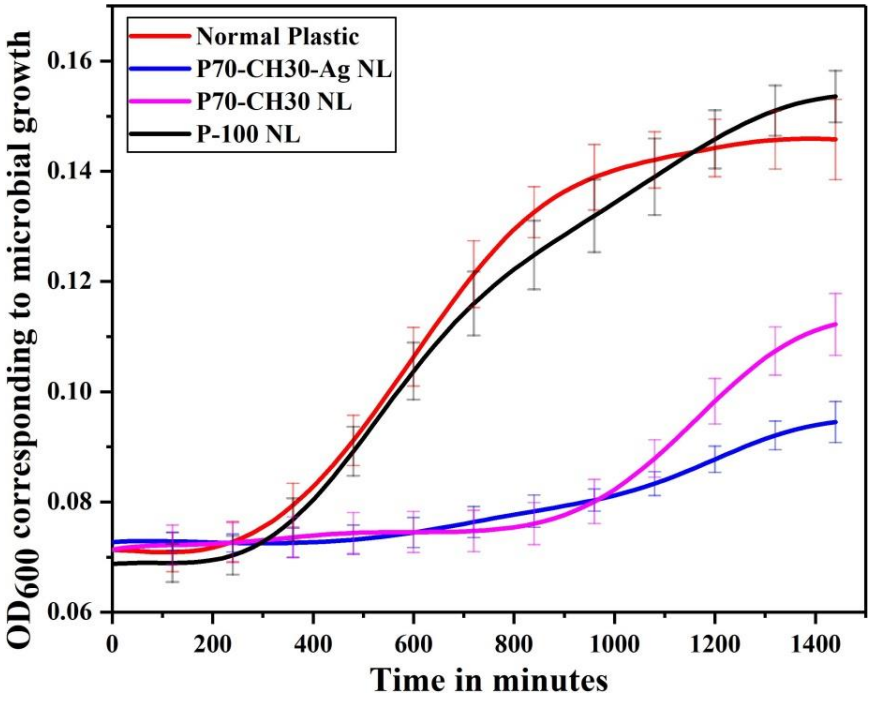

Figure 2.16: Microbial growth pattern in packed meat after 7 days: (a) Normal Plastic; (b) P70-C30-Ag NL; (c) P70-CH30 NL; (d) P-100 NL

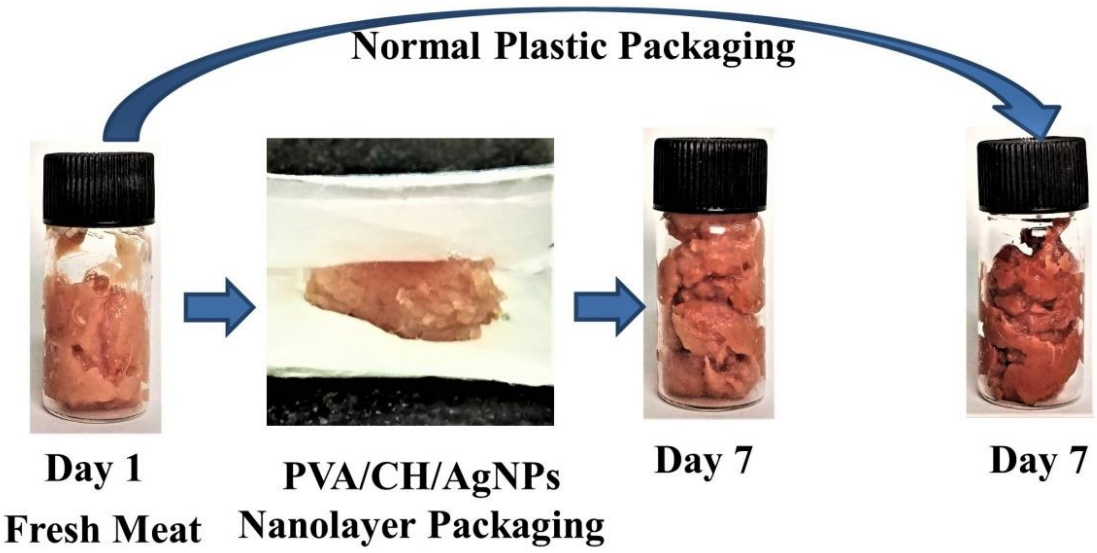

Figure 2.17: Application of PVA/CH/AgNPs composite nano-layer for packaging of fresh meat

The results indicate that the P70-CH30-Ag FCNLs possess better antimicrobial properties among the tested FCNLs. It has extended the product shelf-life and quality due to its antimicrobial action. Antimicrobial activity gets further synergistically enhanced by the addition of green synthesized AgNPs (Table 2.10). The composite nano-layers act as active packaging which can be seen in the antimicrobial action for a prolonged duration.

The strength and barrier properties of the conventional packaging materials in comparison to FCNLs are better than the formed FCNLs as the nano-layers are fibrous and can easily get damaged due to mishandling. Thus, when used with conventional packaging materials, these films could increase the shelf-life of packaged meat and protect consumers from food-borne diseases. One of the major advantages of these electrospun FCNLs is that the electrospinning process occurs at ambient conditions so thermo-labial active constituents can easily be incorporated in these composite nano-layers.

2.6 Conclusion

The AgNPs biosynthesis has been successfully carried out using *O. tenuiflorum* extract in a photocatalyze reaction. Active biomolecules present in the plant extract and the energy associated with the incident light are responsible for the formation of stable AgNPs. The AgNPs, when cast in a polymeric film exhibit the characteristic antimicrobial properties of AgNPs and act as an active film by releasing the extract of medicinal herb Tulsi and AgNPs. In comparison to solution casted films, the electrospun bio-composite fibers P70-CH30 and P70-CH30-Ag FCNLs exhibited good antimicrobial properties against degrading bacteria. Their antimicrobial property is due

to the inherent characteristics of CH, and it gets synergistically enhanced by the addition of green synthesized AgNPs in P70-CH30-Ag NLs. These chitosan-based composite nano-layers can be used to develop novel food packaging materials which can be potentially as functional as the conventional plastics, and therefore may replace conventional plastic packaging whilst leaving a significantly lower environmental footprint.

References

[1] A. Mousavi Khaneghah, S.M.B. Hashemi, S. Limbo, Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions, Food Bioprod. Process. 111 (2018) 1–19.

[2] A.M. Youssef, S.M. El-Sayed, Bionanocomposites materials for food packaging applications: Concepts and future outlook, Carbohydr. Polym. 193 (2018) 19–27.

[3] T.M.W. Mak, X. Xiong, D.C.W. Tsang, I.K.M. Yu, C.S. Poon, Sustainable food waste management towards circular bioeconomy: Policy review, limitations and opportunities, Bioresour. Technol. 297 (2020).

[4] A.A. Shah, F. Hasan, A. Hameed, S. Ahmed, Biological degradation of plastics: A comprehensive review, Biotechnol. Adv. 26 (2008) 246–265.

[5] C.L. Chen, Regulation and management of marine litter, SpringerOpen, 2015.

[6] D. Brennecke, E.C. Ferreira, T.M.M. Costa, D. Appel, B.A.P. da Gama, M. Lenz, Ingested microplastics (>100μm) are translocated to organs of the tropical fiddler crab Uca rapax, Mar. Pollut. Bull. 96 (2015) 491–495.

[7] D. Yang, H. Shi, L. Li, J. Li, K. Jabeen, P. Kolandhasamy, Microplastic Pollution in Table Salts from China, Environ. Sci. Technol. 49 (2015) 13622–13627.

[8] T.S. Galloway, Micro- and nano-plastics and human health, in: Mar. Anthropog. Litter, Springer International Publishing, 2015: pp. 343–366.

[9] C.G. Avio, S. Gorbi, F. Regoli, Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea, Mar. Environ. Res. 111 (2015) 18–26.

[10] V.M. Hernandez-Izquierdo, J.M. Krochta, Thermoplastic Processing of Proteins for Film Formation—A Review, J. Food Sci. 73 (2008) R30–R39.

[11] H. Zhang, P. He, H. Kang, X. Li, Antioxidant and antimicrobial effects of edible coating based on chitosan and bamboo vinegar in ready to cook pork chops, LWT. 93 (2018) 470–476.

[12] L.G.A. Barboza, A. Dick Vethaak, B.R.B.O. Lavorante, A.K. Lundebye, L. Guilhermino, Marine microplastic debris: An emerging issue for food security, food safety and human health, Mar. Pollut. Bull. 133 (2018) 336–348.

[13] T. Huang, Y. Qian, J. Wei, C. Zhou, Polymeric Antimicrobial food packaging and its applications, Polymers (Basel). 11 (2019).

[14] S. Kumar, A. Mukherjee, J. Dutta, Chitosan based nanocomposite films and coatings: Emerging antimicrobial food packaging alternatives, Trends Food Sci. Technol. 97 (2020) 196–209.

[15] S.A. Surwade, K. Chand, Antimicrobial food packaging : An overview, Eur. J. Biotechnol. Biosci. 5 (2017) 85–90.

[16] N.A. Al-Tayyar, A.M. Youssef, R. Al-hindi, Antimicrobial food packaging based on sustainable Bio-based materials for reducing foodborne Pathogens: A review, Food Chem. 310 (2020) 125915.

[17] C.K.S. Pillai, W. Paul, C.P. Sharma, Chitin and chitosan polymers: Chemistry, solubility and fiber formation, Prog. Polym. Sci. 34 (2009) 641–678.

[18] M. Arkoun, F. Daigle, R.A. Holley, M.C. Heuzey, A. Ajji, Chitosan-based nanofibers as bioactive meat packaging materials, Packag. Technol. Sci. 31 (2018) 185–195.

[19] M.J. Firdhouse, P. Lalitha, Biosynthesis of silver nanoparticles and its applications, J. Nanotechnol. 2015 (2015).

[20] J. Pulit-Prociak, M. Banach, Silver nanoparticles - A material of the future...?, Open Chem. 14 (2016) 76–91.

[21] W. Sim, R.T. Barnard, M.A.T. Blaskovich, Z.M. Ziora, Antimicrobial silver in medicinal and consumer applications: A patent review of the past decade (2007–2017), Antibiotics. 7 (2018) 1–15.

[22] T. Faunce, A. Watal, Nanosilver and global public health: International regulatory issues, Nanomedicine. 5 (2010) 617–632.

[23] A.C. Burdușel, O. Gherasim, A.M. Grumezescu, L. Mogoantă, A. Ficai, E. Andronescu, Biomedical applications of silver nanoparticles: An up-to-date overview, Nanomaterials. 8 (2018) 1–25.

[24] K.S. Siddiqi, A. Husen, R.A.K. Rao, A review on biosynthesis of silver nanoparticles and their biocidal properties, J. Nanobiotechnology. 16 (2018).

[25] M. Carbone, D.T. Donia, G. Sabbatella, R. Antiochia, Silver nanoparticles in polymeric matrices for fresh food packaging, J. King Saud Univ. - Sci. 28 (2016) 273–279.

[26] H.Y. Lee, H.K. Park, Y.M. Lee, K. Kim, S.B. Park, A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications, Chem. Commun. (2007) 2959–2961.

[27] X.Y. Dong, Z.W. Gao, K.F. Yang, W.Q. Zhang, L.W. Xu, Nanosilver as a new generation of silver catalysts in organic transformations for efficient synthesis of fine chemicals, Catal. Sci. Technol. 5 (2015) 2554–2574.

[28] S.H. Jeong, H. Choi, J.Y. Kim, T.W. Lee, Silver-based nanoparticles for surface plasmon resonance in organic optoelectronics, Part. Part. Syst. Charact. 32 (2015) 164–175.

[29] M. Kumari, S. Pandey, V.P. Giri, A. Bhattacharya, R. Shukla, A. Mishra, C.S.

Nautiyal, Tailoring shape and size of biogenic silver nanoparticles to enhance antimicrobial efficacy against MDR bacteria, Microb. Pathog. 105 (2017) 346–355.

[30] S. babu Maddinedi, B.K. Mandal, S.K. Maddili, Biofabrication of size controllable silver nanoparticles – A green approach, J. Photochem. Photobiol. B Biol. 167 (2017) 236–241.

[31] R.R. Choudhury, J.M. Gohil, S. Mohanty, S.K. Nayak, Antifouling, fouling release and antimicrobial materials for surface modification of reverse osmosis and nanofiltration membranes, J. Mater. Chem. A. 6 (2018) 313–333.

[32] M. Mohseni, A. Shamloo, Z. Aghababaei, M. Vossoughi, H. Moravvej, Antimicrobial Wound Dressing Containing Silver Sulfadiazine With High Biocompatibility: In Vitro Study, Artif. Organs. 40 (2016) 765–773.

[33] S. Yildirim, B. Röcker, M.K. Pettersen, J. Nilsen-Nygaard, Z. Ayhan, R. Rutkaite, T. Radusin, P. Suminska, B. Marcos, V. Coma, Active Packaging Applications for Food, Compr. Rev. Food Sci. Food Saf. 17 (2018) 165–199.

[34] K. Saravanakumar, R. Chelliah, D. MubarakAli, D.H. Oh, K. Kathiresan, M.H. Wang, Unveiling the potentials of biocompatible silver nanoparticles on human lung carcinoma A549 cells and Helicobacter pylori, Sci. Rep. 9 (2019) 1–8.

[35] J. Ding, J. Zhang, J. Li, D. Li, C. Xiao, H. Xiao, H. Yang, X. Zhuang, X. Chen, Electrospun polymer biomaterials, Prog. Polym. Sci. 90 (2019) 1–34.

[36] A.D. Juncos Bombin, N.J. Dunne, H.O. McCarthy, Electrospinning of natural polymers for the production of nanofibres for wound healing applications, Mater. Sci. Eng. C. 114 (2020) 110994.

[37] M. Abrigo, S.L. McArthur, P. Kingshott, Electrospun nanofibers as dressings for chronic wound care: Advances, challenges, and future prospects, Macromol. Biosci. 14 (2014) 772–792.

[38] B. Schumann, M. Schmid, Packaging concepts for fresh and processed meat – Recent progresses, Innov. Food Sci. Emerg. Technol. 47 (2018) 88–100.

[39] H.A. Yamani, E.C. Pang, N. Mantri, M.A. Deighton, Antimicrobial activity of Tulsi (Ocimum tenuiflorum) essential oil and their major constituents against three species of bacteria, Front. Microbiol. 7 (2016) 1–10.

[40] S. Kumar-Krishnan, E. Prokhorov, M. Hernández-Iturriaga, J.D. Mota-Morales, M. Vázquez-Lepe, Y. Kovalenko, I.C. Sanchez, G. Luna-Bárcenas, Chitosan/silver nanocomposites: Synergistic antibacterial action of silver nanoparticles and silver ions, Eur. Polym. J. 67 (2015) 242–251.

[41] M. Yadav, P. Goswami, K. Paritosh, M. Kumar, N. Pareek, V. Vivekanand, Seafood waste: a source for preparation of commercially employable chitin/chitosan materials, Bioresour. Bioprocess. 6 (2019).

[42] V. Sencadas, D.M. Correia, A. Areias, G. Botelho, A.M. Fonseca, I.C. Neves, J.L. Gomez Ribelles, S. Lanceros Mendez, Determination of the parameters affecting electrospun chitosan fiber size distribution and morphology, Carbohydr. Polym. 87 (2012) 1295–1301.

[43] N. Ben Halima, Poly(vinyl alcohol): Review of its promising applications and insights into biodegradation, RSC Adv. 6 (2016) 39823–39832.

[44] U. UNEP, Marine Plastic Debris and Microplastics: Global Lessons and Research to Inspire Action and Guide Policy Change, (2016).

[45] A. Barron, T.D. Sparks, Commercial Marine-Degradable Polymers for Flexible Packaging, IScience. 23 (2020) 1–13.

[46] T.P. Haider, C. Völker, J. Kramm, K. Landfester, F.R. Wurm, Plastics of the Future? The Impact of Biodegradable Polymers on the Environment and on Society, Angew. Chemie - Int. Ed. 58 (2019) 50–62.

[47] F.K. Mammo, I.D. Amoah, K.M. Gani, L. Pillay, S.K. Ratha, F. Bux, S. Kumari, Microplastics in the environment: Interactions with microbes and chemical contaminants, Sci. Total Environ. 743 (2020) 140518.

[48] B. Malhotra, A. Keshwani, H. Kharkwal, Antimicrobial food packaging: Potential and pitfalls, Front. Microbiol. 6 (2015) 1–9.

[49] X. Wang, Y. Du, H. Liu, Preparation, characterization and antimicrobial activity of chitosan-Zn complex, Carbohydr. Polym. 56 (2004) 21–26.

[50] J.S. Serrano-León, K.B. Bergamaschi, C.M.P. Yoshida, E. Saldaña, M.M. Selani, J.D. Rios-Mera, S.M. Alencar, C.J. Contreras-Castillo, Chitosan active films containing agro-industrial residue extracts for shelf life extension of chicken restructured product, Food Res. Int. 108 (2018) 93–100.

[51] A. Riaz, S. Lei, H.M.S. Akhtar, P. Wan, D. Chen, S. Jabbar, M. Abid, M.M. Hashim, X. Zeng, Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols, Int. J. Biol. Macromol. 114 (2018) 547–555.

[52] R. Priyadarshi, Sauraj, B. Kumar, F. Deeba, A. Kulshreshtha, Y.S. Negi, Chitosan films incorporated with Apricot (Prunus armeniaca) kernel essential oil as active food packaging material, Food Hydrocoll. 85 (2018) 158–166.

[53] Y. Guo, X. Chen, F. Yang, T. Wang, M. Ni, Y. Chen, F. Yang, D. Huang, C. Fu, S. Wang, Preparation and Characterization of Chitosan-Based Ternary Blend Edible Films with Efficient Antimicrobial Activities for Food Packaging Applications, J. Food Sci. 84 (2019) 1411–1419.

[54] S. Bautista-Baños, M. Hernández-López, E. Bosquez-Molina, C.L. Wilson, Effects of chitosan and plant extracts on growth of Colletotrichum gloeosporioides, anthracnose levels and quality of papaya fruit, Crop Prot. 22

(2003) 1087–1092.

[55] A. Perdones, L. Sánchez-González, A. Chiralt, M. Vargas, Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry, Postharvest Biol. Technol. 70 (2012) 32–41.

[56] G.F. Mehyar, A.A. Al Nabulsi, M. Saleh, A.N. Olaimat, R.A. Holley, Effects of chitosan coating containing lysozyme or natamycin on shelf-life, microbial quality, and sensory properties of Halloumi cheese brined in normal and reduced salt solutions, J. Food Process. Preserv. 42 (2018) 13324.

[57] I.K. Sani, S. Pirsa, Ş. Tağı, Preparation of chitosan/zinc oxide/Melissa officinalis essential oil nano-composite film and evaluation of physical, mechanical and antimicrobial properties by response surface method, Polym. Test. 79 (2019) 106004.

[58] L. He, L. Zou, Q. Yang, J. Xia, K. Zhou, Y. Zhu, X. Han, B. Pu, B. Hu, W. Deng, S. Liu, Antimicrobial Activities of Nisin, Tea Polyphenols, and Chitosan and their Combinations in Chilled Mutton, J. Food Sci. 81 (2016) M1466–M1471.

[59] E. Divsalar, H. Tajik, M. Moradi, M. Forough, M. Lotfi, B. Kuswandi, Characterization of cellulosic paper coated with chitosan-zinc oxide nanocomposite containing nisin and its application in packaging of UF cheese, Int. J. Biol. Macromol. 109 (2018) 1311–1318.

[60] A. Khanjari, I.K. Karabagias, M.G. Kontominas, Combined effect of N,O-carboxymethyl chitosan and oregano essential oil to extend shelf life and control Listeria monocytogenes in raw chicken meat fillets, LWT - Food Sci. Technol. 53 (2013) 94–99.

[61] Y. Xing, W. Li, Q. Wang, X. Li, Q. Xu, X. Guo, X. Bi, X. Liu, Y. Shui, H. Lin, H. Yang, Antimicrobial Nanoparticles Incorporated in Edible Coatings and Films for the Preservation of Fruits and Vegetables, Molecules. 24 (2019) 1695.

[62] R. Balti, M. Ben Mansour, N. Sayari, L. Yacoubi, L. Rabaoui, N. Brodu, A. Massé, Development and characterization of bioactive edible films from spider crab (Maja crispata) chitosan incorporated with Spirulina extract, Int. J. Biol. Macromol. 105 (2017) 1464–1472.

[63] J. Jiang, X. Gu, R. Song, X. Wang, L. Yang, Microcystin-LR induced oxidative stress and ultrastructural alterations in mesophyll cells of submerged macrophyte Vallisneria natans (Lour.) Hara, J. Hazard. Mater. 190 (2011) 188–196.

[64] I. Gan, W.S. Chow, Antimicrobial poly(lactic acid)/cellulose bionanocomposite for food packaging application: A review, Food Packag. Shelf Life. 17 (2018) 150–161.

[65] S. Gunalan, R. Sivaraj, V. Rajendran, Green synthesized ZnO nanoparticles against bacterial and fungal pathogens, Prog. Nat. Sci. Mater. Int. 22 (2012) 693–700.

[66] G.D. Venkatasubbu, R. Baskar, T. Anusuya, C.A. Seshan, R. Chelliah, Toxicity mechanism of titanium dioxide and zinc oxide nanoparticles against food pathogens, Colloids Surfaces B Biointerfaces. 148 (2016) 600–606.

[67] K. Saravanakumar, R. Chelliah, S. Shanmugam, N.B. Varukattu, D.H. Oh, K. Kathiresan, M.H. Wang, Green synthesis and characterization of biologically active nanosilver from seed extract of Gardenia jasminoides Ellis, J. Photochem. Photobiol. B Biol. 185 (2018) 126–135.

[68] B. Calderón-Jiménez, M.E. Johnson, A.R. Montoro Bustos, K.E. Murphy, M.R. Winchester, J.R.V. Baudrit, Silver nanoparticles: Technological advances, societal impacts, and metrological challenges, Front. Chem. 5 (2017) 1–26.

[69] S. Ahmed, M. Ahmad, B.L. Swami, S. Ikram, A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise, J. Adv. Res. (2016).

[70] M. Rafique, I. Sadaf, M.S. Rafique, M.B. Tahir, A review on green synthesis of silver nanoparticles and their applications, Artif. Cells, Nanomedicine Biotechnol. 45 (2017) 1272–1291.

[71] T.A. Jorge de Souza, L.R. Rosa Souza, L.P. Franchi, Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity, Ecotoxicol. Environ. Saf. 171 (2019) 691–700.

[72] S. Rajeshkumar, L. V. Bharath, Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity, Chem. Biol. Interact. 273 (2017) 219–227.

[73] S. Pal, Y.K. Tak, J.M. Song, Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli, J. Biol. Chem. (2015).

[74] G.R. Tortella, O. Rubilar, N. Durán, M.C. Diez, M. Martínez, J. Parada, A.B. Seabra, Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment, J. Hazard. Mater. 390 (2020) 121974.

[75] S. Mathew, S. S, J. Mathew, E.K. Radhakrishnan, Biodegradable and active nanocomposite pouches reinforced with silver nanoparticles for improved packaging of chicken sausages, Food Packag. Shelf Life. 19 (2019) 155–166.

[76] L.R. Braga, E.T. Rangel, P.A.Z. Suarez, F. Machado, Simple synthesis of active films based on PVC incorporated with silver nanoparticles: Evaluation of the thermal, structural and antimicrobial properties, Food Packag. Shelf Life. 15 (2018) 122–129.

[77] D. Valerini, L. Tammaro, F. Di Benedetto, G. Vigliotta, L. Capodieci, R. Terzi, A. Rizzo, Aluminum-doped zinc oxide coatings on polylactic acid films for antimicrobial food packaging, Thin Solid Films. 645 (2018) 187–192.

[78] L. Lin, Y. Gu, H. Cui, Moringa oil/chitosan nanoparticles embedded gelatin nanofibers for food packaging against Listeria monocytogenes and Staphylococcus aureus on cheese, Food Packag. Shelf Life. 19 (2019) 86–93.

[79] C. Demitri, V.M. De Benedictis, M. Madaghiele, C.E. Corcione, A. Maffezzoli, Nanostructured active chitosan-based films for food packaging applications: Effect of graphene stacks on mechanical properties, Measurement. 90 (2016) 418–423.

[80] W. Yang, E. Fortunati, F. Dominici, G. Giovanale, A. Mazzaglia, G.M. Balestra, J.M. Kenny, D. Puglia, Synergic effect of cellulose and lignin nanostructures in PLA based systems for food antibacterial packaging, Eur. Polym. J. 79 (2016) 1–12.

[81] C. Swaroop, M. Shukla, Nano-magnesium oxide reinforced polylactic acid biofilms for food packaging applications, Int. J. Biol. Macromol. 113 (2018) 729–736.

[82] X. Niu, Y. Liu, Y. Song, J. Han, H. Pan, Rosin modified cellulose nanofiber as a reinforcing and co-antimicrobial agents in polylactic acid /chitosan composite film for food packaging, Carbohydr. Polym. 183 (2018) 102–109.

[83] P. Wen, D.H. Zhu, K. Feng, F.J. Liu, W.Y. Lou, N. Li, M.H. Zong, H. Wu, Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/β-cyclodextrin inclusion complex for antimicrobial packaging, Food Chem. 196 (2016) 996–1004.

[84] Y.A. Arfat, J. Ahmed, N. Hiremath, R. Auras, A. Joseph, Thermo-mechanical, rheological, structural and antimicrobial properties of bionanocomposite films based on fish skin gelatin and silver-copper nanoparticles, Food Hydrocoll. 62 (2017) 191–202.

[85] Z. Lian, Y. Zhang, Y. Zhao, Nano-TiO2 particles and high hydrostatic pressure treatment for improving functionality of polyvinyl alcohol and chitosan composite films and nano-TiO2 migration from film matrix in food simulants, Innov. Food Sci. Emerg. Technol. 33 (2016) 145–153.

[86] M.P. Indumathi, K. Saral Sarojini, G.R. Rajarajeswari, Antimicrobial and biodegradable chitosan/cellulose acetate phthalate/ZnO nano composite films with optimal oxygen permeability and hydrophobicity for extending the shelf life of black grape fruits, Int. J. Biol. Macromol. 132 (2019) 1112–1120.

[87] A. Jayakumar, H. K.V., S. T.S., M. Joseph, S. Mathew, P. G., I.C. Nair, R. E.K., Starch-PVA composite films with zinc-oxide nanoparticles and phytochemicals as intelligent pH sensing wraps for food packaging application, Int. J. Biol. Macromol. 136 (2019) 395–403.

[88] S.D.F. Mihindukulasuriya, L.T. Lim, Nanotechnology development in food packaging: A review, Trends Food Sci. Technol. 40 (2014) 149–167.

[89] J. Pelipenko, P. Kocbek, J. Kristl, Critical attributes of nanofibers: Preparation, drug loading, and tissue regeneration, Int. J. Pharm. 484 (2015) 57–74.

[90] L. Zhao, G. Duan, G. Zhang, H. Yang, S. He, S. Jiang, Electrospun Functional Materials toward Food Packaging Applications: A Review, Nanomaterials. 10 (2020) 150.

[91] P. Su, C. Wang, X. Yang, X. Chen, C. Gao, X.X. Feng, J.Y. Chen, J. Ye, Z. Gou, Electrospinning of chitosan nanofibers: The favorable effect of metal ions, Carbohydr. Polym. 84 (2011) 239–246.

[92] T.J. Sill, H.A. von Recum, Electrospinning: Applications in drug delivery and tissue engineering, Biomaterials. 29 (2008) 1989–2006.

[93] C.-W. Chen, J. Xie, F.-X. Yang, H.-L. Zhang, Z.-W. Xu, J.-L. Liu, Y.-J. Chen, Development of moisture-absorbing and antioxidant active packaging film based on poly(vinyl alcohol) incorporated with green tea extract and its effect on the quality of dried eel, J. Food Process. Preserv. 42 (2018) e13374.

[94] F. Topuz, T. Uyar, Antioxidant, antibacterial and antifungal electrospun nanofibers for food packaging applications, Food Res. Int. 130 (2020) 108927.

[95] C.D. Soto-Cantú, A.Z. Graciano-Verdugo, E. Peralta, A.R. Islas-Rubio, A. González-Córdova, A. González-León, H. Soto-Valdez, Release of butylated hydroxytoluene from an active film packaging to asadero cheese and its effect on oxidation and odor stability, J. Dairy Sci. 91 (2008) 11–19.

[96] X. He, H. Deng, H. min Hwang, The current application of nanotechnology in food and agriculture, J. Food Drug Anal. 27 (2019) 1–21.

[97] EFSA, Scientific Opinion on the criteria to be used for safety evaluation of a mechanical recycling process to produce recycled PET intended to be used for manufacture of materials and articles in contact with food, EFSA J. 9 (2011).

[98] R. Kalaivani, M. Maruthupandy, T. Muneeswaran, A. Hameedha Beevi, M. Anand, C.M. Ramakritinan, A.K. Kumaraguru, Synthesis of chitosan mediated silver nanoparticles (Ag NPs) for potential antimicrobial applications, Front. Lab. Med. 2 (2018) 30–35.

[99] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, J. Pharm. Anal. 6 (2016) 71–79.

[100] M. Kong, X.G. Chen, K. Xing, H.J. Park, Antimicrobial properties of chitosan and mode of action: A state of the art review, Int. J. Food Microbiol. 144 (2010) 51–63.

[101] A. Cano, E. Fortunati, M. Cháfer, J.M. Kenny, A. Chiralt, C. González-Martínez, Properties and ageing behaviour of pea starch films as affected by blend with poly(vinyl alcohol), Food Hydrocoll. 48 (2015) 84–93.

[102] N. Ahmad, S. Sharma, M.K. Alam, V.N. Singh, S.F. Shamsi, B.R. Mehta, A. Fatma, Rapid synthesis of silver nanoparticles using dried medicinal plant of basil, Colloids Surfaces B Biointerfaces. 81 (2010) 81–86.

[103] T.C. Prathna, N. Chandrasekaran, A.M. Raichur, A. Mukherjee, Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size, Colloids Surfaces B Biointerfaces. 82 (2011) 152–159.

[104] T. Zheng, S. Bott, Q. Huo, Techniques for Accurate Sizing of Gold Nanoparticles Using Dynamic Light Scattering with Particular Application to Chemical and Biological Sensing Based on Aggregate Formation, ACS Appl. Mater. Interfaces. 8 (2016) 21585–21594.

[105] C.M. Maguire, M. Rösslein, P. Wick, A. Prina-Mello, Characterisation of particles in solution–a perspective on light scattering and comparative technologies, Sci. Technol. Adv. Mater. 19 (2018) 732–745.

[106] Y.T. Jia, J. Gong, X.H. Gu, H.Y. Kim, J. Dong, X.Y. Shen, Fabrication and characterization of poly (vinyl alcohol)/chitosan blend nanofibers produced by electrospinning method, Carbohydr. Polym. 67 (2007) 403–409.

[107] S. Sukigara, M. Gandhi, J. Ayutsede, M. Micklus, F. Ko, Regeneration of Bombyx mori silk by electrospinning - Part 1: Processing parameters and geometric properties, Polymer (Guildf). 44 (2003) 5721–5727.

[108] S.B. Qasim, M.S. Zafar, S. Najeeb, Z. Khurshid, A.H. Shah, S. Husain, I.U. Rehman, Electrospinning of chitosan-based solutions for tissue engineering and regenerative medicine, Int. J. Mol. Sci. 19 (2018).

[109] A.T.M. Saeb, A.S. Alshammari, H. Al-Brahim, K.A. Al-Rubeaan, Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria, Sci. World J. 2014 (2014).

[110] V.K. Pandey, K.R. Srivastava, G. Ajmal, V.K. Thakur, V.K. Gupta, S.N. Upadhyay, P.K. Mishra, Differential Susceptibility of Catheter Biomaterials to Biofilm-Associated Infections and Their Remedy by Drug-Encapsulated Eudragit RL100 Nanoparticles, Int. J. Mol. Sci. 20 (2019) 5110.

[111] S. Jabeen, A. Kausar, S. Saeed, B. Muhammad, S. Gul, M. Farooq, Crosslinking of alginic acid/chitosan matrices using bis phenol-F-diglycidyl ether: mechanical, thermal and water absorption investigation, Int. J. Plast. Technol. 20 (2016) 159–174.

[112] Y. Zhang, X. Huang, B. Duan, L. Wu, S. Li, X. Yuan, Preparation of electrospun chitosan/poly(vinyl alcohol) membranes, Colloid Polym. Sci. 285 (2007) 855–863.