CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 Background

The importance of food packaging is to protect the product from physical, chemical, or biological damage from the time of manufacture to the time it is used by the consumer.[1] The most well-known packaging materials are polyethylene- or co-polymer based materials, which comes under non biodegradable plastic, have been in use by the food industry for over 50 years.[1] These are synthetic petrochemical-based polymers, remains in the environment for many hundred years after disposal become a major source of environmental problems due to their poor biodegradability.[2, 3] The problem arises with non biodegradable food packaging films are two fold. First, non biodegradable packaging material ends up in landfills, where they can never degrade. Secondly, shortage of landfill space.[1] Non biodegradable plastic are non renewable in nature and with rising petroleum costs, there is concern with finding cost-effective ways to manufacture packaging materials.[1] Previously mentioned problems have increased the demand of biodegradable, recyclable, bio-based polymers, bio plastic or biopolymer made from raw materials originating from agricultural or marine sources as food packaging materials. These types of packaging materials include starch, cellulose, chitosan/chitin, gelatin , polylactic acid (PLA).[4] But so far the use of biodegradable films for food packaging is limited because of the poor barrier properties, weak mechanical properties shown by natural polymers. For this reason natural polymers are blended with other synthetic polymers.

Sericin a biopolymer is a family of adhesive silk protein which derived from natural resource from the middle silk glands of silkworm. It is a kind of hot water soluble macromolecular globular protein that envelops the fibroin fibre with successive sticky layers. It consists of 18 amino acids, most of which have such strong polar side groups as hydroxyl, carboxyl and amino groups that enable easy cross-linking, copolymerization, and blending with other polymers to form improved biodegradable materials. High hydrophilic nature of sericin arises from the high content of serine amino acids (approximately 34 wt. %). It is a family of naturally occurring gelatinous proteins with a wide range of molecular weights (from 10 to 400 kDa). [5-7]

The excellent hydrophilicity and water retention/release properties, sericin has been used as a biomaterial to make wound dressing and hydrogels for various applications.[6] Sericin has shown to be a promising biomaterial for cell culture, drug delivery, and functional coatings. It is also known to accelerate proliferation of various cell lines including mammalian and hybridoma as an additive in serum free media. Sericin can be utilised in skin care, food, antioxidant and anti-apoptotic, tumour suppression, anticoagulant and wound healers these applications are shown in work of Dash et al., 2008a,b; Zhang, 2002; Sasaki et al., 2000a,b,c; Kato et al., 1998; Zhaorigetu et al., 2001, 2003; Kundu et al., 2008. Because of its properties, sericin is particularly useful for improving artificial polymers such as polyesters, polyamide, polyolefin, and polyacrylonitrile.[7, 8]

Sericin can be utilised in various field like food packaging in the form of films. However it is difficult to fabricate pure sericin films. This is because sericin is amorphous by nature due to its wide range of molecular weights, ranging from 10 to over 400 kDa.[6] Also during its extraction from cocoons the harsh treatment seems to make the protein denature that

eventually leads to inferior biophysical properties. Due to this reason researchers have blended sericin with other natural/synthetic polymers to fabricate films, membranes and 2D and 3D matrices with improved properties. High molecular weight of sericin (more than 200 k Da) can produce strong blends with polymers, like PVA, gellatin,[9] whey protein,[10] glucomannan,[11] polysulfone (PSF)[12], chitosan[13] etc . Cross linking agents like PEG-DE[14], epoxides[15], glutraldehyde (GA), dimethylolurea (DMU) [6, 16], genipin[17], citric acid[18] , are used to improve the thermal, chemical and mechanical stabilities of the blends.

With citric acid as the cross linking agent, surface structure of the sericin/PVA/citric acid solidified film is made[18], mechanical strength of the film is reinforced with 0.1% sericin by the hydrogen bonding between the sericin and whey protein these films are edible in nature [9], Bombyx mori Sericin films were prepd. with PEG-DE as cross linking agent [13], Sericin films with good mechanical properties were prepared by using epoxides as cross linking agent. Cross linking agent has changed crystal structure of sericin and improved the physical and mechanical properties of sericin films[14], films of originally high molecular weight sericin, cross linked with dimethylolurea were prepared[19], native sericin membrane prepared without using any cross-linking agent for biomedical application[7], 3-D sericin/gelatine scaffolds and 2-D films using non-mulberry Antheraea mylitta silk cocoon sericin protein for cell culture applications.[9] The films were manufactured with silk sericin, using different dimethylolurea (DMU) concentrations as cross-linking agent and glycerol as plasticizer [16], polysaccharide polymers, such as glucomannan, were incorporated with sericin, glycerol as plasticiser to form a flexible film used for food coatings to prevent water and oxygen permeation between food products and the atmosphere.[11]

Polyvinyl alcohol (PVA) is widely used for packaging in the form of films which are water soluble biodegradable in nature.[20] It is synthetic and non-toxic polymer.[21] Because of its good film forming and highly hydrophilic water-soluble with outstanding chemical stability it was blended different synthetic and natural polymers.[21] PVA water-soluble film used for food packaging has good air barrier and resistance to oil. It could preserve food for longer time and keep fresh. This film can also be used to deliver savoury flavours in film to apply flavour and spices to meat products during cooking. Antimicrobial film was prepared by blending chitosan (CS) and PVA for food packaging application.[22] Blends of polyvinyl alcohol (PVA) and angico gum (AG) and/or cashew gum (CG) were used to produce films by casting method (pva/polysaccharide). Antimicrobial film was prepared by blending chitosan (CS) and poly(vinyl alcohol) (PVA) with glutaraldehyde (GA) as the cross-linker.[22] Gas permeation through water-swollen sericin / PVA membranes[23], hydrophilic membranes were prepared from sericin and poly(vinyl alcohol) (PVA) for applications in alcohol dehydration,[6] silk sericin–PVA scaffold cross linked with genipin provide a framework for cells to attach, proliferate and form an extracellular matrix,[17] blends of polyvinylalcohol (PVA) containing chitosan (CS) for active food packaging[21].

Natural polymers shows weak mechanical properties, high gas water vapour permeability, susceptibility to microorganisms which can be altered by using bioactive agents like nanoclay and silver salt. The use of nanoclay in the polymer matrix will enhance its mechanical and barrier properties which is useful for its potential application in the area of packaging.[24] The clay is good antimicrobial agent. The nanoclay can be used to produce bioactive sericin/clay nanocomposite[25]. Bionanocomposite film was developed by nanoclay incorporation to chitosan films for food packaging application.[26] Silver salt is also antimicrobial in nature. Silver and silver ion based materials are widely known for their

bactericidal and fungicidal activity. Their antimicrobial effect is due to blockage of respiratory enzyme pathways, alteration of microbial DNA and the cell wall[27].

In the present study bioactive sericin/PVA blend films are developed using silver salt and cloiste 30B as bioactive agents, glycerine as plasticizer and glutraldehyde as cross linking agent. The developed bioactive films are characterized for its structural and performance properties for food packaging application.

1.2 Objectives:

- Preparation of biodegradable sericin/PVA blended films.
- Preparation of bioactive, biodegradable nanocomposite sericin/PVA blended films.
- Characterization of blended films for its structural and performance properties for food packaging application.

CHAPTER 2

LITERATURE REVIEW

Food packaging is one the largest growing sector in market. Polymers which are mainly used are petrochemical-based polymers, due to their availability in large quantities at low cost and favourable functionality characteristics, such as, good tensile and tear strength, good barrier properties to O_2 and heat selability[28, 29]. These are synthetic polymers, remains in the environment for many hundred years after disposal become a major source of environmental problems due to their poor biodegradability and difficult to recycle or reuse [2, 3]. Recently, significant progress has been made in the development of biodegradable plastics , capable of degrading after disposal in bioactive environments by the enzymatic action of microorganisms such as bacteria, fungi, and algae[3] which are largely from renewable natural resources, to produce biodegradable materials with similar functionality to that of petrochemical-based polymers[30]. These polymers bring a significant contribution to the sustainable development in view of the wider range of disposal options with minor environmental impact. As a result, the market of these environmentally friendly materials is in rapid expansion, 10–20 % per year.[31]

2.1 Biodegradable Polymers Classifications

According to ASTM standard D-5488-94d and European norm EN 13432, ‘‘biodegradable’’ means ‘‘capable of undergoing decomposition into carbon dioxide, methane, water, inorganic

compounds, and biomass''. A vast number of biodegradable polymers (e.g. cellulose, chitin, starch, polyhydroxyalkanoates, polylactide, polycaprolactone, collagen and other polypeptides) have been synthesized or are formed in natural environment during the growth cycles of organisms.[32] Some microorganisms and enzymes capable of degrading such polymers have been identified [33][30].

Different classifications of various biodegradable polymers have been proposed. We propose to classify the biodegradable polymers according to their synthesis process (Fig.1):

(i) Polymers from biomass such as agro-polymers from agro-resources (e.g., starch or cellulose),

(ii) Polymers obtained by microbial production such as the polyhydroxyalkanoates (PHAs),

(iii) polymers conventionally and chemically synthesized from monomers obtained from agro-resources, e.g., the polylactic acid (PLA), and

(iv) Polymers obtained from fossil resources.

Only the first three categories (i–iii) are obtained from renewable resources. We can further classify these biodegradable polymers into two main categories: the agro-polymers (category i) and the biodegradable polyesters or bio polyesters (categories ii–iv)[30].

2.1.1 Agro-Polymers

Main agro-polymers are the polysaccharides and the proteins. Polysaccharides are the most abundant macromolecules in the biosphere. These complex carbohydrates constituted of glycosidic bonds are often one of the main structural elements of plants and animals exoskeleton. The polysaccharides includes cellulose, starch, chitosan, and pectins etc [30]

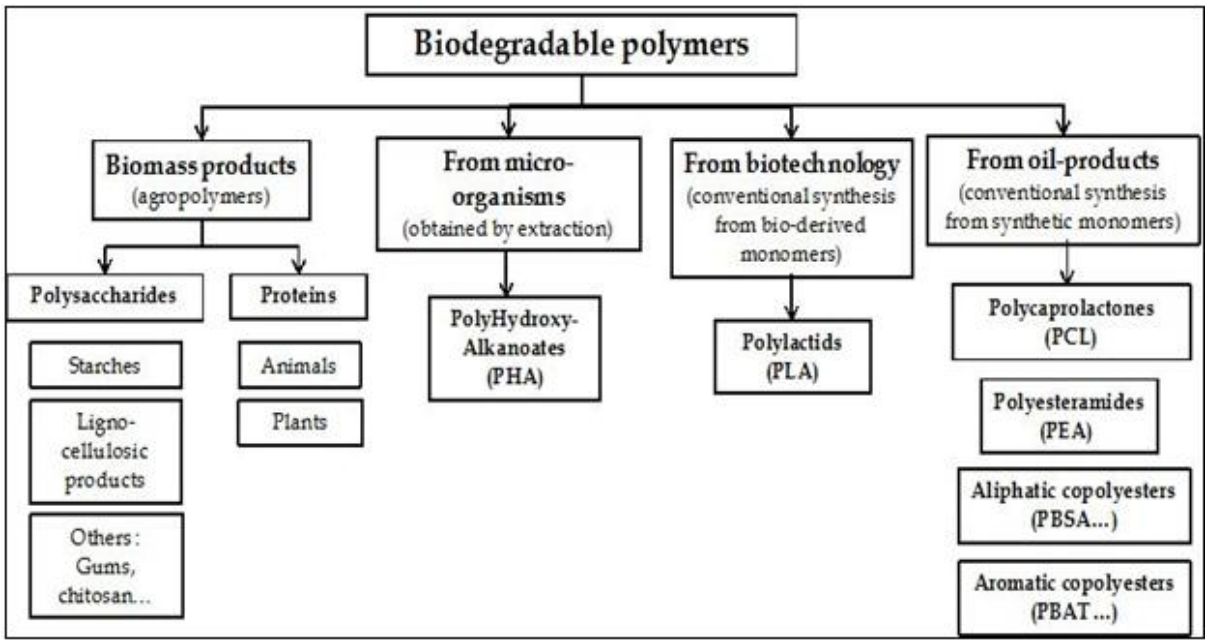

Figure 2.1 Classification of biodegradable polymers [30]

Cellulose is a well-known natural polymer, which after chemical modification is applied in a remarkably diverse set of applications cellulose acetates with degrees of substitution of up to 2.5 are biodegradable[34]. A decrease in the degree of substitution of cellulose acetate from 2.5 to 1.7 results in a large increase in the rate of its biodegradation. Eastman Chemical Company has developed fully biodegradable cellulose acetates, which are commercially available and find application in osmotic drug delivery and taste-masking, coatings, pressure sensitive tapes, packaging and wood sealers.[3]

Starch is an abundant, inexpensive, and annually renewable material available from potatoes as well as corn and other crops. It is composed of amylose, a mostly linear *alpha*-D-(1-4)-glucan and amylopectin, a branched *alpha*- D-(1-4)-glucan, which has *alpha*-D-(1-6) linkages at the branch point. Ratios of amylose and amylopectin vary with the starch source[34, 35]. Starch-based biopolymers can be obtained by blending or mixing starch with synthetic polymers. The properties and morphology of these blends can be adjusted easily and

efficiently. Full advantage of this phenomenon was taken by Novamount Company to produce Mater-Bi® material. It is mainly formed into films and sheets, which found application in agriculture, waste management, packaging, personal care & hygiene, accessories for animals.[3] Numerous intermolecular hydrogen bonds existing between the chains, the melting temperature (Tm) of starch is higher than its degradation temperature. [21, 22] Consequently, to elaborate a plastic-like material, it is necessary to introduce high water content or/and some non-volatile plasticizers (glycerol) which decrease the glass transition temperature (Tg) and the Tm[36]. These plasticized materials are currently named ‘‘thermoplastic starch’’ or ‘‘plasticized starch’’ [30].

Deacetylation of chitin results in chitosan. It is a random linear chain of N-acetyl-D-glucosamine units (acetylated unit) and D-glucosamine (deacetylated unit) linked by β(1,4) linkages. Amino group present in chitosan shows some particular properties. In acid conditions, when the amino groups are protonated, it becomes a water soluble polycation. The chitosan is characterized by its acetylation degree and by its molecular weight. These last parameters influence its viscosity and solubility. Depending on the source (shrimp, crab, mushrooms…), industrial chitosan has molecular weight varying from 5,000 to 1,000,000 g.mol-1 and acetylation degrees from 2 to 60 %. Chitosan can be plasticized with glycerol to obtain a kind of thermoplastic material. [30]

Gelatin is a natural material based on animal proteins. Gelatin is commonly used for biomedical applications due to its biodegradability and biocompatibility in physiological environments, in contact with living tissues[37]. Gelatin has shown to be useful in a great number of fields such as adhesives, and pharmaceutical and biomedical applications. [30]

2.1.2 Biodegradable Polyesters

Poly(ε-caprolactone) (PCL), polyhydroxylalkanoates (PHAs) and poly(lactic acid) (PLA) are the biodegradable polymers which belong to polyesters. They are water resistant and may be processed with melt-extrusion method into sheets, bottles, and diverse shaped products, which makes these plastics very promising for use as biopolymers. We can classify further into bacteria based (PHA), biobased (PLA) and non-renewable polyesters (PCL, PBSA, PBAT)

2.1.3 Bacteria based

Polyhydroxylalkanoates (PHA) are produced from renewable resources by bacterial fermentation of sugar and lipids.[3] PHAs are considered as biodegradable and thus suitable for e.g., short-term packaging, and also considered as biocompatible in contact with living tissues and can be used for biomedical applications (e.g., drug encapsulation, tissue engineering). PHA can be degraded by abiotic degradation, i.e., simple hydrolysis of the ester bond without requiring the presence of enzymes to catalyze this hydrolysis. During the biodegradation process, the enzymes degrade the residual products till final mineralization (biotic degradation). [30] They are thermoplastic or elastomeric polymers, depending on the monomer used in the synthesis. These materials, alone or in a blend with synthetic polymer or starch, give packaging films [3]. The main biopolymer of the PHA family is the polyhydroxybutyrate homopolymer (PHB). PHB can be plasticized with citrate ester. The production of PHA is intended to replace synthetic non-degradable polymers for a wide range of applications: packaging, agriculture, leisure, fast-food, hygiene as well as medicine and biomedical[38] since PHA is biocompatible. [30]

2.1.4 Bio based

Poly(lactic acid) can be synthesized by biological and chemical methods. The first one is more environmentally friendly, due to its renewable character. It is based on starch and other polysaccharides fermentation. It can be produced from corn, sugar beet, sugar cane, potatoes, and other biomasses [3][39]. PLA can be plasticized using oligomeric lactic acid (o-LA), citrate ester, or low molecular weight polyethylene glycol (PEG). The effect of plasticization increases the chain mobility and then favours the PLA organization and crystallization. After plasticization, a crystallinity ranging between 20 and 30 % is obtained. PLA presents a medium water and oxygen permeability level comparable to polystyrene[40]. These different properties associated with its tunability and its availability favour its actual developments in different packaging applications (trays, cups, bottles, films).[30] High molecular weight poly (lactic acid) is water insoluble. If water penetrates into the bulk of the polymer matrix, PLA is subjected to degradation as a result of hydrolysis on the ester group. The chemical bonds in the amorphous phase are attacked and long polymer chains are fragmented into shorter ones.[3]

2.1.5 Non-renewable polyesters

A large number of biodegradable polyesters are based on petroleum resources, obtained chemically from synthetic monomers. Examples include polycaprolactone, aliphatic copolyesters, and aromatic copolyesters. Ring Opening polymerization of e- caprolactone in the presence of metal alkoxides (aluminium isopropoxide, tin octoate) gives PCL poly (e-caprolactone). Some applications based on its biodegradable character in domains such soft compostable packaging. PCL is generally blended or modified (e.g., copolymerisation, crosslinking. Degradation of PCL is hydrolysis and biodegradation by fungi. Tokiwa et al. have shown that PCL can easily be enzymatically degraded.

2.2 PVA

PVA has such physical properties as viscosity, emulsifying, dispersing power, adhesive strength, tensile strength, and flexibility which make it suitable for forming films for the application of food packaging. It is water soluble synthetic polymer. PVA is resistant to oil, grease, and solvent. It was widely used, especially in fabric and paper sizing, fibre coating, adhesives, emulsion polymerization, films for packing and farming and the production of poly (vinylbutyral). Poly (vinyl) alcohol is a vinyl polymer in which the main chains are joined by only carbon-carbon links[41]. It is known to be used as a stabilizer in nanoparticles synthesis. PVA are well known polymer with excellent absorption capacities for a number of metal ions due to the presence of hydroxyl (-OH) group in its structures [16, 20, 21, 26].

2.2.1 PVA Biodegradation

The chemical structure of (PVA) is composed mainly of head-to-tail 1,3-diol units. However, only 1-2% of head-to-head 1, 2-diol units exist in PVA. The 1,2-diol content influences some properties of PVA, interlay its biodegradability. Factors affecting PVA biodegradability include the degree of polymerization (DP), the degree of saponification (hydrolysis; DS), tacticity of the main chain, ethylene content, and 1, 2-glycol content. In general, DP and DS did not have a significant influence on the biodegradation of PVA[42]. Poly (vinyl) alcohol historically has been produced industrially by the hydrolysis of poly (vinylacetate). As the vinyl alcohol monomer it cannot exist due to its tautomerization into acetoaldehyde. . The history of PVA biodegradation extends back more than 70 years, since the first report of degradation by Fusarium lini B[42]. Recently, it was reported that 55 species of microorganisms (including bacteria, fungi, yeast and mould) participate in degradation of poly (vinyl) alcohol. Scientists have isolated the Pseudomonas bacteria from soil bacterium

growing on PVA as the source of carbon. Pseudomonas is the main PVA degrader. This bacterium produces and secretes an enzyme that degrades PVA. This enzyme was isolated, purified and characterized. Polivinyl alcohol dehydrogenase (PVADH) from Pseudomonas ssp. 113P3 catalyzed an oxidation reaction of PVA to produce beta-diketone structure on PVA. The degradation mechanism included two steps: the conversion of the 1, 3-glycol structure of two successive repeating units to a β-diketone by a random oxidative dehydrogenation reaction or oxidation of one hydroxyl group yielding monoketone structures. This process was catalyzed by an extracellular secondary alcohol oxidase enzyme. And the second step was a reaction that broke the carbon-carbon bond and converted one of the ketone groups to a carboxylic group. This results in chain scission[43]. But according to the products produced by the first step of PVA degradation, there are two possible pathways for this second step: either hydrolysis of β-diketone structures of oxidized PVA (oxiPVA) by a β-diketone hydrolase (oxiPVA hydrolase) or the aldolase reaction involving the monoketone structures of oxiPVA [16, 26, and 31]

2.3 Biodegradation of Polymer Blends

The degradation of the more readily biodegradable component controls the rate of degradation of polymer blends[44]. Blending has become an economical and versatile route to obtain polymers with a wide range of desirable properties. Partially biodegradable polymers obtained by blending biodegradable and non-biodegradable commercial polymers can effectively reduce the volume of plastic waste by partial degradation. They are more useful than completely biodegradable polymers due to the economic advantages and superior properties imparted by the commercial polymer used as a blending component [30, 31].

2.3.1 Starch/PVA Blends

PVA is compatible with starch and blends are expected to have good film properties. If both components are biodegradable in various microbial environments, PVA and starch blends are biodegradable. The hydrophilic nature of PVA enhances compatibility with starch, making it suitable for the preparation of polymer blends. The use of PVA with starch improves the mechanical properties of the blends. Starch/polyvinyl alcohol blends are one of the most popular biodegradable plastics, and are widely used in packaging and agricultural applications [31][45]

2.4 Sericin

2.4.1 Structural information

Silk sericin is a natural macromolecular protein derived from silkworm *Bombyx mori*. Sericin is represented by a family of proteins with their molecular weight distributed between 10 and 300 kDa[7, 46]. When subjected to alkaline degumming process, sericin is degraded into sericin peptides or hydrolyzed sericin with molecular weight less than 20 kDa [6]. The sericin solution at room temperature is a partially gelled liquid with high viscosity dependent on temperature and pH. The sericin solution is a non-Newtonian fluid whose viscosity depends on the velocity of the flow [9, 12]. The sericin peptides having molecular weights of less than 60 kDa, commonly less than 5 kDa, are soluble in cold water. These are characterized by excellent moisture absorption and release, and a lot of biological activities such as antioxidation, tyrosinase activity inhibition[8], and pharmacological functions such as anticoagulation, anticancer activities[46], cryoprotection and promotion of digestion[46]. The rest, a higher range of molecular weight ranging 29 from 60 to more than 300 kDa, is poorly soluble in cold water but soluble in boiling water[46, 47]. Sericin is a highly hydrophilic

protein and classified into at least six proteins of different lengths generated by alternatively splicing the primary transcripts of two sericin genes, Ser1 and Ser2. In ExPASy protein databases, sericin is defined as Ser1 and Ser2. The Ser1 gene encodes for various polypeptides which contain several repeats of a 38 amino acid motif with a high content of hydroxyl amino acids, whose composition is very close to the average composition of sericin. In a study, chemical properties of oxidized sericin were determined by amino acid analysis. The amino acidic pattern of sericin is dominated by the presence of hydroxyl (serine, threonine, tyrosine), acidic (aspartic acid, glutamic acid), and basic (lysine, histidine, arginine) amino acid residues, which totally account for about 73 mol%. Glycine and alanine are minor components, with a total concentration of only 20 mol%. Other amino acids (proline, methionine, isoleucine, leucine, phylalanine, cysteine, valine and tryptophane) are present in very small amounts. Sericin is a globular protein. Its molecular formula is shown in fig(2.2). Serine and threonine are important because both are related to some mechanisms of sericin functionality such as the antioxidant activities and the tyrosinase-inhibitory effect[8]. In addition, Wu et al. (2008) showed that the amount of the hydrophilic amino acids was up to 76%, and could explain why sericin possesses the water absorbability and good solubility. Some of the amino acid residues of sericin macromolecule have polar side groups whereas others have non-polar side groups[48].

So, sericin macromolecule has both hydrophilic and hydrophobic elements. The isoelectric point (pI) of sericin purified from the cocoon shell of silkworm and that of silk fiber have been found as 4.3 and 5[23].

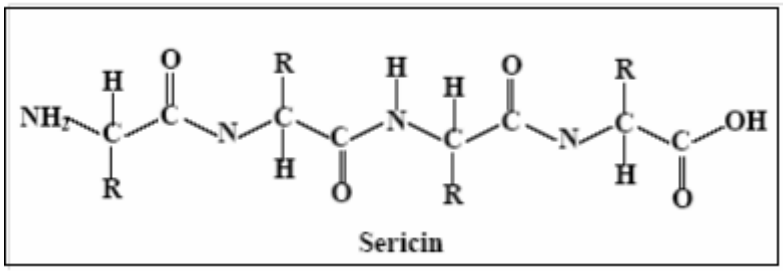

Figure 2.2 Structure of Sericin[23].

In a study of Whewell, it was found that C, H and N contents of sericin were 42.6%, 5.8% and 16.5%, respectively[23].

Proteins generally have two absorbance peaks in the UV region, one between 215-230 nm, where peptide bonds absorb, and another at about 280 nm due to light absorption by aromatic amino acids. The silk sericin shows a peak absorbance at around 280 nm of wavelength[23]. The range of molecular weight of sericin can be determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Wu et al. found that MW of sericin was 14-467 kDa. These results indicate that MW of sericin is affected by the conditions of applied methods.[23, 49]

2.4.2 Sericin extraction

Silk processing, covers activities such as production of cocoons, spinning of the silk yarn, dyeing and manufacturing of the final products. These activities lead to the generation of cocoon cooking, silk degumming and dyeing wastewaters. In silk processing, firstly, the cocoons are cooked to kill off the insects and unwind the silk fibers. The silk fibers are enveloped by the silk gum, namely sericin, which must be removed prior to dyeing. The degumming process is used to remove the external sericin, which consists of boiling silk fibers in a hot water bath containing soap and sodium carbonate, where the silk fiber loses

25-27% of its original weight, corresponding to the amount of sericin discarded in the wastewater CW (cocoon cooking wastewaters) and SDW (silk degumming wastewaters) have quite high COD, total solids, colour and turbidity. The COD and sericin contents of CW are as high as 11-17 g/L and 5-8 g/L, respectively. On the other hand, SDW contains much higher amounts of organic matter, i.e., 55-63 g/L of COD and 27-34 g/L of sericin. Therefore, they need to be treated properly prior to discharge into the receiving environment[50].

The sericin in silk processing wastewaters is a valuable protein; however, it is currently discarded as a waste. Sericin can be used in food, cosmetics and pharmaceutical products as well as for manufacturing biomaterials because of its unique properties such as moisture absorption/desorption, antibacterial and antioxidant properties, and UV resistance. The commercial value of sericin is high, as evidenced from the price of about 80-90 € per its gram on the market. The cocoon production in the world is about 1 million tons, which is equivalent to 400000 tons of dry cocoon, and processing of the raw silk produces about 50000 tons of sericin[47]. Therefore, the recovery of sericin from cocoon cooking wastewaters would provide economical benefits. It would also significantly reduce the environmental impact of silk production processes and help sustainable development.

Conventionally, removal of sericin is achieved in boiling water or in degumming solution containing soap. However, since boiling water alone is ineffective, the process could be catalyzed by the addition of acid or alkali but acids or alkali are considered as toxic chemicals and the severe conditions used make the process unfavourable[50]. The development of an effective degumming process based on enzymes as active agents would entail savings in terms of water, energy, chemicals, and effluent treatment[50]. However, the higher cost of

enzymes themselves has so far limited the development of industrial processes. It is found that without addition of toxic chemicals, silk waste could be hydrothermally decomposed into protein and amino acids. An alternative degumming technique using water at temperature of 120-130^{o}C and pressure of 300 - 400 kPa.[50]

Two types of wastewaters are generated in sericin removal processes; first, the wastewaters from the cocoon cooking process (CW) and second, the wastewaters from the silk degumming process (SDW). These wastewaters contain high COD, colour and turbidity. Cocoon cooking and silk degumming wastewaters have much higher COD, total solids, colour and turbidity than the other types of wastewaters have[23]

2.4.3 Extraction methods

Removal of the sericin from silk fibroin is accomplished by a process called “degumming”, usually by one of the following methods:[23]

(1) Extraction with water at a high temperature,

(2) Extraction with water at a high temperature and under pressure,

(3) Extraction with a soap solution,

(4) Extraction with a synthetic detergent,

(5) Extraction with an acid, and

(6) Removal by enzymes.

The degumming process is generally used to remove the silk sericin from the fibres in the silk industry.[50]

2.5 Bioactive Agents

Nanoclay is is used to improve modulus and tensile strength, barrier properties, flame resistance, and thermal properties of natural occurring polymers. It is derived from montmorillonite (MMT), a mineral deposit that has negatively charged silicate layers , typically have a stacked arrangement of with a platelet thickness of about 1 nm and a high aspect ratio (ratio of length to thickness). The layered silicate filled polymer composites exhibit enhanced mechanical, thermal, and other physicochemical properties at low filler content when compared with the pure polymer and conventional microcomposites and have excellent barrier properties due to the presence of the non-permeable clay layers that are dispersed in the polymer matrix. It has been also reported that biodegradability of nanocomposite materials increases after compositing with nano-sized clays[25]. The transfer of substances across a polymeric matrix involves three stages: dissolution of the substance in the polymeric matrix; diffusion across the polymeric matrix, and posterior release of the substance at another part of the polymeric matrix. Packaging used for food must prevent or delay one or all of the stages involved in this process to increase the shelf life of the food and to increase the safety of the food to consumers. Nanoclay act as a physical barrier that delays the passage of oxygen across the polymeric matrix of the nanocompound. The resulting delay in the speed of diffusion allows the food to remain fresh[51]. In natural form, clay can disperse only in hydrophilic polymers like Poly (ethylene oxide) and PVA[24]. The parameters that affect the development of a nanocomposite are, amongst others: the method used to elaborate the nanocomposite; the type of resin; the modifications used in producing the nanoclay; the content and the source of the nanoclay[51].

MMT is widely used as reinforcement for the polymer–clay nanocomposite synthesis because it is environmentally friendly, readily available in large quantities at a relatively low cost, and its intercalation chemistry is well understood. Montmorillonite is extremely fine-grained, do

not form macroscopic crystals and swell on addition of water or organic liquids. Montmorillonite is a 2:1 type consisting of two silicon oxygen sheets held together by intervening cations with water molecules in the interlayer spaces. Based on the extent of dispersion of platelets in matrix, polymer nanoclay based composites are classified in to three widely known morphologies: intercalated, exfoliated and delaminated. The term intercalation describes the case where a small amount of polymer chain seep in between clay galleries and cause about 20 – 30Å separation between the platelets. Un-separated clay layers and agglomerates in polymer matrix are often referred to as tactoids. Exfoliation or delamination occurs when polymer further separates the clay platelets, e.g., by 80 – 100Å or more. Delaminated structure refers to dispersion and homogeneous spreading of platelets in the polymer matrix beyond 100Å[24].

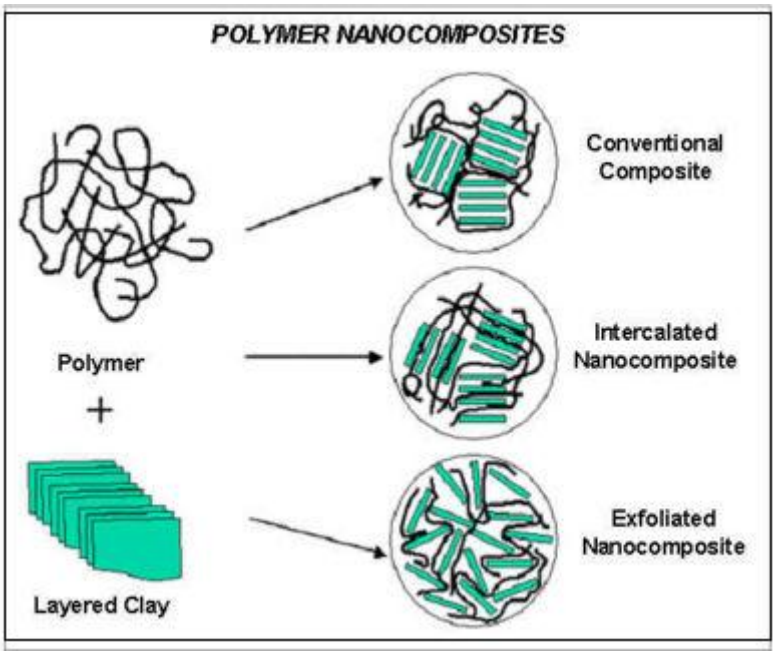

Figure 2.3 Schematic drawing of exfoliation and intercalation states[52].

Silver and silver ion based materials are widely known for their bactericidal and fungicidal activity. Their antimicrobial effect is due to blockage of respiratory enzyme pathways, alteration of microbial DNA and the cell wall. Therefore, silver and silver ion containing

materials are used as prostheses, catheters, vascular grafts and as wound dressings[27]. The silver exert their antimicrobial property by interacting with the sulphur containing proteins present in bacterial cell membrane as well as with phosphorous containing DNA. In addition, silver nanoparticles based antimicrobials have many advantages due to their thermal stability, health and environmental safety. As a result, the usage of silver based commercial products including topical ointments, bandages, augmentation devices, tissue scaffolds, antimicrobial filters and gels have increased for improving public health care. The combination of silver salt or nanoparticles with water soluble biopolymers will produce new antimicrobials. Based on this, various natural polymers such as gum acacia, starch, gelatine, sodium alginate, carboxy methyl cellulose etc., have been employed to prepare biocompatible polymeric silver composites[53, 54].

CHAPTER 3

EXPERIMENTAL METHODS

3.1 Materials

Sericin was extracted from cocoons, *Bombyx mori*, Polyvinyl alcohol powder (M.W. 14,000) from Central Drug House (P) LTD, Glutaraldehyde (GA) solution ($C_5H_8O_2$) (M.W. 100.12, 25 wt% aqueous solution) was purchased from Central Drug House (P) LTD, Glycerol Anhydrous (glycerine) pure ($C_3H_8O_3$) (M.W. 92.10) from SRL , Silver Nitrate Pure ($AgNO_3$) (M.W. 169.87 g/mol) from MERCK , Nanoclay (Closite 30 B) from Southern clay USA and Distilled water was used for all the experiments.

3.2 Methods

3.2.1 Sericin extraction and characterization

Sericin was extracted from the cocoons of *Bombyx mori* silkworm. The cocoons were cut into small pieces (about 1 cm^2), rinsed thoroughly and then immersed in distilled water in ratio 1:15 (w/v). In order to obtain sericin protein having relatively large molecular weights, the extraction was carried out using an autoclave. Aqueous Sericin solutions (SS) were extracted with pressure at 120º C for 90 minutes. In the next stage, the solution obtained from boiling was purified by paper filtering to remove fibroin and other suspended impurities. The SS solution was quite turbid and viscous at room temperature and converts to gel in low

temperature. To separate sericin from water SS is heated at 40^{o} C until sericin in solid form is obtained.

3.2.1.1 Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis test (SDS-PAGE)

The sericin solution after filtration was mixed with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (0.1 M Tris-HCl (SRL) of pH 6.8, 4% SDS (Sisco, Mumbai), 12% 2-mercaptoethanol (SRL), 20% glycerol (SRL)) and boiled for 5 min. The sample was adjusted to pH 6.8 with dilute HCl (SRL) solution immediately before electrophoresis. The range of molecular weight of sericin was determined by SDS-PAGE according to the method of Laemmli. [66] Samples ($20\mu L$) were electrophoresed in running buffer (1% SDS; 192 mM glycine (SRL); 25 mM Tris-base (SRL); pH 8.3) on a 10% resolving gel (10% acrylamide/bisacrylaminde (SRL), 375 mM Tris-HCl, 0.1% SDS, pH 8.8) at 100-130V for 1.5h using a Biogenic Vertical Dual Midi Gel system. The molecular weights of the sample were estimated using a molecular weight marker. The gels were stained with Bromophenol Blue (Sisco , Mumbai) and destained with 45% methanol, 10 acetic acid (SRL) to visualize.

3.2.1.2 UV/visible spectroscopy

Silk sericin solution obtained from the extraction was examined by using UV/visible Spectrophotometer (Agilent, CARY 300).

3.2.2 Preparation of biodegradable blend films

3.2.2.1 Pure sericin/PVA blend films

Sericin/PVA (10% wt.) blend films were prepared by solution casting method. Different blends were created by varying the variables glutraldehyde (GA) as crosslinking agent, glycerol as plasticizer.

1) Aqueous solution of Sericin/PVA in 1:1 ratios were prepared first, by dissolving PVA in distilled water for 90^{o} C for 45min. Then PVA solution is added with aqueous Sericin solution and mechanically stirred for about 1 h to ensure homogeneity at 40^{o} C. The pure Sericin/PVA film was poured on a Petri dishes (d = 90mm) and dried at room temperature for at least 24 hours.

2) Different blends were prepared by varying the concentration (0.2gm, 0.4gm. 0.8gm/gm of sericin) of cross linking agent. After the preparation of aqueous solution of Sericin/PVA glutaraldehyde was added into the solution and stirred for 15mins at 40^{o} C to ensure homogeneity. The cross linked film forming solution was poured into Petri dishes (d = 90mm) and dried at room temperature. After drying, the film was heat treated at 120^{o} *C* for 1 hour in order to crosslink films.

3) By varying the concentration (0.4, 0.6, 0.8gm/gm of sericin) of glycerine as plasticizer films were prepared. After the preparation of aqueous solution of Sericin/PVA glycerine was added into the solution and stirred for 15mins then GA was mixed, and again mixture is kept for stirring for 15mins at 40^{o} C to ensure homogeneity. Same procedure is followed as above to crosslink the blended films.

3.2.2.2 Bioactive sericin/PVA blend films

1) Silver salt is added in different concentrations (0.5%, 1%, 1.5 % of Sericin) to prepare films. In aqueous solution of Sericin/PVA silver salt is dissolved then glycerol is added and kept of stirring for 15mins. GA is added the film was cast on a Petri plate, as described before.

2) Nanoclay (closite 30 B) in different concentrations (0.5%, 1%, and 1.5 % of Sericin) was added to film forming solution. After mixing of glycerine and GA to aqueous solution of Sericin/PVA , closite 30 B is added and sonicated for 30mins. Film forming solution was poured into Petri dishes (d = 90mm) and dried at room temperature. After drying, the membrane was heat treated at 120^{o} *C* for 1 hour in order to crosslink films.

The dry films were stored in plastic bags before all subsequent characterization procedures.

3.3 Characterization

3.3.1 Mechanical testing

For the development of biodegradable blended material for food packaging, films of high tensile strength is desired. So blended films with different concentrations of GA, glycerine and bioactive agents were optimised by using Universal Testing Machine (INSTRON 3369) running at a crosshead speed of 5 mm/min. The sample films are cut into 10mm x100mm size and the gauge length is about 50mm. After optimisation tensile parameters of blended Sericin/ PVA films were investigated.

3.3.2 Structural properties

3.3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize the presence of specific chemical groups in the materials. Biodegradable Sericin/PVA blended films were obtained as 0.03-0.05 mm thick films and analyzed by FTIR using Transmittance Mode on

Thermo Scientific Nicolet 380 Spectrophotometer (Nicolet) USA. FTIR spectra were obtained in the range of wave number from 4000 to 650 cm^{-1}

3.3.2.2 X Ray Diffraction (XRD)

X-ray diffraction intensity curves were obtained at a $\lambda = 1.5$ Å for 2θ from 10 to 80º with a diffractometer Bruker D S Advanced (Germany) using CuKα radiation.

3.3.3 Thermal properties

3.3.3.1 Thermo gravimetric analysis (TGA)

TGA has been used extensively in the study of polymeric systems. This analytical technique used to determine materials thermal stability and its fraction of volatile components by monitoring the weight loss of the sample in a chosen atmosphere as a function of temperature. The samples were measured in the temperature range from 30°C to 600°C with a constant rate of 10°C/min under nitrogen atmosphere using TGAQ50 V20.10 Build 36 (TA instruments).

3.3.3.2 Dynamic mechanical testing (DMA)

The dynamic-mechanical thermal analysis of polymer materials is of great interest and importance, resulting from its great sensitivity in detecting changes of internal molecular mobility and in probing a phase structure and morphology of polymers. DMA was done on a DM 8000 PerkinElmer , using film testing fixture. Specimens of 40×10 mm was run at tensile mode at a frequency of 1Hz with heating rate 5° C/min. Storage modulus and tanδ were determined as a function of temperature from 0-250° C.

3.3.4 Morphological testing

3.3.4.1 Scanning electron microscopic (SEM)

Surface morphology of Sericin/PVA or bioactive Sericin/PVA blend films was observed by a scanning electron microscope (S-3700N SEM, Germany) at a voltage of 20 kV. Surface of specimen was coated with gold before analysis.

3.3.5 Measurement of Light Transmission

The ultraviolet (UV) and visible light barrier properties of the films were evaluated between 200 and 800 nm using a UV Spectrophotometer (Agilent, CARY 300). Transparency of the films was expressed as A600/x, where A600 is the absorbance at 600 nm, and x is the film thickness.

3.3.6 Antimicrobial Testing

The antimicrobial and activity of the developed silver salt incorporated films are tested by disc diffusion method against gram negative bacteria Neisseria. For disc diffusion method, the films are cut into a disc shape with 5 mm diameter, sterilized by autoclaving for 30 min at 120^{o}C, and placed on LB (Luria broth) cultured media plates. The plates are incubated for 2 days at 37^{o}C in an incubation chamber maintaining with 5% CO_2 flow and the inhibition zone is then measured.

CHAPTER 4

RESULTS AND DISCUSSION

For the development of biodegradable Sericin/PVA blend films with or without bioactive agents (silver salt and nanoclay), biopolymer Sericin protein was first extracted from cocoons by simply using autoclave for 90min. Extracted sericin was then mixed with water soluble PVA in 1:1ratio. Blended films are prepared by solution casting method. Steps involved in preparation of Sericin/PVA blended films are shown in fig (4.1) below:

The effect of different concentrations of cross linking agent GA (0.2gm, 0.4gm. 0.8gm/gm of sericin), glycerine as plasticizer (0.4, 0.6, 0.8gm/gm of sericin), silver salt (0.5, 1, and 1.5 % of Sericin) and nanoclay (0.5, 1, and 1.5 % of Sericin) as bioactive agents was optimised with maximum tensile strength. Glycerine is added to give flexibility to films. Preparation of bioactive Sericin/PVA blended films is shown in fig (4.2). Optimised biodegradable blended films are then characterised for its structural and performance properties for food packaging application.

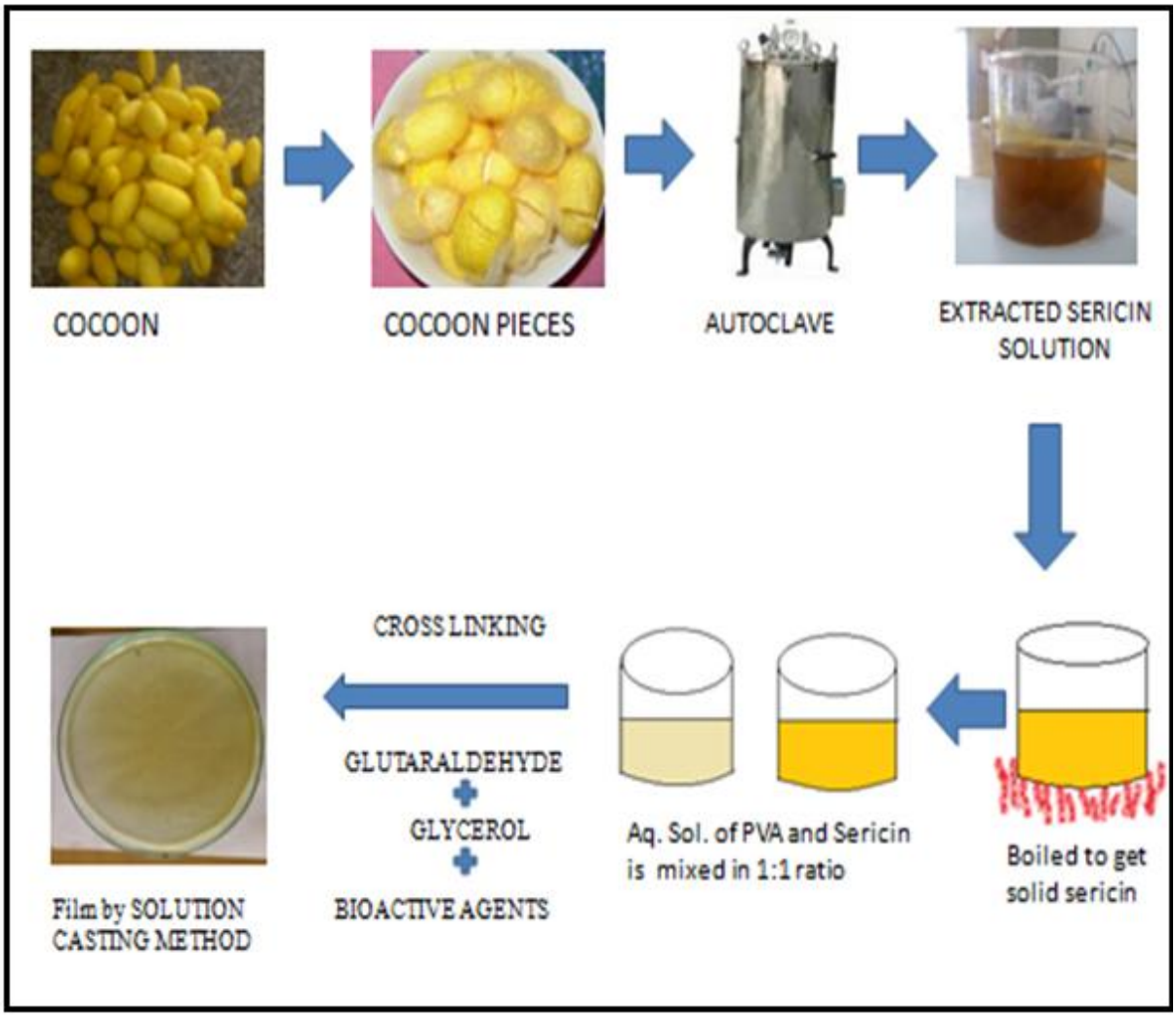

Figure 4.1 Diagrammatic representations of biodegradable Sericin/PVA blend films by solution casting method.

The films were chemically crosslinked with GA to improve their mechanical strength and chemical stability. Yeom et al. [55] studied the cross linking reaction of PVA with GA. In cross linking reaction between the hydroxyl groups of PVA and the aldehyde groups of GA hydroxyl groups are reacted and more acetal rings and ether linkages are formed. Three possible reactions are proposed and shown in fig (4.3)

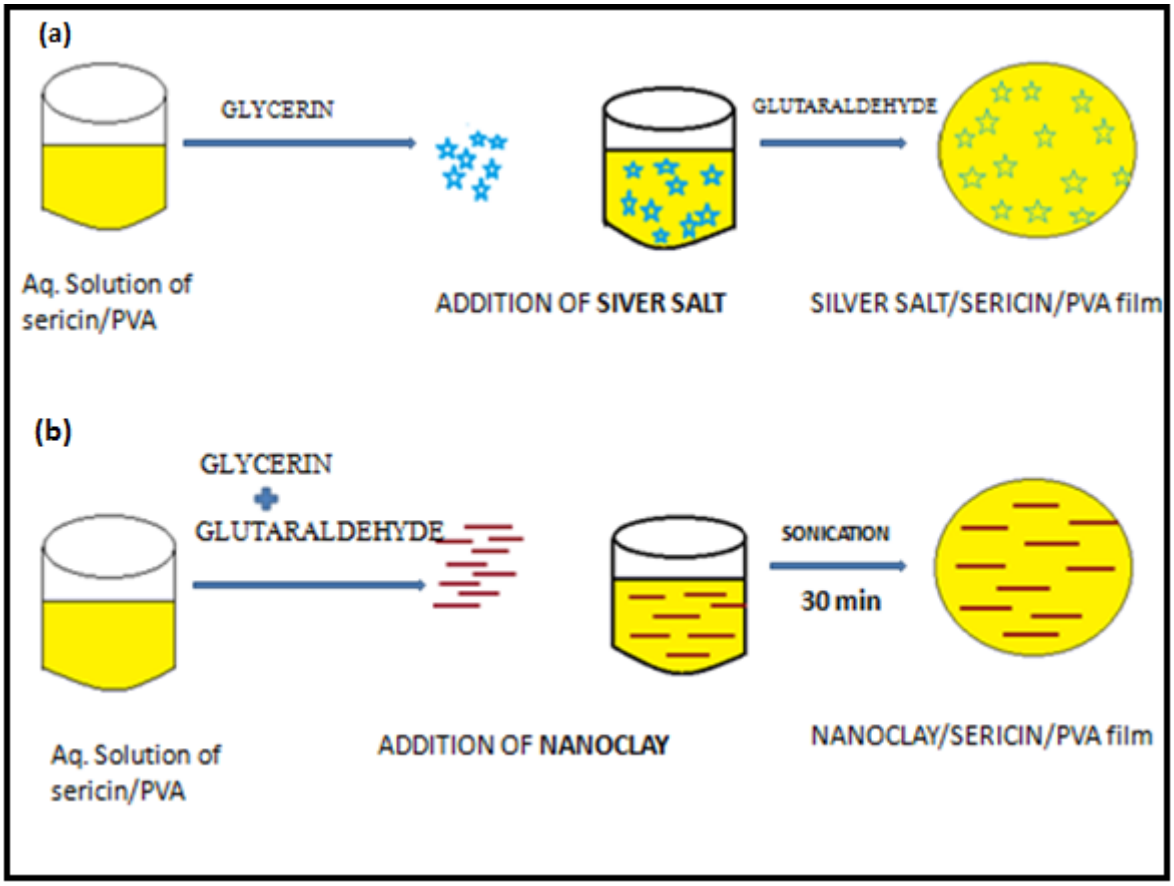

Figure 4.2 Preparation of biodegradable bioactive Sericin/PVA blend films

The cross linking reaction of sericin and GA has not been well studied yet because silk sericin has 18 amino acids, which means there may be lots of possible reactions for the cross linking. Little work can be found in the literature on the cross linking of sericin. Polypeptides have amine and carboxyl groups at the end of the chain. However, -OH from the carboxyl group at the end of the polypeptide chain can react with GA. Considering that the hydroxyl groups in PVA are readily cross linked by GA, it can be thought that the hydroxyl groups in sericin are highly likely to react with the cross linker. GA can crosslink both amino and hydroxyl groups [6, 56] thus the use of GA to crosslink sericin/PVA blend may result in an intense crosslink in the blended films to provide required tensile strength for the application in food packaging. The hypothetical reaction of PVA / sericin blend with glutaraldehyde (GA) is shown fig (4.4) below:

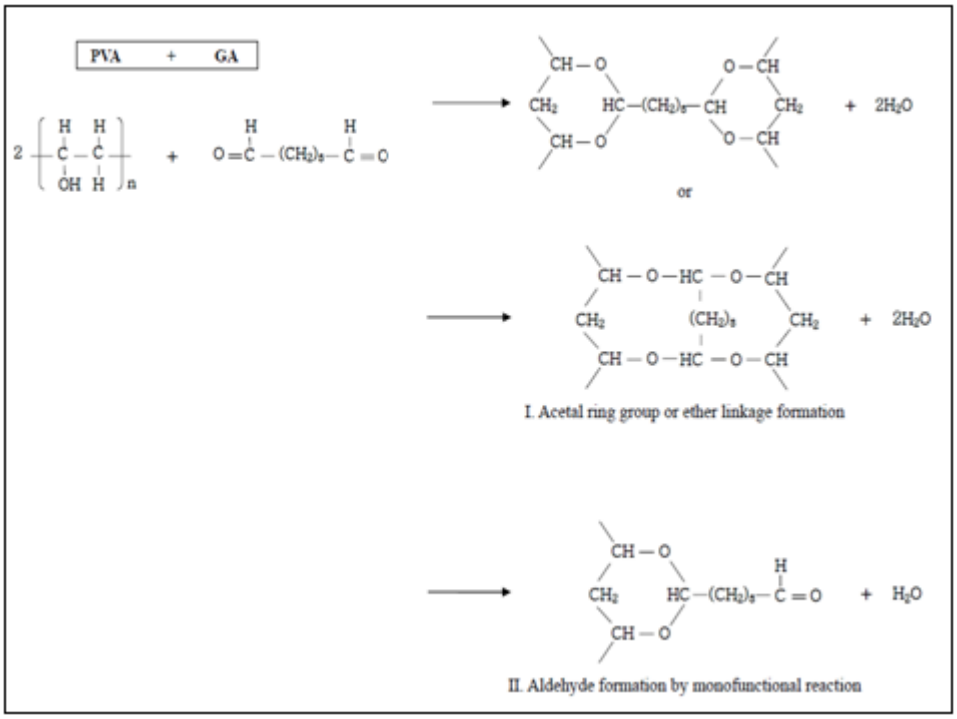

Figure 4.3 Cross linking of PVA and GA[23]

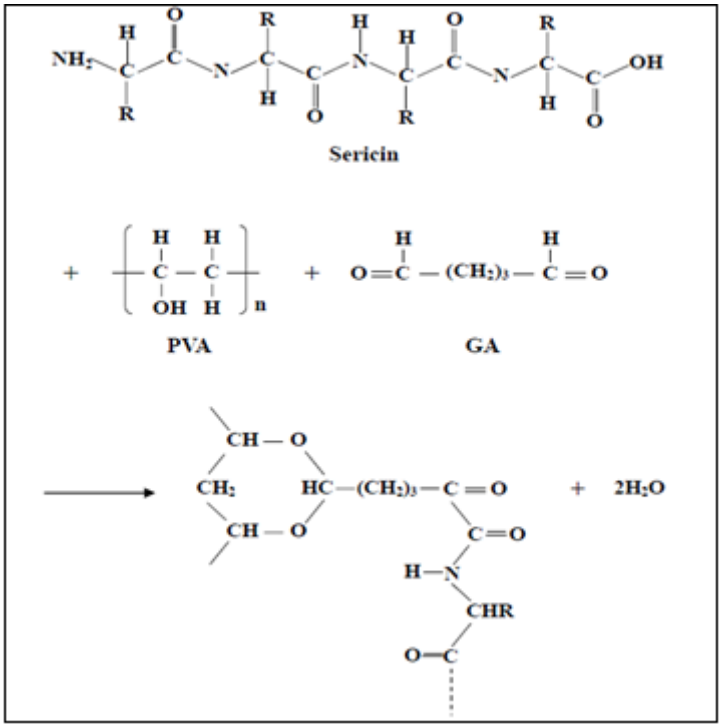

Figure 4.4 Cross linking of sericin/PVA and GA[23]

4.1 CHARACTERISATION OF EXTRACTED SERICIN

4.1.1 MOLECULAR WEIGHT

The Sericin extraction was carried by water extraction using an autoclave at 120^{o} C for 90 minutes. Figure (4.5) shows the molecular weights of the sericin as defined by SDS-PAGE. From figure it can be concluded that molecular weight of extracted Sericin more than 205 kDa.

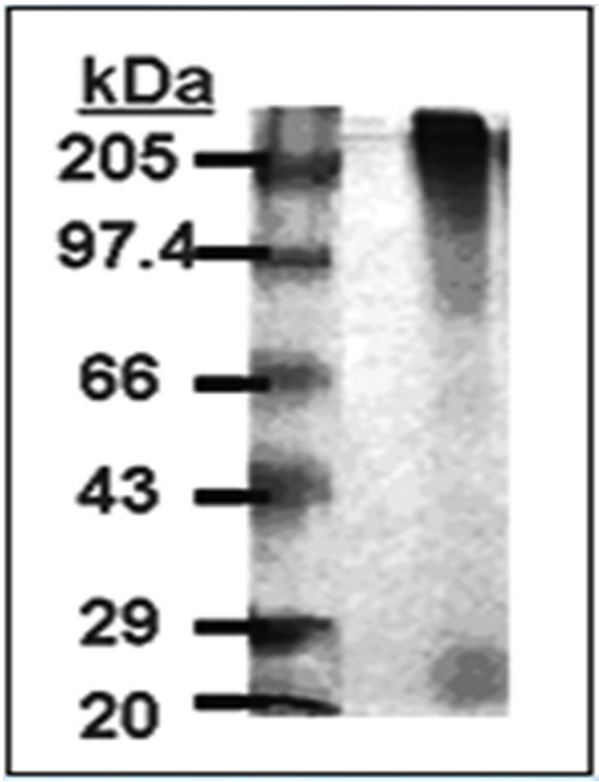

Figure 4.5 SDS – PAGE of Sericin

4.1.2 U V ABSORPTION SPECTRA

UV spectrum of the extracted sericin is presented in fig (4.6) below. The absorption spectrum shows a characteristic peak of sericin in region of 278 nm, which means the absorption peaks

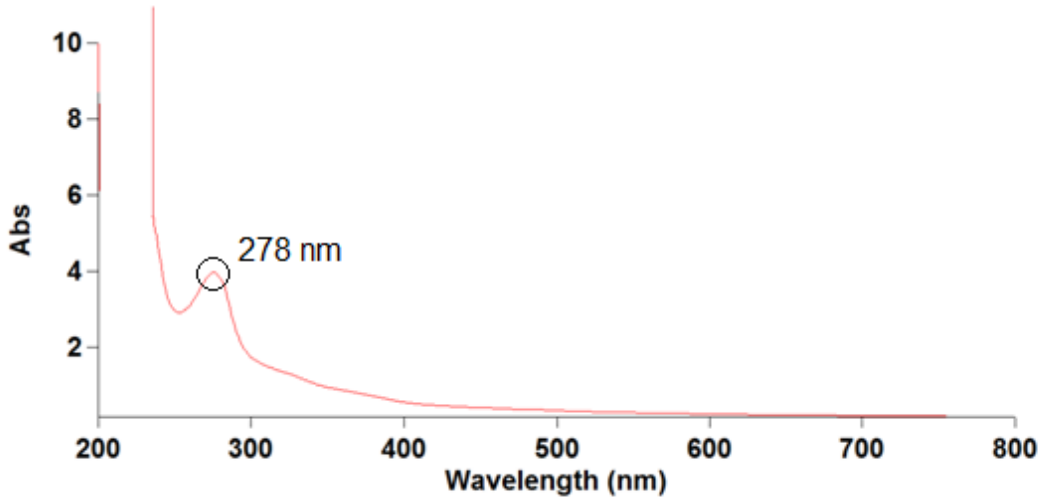

Figure 4.6 UV absorption spectrum of the extracted sericin solution

of peptides and amino acids in sericin are at around 280nm, similar results can be compared with M.L.Gulrajani et. al. [50] Peak is more apparent which confirms the property of sericin to absorb UV light, so it may be used as UV protective agent.

4.2 CHARACTERISATION OF SERICIN/PVA BLENDED FILMS

4.2.1 Mechanical Testing

4.2.1.1 Optimisation of Blended Films

With different concentrations of GA, glycerine and bioactive agents optimisation are carried out on the basis of their tensile strength. A tensile test was performed to investigate the tensile properties of the different Sericin/PVA blend films. Because packaging materials may be subjected to various kinds of stress during use, the determination of the mechanical properties involves not only scientific but also technological and practical aspects. Different

concentrations of cross linker, plasticizer and bioactive agents are added to blended films are prepared in stages. In each stage films are optimised on the basis of their mechanical strength.

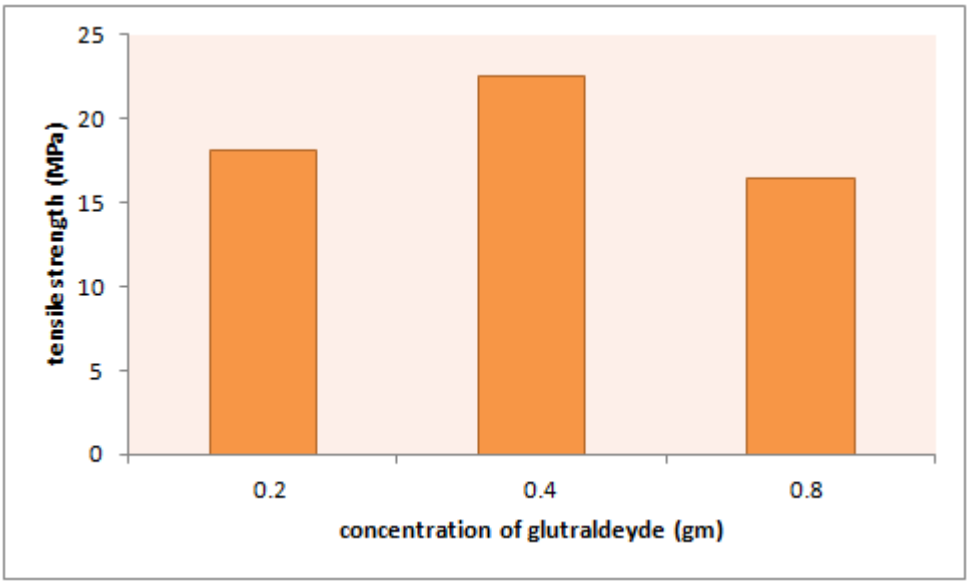

Figure 4.7 Graph showing effect of concentration of cross linking GA on tensile property

Above fig (4.7) shows the tensile strength of films when cross linker GA is taken in 0.2, 0.4, 0.8 gm/ gm Sericin. We can observe that 0.4gm of GA /gm of sericin give maximum strength to Sericin/PVA blend films.

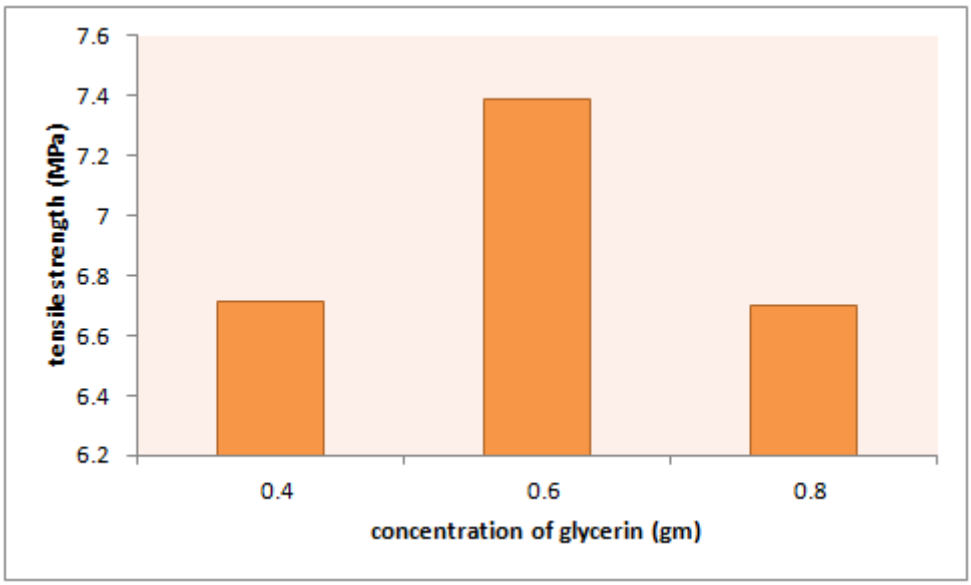

Figure 4.8 Graph showing effect of concentration of glycerine on tensile property

Glycerine is used as plasticizer in concentration of 0.4, 0.6, 0.8 gm/gm of Sericin, but its presence also affects the mechanical property of blended films. 0.6 gm of glycerine/ gm of sericin give the maximum strength to the Sericin/PVA films.

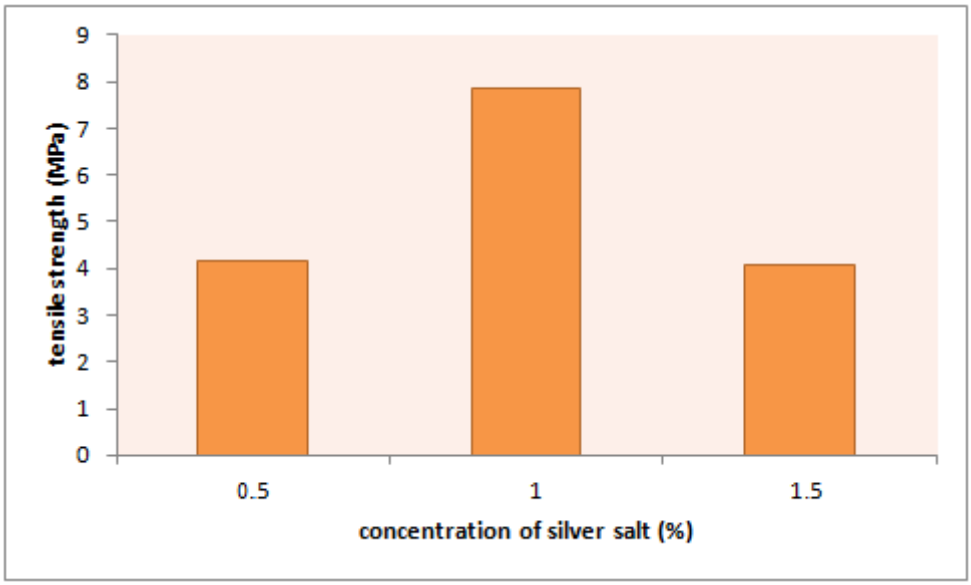

Figure 4.9 Graph showing effect of concentration of silver salt on tensile property

Bioactive agents used here are taken in 0.5, 1, 1.5 % / gm of Sericin. Silver salt gives maximum tensile strength on 1% of $AgNO_3$/gm of sericin.

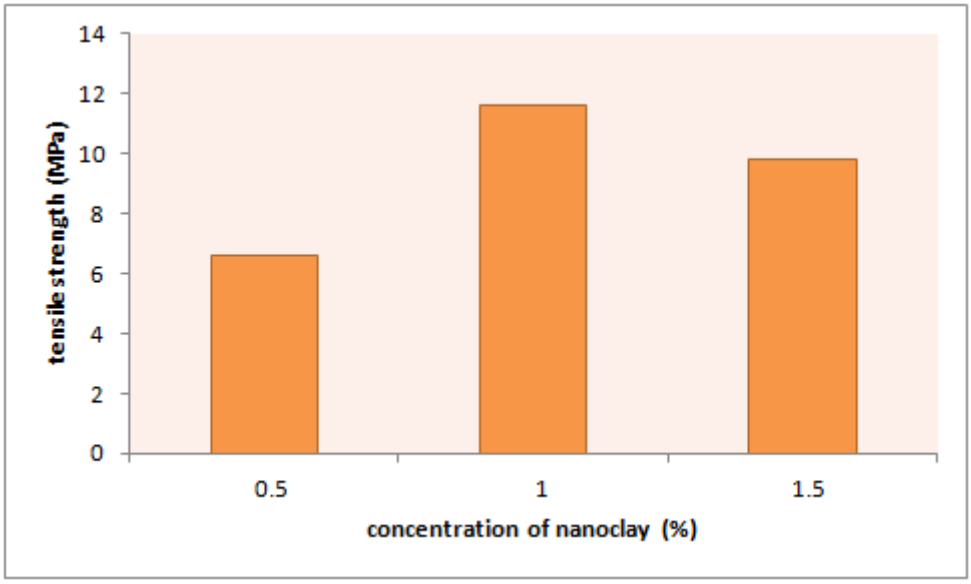

Figure 4.10 Graph showing effect of concentration of nanoclay (closite 30 B) on tensile property

Nanoclay also show maximum tensile on 1% of nanoclay/gm of Sericin. So by observing mechanical properties we can conclude that for preparation of biodegradable bioactive Sericin/PVA blends we require 0.4gm of GA/ gm of Sericin, 0.6gm of glycerine/ gm of Sericin and 1% of bioactive agents/gm of sericin to have maximum tensile strength for the application of food packaging.

4.2.1.2 TENSILE STRENGTH OF BLENDED FILMS

Polymer materials used for packaging such as films are subjected to various kinds of stress during their application as a consequence the study of the mechanical properties is of primary importance for determining the performance of the materials. Tensile strength (TS) is plotted in fig (4.11, 4.12) as a function of cross linker, plasticizer and bioactive agents. It has been observed from fig (4.11) that the TS of the film increases initially from 16.08MPa with addition of cross linker but it get reduced to a significant value from 22.08 MPa to 7.39 MPa when glycerine is added. Glycerine is used to add flexibility in film so it is a desirable property to give application to films for packaging. So to increase the mechanical strength to films nanoclay is added. Fig (4.12) shows the comparison of films tensile strength after addition of bioactive agents. Silver salt hardly affect the tensile properties of films but nanoclay has resulted in improvement of tensile strength of glycerine incorporated films.

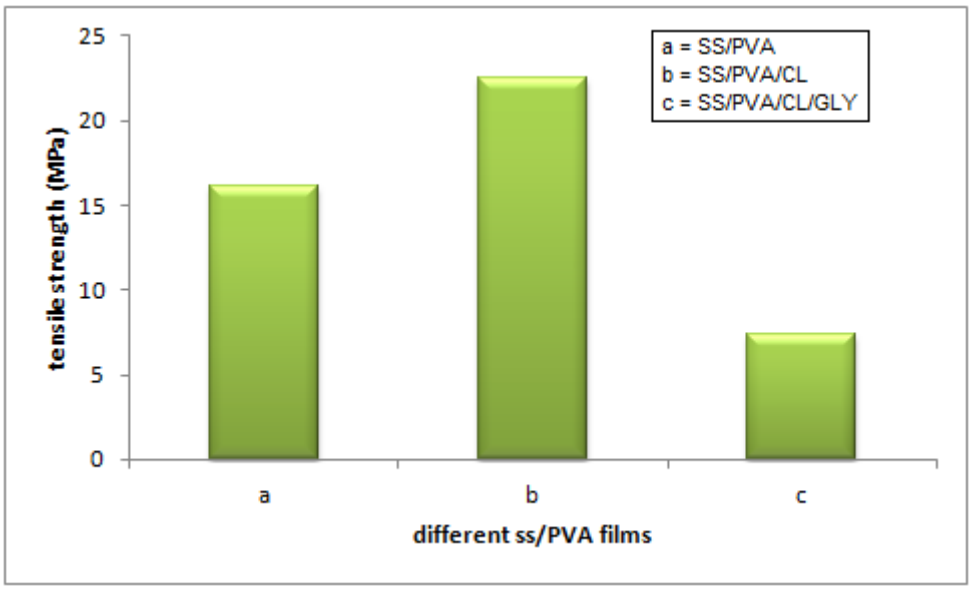

Figure 4.11 Effect of cross linker and glycerine on tensile strength of Sericin/PVA blends

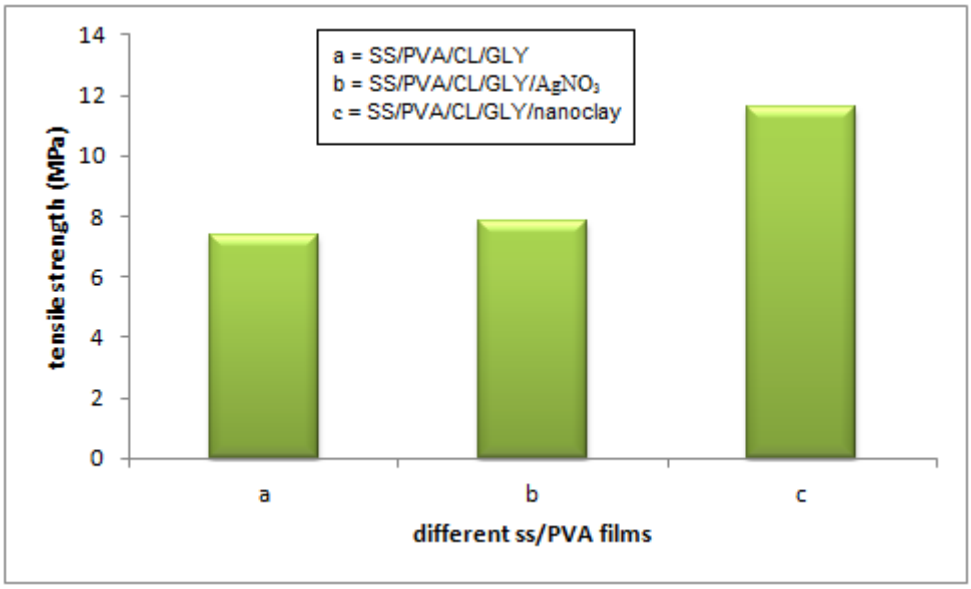

Figure 4.12 Effect of bioactive agents on tensile strength of Sericin/PVA blend

4.2.2 Structural Properties

4.2.2.1 FTIR

4.2.2.1.1 Effect of GA

The secondary structure of Sericin/PVA blended films were characterized by Fourier Transform Infrared. Fig (4.13, 4.14) shows the FTIR spectra of pure PVA, Sericin and Sericin/PVA blend to compare the effect of cross linking agent. All major peaks related to hydroxyl and acetate groups were observed. The large bands observed between 3550 and 3200 cm^{-1} are linked to the stretching O–H from the intermolecular and intramolecular hydrogen bonds. The reaction of PVA with GA results in a considerable reduction of the intensity of O–H peaks indicating the formation of acetal bridges similar work is shown by Herman S. Mansur et.al [57].

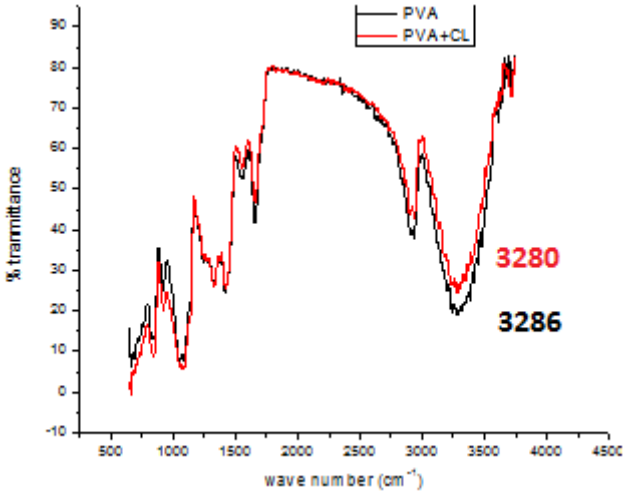

Figure 4.13 FTIR spectra of PVA and PVA cross linked GA films

Broad absorbance peak at (3550-3200 cm^{-1}) shows reduction in both the cases, 3286 cm^{-1} to 3280 cm^{-1} and 3268 cm^{-1} to 3263 cm^{-1} due to the formation of acetal bridges. But when

sericin reacts with GA, there is no reduction in this peak so it shows absence of acetal bridges between Sericin and GA films shown in fig. (4.14)

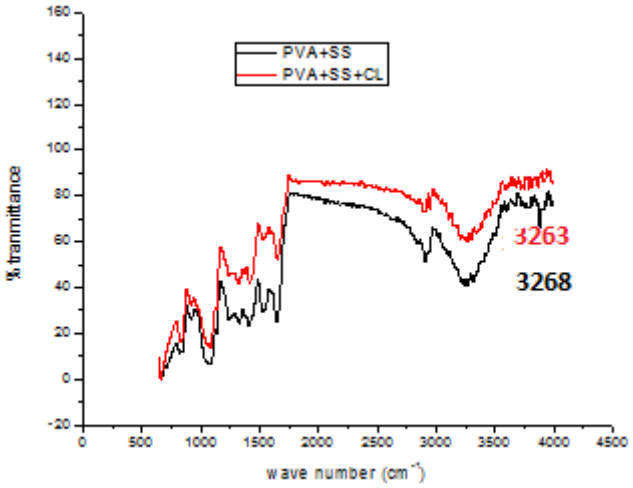

Figure 4.14 FTIR spectra of Sericin/PVA and Sericin/PVA cross linked GA films

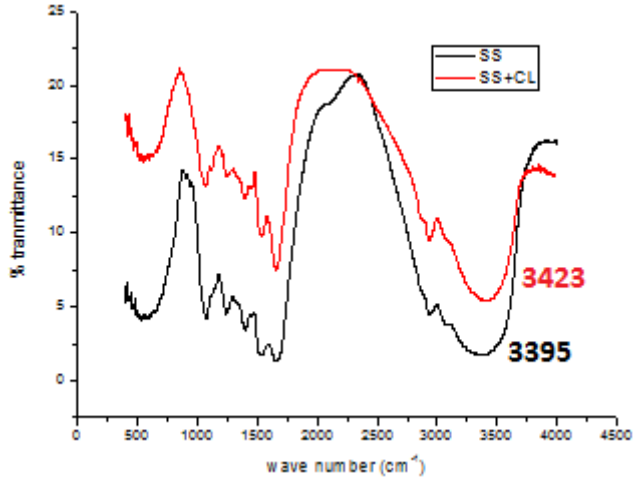

Figure 4.15 FTIR spectra of Sericin (SS) and Sericin cross linked GA films (SS+CL)

Absorption peak shows increase from 3395 cm^{-1} to 3423 cm^{-1} in fig (4.15) so we can conclude that there is formation of intermolecular and intramolecular hydrogen bonds between Sericin and GA.

4.2.2.1.2 Secondary structure analysis

There are four different types of distinguishable vibration peaks associated with protein amide—amide I (1710–1590cm^{-1}), amide II (1570–1480cm^{-1}), amide III (1270–1200cm^{-1})[29]. Amide bonds form the polypeptide backbone and due to specific vibrational frequencies result in conformational changes in protein molecules[7]. Major secondary structure can be estimated from the amide I peak position. According to Hidetoshi Teramoto absorption from α helix, β sheet and random coil is usually observed at 1655, 1630 and 1645 cm^{-1} [29]

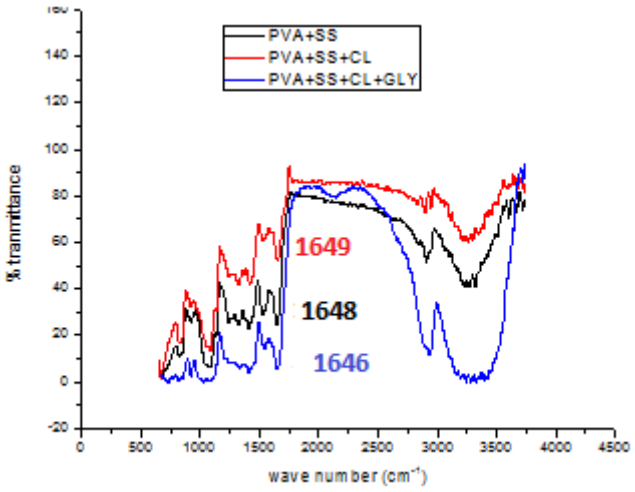

Figure 4.16 FTIR spectra of Sericin/PVA and Sericin/PVA/GA and Sericin/PVA/GA/GLY

Structure of Sericin in Sericin/PVA blended films are random coiled structure and absorption peak at 1648 , 1649 and 1646 cm^{-1} for Sericin/PVA, Sericin/PVA/GA and Sericin/PVA/GA

containing glycerine. This confirms the random coiled structure of sericin in these blended films. It can be concluded from literature that this random coiled structure is responsible for large deformation observed in cast film upon wetting because water molecules easily intrude into the loosely interacting random coil region[29].

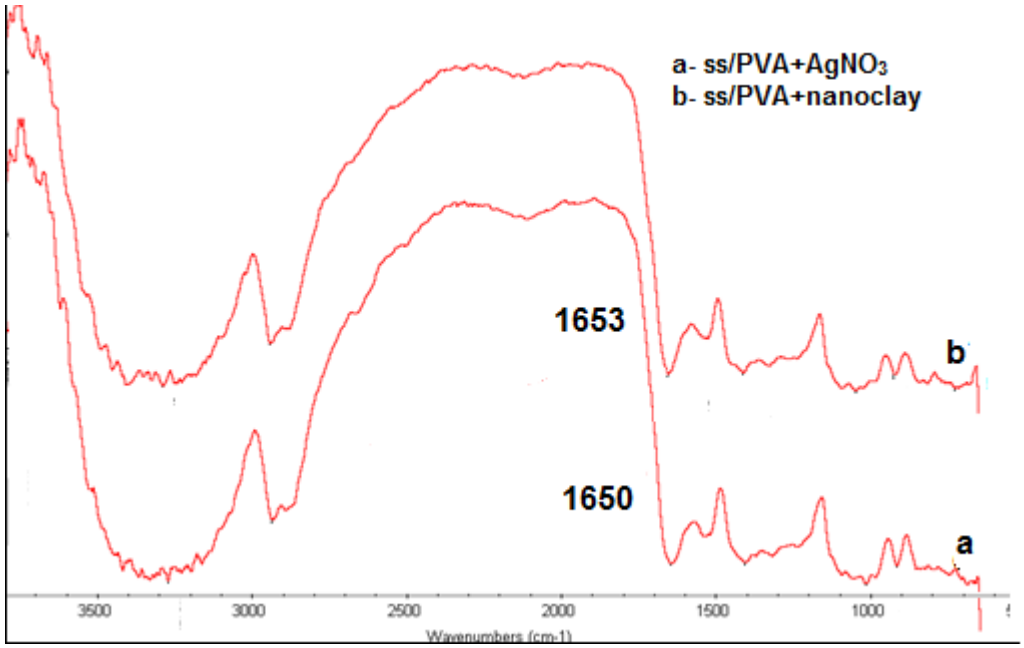

Figure 4.17 FTIR spectra of bioactive blended films (a) silver salt blended film (b) nanoclay incorporated film

But bioactive Sericin / PVA films containing silver salt and nanoclay shows absorption peak at 1650 and 1653 cm^{-1}. This confirms the transformation of random coil structure towards α helix structure of Sericin in bioactive blended films.

4.2.2.1.3 Effect of glycerine

According to the studies of Pornanong Aramwit et.al the amide II band which is 1570 - 1480 cm^{-1} responds to differences in the hydrogen bonding environment[17]. Thus, glycerine

presumably has an effect on the hydrogen bonding of the amide groups such that amide–amide interactions are reduced by an increase in amide–plasticizer interactions. According to the studies the amide II band in Sericin/PVA blend film containing glycerine has a reduced intensity compared with that of the sericin/PVA blends[17]. Here in present study also amide glycerine interaction has reduced the amide II peak i.e. 1646 cm^{-1}. When glycerine was added to the sericin/PVA as a plasticizer, the main change was the appearance of a broad peak at 3200-3500cm^{-1}, indicates presence of water in the sample. This was due to the moisture sorption properties of the glycerine plasticizer this can be concluded by studies of Pornanong Aramwit . In this study this broad peak can be seen at 3328cm^{-1}.

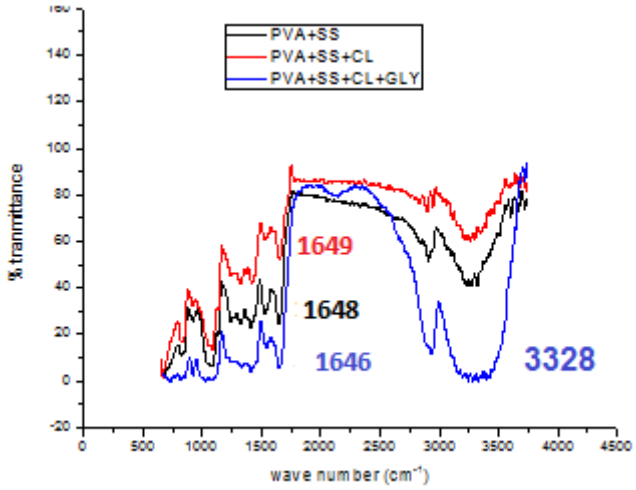

Figure 4.18 FTIR spectra of showing glycerine effect on Sericin/PVA blended films

From literature we know that 3550-3200 cm^{-1} band represent OH stretching. There is a continuous increase in the width of the peak. From fig (4.19) we can conclude that peak is getting broader which confirms that films are getting more hydrophilic in nature.

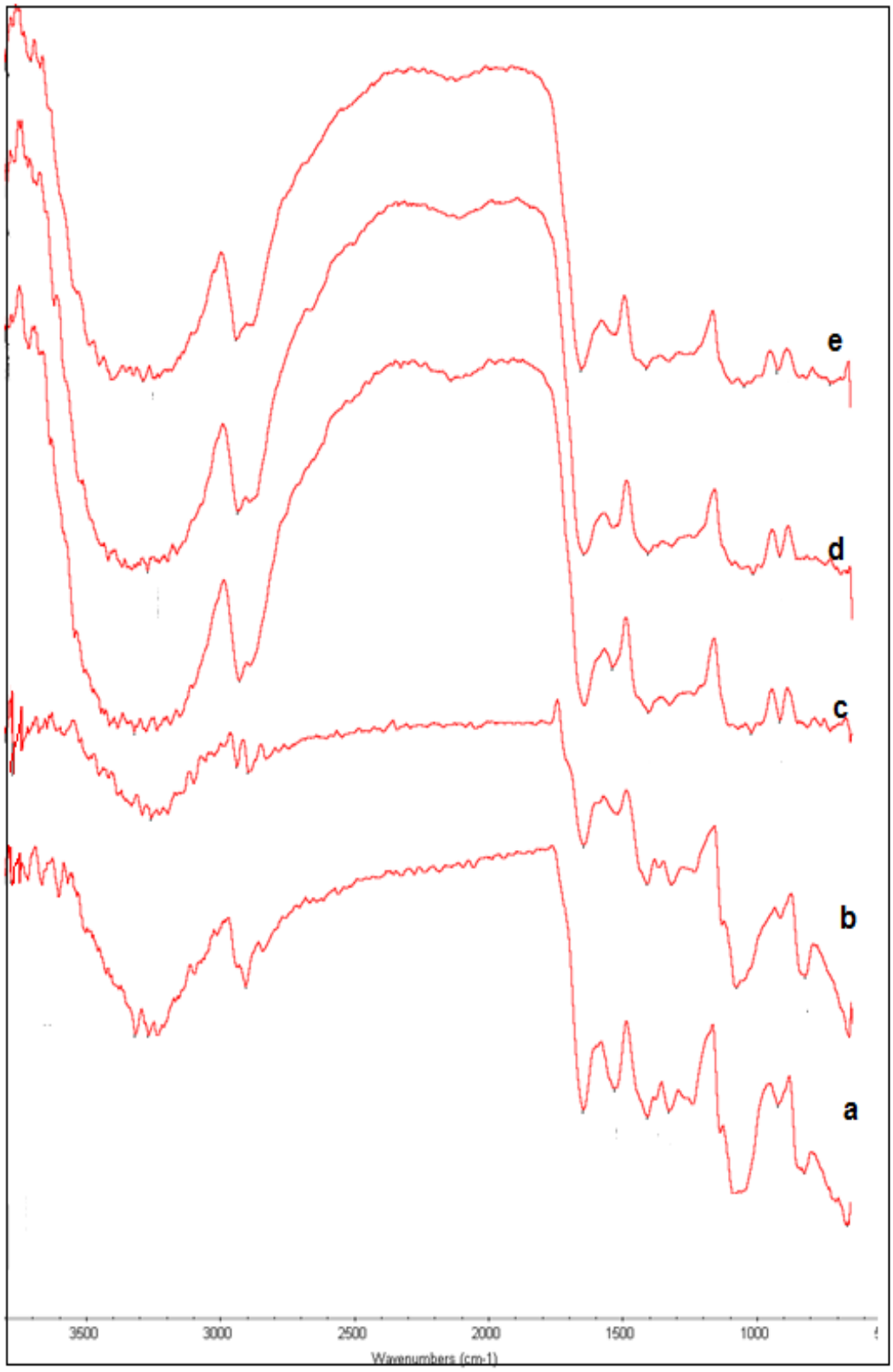

Figure 4.19 FTIR spectra of showing (a) Sericin/PVA (b) Sericin/PVA/CL (c) Sericin/PVA/CL/gly (d) Sericin/PVA/CL/gly/$AgNO_3$ (e) Sericin/PVA/CL/gly/nanoclay

4.2.2.2 X ray Diffraction (XRD)

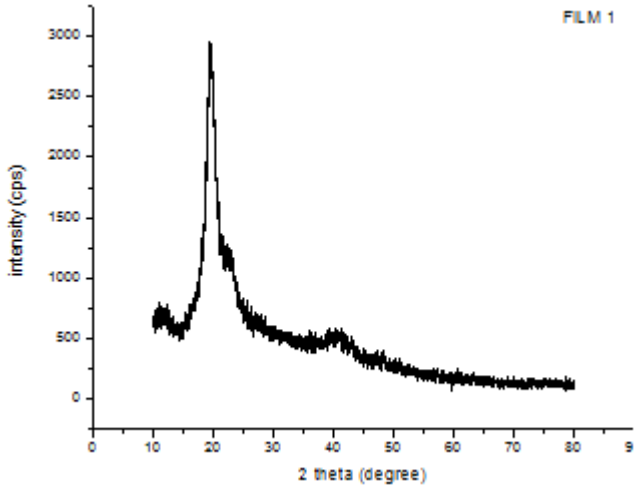

Figure 4.20 X-ray diffraction pattern of Sericin/PVA film

Structural transformations in sericin protein were analyzed using X-ray diffraction. According to Miyake et. al. high molecular weight sericin show major peak at 2Θ = 20^{o} C and shoulder peaks at 2Θ = 12^{o} C , 28^{o}C and 43^{o} C[16]. Here, Sericin/PVA film shows the same peaks of high molecular weight Sericin, major peaks at 2Θ = 20^{o} C and shoulder peaks 12^{o} C , 23^{o}C and 42^{o} C. So it confirms that in Sericin/PVA film sericin shows the original structure. According to literature pure PVA shows an intense reflection peak at 2θ = 19.7˚ [58]. Dash et al., Tsukada et al, Teramoto et al. proposed native sericin contains both random coils and β-sheets representing amorphous and crystalline regions respectively[7]. In current work Sericin/PVA blend shows major peak at 20^{o} C of high intensity which corresponds to crystallinity of both β-sheets of Sericin protein and PVA. Hence this sericin/PVA film is highly crystalline in structure.

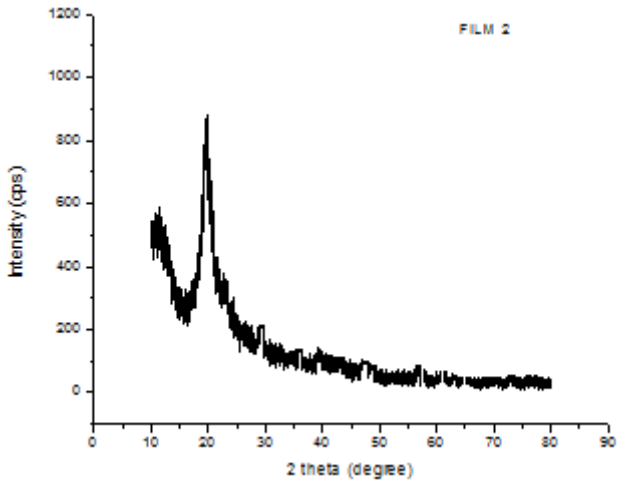

Figure 4.21 X-ray diffraction pattern of cross linked Sericin/PVA film

Earlier studies suggested native sericin containing both random coils and β-sheets representing amorphous and crystalline regions respectively and upon cross linking caused a transformation of β-sheet and aggregated β-sheet structure to random coil due to intermolecular hydrogen bonding. This resulted in a decrease in the amount of crystallinity of X ray diffraction curve[16]. Hence, cross linking with GA, sericin/PVA films showed a sharp peak but decrease in peak intensity at $2\Theta = 20^{o}$ and disappearance of shoulder peaks at $23^{o}C$ and 42^{o} C. This confirms the presence of both amorphous and crystalline structure and makes crosslinked Sericin/PVA film a semi crystalline in nature.

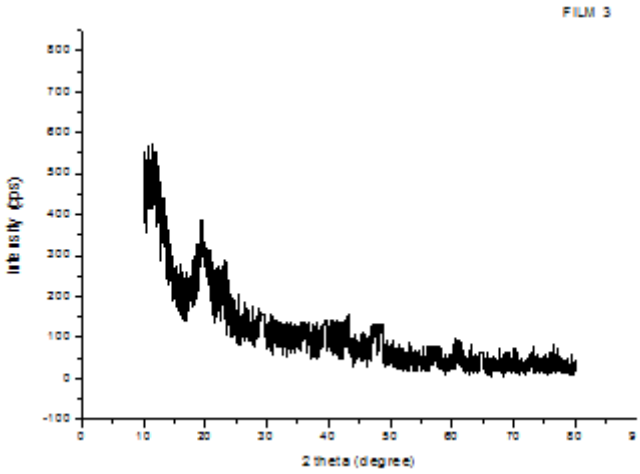

Figure 4.22 X-ray diffraction pattern of cross linked glycerine containing Sericin/PVA film

In glycerine containing film diffraction intensity of peak 2Θ = 20º has reduced to half as compared to cross linked Sericin/PVA film. Broad peak depicts the amorphous nature of the glycerine containing film and confirms the random coil structure of sericin.

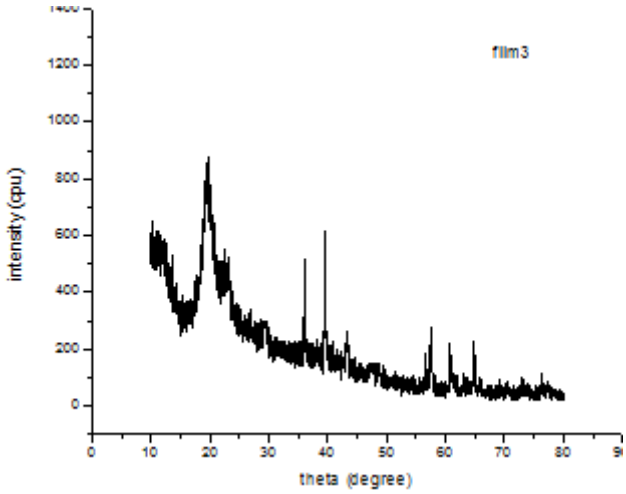

Figure 4.23 X-ray diffraction pattern of bioactive silver salt containing Sericin/PVA film.

Diffraction peak intensity of silver salt containing film has increased as compared to glycerine containing film and reaches comparable to cross linked film. Sharp peak confirms the crystalline nature of film. According to Kanikireddy Vimala et. al. small shoulder peaks at 38^{o}C is characteristic of face centered cubic (FCC) of the silver nanoparticles or it is identical with the Ag (111) excitation[27]. From the studies of F. Heidarpour peaks at 2 Θ of 38.2º, 44.3º and 64.5º, 77.6º, and 83.3º can be attributed to the (111), (200) and (220), (311), and (331) crystallographic planes of face-centered cubic (FCC) silver crystals. XRD pattern for silver salt containing Sericin/PVA shows the presence metallic silver can be concluded.

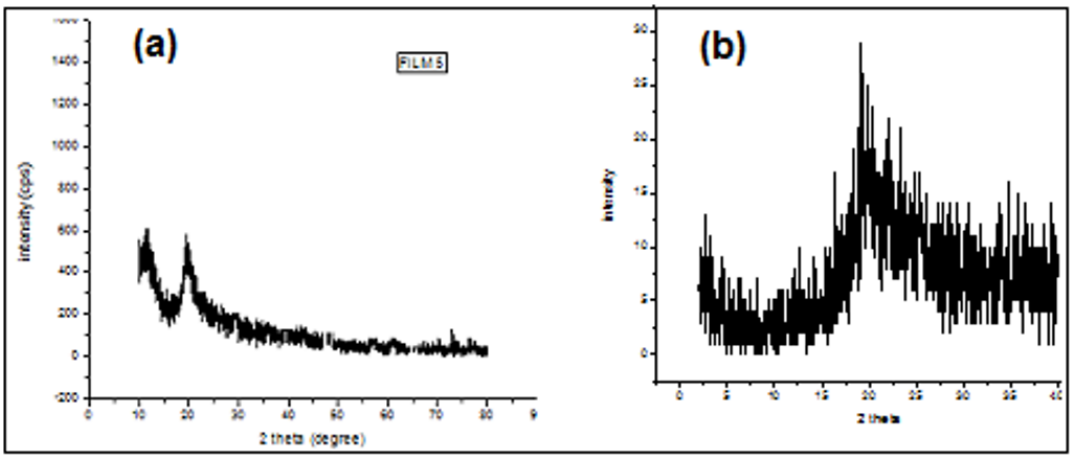

Figure 4.24 X-ray diffraction pattern of bioactive nanoclay (closite 30B) containing Sericin/PVA film

The XRD spectrum (Fig. 4.24 b) shows the peaks typical of nanoclays in the range 2–10 Θ, major peak at 2.5 Θ. The presence of Cloisite 30B contributes to intercalation by raising the gallery spacing in blend nanocomposite samples implying the facilitated insertion of polymer chains within the interlayer space in virtue of polymer-nanoclay affinity. Diffraction pattern shows that nanoclay incorporated bioactive Sericin/PVA film is semi crystalline in nature and more amorphous than silver salt containing film.

By studying above all diffraction pattern shows that pure sericin/PVA film is more crystalline in nature than rest of all the films. Glycerine containing film contains maximum amorphous regions.

4.2.3 Thermal Properties

4.2.3.1 TGA

Tudorachi *et. al.*, (2000) [17] in their study on PVA the first stage of presents multi stage degradation, firstly the loss of bound water (moisture vaporization) while second stage exhibits the decomposition of side chain of PVA. The third stage shows the decomposition of main chain of PVA.

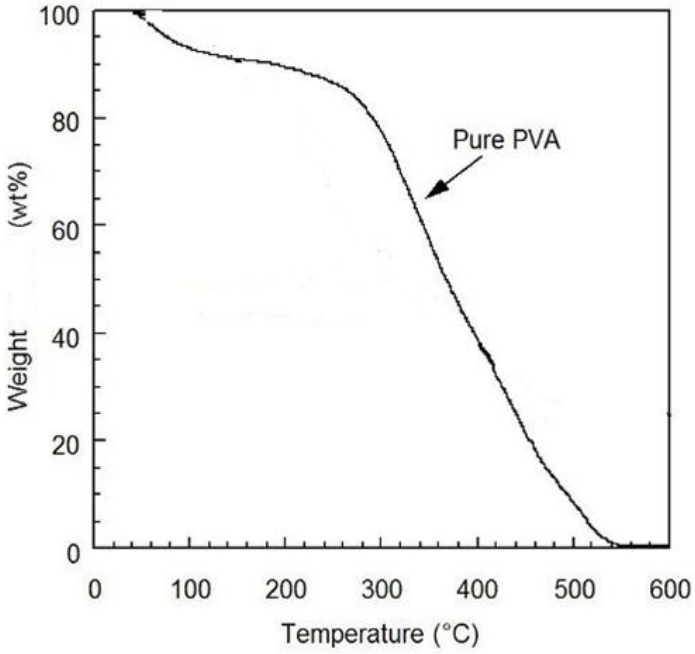

Figure 4.25 TGA curves for the PVA[59]

Thermograms of PVA/Sericin blends show 3 stages of degradation. Mudigoudra B.S. et.al shows the same work which showed thermal behaviour of Poly (vinyl alcohol)/ Poly (vinyl pyrrolidone)/Chitosan Ternary Polymer Blend films which helped in concluding from the figure (4.25) that the initial weight loss for samples starts around 62^{o}C and shows weight loss about 10% initially. This may corresponds to the loss of bound water from the blend. The second weight loss starts around 180^{o} C and continues up to 360^{o}C which is a region of melting temperature, Tm = 310^{o} C, during which there was 45% weight loss. The third weight loss was observed in the range from 370-410^{o}C which may be correspondent to the structural

decomposition of the blend during which there was 15% weight loss. This clearly shows that there is very less influence of sericin on thermal stability of PVA[60].

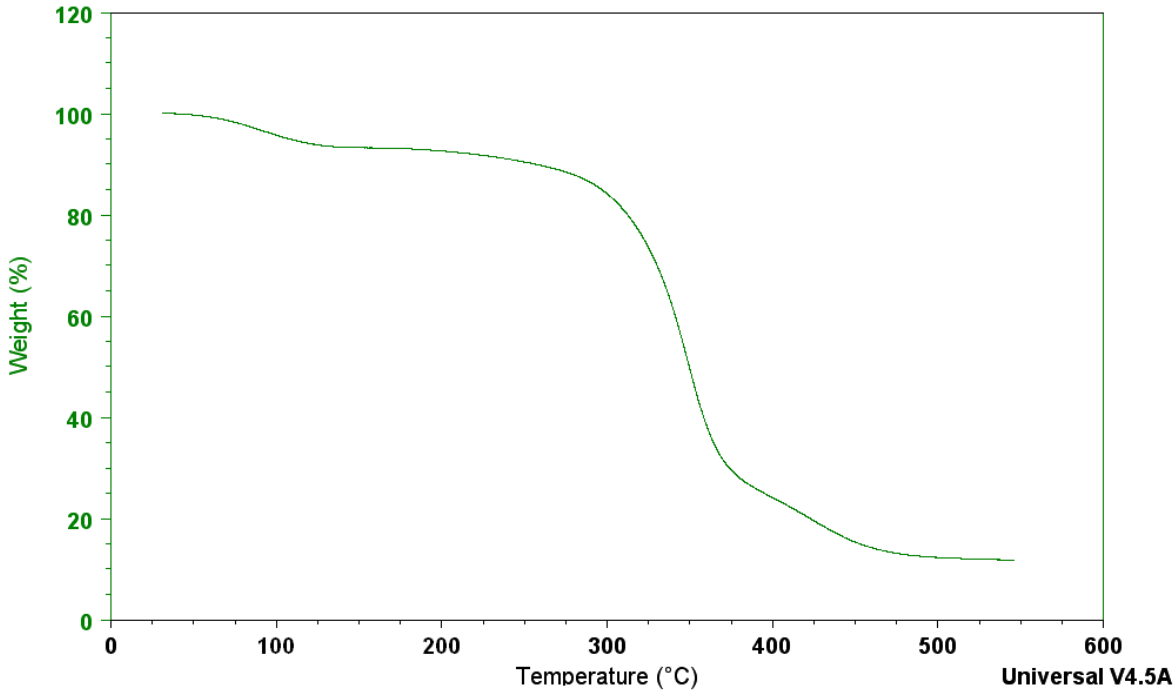

Figure 4.26 TGA curves for the Sericin/PVA blend film.

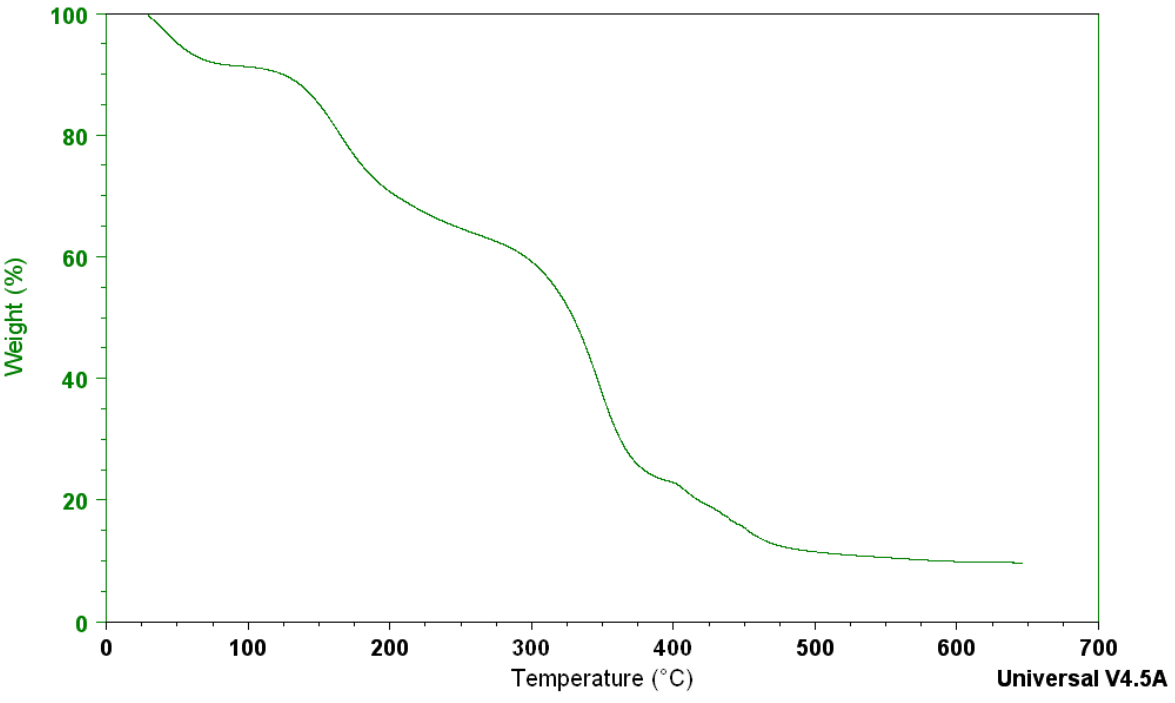

Figure 4.27 Thermogram for Sericin/PVA/glycerine cross linked film

Thermogram fig (4.27) indicates that sericin/PVA films with glycerol decomposed more quickly. Glycerol can accelerate the decomposition of sericin films which is also concluded by Haiping Zhang et al[61].

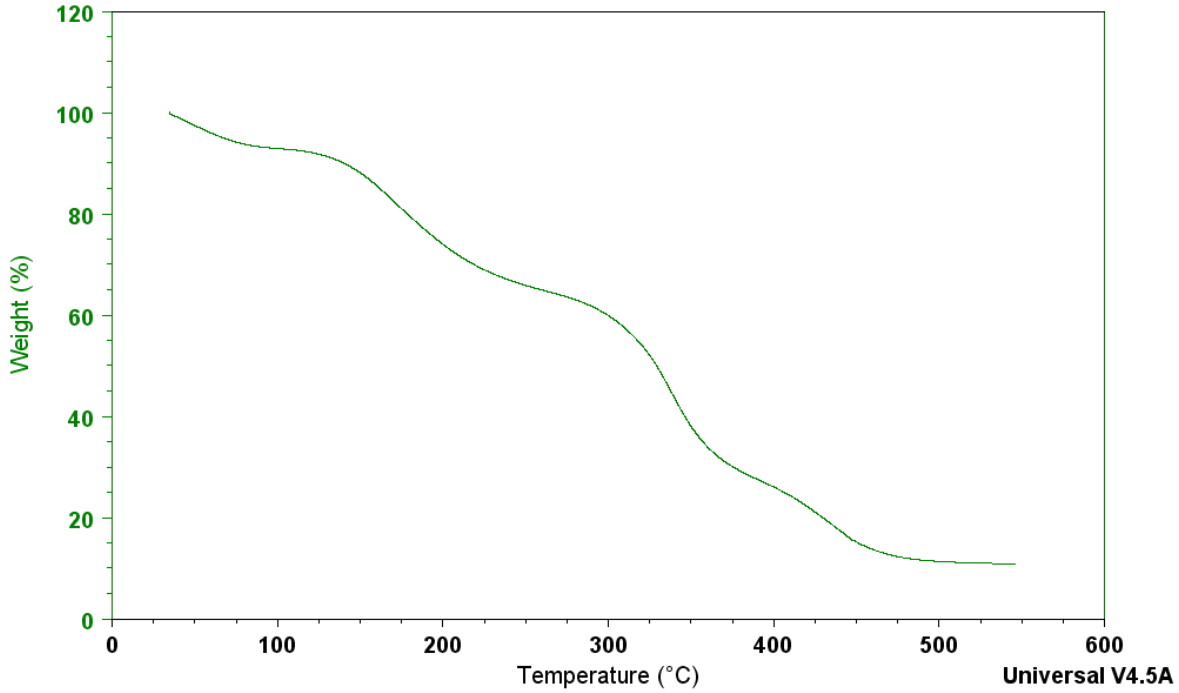

Figure 4.28 Thermogram for nanoclay incorporated Sericin/PVA/glycerine cross linked film.

The addition of clay improves the thermal degradation of Sericin/PVA film. The residue obtains in Sericin/PVA/ nanoclay film is 15% as compared to the residue obtained in Sericin/PVA film was 10%. We can conclude that pure PVA has a higher thermal stability than that of different blends of plasticized PVA/Sericin films. The thermal decomposition of plasticized and bioactive Sericin/PVA blends shifted slightly toward lower temperature compared to PVA or Sericin/PVA blends. This can be concluded that Sericin/PVA blends with glycerol suppress the thermal stability of PVA this similar work is carried by nadras othman et al. [62] , there is a molecular interaction between PVA and glycerol. But incorporation of nanoclay has improved thermal degradation.

4.2.3.2 DMA

The given fig (4.29) shows the effect of cross linker GA on storage modulus of pure PVA or Sericin/PVA film. The storage modulus measures the stiffness of the polymer.

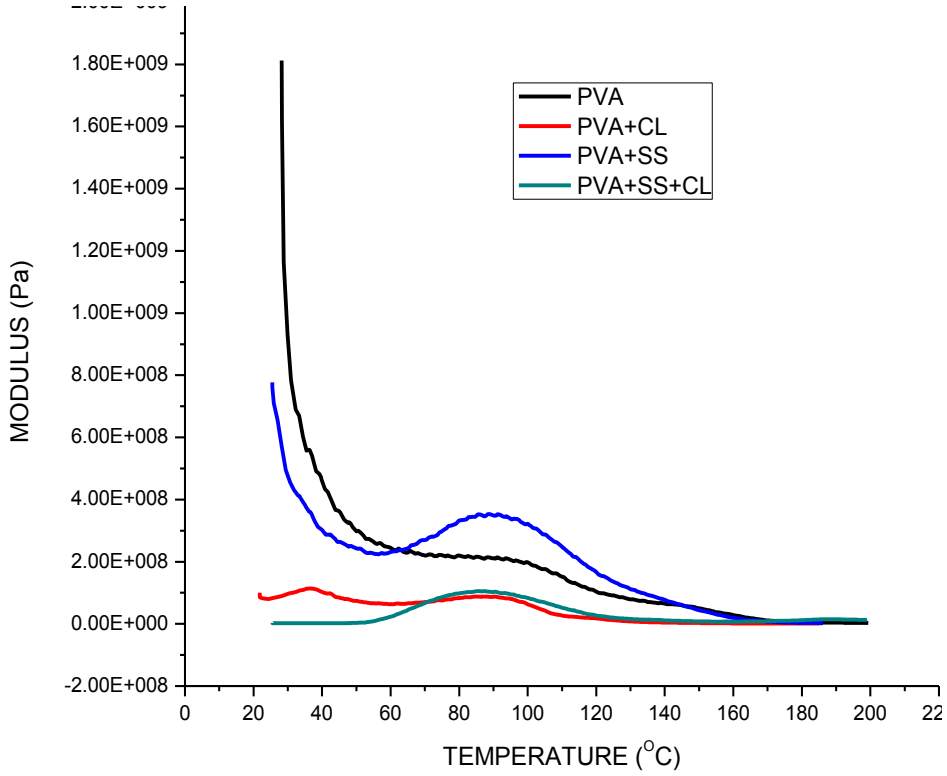

Figure 4.29 Storage modulus of PVA and different blends.

A sharp decrease in storage modulus was observed for the pure PVA or Sericin/PVA when cross linked with glutraldehyde in the glass transition region, which later reached a plateau. Storage modulus of Sericin/PVA film has increased after blending with sericin as compared to pure PVA film which indicates an increase in the rigidity of PVA chains in Sericin/PVA blend, this kind of similar work was carried by vijaya et. al[63].

Figure (4.30) shows that there is no dual peak in the region of Tg which shows that there is no phase separation in the blended films. Films are homogenous and compatible in nature.

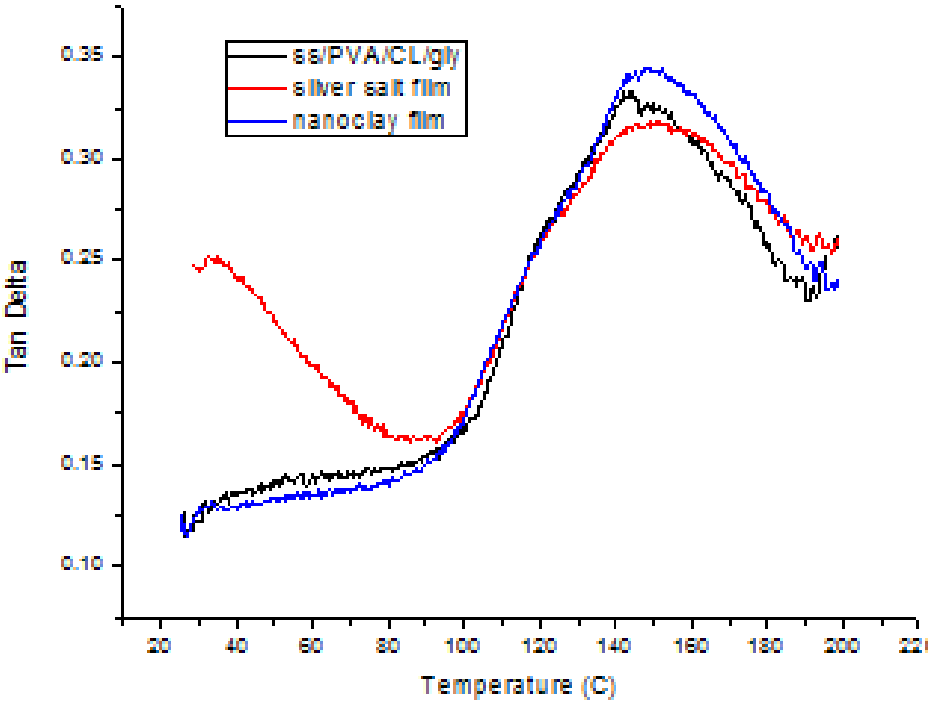

Figure 4.30 Tan δ graph of different blends

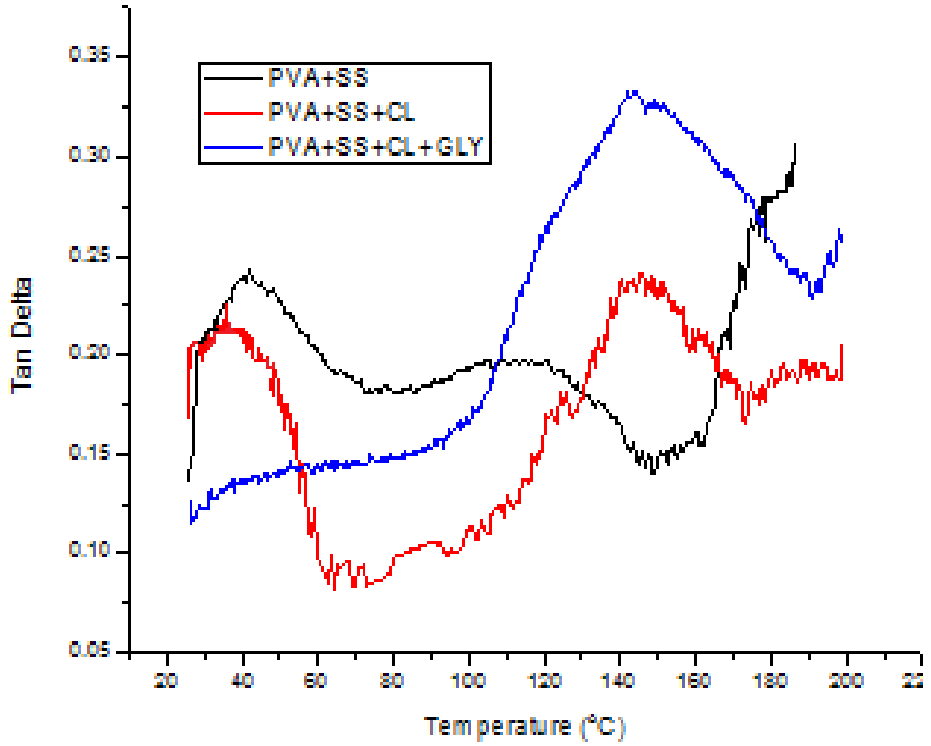

Figure 4.31 Tan δ curve for different blends

After adding glycerine is used as plasticizer Tg decreased due to the increase in slippage between the chains. The reduction in Tg was attributed to the reduction in the cohesive forces of attraction between the polymer chains. The plasticizer molecules penetrated the polymer matrix because they were smaller than the polymer molecule. As a result, polar attractive forces were established between the plasticizer and chain segments, which were responsible for the reduction of the cohesive forces and, therefore, a reduction of Tg. The size of the tan δ peak is believed to relate to the volume fraction of the material undergoing the transition. The peak size of tanδ increased larger after adding glycerine which meant the plasticization is taking place in Sericin/PVA films fig (4.30).

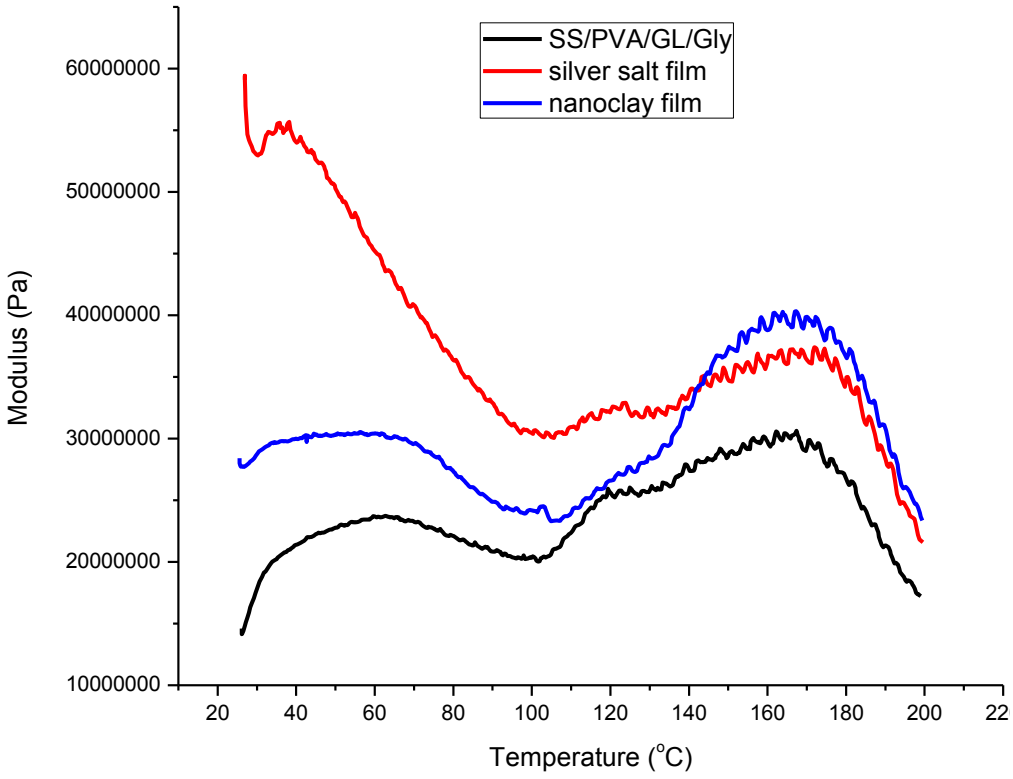

Figure 4.32 Storage modulus of bioactive blended films.

In case of nanoclay with effect of temperature modulus is rising with temperature and in silver salt film modulus is highest in Tg region but it is getting reduced with temperature increase as compared to glycerine incorporated film. The storage modulus curve has

increased value of storage modulus in temperature range of 160^{o} C from 3×10^{7} for Sericin/PVA containing glycerine film to 3.8×10^{7} (Pa) and 4×10^{7} for silver salt or nanoclay blended films respectively. The increase of storage modulus indicates a good stability of the blends owing to the compatibility of the components. This increase in storage modulus results with temperature increase shows the stiffness of nanoclay film.

4.2.4 Morphological Testing

4.2.4.1 Scanning electron microscopic (SEM)

The morphology change of sericin films after the addition of GA, glycerol, $AgNO_3$ and nanoclay can be interpreted by SEM images.

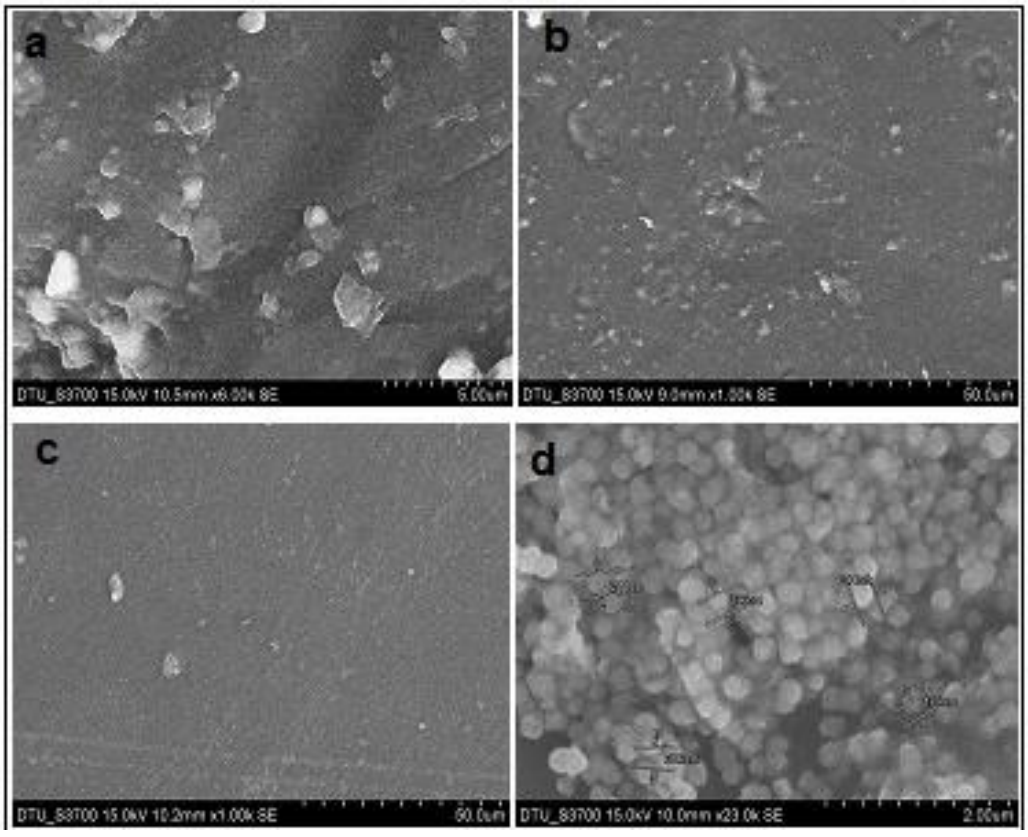

Figure 4.33 SEM images blends (a) Sericin/PVA (b) Sericin/PVA/GA (c) Sericin/PVA/GA/gly (d) Sericin/PVA/GA/gly/$AgNO_3$

Sericin/PVA blend shows cracks in its structure which is reduced by incorporation of GA and glycerine. Both are responsible for its structure integrity and dense morphology. Sericin/PVA blends film shows evidence of more roughness which might be due to the phase separation of sericin and PVA also. Addition of glycerine shows more smoothness in surface of films. Granular structure of $AgNO_3$ represents the formation of silver in the form of particles which is also confirmed by XRD. SEM confirms the uniform presence of silver particles on the surface of bioactive film. Particle size of silver granules observed through SEM is 189-244 nm.

4.2.5 Light transmittance and film transparency

Light transmittance (%T) in UV Visible range and transparency value of Sericin/PVA blended films is evaluated. PVA film showed the decreased %T at UV range 200 nm to 280nm[25]. Sericin shows a peak absorbance at around 280 nm of wavelength due to the presence of phenyl groups of tyrosine, phenylalanine and tryptophan residues contain conjugated double bonds, the protein films exhibited great barrier properties to UV light in the region from 200 to 300 nm [10] that absorb UV light so Sericin/PVA blends show excellent barrier property for UV light as compared to the PVA film. The value of the transparency of the films is shown in fig (4.34)

Greater value results in lower transparency of the film. Silver salt incorporated blended film shows lowest transparency. And nanoclay blended film is most transparent. Incorporation of silver salt makes film brown in colour as compared to other blended films which are yellow and transparent in nature. Addition of glycerine and GA makes film more transparent as compared to Sericin/PVA film as it is responsible for homogenising the Sericin with PVA

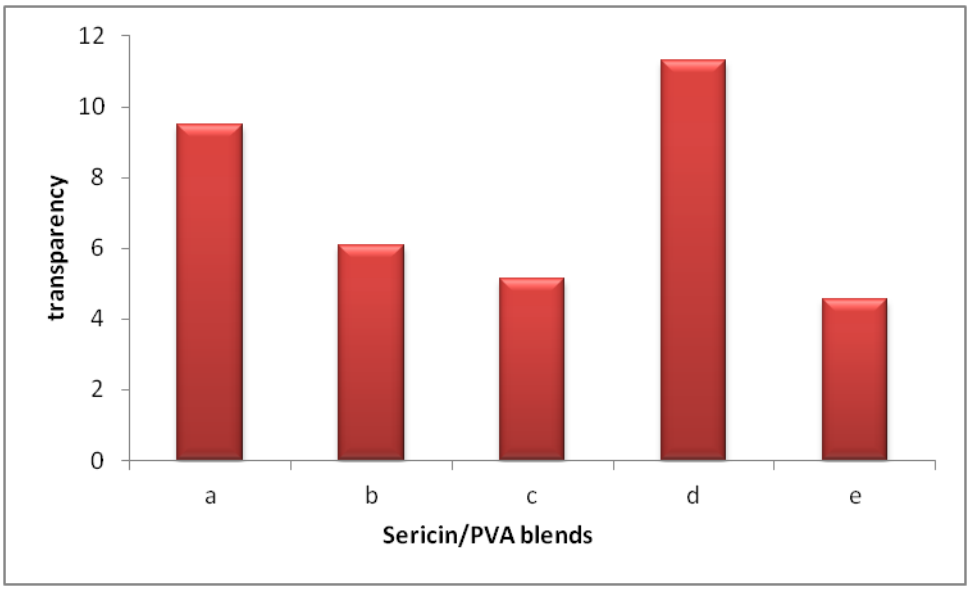

Figure 4.34 Variation of light transparency of (a) Sericn/PVA (b) Sericin/PVA/GA (c) Sericin/PVA/GA/ gly (d) Sericin/PVA/GA/ gly/ $AgNO_3$ (e) Sericin/PVA/GA/gly/nanoclay

and for their structural integrity. Hence glycerine and nanoclay has maximum contribution in transparency of films above others.

4.2.6 Antimicrobial Testing

The antimicrobial of the developed silver salt incorporated films are tested by disc diffusion method.

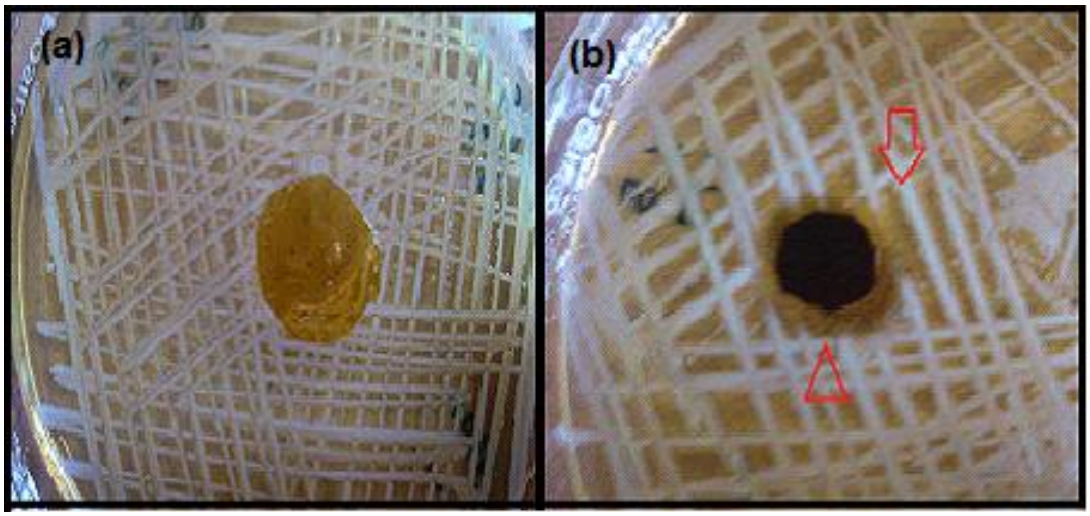

Figure 4.35 Anti-microbial activity of (a) Sericin/PVA blended film (b) silver salt incorporated Sericin/PVA film against gram negative bacteria.

Figure (4.35) exhibits the typical antimicrobial test results of films by the disc method. It is found that the silver salt incorporated films have exhibited an inhibition zone (red colour arrows) whereas Sericin/PVA film does not involve in the inhibition zone process. So silver salt films are very much anti microbial in nature.

CHAPTER 5

CONCLUSION AND FUTURE SCOPE

5.1 Conclusion:

Sericin was extracted from silkworm cocoons and characterised. Blended films of Sericin / PVA with or without bioactive agents were prepared and tested for their food packaging application. The following conclusions can be drawn from the studies:

Silk sericin can be obtained by extraction with using autoclave. This is the simplest way to extract sericin from silkworm cocoons without using any chemicals.

UV absorption spectra show the characteristic peak around 278nm so Sericin may be used in the form of blended films for packaging of food which requires UV resistance.

By studying the mechanical properties of films with different concentrations of GA , glycerine and bioactive agents, blended films with 0.4gm/ gm of Sericin , 0.6 gm glycerine/gm of Sericin and 1% of bioactive agents /gm of Sericin gives maximum tensile strength to Sericin/PVA films.

Nanoclay has resulted in increase of tensile strength of glycerine incorporated films which gives flexibility to films but reduces tensile strength of films.

FTIR confirms the formation of acetal bridges between PVA and GA and formation of intermolecular or intramolecular hydrogen bonds between Sericin and GA which confirms the structural integrity of blended films with incorporation of crosslinker GA. Broadening of band in the range of 3550-3200 cm^{-1} confirms the hydrophilic nature of films.

Diffraction patterns of XRD confirm the amorphous nature of films with addition of glycerine. Glycerine is incorporated as plasticizer which gives flexibility to films.

Study of thermogram has concluded that addition of clay improve the thermal dergradation of Sericin/PVA film as residue obtained is more as compared to the residue obtained in Sericin/PVA/ film.

DMA shows that Sericin/PVA blend films are completely miscible in nature. Addition of nanoclay enhances modulus of film at higher temperature.

SEM images show that addition of GA has resulted in Sericin/PVA films without phase separation and glycerine has increased the smoothness of films. Both are responsible for dense morphology. Granular like appearance confirms the uniform presence of silver salt on the surface of Sericin/PVA films.

Superior antimicrobial properties are shown by silver salt incorporated films as compared to Sericin/PVA blended films, which make these films for application against microbes in field of food packaging.

5.2 Future scope

Sericin exhibit strong ability to form stable cross link network with other macromolecular materials owing to its polar side groups. Blended sericin films can be produced along with whey protein, glucomannan, gellatin, polysulfone (PSF) , chitosan , starch , carboxymethyl cellulose, xanthan , dextrin etc with cross linking agents like glutraldehyde (GA), dimethylolurea (DMU), genipin, citric acid to improve the thermal, chemical and mechanical stabilities of the blends as per the application. These films can be made bioactive with incorporating bioactive agents like montmorillonite clay, silver nanoparticles in sericin matrix which has not been exploited. These sericin blended films can be used in the area of biomedical for wound dressing, scaffold.

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