"BIODEGRADABLE POLYMERIC FILMS FROM GELATIN AND SILK SERICIN: DEVELOPMENT AND CHARACTERIZATION FOR PACKAGING APPLICATIONS"

A Major Project Dissertation

Submitted in partial fulfillment of the requirement for degree of

Master of Technology *in* **Polymer Technology**

Submitted by

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DECLARATION

This is to certify that the work presented in this thesis entitled "**Biodegradable polymeric films from gelatin and silk sericin: Development and characterization for packaging applications"** is original and has been carried out by me for the degree of **Master of Technology** under the supervision of Dr. Roli Purwar, Assistant Professor, Department of Applied Chemistry. This thesis is a contribution of my original research work. Wherever research contributions of others are involved, every effort has been made to clearly indicate the same. To the best of my knowledge, this research work has not been submitted in part or full for the award of any degree or diploma of Delhi Technological University or any other University Institutions.

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CERTIFICATE

This is to certify that the M.Tech thesis entitled "**Biodegradable polymeric films from gelatin and silk sericin: Development and characterization for packaging applications"** submitted to the Delhi Technological University, Delhi-110042, in fulfillment of the requirement for the award of the degree of **Master of Technology** by the candidate **Anjali Verma** under the supervision of **Dr. Roli Purwar, Assistant Professor**, Department of Applied Chemistry. It is further certified that work embodied in this thesis has neither partially nor fully submitted to any other university or institution for the award of any degree or diploma.

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ABSTRACT

This project work describes the preparation and characterization of flexible cross linked gelatin/sericin/clay blend films. Gelatin/sericin (3:1, 1:1, 1:3 w/w) films are prepared by using glutaraldehyde as crosslinking agent and glycerol as plasticizer by solution casting method. The concentrations of gelatin and sericin are optimized on the basis of mechanical strength. Closite 30B and copper modified sodium montmorillonite are used as antibacterial nanofiller additive for gelatin/sericin blend films. It is observed that tensile strength of the crosslinked flexible gelatin/sericin films is enhanced on addition of clay. XRD spectra shows clay is exfoliated in gelatin/sericin matrix. Fourier transform infrared spectra showed glutaraldehyde crosslinked with gelatin and sericin .Scanning electron micrographs shows surface morphology of films. The films show smooth morphology and no phase separation. Biodegradability test in soil and antibacterial test shows biodegradable behavior and antibacterial activity of flexible gelatin/sericin and gelatin/sericin/clay films respectively. TGA thermograms represent the thermal stability of films.

LIST OF ABBREVIATIONS

S	Sericin
GL	Gelatin
GLU	Glutaraldehyde
MMT	Montmorillonite
NaMMT	Sodium montmorillonite
COMMT	Copper modified MMT
XRD	X-Ray diffraction
UTM	Universal testing machine
TGA	Thermo gravimetric analysis
DMA	Dynamic mechanical analysis
WVP	Water vapor permeability
FTIR	Fourier transform infra-red spectroscopy
S.R	Swelling rate
SEM	Scanning electron microscopy

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CHAPTER NO.1

1. INTRODUCTION

1.1. Background

Now days, the use of plastic has increased drastically because of its many applications such as packaging, wrapping etc. Generally petrochemical polymers such as polyethylene (PE) polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) etc. are synthetic in nature and have excellent chemical and weather resistance. These two resistive properties of synthetic polymers make them non-degradable in normal environmental conditions which is beneficial in one aspect as it makes life long use of synthetic polymer products and in other aspect it generate waste which is harmful for our environment. Synthetic polymer cannot be degrade in short period of time by natural process such as burying , For their destruction a special technique called **Pyrolysis** [1-7] is used, By this technique polymeric wastes are reduced into ash, toxic and harmful gases, released to the atmosphere leads air pollution. One of the best alternatives to overcome this serious problem is the use of biodegradable polymers in place of petrochemical polymers. Biodegradable polymers are derived from replenishable agricultural feed stocks, animal sources, marine food processing industry wastes, or microbial sources. These can be easily and harmlessly degrade back into earth under normal environmental conditions. Biodegradable materials break down to produce environmentally friendly products such as carbon dioxide, water, and biomass. In addition their degradation conserves energy as the biodegrading process does not require external energy. Because of these properties biodegradable polymers such as starch, cellulose, gelatin etc are frequently used as packaging material [8].

Controlled hydrolysis of fibrous insoluble protein, collagen forms gelatin. Gelatin is the major constituent of skin, bones and connective tissue. Gelatin contains higher content of the amino acids such as glycine, proline and hydroxyproline [9]. The annual world out-put of gelatin is nearly 3,26,000 tons and its demand increases day by day [10]. Gelatin has been used as biomaterial for tissue engineering, in the last ten years. The results show that gelatin helps to increase of migration and cell adhesion. Gelatin has also applied in some other areas such as wound dressing, controlled drug released and health caring devices [11].

Gelatin-based edible films and coatings have already been proposed to protect, maintain or extend the shelf-life of food products [12].

The use of antimicrobial additives in gelatin-based films or coatings for food packaging applications is a promising area, with the main goal being the prolongation of food shelf-life based on retarding deterioration mechanisms inside the package by using natural additives. A wide range of agents with antimicrobial properties has been proposed, e.g., organic acids, bacteriocins, spice extracts, thiosulphates, enzymes, proteins, isothiocyanates, antibiotics, fungicides, chelating agents, parabens and metals [13]. Other biopolymers have been blended with gelatin with an improvement in gelatin film properties such as lignin [14], chitosen [15], lignosulphonates isolated from sulphite liquors [16] or fish isolate [17].

Silk worm has very important place in field of biomedical because of two powerful proteins sericin and fibroin. Fibroin is widely used in textile industries due to its lustier property. Another protein which is sericin is gummy in nature, it binds fibroin together. Sericin is waste material of degumming process in textile industry and discarded in water, which enhance biochemical and chemical oxygen demand of water leads water pollution. That’s why the use of sericin could be

very much economic and put an environmental impact especially in countries where sericulture is practiced, such as India, China and Brazil [18]. Sericin contains 18 amino acids where serine (32%), aspartic acid (18%), and glycine (16%) are in significant amount Additionally, this protein is composed of 45.8% of hydroxyl amino acids (serine and threonine) [19]. Molecular weight of sericin protein ranges from 24 to 400 kDa [20]. Sericin protein is useful because of its properties such as UV resistance, antibacterial properties, absorbs and release moisture easily, etc [21].

Sericin based films are also very important in food packaging applications as well as in medical applications. Silver nano particles coated sericin/PVA films are used for antimicrobial action [22]. sericin can be coated onto the synthetic polymers such as nylon fibre and polyester for air filtration to reduce free radical exposure and inhibit microbial growth in the air filter [23]. sericin and glucomannan composite films are used for food coatings [24]. Recently, Silk sericin/chitosan composite film was prepared by mixing different ratios of silk sericin and chitosan resulting in homogeneous composite film that has wound dressing applications [25].Genipin, a natural compound extracted from gardenia fruit, has been successfully cross-linked to sericin forming a scaffold with good physical properties [26]. Due to its hydrophilicity that assists to maintain a moist environment and to absorb excess exudates from wounds, sericin has been used as wound dressing agent [27, 28]. Sericin in two-dimensional form, like films and membranes or in three-dimensional form, like hydrogel and porous scaffolds matrices have been studied [29]. Sericin can form a film, casting film or gel analogue. Acrylonitrile was copolymerized with sericin to prepare a protein containing synthetic polymer film that used for separating water from organics [30].

Montmorillonite nano clays

Montmorillonite nano clays are unique clays having a platy structure with a unit thickness of one nano meter or less. This clay also has an aspect ratio in the 1000:1 range. It can easily modify for increasing its compatibility with most of the polymers [31].Clays invariably contain exchangeable cations and anions held to the surface. The prominent cations and anions found on clay surface are Ca^{2+}, Mg^{2+}, H^{+}, K^{+}, NH^{4+}, Na^{+}, SO_4^{2}-, Cl^{-}, PO_4^{3-}, and NO_3^{-}. These ions can be easily exchanged with other ions without affecting the clay mineral structure [32]. This ion exchange process is known as clay modification. MMT act as reinforcement for polymer-clay nanocomposites [33].

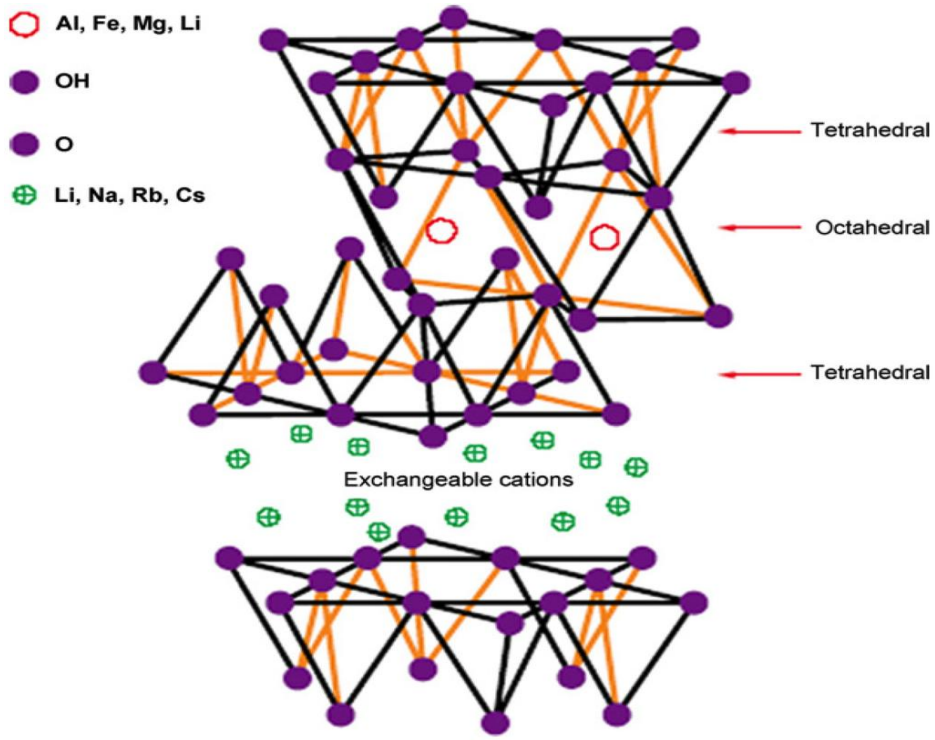


Fig.1 Structure of MMT(www.googleimages.com)

Na-MMT is commonly used as nanofiller in the preparation of polymer composite. The modified Na-MMT is used for making eco-friendly polymer clay nanocomposite with improved physical and mechanical properties [34].copper loaded MMT is widely used for fabricating the packaging material because it shows good antibacterial properties as well as good mechanical properties due to presence of copper ion [35].

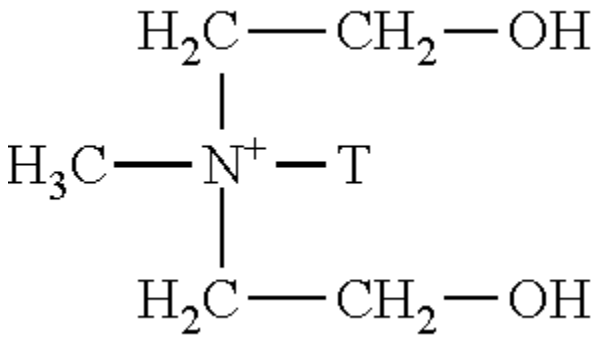


Fig.2 Structure of closite 30B (www.googleimages.com)

Cloisite 30B is another nanoclay used in this study is an organoclay derived from montmorillonite by modification with methyl, tallow, bis-2-hydroxyethyl, quaternary ammonium, where tallow is 65% C18, 30% C16, 5% C14. cloisite 30B also impart antimicrobial activity [36].

RESEARCH GAP

With extensive literature search, it was observed that gelatin/sericin/clay blend films have not been reported and has not been exploited as antibacterial food packaging material.

OBJECTIVE

The objective of this project is to prepare biodegradable and antibacterial polymeric films by the combination of two natural polymers gelatin and sericin and nanoclay for packaging application.

CHAPTER NO.2

REVIEW OF LITERATURE

2.1. Biodegradable film for food packaging

Biodegradable films and coatings are very good replacement of synthetic polymeric films and coatings. Generally food packagings are made by synthetic polymers which are non biodegradable in nature; shows harmful effect on environment [37]. Biodegradable packaging is preferable in comparison to recyclable packaging material because they need energy in recycling process which is not much economical [38]. Over the last decades; there have been interests in films which are made from natural polymers. Natural polymers degraded easily in the effect of environmental conditions such as in the presence of soil, sun radiation, water and microorganism.

2.2. Proteins as film forming material

Proteins are thermoplastic containing amino acids .They are macromolecule with specific amino acid sequences and there are limitless number of Sequential arrangements with a wide range of interactions and chemical reactions [39]. Structures of proteins can be easily transform by irradiation, pressure, mechanical treatment, heat and acids, alkaline, metal ions, salts, chemical hydrolysis, enzymatic treatment and chemical cross linking [40]. Proteins are commonly used as film-forming materials. The most favorable characteristics of proteins compared to other film-forming materials are conformational denaturation and electrostatic charges [41]. Proteins can be derived from natural sources such as animal tissues, milk, grains, eggs, and oilseed [42].

2.3. Gelatin

Gelatin is the denatured product of collagen has very good mechanical strength and film forming properties that is why it is combined with other polymer to enhance the mechanical ability of end product.

2.3.1. Chemical structure of gelatin

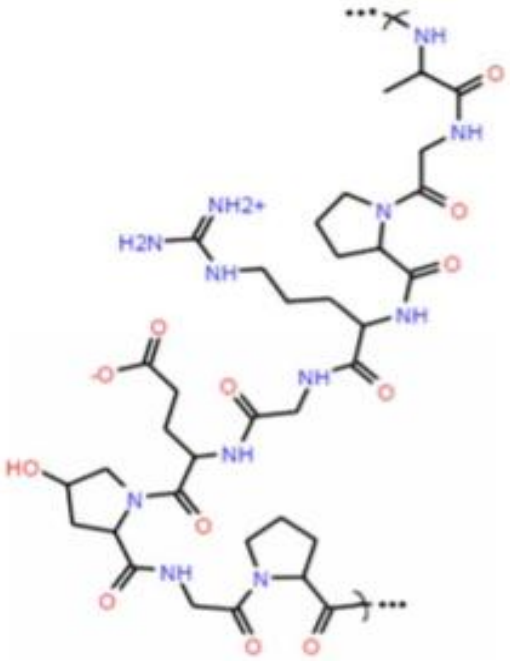


Fig.3 Structure of gelatin (adapted from N. Hanani et al.)[43]

2.3.2. Chemical composition of gelatin

The dominating amino acid sequence is glycin- proline- hydroxyproline [44].Gelatin contains glycin (Gly) 26-34%, proline (Pro) 10-18% and hydroxyproline (Hyp) 7-15%. Other significant amino acids include alamine (Ala) 8-11%, arginine (Arg) 8-9%, aspartic acid (Asp) 6-7% and

glutamic acid (Glu) 10-12% [45]. It does not contain tryptophan and also deficient in isoleucine, threonine and methonine [46]. Sulfur containing amino acids are also deficient in gelatin [47]. It is non-immunogenic, non-antigenic, biocompatible and biodegradable. There are two types of Gelatin ; type A and type B. basic difference between these two types is that type A is produced from acid treatment while type B is form by alkali-treated processes[48]. 1% sugar is also present in gelatin. The type, nature, and amount of sugars depend on the source of the gelatin and the method of determination. Reported sugars are mannose, glucose, lactose, galactose, and xylose [49].

2.3.3. Properties of gelatin

Gelatin has multiple properties:

2.3.3.1. Solubility

Gelatin is soluble in hot water, polyhydric alcohols such as propylene glycol, glycerol and also in hydrogen bonding solvents such as acetic acid and formamide. Gelatin is insoluble in less polar organic solvents such as dimethylformamide and acetone [50].

2.3.3.2. Thickening ability

When gelatin added to solution, its viscosity increases [51].

2.3.3.3. Gelling ability

Gel obtained from gelatin is thermo reversible in nature when temperature of gelatin solution is decreases its converts from sol to gel and vice versa [52].

2.3.3.4. Film-forming property

When a gelatin solution is spread in a thin layer over a surface and converts from sol into gel, it forms a film [53].

2.3.3.5. Emulsifying ability

The emulsifying ability of gelatin makes a homogenous dispersal in a mixture of constituents which are not normally miscible, such as mixture of oil and water [54].

2.3.4. Applications of gelatin

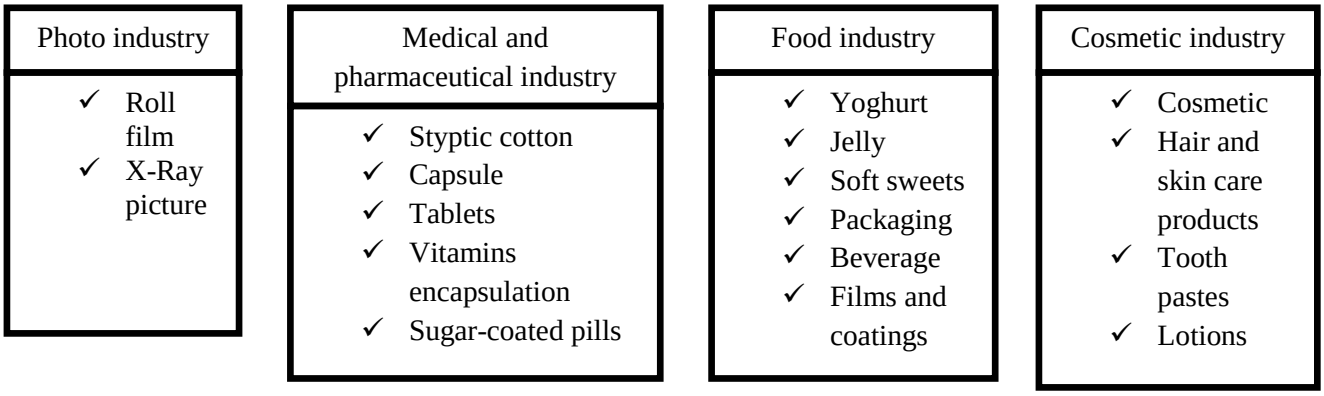


Fig.4 applications of gelatin in various fields (adapted from Ramos et al.) [55]

2.3.5. Gelatin composites

Films fabricated from gelatin or its combination with other polymers used in various areas such as drug delivery system, food industry and in optical devices such as in holography. Kim et al. [56] was produced Hydroxyapatite (HA) and gelatin composites in a foam type by novel freeze-drying and crosslinking technique. HA addition makes foam stronger. This foam has great prospective for use as tissue regeneration scaffolds. Similarly Mohamed et al. [57] was prepared nano-hydroxyapatite/ chitosan–gelatin composites for bio-applications and he found that water

absorption capacity of composite increases on increasing the content of chitosan-gelatin content due to increase in hydrophilic groups.

Table.1 some gelatin composites

Composite Materials	Ratio	Reference
Gelatin/Soy protein isolate(SPI) blend	SPI: Gelatin(0,25,50,75,100%)[w/w]	Denavi et al.2009
Gelatin/sunflower oil	Sunflower oil:0,0.3,0.6 and 10% of gelatin	Perez-Mateos et al.2009
Gelatin/corn oil	Gelatin powder is dissolve in distilled water (4-8% w/v) and in this solution add corn oil to make solution concentration to 55.88 % w/v	Hanani et al. 2013
Gelatin/polyvinyl alcohol	2 g (PVA + Gelatin)/100 g of solution	Silva et al.2008
Gelatin/Nanoclay	Nanoclay 5 % (w/w) of gelatin	Bae et al .2009
Gelatin/PVA	10,20,30 and 40 g of PVA/100g of macromolecules (Gelatin + PVA)	Mendieta-Taboada et al.2008
Konjac glucomannan/Gelatin	KGM/GEL: 1/9,2/8,3/7,4/6,5/5,6/4,7/3,8/2 and 9/1 (w/w)	Li et al.2006

Gelatin/casein	Casein-gelatin blend (100:0,75:25,50:50,25:75 and 0:100)	Chambi and Grosso 2006
Sodium caseinate (NaCas)/Whey protein isolate (WPI)	NaCas/WPI (100:0,75:255,50:50,25:75,0:100)	Longares et al.2005
Gelatin/Soy protein isolate	SPI to gelatin (10:0,8:2,6:4,4:6,2:8,0:10)	Cao et al.2007
Gellan/Gelatin	Gellan to gelatin (100:0,80:20,60:40,40:60 and 20:80(V))	Lee et al.2004

The commercial quality of gelatin is shown by its physic-chemical properties such as composition, color, transparency, tasteless and odorless. Due to the highly hygroscopic nature of gelatin, it has a tendency to swell when putting in contact with the surface of foodstuffs with high moisture content. Zhao et al. [58] demonstrated the viability of using a natural extract as a new natural crosslinker for the modification of gelatin (type B, from bovine bone) by hydrogen bonding formation between water and free hydroxyl groups of amino or poly-phenol groups. The results showed that the fusion of this extract into gelatin significantly increased gel strength compared to the untreated gelatin. The combination of gelatin with other biopolymers, such as whey proteins [59], starch [60], pectin [61], could be a good scheme for the development of films with higher mechanical and excellent water resistance properties.

2.3.6. Gelatin based films and coatings for food packaging

Recent researches have focused on techniques to develop active packaging films and coatings which contain antimicrobial, antioxidant and other agents to enhance the biological features of food [62-63]. Martucci et al. [64] developed gelatin based film by using lavender or oregano essential oils and a mixture of them (50:50) at concentrations ranging between 0 and 6000 ppm. Antimicrobial activity of films was determining against *Escherichia coli* and *Staphylococcus aureus* bacteria. Film showing lower values of inhibition zone for S. aureus compared to E. coli. Recent trends in the use of nanoclays as reinforcement agents in gelatin have been reported in the literature, such as montmorillonites [65-67] and laponites [68].

2.3.7. Edible film and coating applications

2.3.7.1. Meat Products

Due to gradual accumulation of metmyoglobin on the surface of meat color of meat converts from red to brown. In order to reduce this color deterioration Cardoso et al. [69] was produced Gelatin-chitosan blend film and this film was successfully increasing the shelf life of beef steak. In other work Liu et al. [70] was fabricated the sodium alginate, pectin, gelatin blend film for packaging of pork meat.

2.3.7.2. Fishery Products

Fish is one of the putrescible food products mainly due to microbial spoilage and chemical reactions [71]. Maintaining the freshness and quality of fish is a challenge for Food Processing Industries. Preservation techniques can upgrade the quality of Fish products. To improve the life of fish products research has been focused on development of edible films and coatings. Films

and coating which are based on cold water fish gelatin-chitosan shows excellent antioxidant properties, more over coatings of cold water fish gelatin-chitosan combination shows resistance against lipid oxidation [72].

2.3.7.3. Other Food Products

Not only for meat and fishery products, some food products also coated or packed in to gelatin based films. Potential applications of gelatin edible films in the food industry may include the transport of gases (O_2 and CO_2), water vapor, and flavours for fruits and vegetables [73]. Carrots [74], cherry tomatoes [75], calyx [76], oranges [77], banana and eggplant epicarps [78], fresh-cut melons [79], peppers [80], strawberries [81], blueberry fruit [82], pineapple fruit [83] and minimally processed persimmon [84] are packed or coated in gelatin films.

2.3.8. Gelatin fibers

Chaochai et al. [85] was prepared gelatin fiber using by N-acetyl-D-glucosamine and glutaraldehyde as crosslinkers which gives remarkable increase in tensile strength and water resistance. These fibers also exhibited good water resistance. Midorikawa et al. [86] was fabricated gelatin fiber by gel-spinning using ethylene glycol as solvent. Gelatin fiber prepared by extrusion of the gelatin (15 wt %) ethylene glycol into methanol at 20^0C and drawn up to seven times its original length at 5^0C had a Young modulas of 11 GPa, highest tensile strength (405 MPa), and storage modulus of 7.9 GPa at 200^0C.

Choktaweesap et al. [87] was also prepared gelatin fiber with different solvents Glacial acetic acid or mixed solvent system such as (acetic acid/2, 2, 2 trifluoroethanol, acetic acid/dimethyl sulfoxide (DMSO), acetic acid/formamide) and he found that contribution of DMSO and Ethylene glycol produced smooth and fine fiber. When gelatin cross-linked with sugars than its

mechanical strength as well as water resistance enhanced rapidly [88]. The reaction between sugars and gelatin referred as the Maillard reaction [89]. Huang et al. [90] also produced electrospun gelatin fiber with 2,2,2 trifluoroethanol as solvent and he observed that at 7.5 % mass concentration, fiber is smooth, fine and show very good tensile modulus and strength.

2.4. Sericin (silk protein)

Silk cocoon is composed of two protein material fibroin and sericin. Sericin is a gummy material which combined two fibroin and form cocoon. In textile industries for obtaining the fibroin sericin is removed by the degumming process and released in to hot water stream which leads to water pollution.

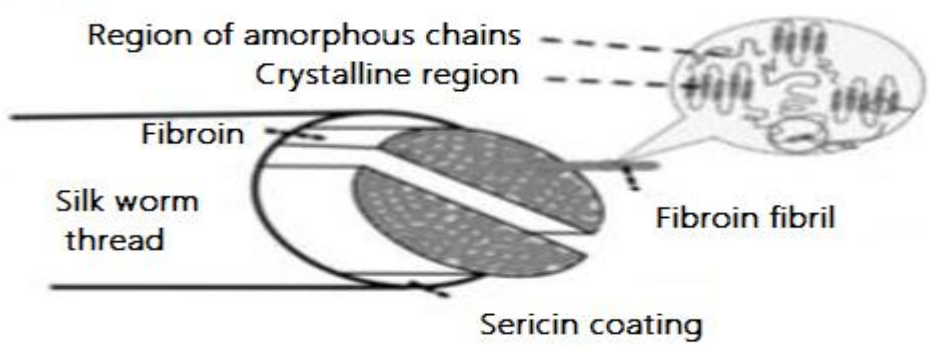


Fig.5 Schematic presentation of the silk fibroin structure (adapted from Volkov et al.) [91]

2.4.1. Degumming methods to obtain sericin and fibroin

2.4.1.1. Degumming in distilled water

Take small pieces of cocoon in distilled water in 1:15 ratio (for 1g of cocoon 15 ml water is required) and boil it at very high pressure (15 psi) and temperature (120^0C). So that sericin is hydrolyzed in to water.

2.4.1.2. Degumming in the presence of sodium carbonate

Take small pieces of cocoon in distilled water in 1:30 ratio and boil it in the presence of sodium carbonate.

2.4.1.3. Sedimentation method

For the formation of deposition to be obtained degumming soluble in any processes by added 40g of ammonium sulfate for each 100 ml solution and the resulting sedimentation is filtered and dried to powder sericin at ambient temperature [92].

2.4.2. Properties of sericin

2.4.2.1. Gelling property

Sericin have random coil as well as β- sheet structure , random coil structure is soluble in hot water but up on cooling this random coil structure is converts in to β- sheet structure which form gel [93].

2.4.2.2. Sol- gel transition

Sericin is easily dissolved in hot water but when temperature gets down it converts into gel [94].

2.4.2.3. Iso electric pH

Sericin contains more acidic amino acids in comparision to basic amino acids, isoelectric point of sericin is about 4 [95].

2.4.3. Amino acids in sericin

Sericin contains 18 amino acids including essential amino acids and is characterized by the presence of 32 percent of serine. The total amount of hydroxyl amino acid in sericin is 45.8 percent. There are 42.3 percent of polar amino acid and 12.2 percent of non polar amino acids.in presence of sericin fibers are hard and become soft and lustrous after its removal [96].

2.4.4. Availability of sericin

Karnataka is the silk bowl of the country. The sericulture area has been divided into, Traditional and Non-traditional. The highest production of silk cocoons has taken place in traditional areas. Now there are 56 cocoon markets established over different parts of the state. Among them Three (3) are Big Markets at Sidlaghatta, Ramnagara and Vijayapur and these 3 markets contributes 75% of the total cocoons transactions in the state. Hence, the market price of cocoons in Karnataka is determined on the basis of these 3 markets [97].

2.4.5. Application of sericin in market

Sericin is a matchless member of cosmetic area that own moisture absorption and preservative ability. The hydrophilicity of sericin renders it to absorb water 50 times high than that of glycerin. Also it was shown that sericin has a capability to inhibit tyrosine kinase activity [98], which is responsible for the production of skin melanin. When sericin is applied on skin and hairs it makes skin and hair soft. Sericin is a less irritant moisturizer and is gentle on a delicate skin. Sericin cream is available in the market with wound healing properties [99]. Dermal and corneal wound healing have been tried by many researchers using sericin. sericin is known to accelerate the proliferation of several mammalian cell lines and protect insect cells from death

caused by acute serum deprivation in culture and has protective and healing activities on the skin. As sericin has been shown to activate cell proliferation and protect against cell death, this protein may be a useful additive to serum-free media for culturing mammalian tissues and cell lines [100].

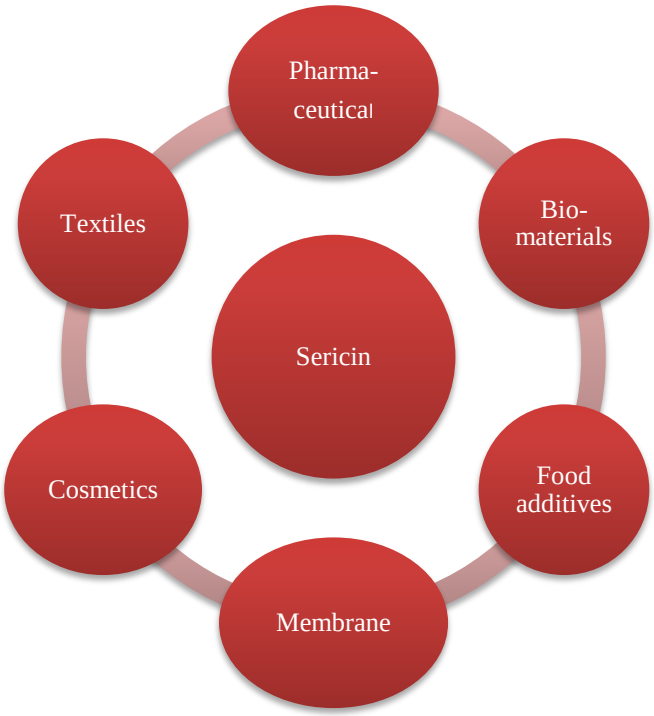


Fig.6 Application of silk sericin

2.4.6. Sericin as film and membrane material

Zhang et al. [101] was prepared sericin film with and without using glycerol for investigating the effect of glycerol on tensile strength of sericin film and he found that at 10 wt % concentration of glycerol film shows great tensile strength and due to presence of glycerol flexibility of film was also increased. Similarly Yun et al. [102] was also fabricating the sericin and glycerol blend film but he varies glycerol content 50 to 70 wt % and found that when glycerol content increases from 50 wt %, moisture content will also increases due to hygroscopic nature of it and give

contribution in plasticization of films. Namviriyachote et al. [103] was fabricating sericin and polyvinyl alcohol blend film for wound dressing with various concentration of silk sericin (1-3 % w/v) and he reported that film composed of 2 % polyvinyl alcohol and 3 % silk sericin represent very high tensile modulus. Purwar et al. [104] was also fabricating the sericin, polyvinyl alcohol and clay blend films for packaging application.

Sericin is also used to make membrane for separation process. Mizoguchi et al. [105] reported the method of formation of cross-linked thin film for separating the water and ethanol. The membrane was made by mixing aqueous solution of a cross-linking agent such as formaldehyde, hydrochloric acid, water-soluble urethane resin copolymer, and aqueous sericin. The prepared solution was spread on a smooth plate at room temperature and allowed to stand at room temperature to obtain the cross-linking The resulting film was treated with hot air at 120 ^{0}C for 10 min to cross-link the urethane. Yamada et al. [106] were prepared sericin membrane for the separation of racemic mixtures.

CHAPTER NO.3

EXPERIMENTAL WORK

3. Materials and methods

3.1. Material and equipment

The materials and equipments used are listed below:

Table.2 List of Materials used

S.No.	Chemical	Source
1	Gelatin	Thermo Fisher Scientific India Pvt. Ltd
2	Sericin	Central Silk Research Board, Bahraich, India
3	Glutaraldehyde	Spectrochem Pvt. Ltd.
4	Glycerol	Sisco research laboratories Pvt. Ltd
5	Sodium MMT	Sisco Research Laboratories Pvt. Ltd., Maharashtra.
6	Closite 30B	Southern Clay USA
7	Distilled water	----------
8	Luria broth	Titan biotech Ltd.
9	Cupric chloride	British Drug House
10	Agar Agar	Central Drug House

Table.3 List of Equipments used

S. No.	Name of equipment	Model	Make
1	FTIR Spectrometer	Thermo scientific spectrophotometer Nicolet 380	USA
2	Universal testing machine	INSTRON model no 3369	USA
3	X-Ray diffractometer	Bruker D8	Bruker, Germany
4	Dynamic mechanical Analyzer	8000 Perkin Emuler	USA
5	Thermo gravimetric Analyzer	4000 Perkin Emuler	USA
6	Scanning electron microscope	S-3700N	Germany

3.2. METHODS

3.2.1. Isolation of sericin

Cocoons were cut in to very small- small pieces and sericin was removed using high temperature high pressure degumming technique. For extraction of aqueous solution of sericin, small pieces of cocoons boiled at 105^0C for one hour in autoclave keeping material to liquor ratio 1:15. Extracted aqueous solution of sericin filtered with muslin cloth to separate sericin and fibroin. Solid sericin was obtained by heating sericin solution at 40^0C.

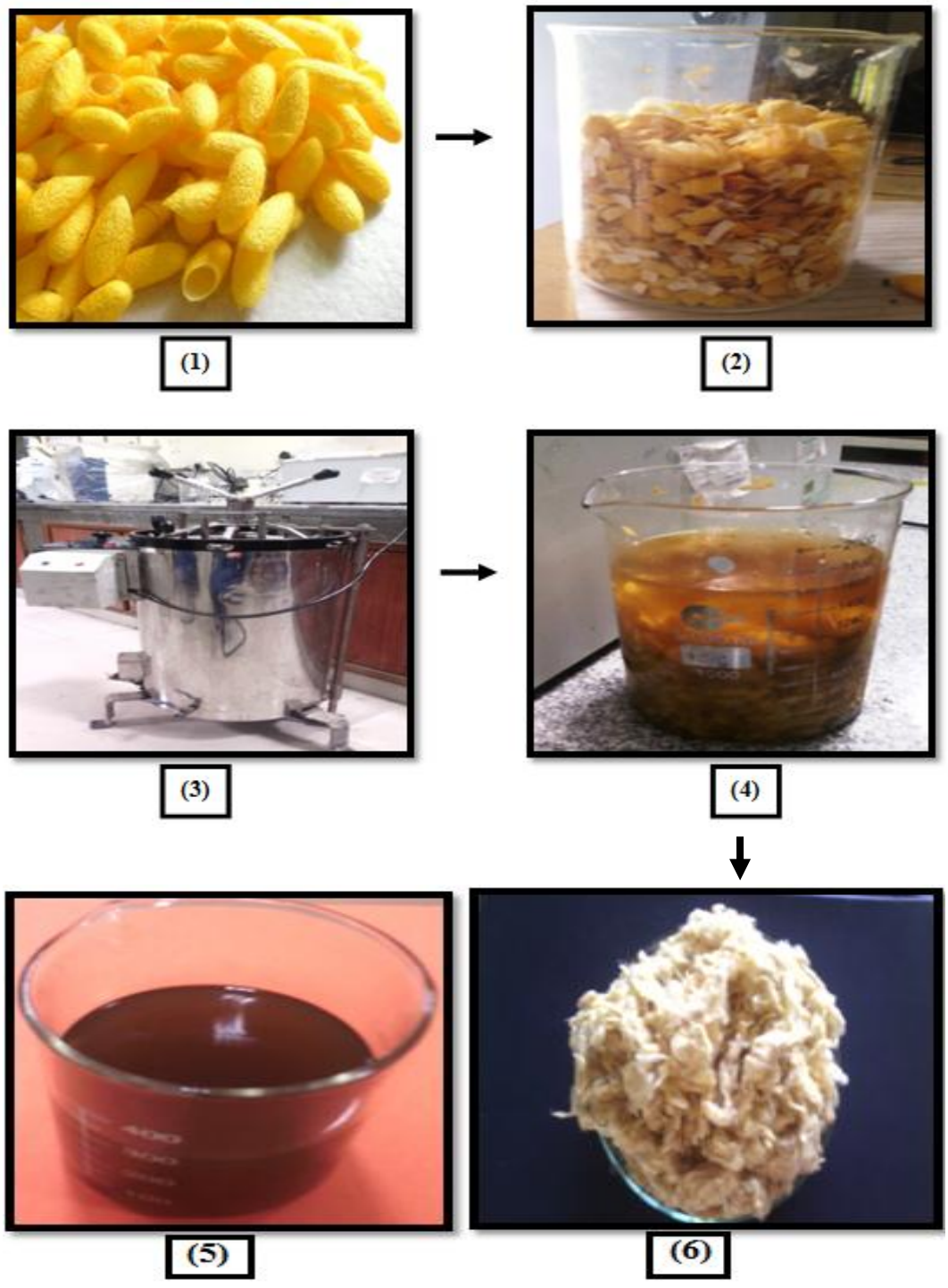


Fig. 7 Schematic diagram of isolation of sericin; (1) cocoons, (2) cocoons cut into small pieces, (3) autoclaving of cocoons, (4) mixture of sericin and fibroin, (5) sericin (6) fibroin

3.2.2. Preparation of gelatin/sericin blend films

Gelatin/sericin (solid weight 10 wt. %) blend films were prepared by solution casting method. Aqueous solution of gelatin/sericin in 1:3, 1:1, 3:1 (w/w) ratios were prepared in hot water with continuous stirring. 5% (w/w) glutaraldehyde and 30% (w/w) glycerol were added in gelatin and sericin solution .these prepared solutions were poured in petri-dishes and dried at 40^0C temperature at least for 24 hours. After heating the films were treated at 120^0C for 1 hour. These film samples are considered as controlled sample.

3.2.3. Modification of clay

For modification of clay 0.2 molar solution of cupric chloride dihydrate in 100 ml of distilled water were prepared and 3 g of NaMMT were dispersed in cupric chloride solution under continuous stirring of 7 days.

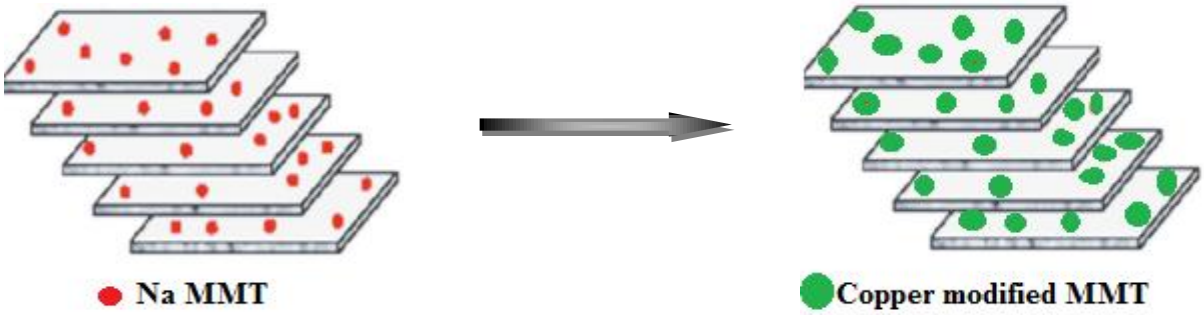


Fig.8 Modification of clay

3.2.4. Preparation of gelatin/sericin/clay blend films

Aqueous solution containing gelatin/sericin (3:1 w/w ratios), 5% (w/w) glutaraldehyde and 30% (w/w) glycerol were prepared in hot water. Different concentrations of clays ranging from 1 to 5% (w/w) were dispersed in the solution.

The solution were ultrasonicated for 30 min and poured in petri dishes. After drying, the films were heat treated at 120^0C for 1 hour.

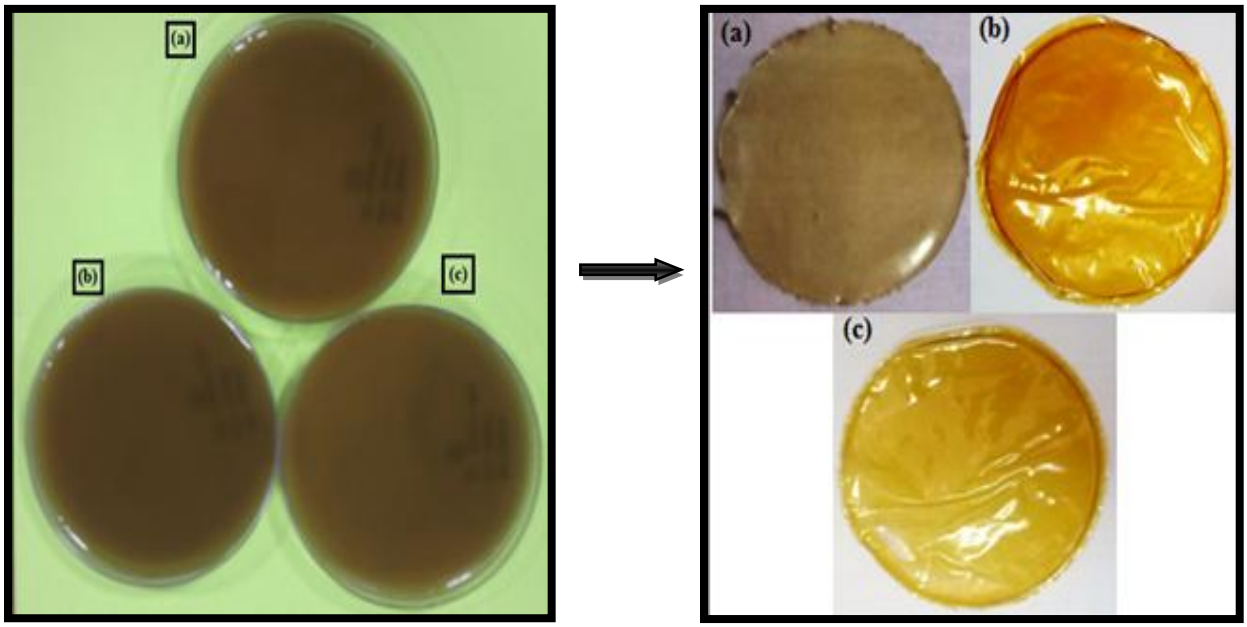


Fig. 9 casting of various gelatin/sericin and gelatin/sericin/clay blend film (a) gelatin/sericin film (b) gelatin/sericin/closite 30B film (c) gelatin/sericin/COMMT

3.3. CHARACTERIZATION TECHNIQUES

3.3.1. Mechanical Testing

Mechanical properties of gelatin/sericin and gelatin/sericin/clay blend films were determined by using an Instron Universal Testing Machine (Model no 3369) running at a crosshead speed of 5 mm/min and gauge length of 50mm. samples were cut into 70mm×10mm rectangular pieces. six samples were analyzed and average values are reported.

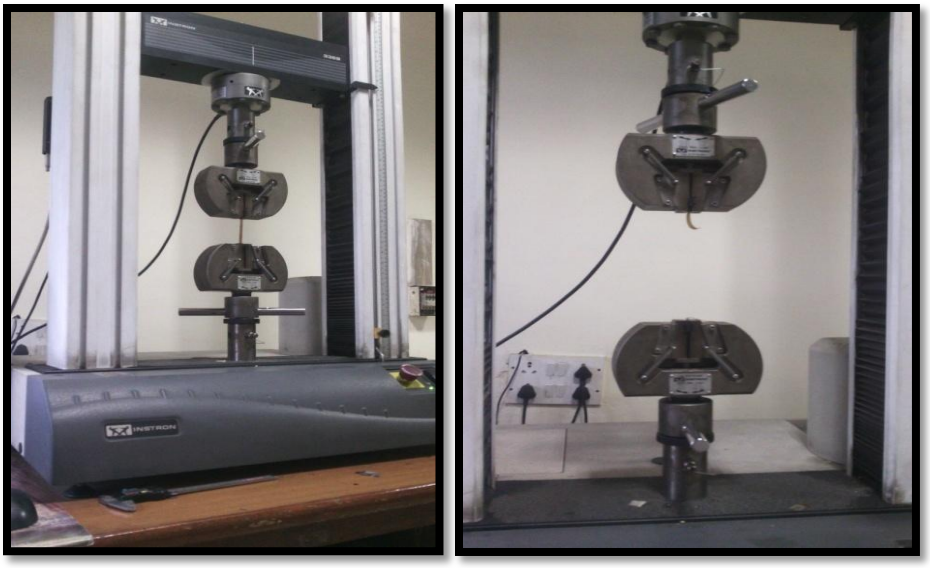


Fig.10 tensile testing of films

3.3.2. Water Vapor Transmission

Water vapor transport rate was measured by the method described by Kang et al. [107] with some modifications. The specimens were placed in test containers with distilled water, which were placed in an oven at a constant temperature (37^{o}C). Water vapor transmission was calculated from the weight of test container before and after the test.

WVT (water vapor transmission rate) = $\frac{G}{t\,A}$

Where, G: weight change (g)

t- Time during which G occurred (h)

A- Test area (m^2).

3.3.3. Swelling Behavior

Swelling properties of the films were tested by the method described by Aadil et al. [108] with some modification. Films (1 × 1 cm in size) were immersed in 25 ml of distilled water at room

temperature (25 °C). The weight gain of swollen films (Ws) was measured at selected times, after blotting the surface with filter paper, until equilibrium was reached. The swelling ratio (SR) was calculated using the following equation:

$$\text{Swelling Rate (\%)} = \frac{Ws - Wd}{Wd} \times 100$$

Where W_s is the weight of swollen samples (g); W_d is the weight of dry samples (g). The measurements were repeated three times for each type of film and an average was taken as the result.

3.3.4. Biodegradability in soil

Biodegradable behavior of film samples were tested by the method described by kermani et al. [109] with some modifications. In this method, gelatin/sericin and gelatin/sericin/clay films were cut in to (7cm×1cm) small pieces, weighted and placed into the soil in a pot. The pot was covered with a plastic net and exposed to atmospheric conditions. In this study the degradation of films was evaluated by measurement of their weight loss and weight loss in films were recorded in time interval of 48 hrs.

3.3.5. Thermal Analysis

Thermal property of gelatin/sericin films was analyzed by the thermal gravimetric analysis. TGA measures the change in the mass of a sample as a function of temperature in inert (in presence of nitrogen) atmosphere. TGA analysis was done by the TGA 4000 Perkin Emuler. Samples were analyzed at heating rate of 5^0C in the range of 30-1000^0C in above mentioned atmosphere.

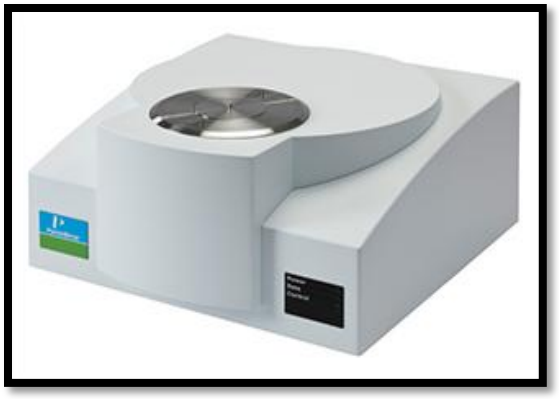


Fig. 11 Thermogravimetry analyzer (www.googleimage.com)

3.3.6. FTIR Analysis

FT-IR spectroscopy was used to confirm that a reaction was occurred between gelatin, sericin and glutaraldehyde or not. FTIR spectrophotometer (Thermo Scientific Nicolet 380 Spectrophotometer, US) was used in transmittance mode.

Fig.12 FT-IR spectrometer (www.googleimage.com)

FTIR spectra of gelatin/sericin and crosslinked gelatin/sericin films were recorded in the range of 400 - 4000 cm^{-1}.The samples were grounded into powder, mixed with KBr and then converted into thin pellets.

3.3.7. Antibacterial Testing

Antibacterial activity of samples determine by the method which is described by Romainor et al. [110] with slight modifications. The antimicrobial activity of gelatin/sericin and gelatin/sericin/clay films was investigated against the growth of E. coli (Escherichia coli) bacteria. Bacteria were cultured in Luria broth followed by incubation in incubator shaker for 24 hours. 20 µL of bacterial suspension was uniformly spread on the sterile Petri dishes of Muller-Hinton Agar using sterile spreader and pieces of antimicrobial films were placed on the bacterial culture. Plates were sealed and incubated at 37°C for 24 hours. Antibacterial activity of samples was determined on the basis of minimum bactericidal concentration (MBC).For determining the MBC 20 µL of mixture from serial dilution test tubes was transferred and spread on the Muller-Hinton Agar (MHA) plates. The MBC point was determined as the lowest concentration in serial dilution series that shows colonies growth after 24 hours incubation at 37°C.

$$\text{Percent of inhibition (\%)} = \frac{No\ of\ colonies\ \ in\ control\ \ plates - No\ of\ colonies\ \ in\ sample\ \ plates}{No\ of\ colonies\ \ in\ control\ \ plates} \times 100$$

Fig.13 Laminar flow used for antibacterial testing

3.3.8. XRD Analysis

X- ray diffraction intensity curves of gelatin/sericin and gelatin/sericin/clay blend films and nanoclays were obtained at a λ=1.5 Å for 2θ from 3 to 40^0 with a diffractometer Bruker D S advanced using CuK α radiation.

Fig. 14 X- Ray Diffractometer (www.googleimage.com)

3.3.9. Morphology

The surface morphology of film samples was analyzed by the scanning electron microscope (model No.S-3700N, Germany) at 20kV voltage.

Fig. 15 Scanning Electron Microscope (www.googleimage.com)

CHAPTER NO.4

4. RESULT AND DISCUSSSION

4.1. Mechanical analysis

4.1.1. Effect of gelatin (GL)

The effect of gelatin on tensile strength, tensile modulus and elongation at break of gelatin/sericin films is shown in table.4. It is observed that as concentration of gelatin is increases from 25 to 75 %, the tensile strength and elongation at break increases. Increase in tensile strength is due to crystalline structure of gelatin. Sericin is globular protein so that it cannot show the strength. On the basis of mechanical strength, concentration of gelatin and sericin is optimized. Prodpran et al. [111] was also prepared film of gelatin and chitosan in 8/2 ratio shows good mechanical strength, this is only due to the good miscibility of gelatin and chitosan as well as crystalline structure of gelatin.

Table.4 Tensile properties of flexible crosslinked gelatin/sericin films with different concentrations **(Value given in the bracket shows standard deviation)**

Sample	Tensile strength(Mpa)	Tensile modulus(Mpa)	Elongation at break (%)
25%gelatin+75%sericin	1.53(0.09)	1.25(0.13)	350
50%gelatin+50%sericin	2.31(0.13)	1.06(0.25)	443
75%gelatin+25%sericin	3.12(0.48)	1.02(0.34)	448

4.1.2. Effect of nano clays

Nano clays are act as reinforcing filler. As concentration of nanofiller increases, tensile strength will also increases which shown in table no.5 and table no.6 but after 3% concentration of closite 30B tensile strength decreases this is due to lower dispersion and agglomeration[112].similarly at 1%COMMT film shows high tensile strength after this tensile strength decreases.

Table.5 Tensile properties of flexible crosslinked gelatin/sericin films with different closite 30B content

Sample	Tensile strength(Mpa)	Tensile modulus(Mpa)	Elongation at break (%)
1% closite 30B	4.64(0.64)	0.99(.14)	678
3% closite 30B	8.1(0.57)	1.50(0.39)	820
5% closite 30B	2.37(0.28)	3.17(.10)	560

Table.6 Tensile properties of flexible crosslinked gelatin/sericin films with different copper modified Na MMT content

Sample	Tensile strength(MPa)	Tensile modulus(MPa)	Elongation at break (%)
1% COMMT	4.23(0.34)	0.63(.26)	830
3%COMMT	2.70(0.48)	0.84(0.05)	632
5%COMMT	2.13(0.51)	1.71(0.74)	390

Results shows that 75%gelatin/25%sericin, 75%gelatin/25%sericin/3%closite30B, 75%gelatin/25%sericin/1%COMMT shows good values of tensile strength in comparison to other samples. Hu et al. [113] was prepared chitosan-agrose film composite for packaging purpose and his best sample (40% mass concentration of agrose +60 % mass concentration of chitosan) show 5.31 MPa tensile strength. Yoshida et al. [114] was also reported the tensile strength of whey protein film 2.30 MPa for the food packaging applications. Similarly Jose et al. [115] was mentioned tensile strength of polyvinyl alcohol starch and carbon nano tube composites is 8.09 MPa for packaging industries applications.

4.2. Water Vapor Permeability/Transmission

Water vapor permeability of gelatin/sericin and gelatin/sericin/nanoclay are shown in figure.16.

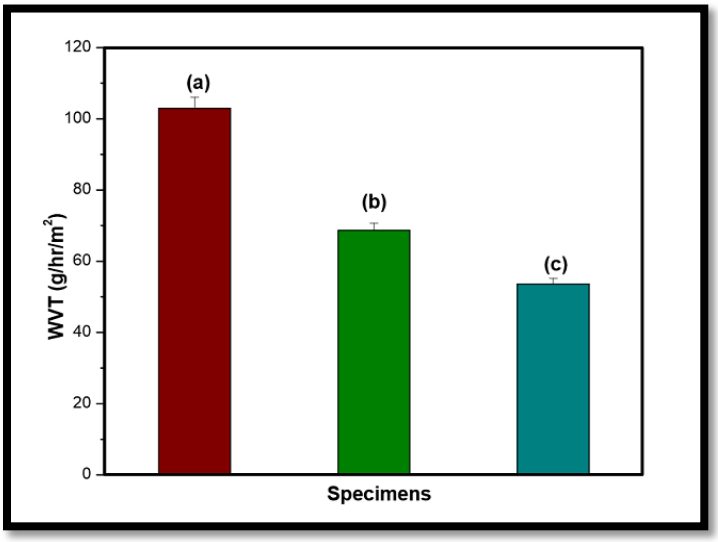


Fig.16 (a)75%GL/25%S,(b) 75%GL/25%S/1%C0MMT,(c)75%GL/25%S/3%closite 30B

Water vapor permeability for the gelatin/sericin film which is considered as control sample is 102.94 g/hr/m^2. As clay content is increases water vapor permeability value is decreases. Farahnky et al. [116] was also reported significant reduction in permeability of gelatin and

nanoclay films for water vapor. The minimum and maximum WVPs of 0.42 and 0.86 g mm/kPa m^2 h were found for the control (with no clay) and gelatin film containing 18% clay, respectively. The barrier properties of polymers can be significantly enhanced by insertion of impregnable lamellar fillers, such as clay particles with sufficient aspect ratio to alter the diffusion path of gas-penetrant molecules. These nanofillers can impede gas molecules diffusion and increase the tortuosity so that diffusion of gas molecules in such tortuous path becomes difficult [117].

4.3. Swelling properties

As clay content is increases; swelling behavior of polymer composite is increasing due to hydrophilic nature of clay. Different metal cations such as Na^+, Ca^{++} present between the layered structures of clay [118-119]. These ions are responsible for the hydrophilic behavior of clays. Swelling behavior of various gelatin/sericin and gelatin/sericin/clay films are shown in figure.17.

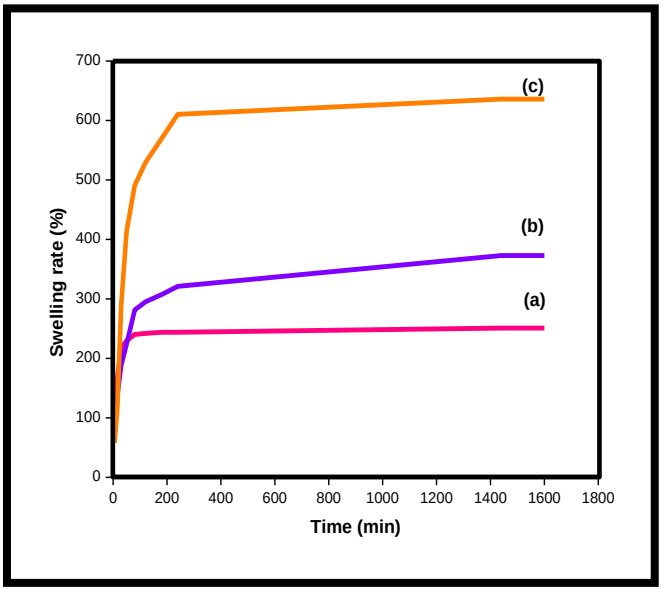


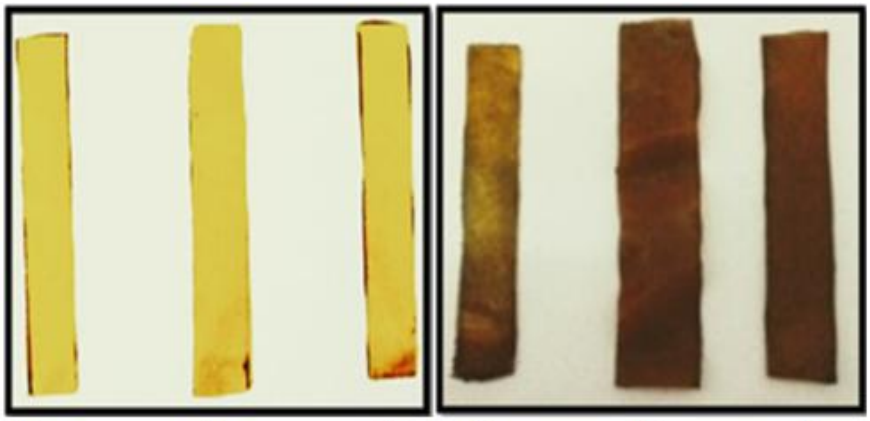
Fig. 17 Swelling behaviour of various gelatin/sericin films (a) 75%GL/25%S, (b) 75%GL/25%S/1%CO MMT (c) 75%GL/25%S/3%closite 30B

4.4. Biodegradability in soil

It was observed that the film samples swells due to moisture absorption from soil after that weight loss was recorded. The weight loss occurs due to adhering of soil particles on the film surface [120] and enzymatic activity of micro-organism present in soil [121]. But degradation occurred faster in gelatin/sericin matrix in comparison to gelatin/sericin/clay nanocomposite, it means presence of nanoclay reduces the degradation process, this is only due to reduction in enzymatic activity by micro-organism present in soil. The weight loss in 75%GL/25%S (sample a), 75%GL/25%S/3%closite30B (sample b), 75%GL/25%S/1%COMMT (sample c) was shown in table.7.

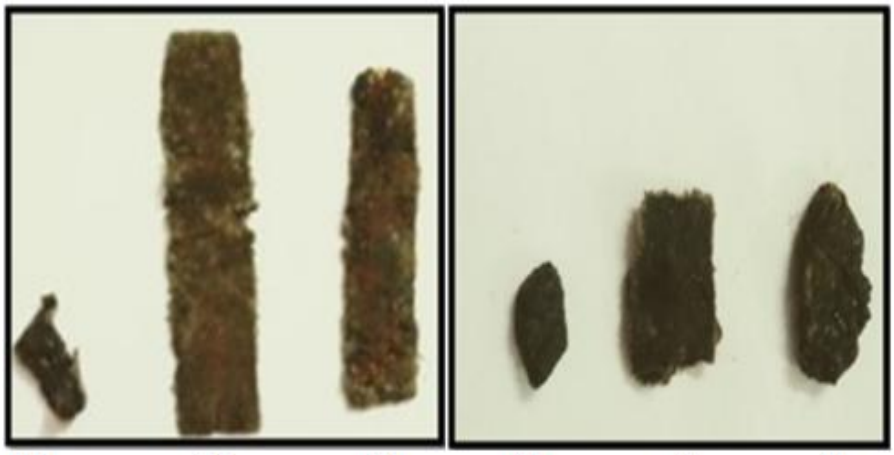
Table.7 Weight loss in gelatin/sericin and gelatin/sericin/clay films

Time	sample a	sample b	sample c
Day 1	0.29	0.46	0.59
Day 3	0.67	0.99	1.17
Day 5	0.90	1.27	1.62
Day 7	0.93	1.45	1.77
Day 9	0.56	1.48	0.41
Day 11	0.26	1.24	0.33
Day 13	0.06	1.05	0.19
Day 15	0.03	0.96	0.15
Day 17		0.62	0.05
Day 19		0.45	0.04
Day 21		0.21	
Day 23		0.09	
Day 25		0.04	



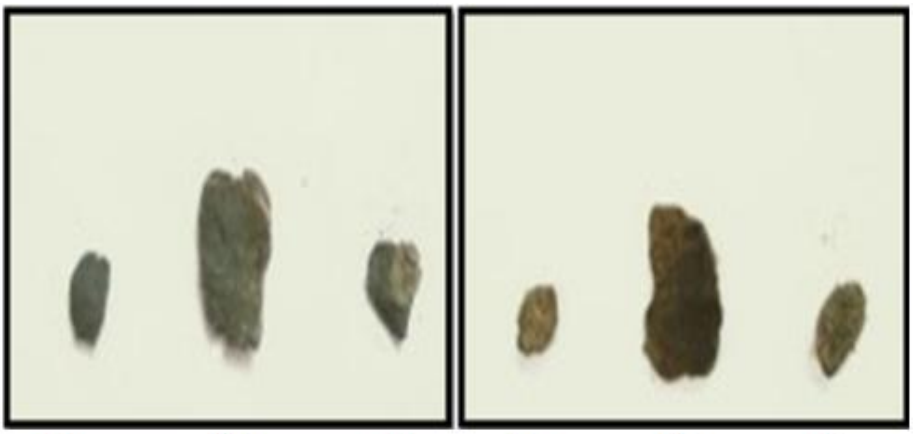
(a) (b) (c) (a) (b) (c)

Day 1 Day 3



(a) (b) (c) (a) (b) (c)

Day 5 Day 7



(a) (b) (c) (a) (b) (c)

Day 9 Day 11

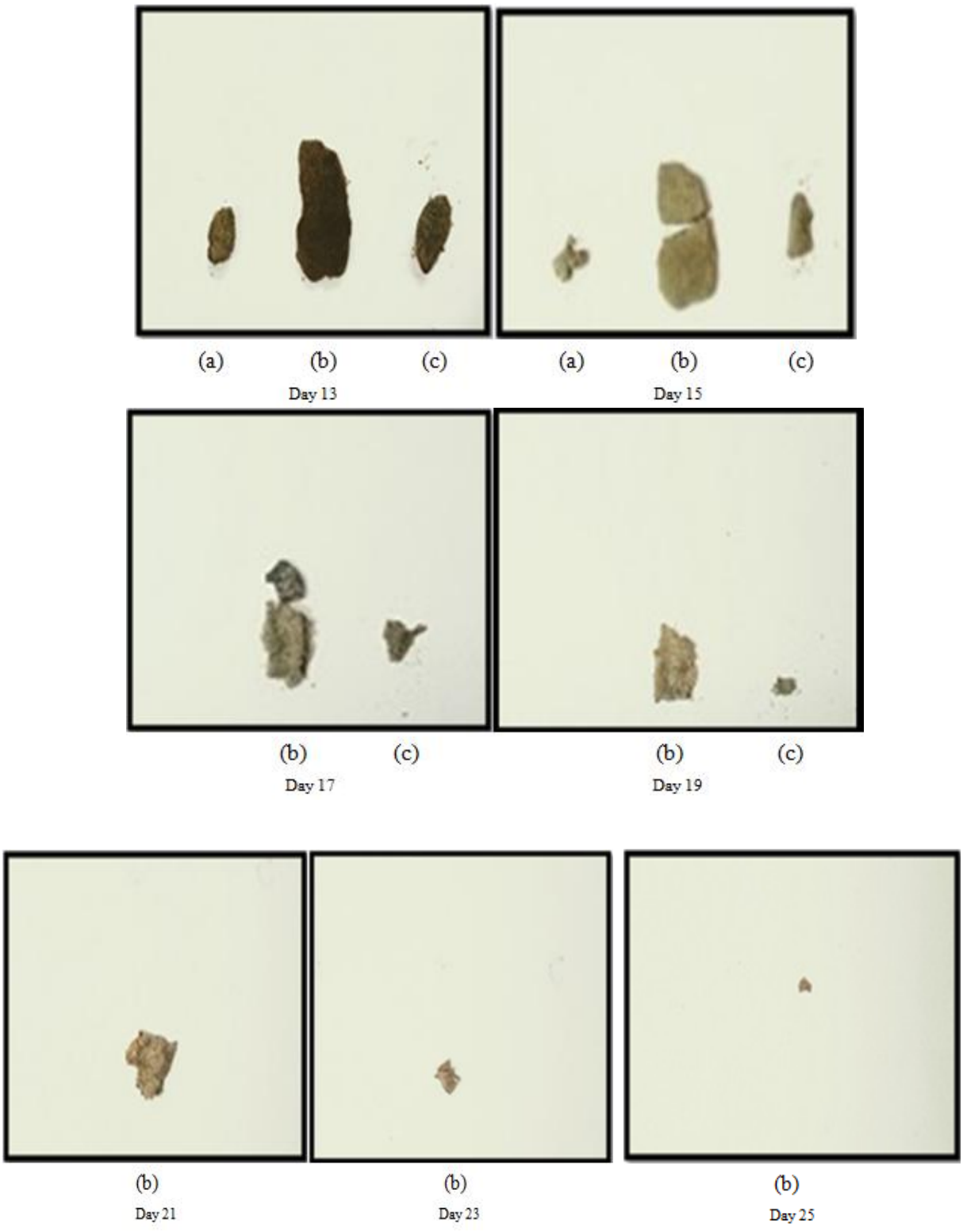


Fig.18 Degradation of various films (a) 75%GL/25%S (b) 75%GL/25%S/3%closite 30B (c) 75%GL/25%S/1%COMMT

4.5.Thermal analysis

Thermal properties of gelatin/sericin and crosslinked gelatin/sericin films were analysed by TGA technique. TGA is an analytical technique used to determine a material's thermal stability. Thermogravimetric analysis (TGA) of film samples shown in Figure.19.

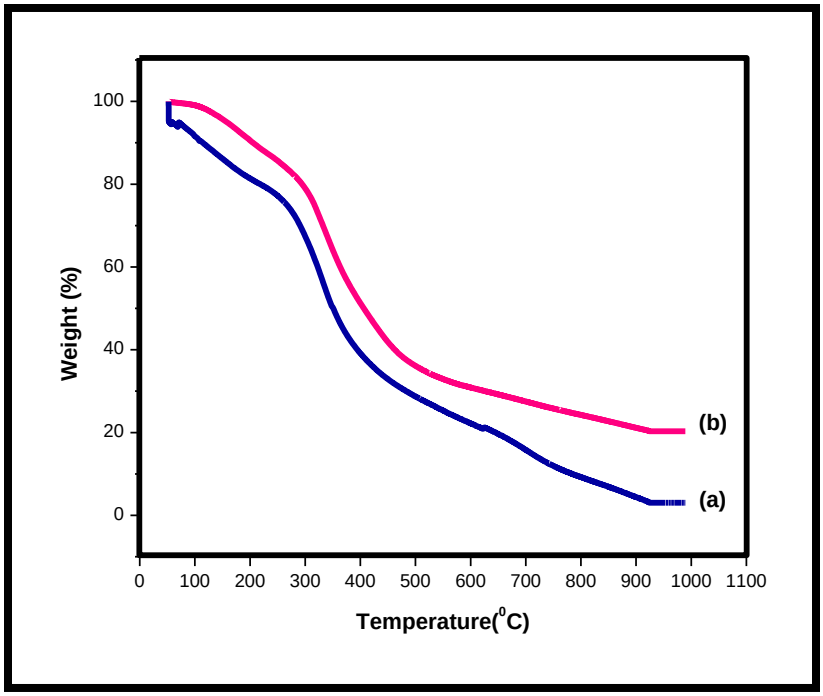


Fig.19 TGA Thermograms of (a) GL/S and (b) GL/S/GLU

thermograms of gelatin/sericin and gelatin/sericin/glutaraldehyde film presents multistep degrdation. initial weight loss, which is up to 100^0C, is due to the some moisture content in film samples. The initial degradation temperature of gelatin/sericin film sample is 280^0C and it is completely degraded at 900^0C with 99% weight loss. figure.19(b) shows the thermogram of gelatin/sericin/glutaraldehyde film. Moisture content is responsible for the initial weight loss up to 100^0C. it was observed that initial degradation temperature of gelatin/sericin/glutaraldehyde is

310^0C and total weight loss is 80% at 900^0C. this prooves that glutaraldehyde is chemically crosslinked with gelatin and sericin.

4.6.FT-IR Analysis

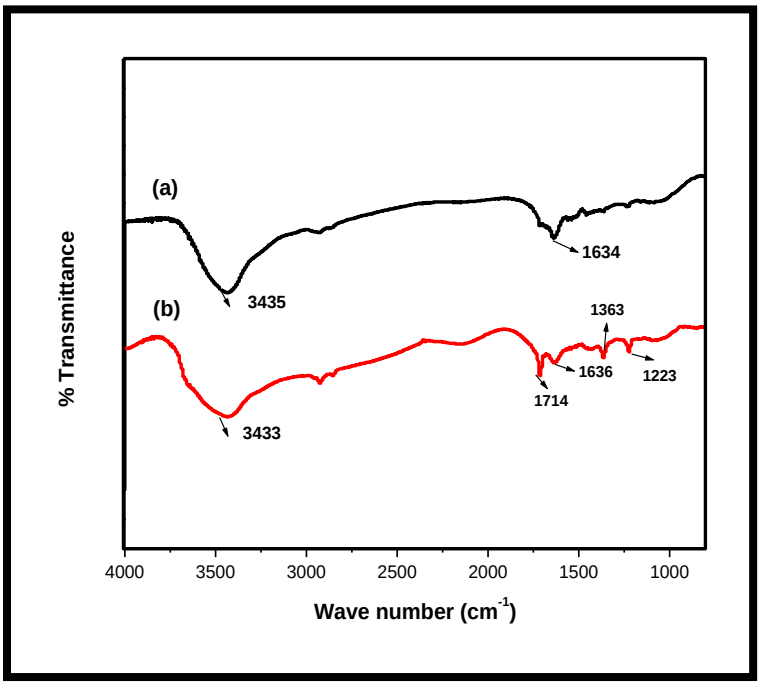


Fig.20 FTIR spectra of various films; (a) S/GL/GLU (b) S/GL

The structural properties of the films were analysed by fourier transform infrared spectroscopy. the FTIR of the films are shown in figure.20. figure 20(b) shows the FTIR spectra of sericin/gelatin blend. Peak at 3433 cm^{-1} and 1714 cm^{-1} assigned for N-H streaching vibrations and C = O stretch of COOH respectively. Peak at 1636 cm^{-1} represents the amide I (C = O) stretching vibrations. While peak at 1363 cm^{-1} for C – H bending and peak at 1223 cm^{-1} shows the amide III band. Figure 20 (a) shows the FTIR of crosslinked gelatin/sericin film. Glutaraldehyde undergoes a ***Schiff's base*** reaction with the amino groups of both proteins which are present in gelatin as well as in seicin. In ***Schiff's base*** reaction imine is formed which

represent by the peak at 1634 cm^{-1} [122] but due to some unreacted amino groups peaks at 3436 cm^{-1} is observed.

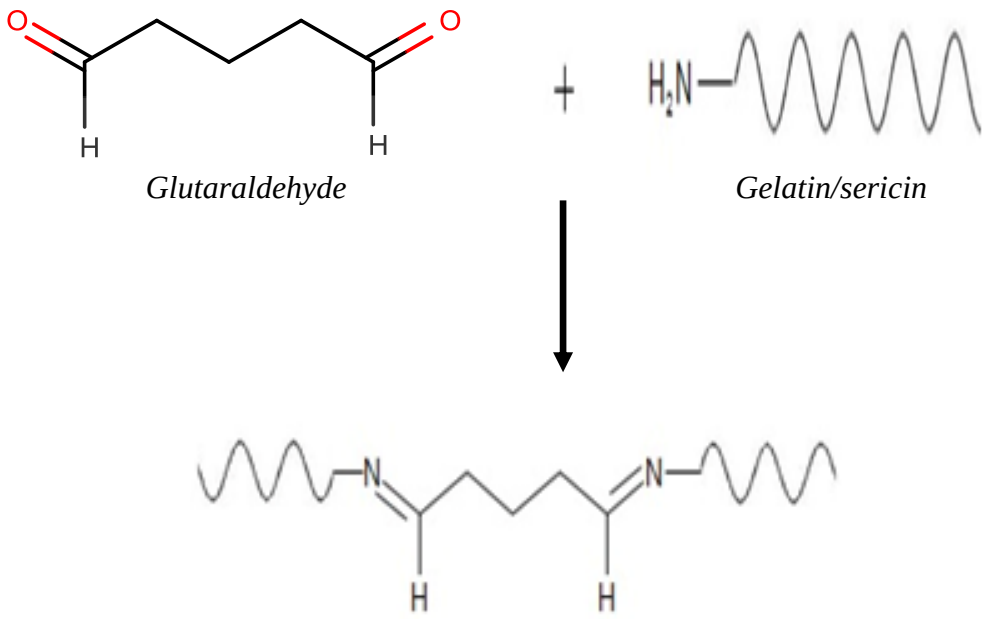


Fig.21 Praposed reaction between gelatin sericin and glutaraldehyde

4.7. Antibacterial Testing

Antibacterial activity of films determine against E.Coli bacteria on the basis of minimum bactericidal concentration (MBC).on the basis of no of colonies growth, percentage of inhibition was calculated. Divya et al.[123] prooves sericin have excellent antibacterial properties that is why it is used for wound healing purpose and biomedical applications. Savitha et al.[124] says that sericin is a promising futuristic antibacterial protein. so that gelatin/sericin composite which is considered as controlled sample, shows antibacterial action and due to insertion of antibacterial agent (closite 30B and copper modified NaMMT) antibacterial property of films is enhanced.

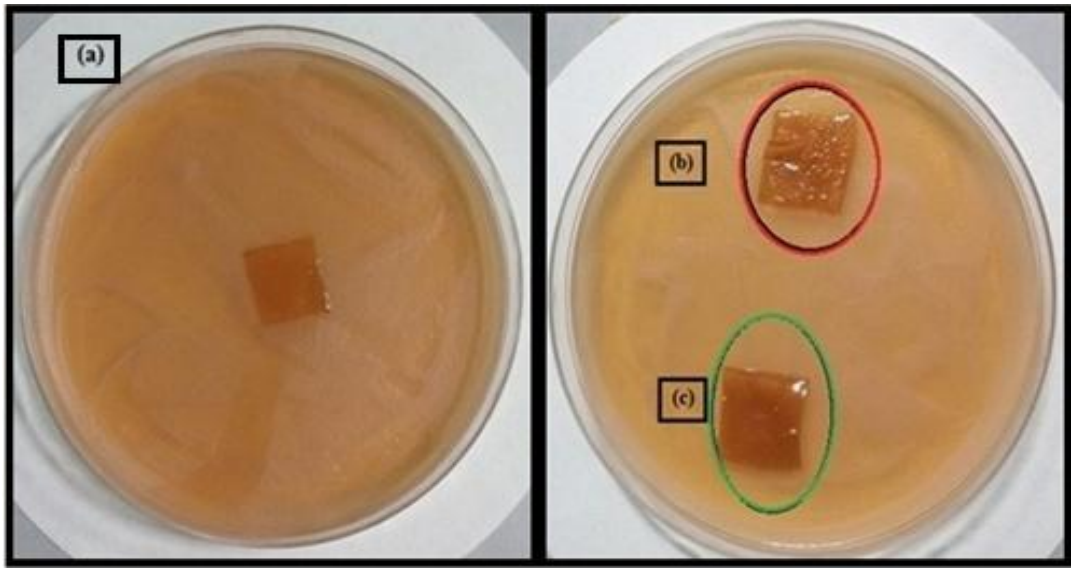


Fig.22 shows antibacterial testing of various gelatin/sericin and gelatin/sericin/clay blend films (a)75%GL/25%S, (b)75%GL/25%S/1%COMMT, (c)75%GL/25%S/3%closite30B

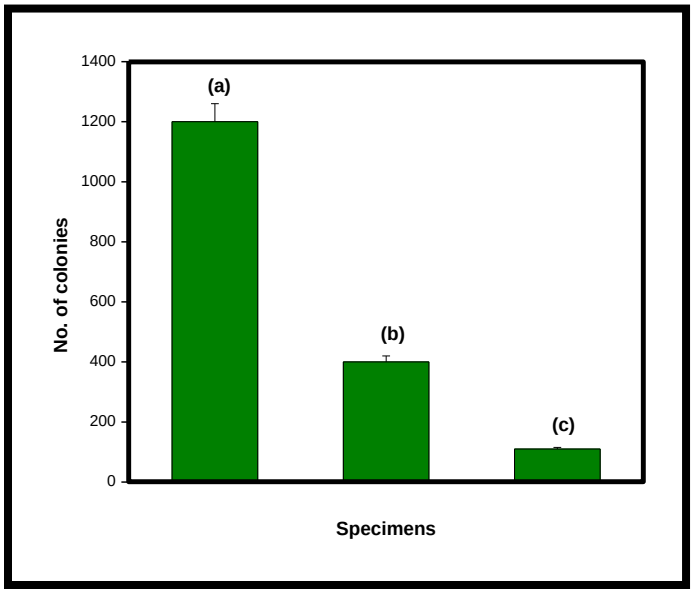


Fig.23 shows growth of colonies on film surface after serial dilution (a)75%GL/25%S, (b)75%GL/25%S/1%COMMT, (c) 75%GL/25%S/3%closite 30B

Medical applications of montmorillonite include the treatment of irritable bowel syndrome, prevention of constipation and aflatoxin. It also inhibits the intestinal adsorption of cholesterol

[125]. Abreu et al. [126] described that closite 30B shows its antimicrobial effect against bacteria due to release of ammonium salts from the nanoclay which affects the bacteria sensitive portions while copper Modified clay shows its toxic effect against bacteria due to the copper ion [127].copper ion entered in to cell of bacteria and obstruct the metabolic activity. Antibacterial films can easily used for food packaging purpose. Figure.23 shows that 3% closite 30B content reduce the growth of bacteria by 90.8 % while 1% COMMT reduce the growth of bacteria by 67%. sample which do not contain clay considered as control sample so that reduction in bacterial growth measured on the basis of it.

4.8.X-ray Diffraction(XRD)

The crystallographic analysis of gelatin/sericin films were analysed by X-ray diffraction.

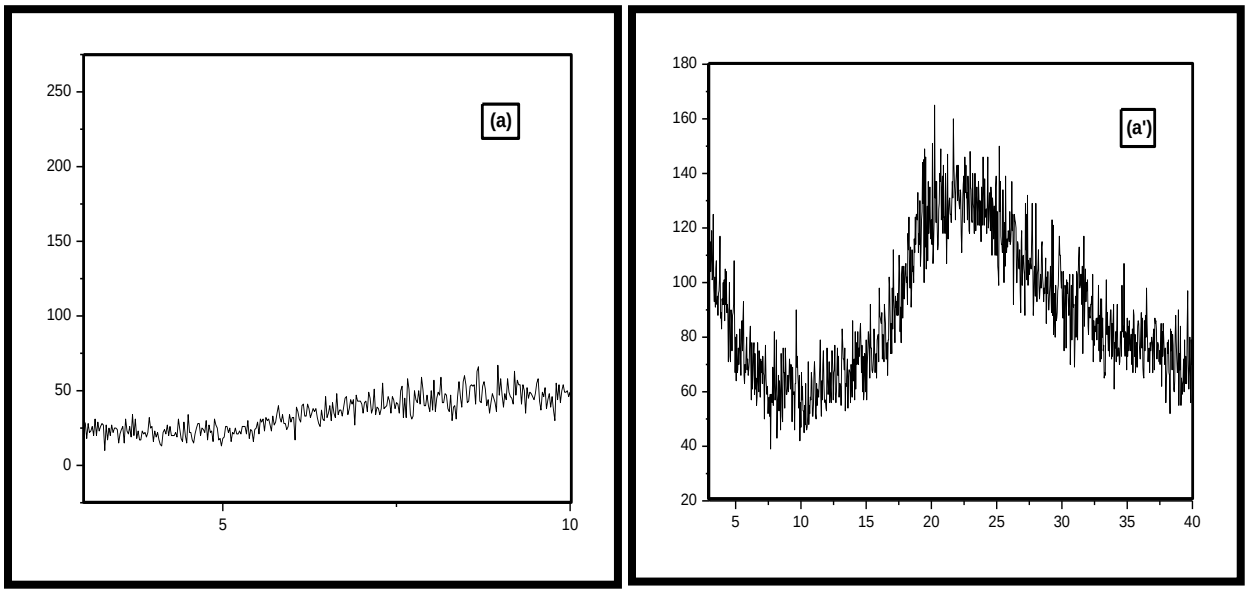


Fig.24 (a) and (a') shows X- ray diffraction of crosslinked plasticized gelatin/sericin film in range of 3 to 10^0 and 3 to 40^0 respectively

Figure.24 shows the XRD diffractogram of cross linked plasticized gelatin/sericin film in the range of 3 to 10^0 and 3 to 40^0 respectively. pure sericin shows a diffraction peak at near $2\theta = 20^0$ and shoulder peak at near $2\theta = 12^0, 28^0$ and 43^0, observed diffraction indicating that the high molecular weight of sericin and β-sheet structure in sericin [128]. similarly gelatin also shows sharp peak at $2\theta = 20^0$ due to its crystalline structure [129]. (Nair et al. 2015)[130] was observed peak at $2\theta = 20^0$ of gelatin and sericin blend. on the addition of crosslinking agent peak at $2\theta = 20^0$ is completely dimnises and broder peak at $2\theta = 22.5^0$ is observed (fig.24 (a), 24(a')) which leads to the decrease in crystallinity.

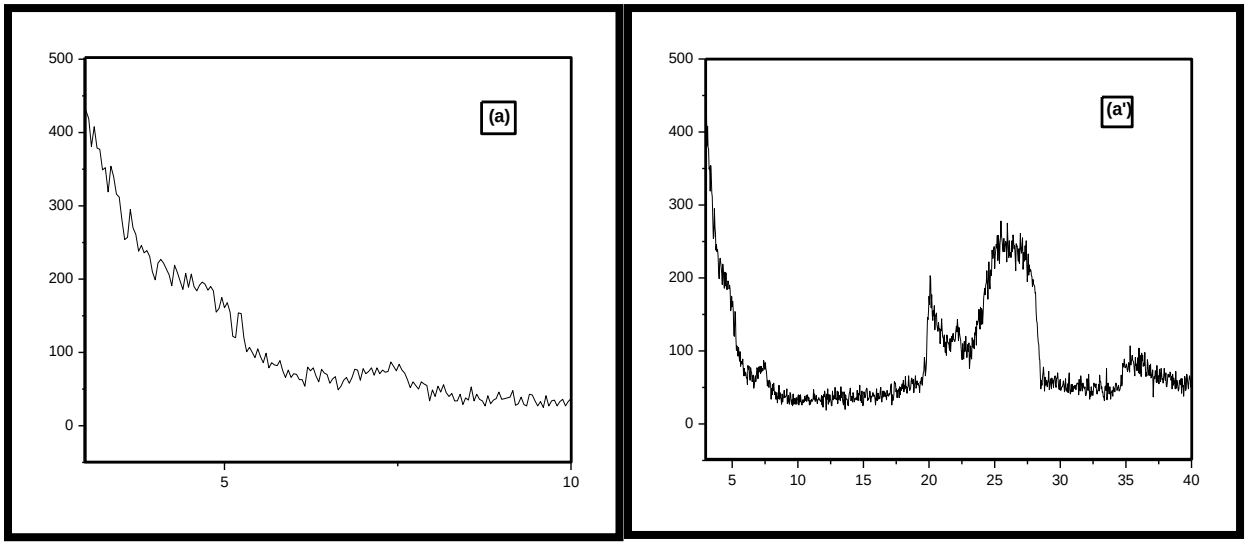


Fig.25 (a) and (a') shows X- ray diffraction of NaMMT in range of 3 to 10^0 and 3 to 40^0 respectively

Figure. 25(a),25(a') and 25(b),25(b') shows the X-ray diffractogram of NaMMT and copper modified NaMMT in the range 3-10^0 and 3-40^0 respectively. The bassal spacing of Na MMT and copper modified NaMMT are shown in table.8. X-Ray diffractograms of NaMMT and COMMT exhibits an increase in d spacing compared to the original unmodified clay that is NaMMT indicated that clay particles completely entered in to polymer galleries. high intensity

reflections for copper modified NaMMT confirms a high degree of order for a lamellar stacking of layers of the originally modified clays [131].

X-ray diffractogram of copper modified NaMMT (figure. 25 (b')) shows a diffraction peak at 2θ = 16.5^0, corresponding basal spacing 0.53 nm between clay platelets. Ahmes et al.[132] was observed closite 30B nanoclay shows chracteristic peak at 2θ = 4.62^0 and d spacing for nanoclay was 1.91nm.

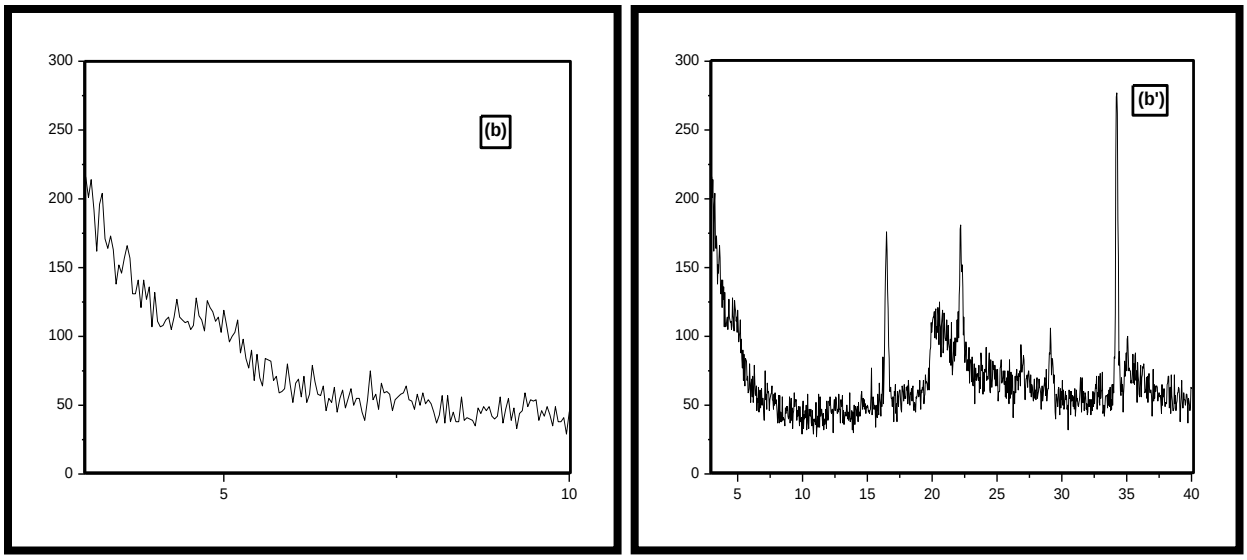


Fig. 25 (b) and (b') shows X- ray diffraction of copper modified NaMMT in range of 3 to 10^0 and 3 to 40^0 respectively

Table .8 final basal spacing recorded for NaMMT and Copper modified NaMMT

S.No.	Sample	Final basal spacing	
		nm	2θ
1.	**NaMMT**	**0.44**	**20**
2.	**COMMT**	**0.53**	**16.5**

Figure 26(a), 26(a') and 26(b), 26(b') show the X-ray diffractogram of gelatin/sericin/COMMT and gelatin/sericin/closite30B blend films in the range 3-10^0 and 3-40^0 respectively.

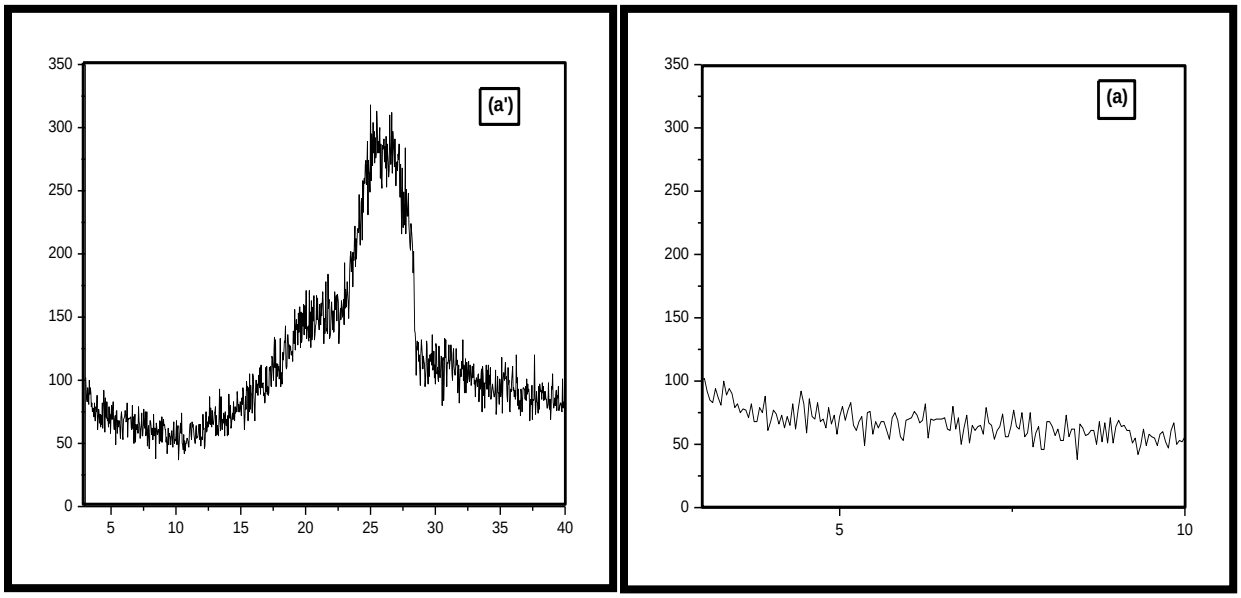


Fig. 26 (a) and (a') shows X- ray diffraction of gelatin/sericin/COMMT film in range of 3 to 10^0 and 3 to 40^0 respectively

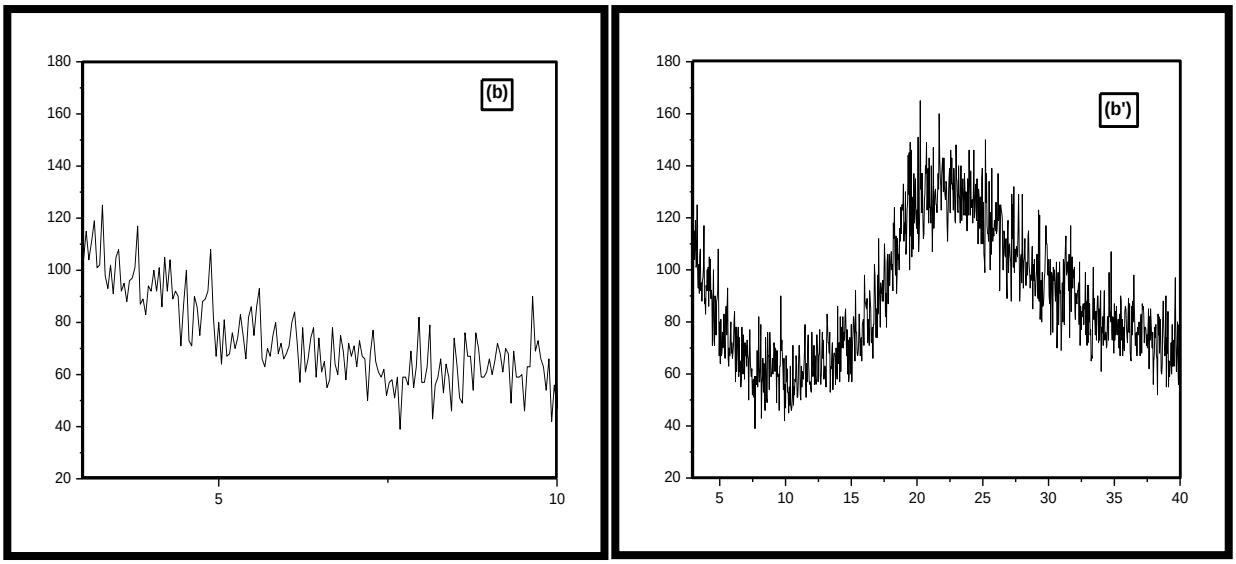


Fig. 26 (b) and (b') shows X- ray diffraction of gelatin/sericin/closite 30B film in range of 3 to 10^0 and 3 to 40^0 respectively

Both film do not show any peak in $3\text{-}10^0$ which leads to the exfoilation of clay in polymer matrix and gelatin/sericin/COMMT shows peak at $2\theta = 26.25^0$ while gelatin/sericin/closite 30B shows peak at $2\theta = 22.5^0$. the shift in the diffraction peak towards the higher side indicated amorphous structure.

4.9. Morphology

Surface morphology of crosslinked plsticized gelatin/sericin film and crosslinked gelatin/sericin/clay blend film are analysed by the scanning electron microscopy. Figure 27 show SEM images of film samples.

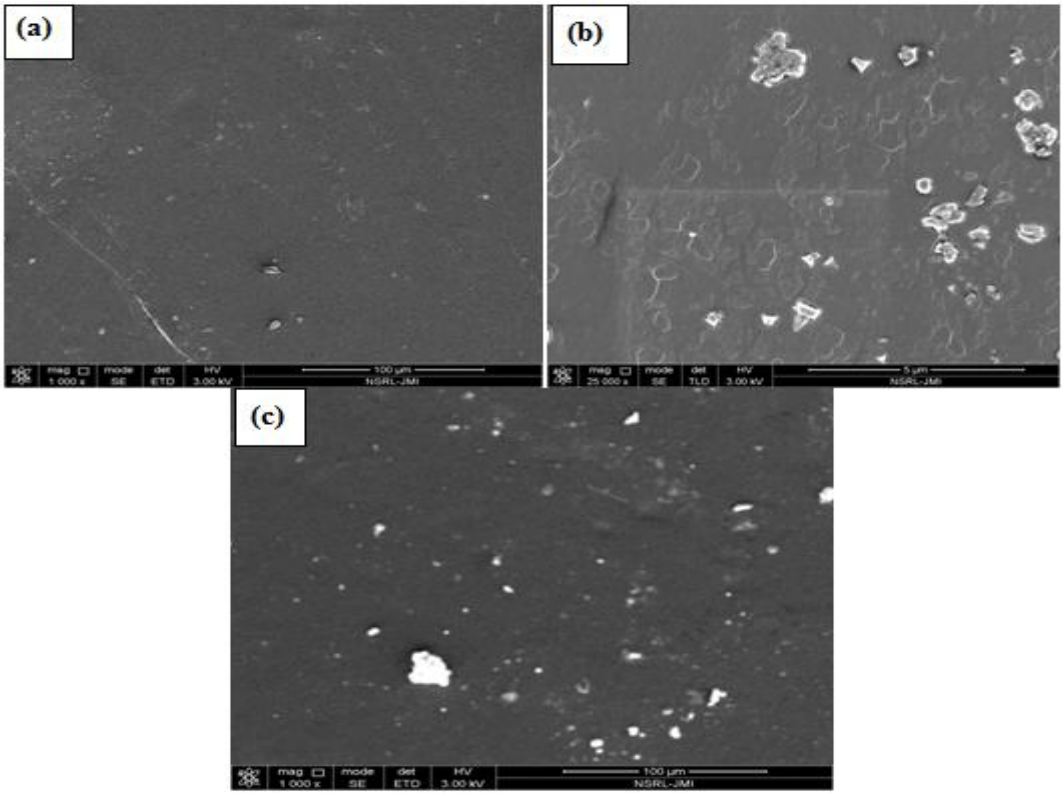


Fig.27 SEM images of various films (a) plasticized crosslinked gelatin/sericin film (b) plasticized crosslinked gelatin/sericin/closite 30B (c) plasticized crosslinked gelatin/sericin/COMMT

CONCLUSION

From this work we conclude that 3:1%(w/w) gelatin/sericin film shows higher mechanical strength incomparision to other concentrations of gelatin and sericin blend.closite 30B and copper modified NaMMT clays are added as antimicrobial as well as reinforcing material so that 3 % (w/w) of closite 30B concentration in 3:1 % (w/w) gelatin/sericin combination shows higher mechanical strength similarly 1 %(w/w) COMMT concentration in same combination shows enogh mechanical strength. all other properties are determine for this concentration of clays and compared with respect to 3:1%(w/w) gelatin/sericin film and we get excellent results shwon below:

1] water vapor transmission results

i. Sample (a) 75%GL/25%S – 102.94 g/hr/m^2
ii. Sample (b) 75%GL/25%S/1%COMMT – 68.65 g/hr/m^2
iii. Sample (c) 75%GL/25%S/3%closite 30B – 53.60 g/hr/m^2

2] Swelling behaviour results

i. Sample (a) 75%GL/25%S – 250% (swelling %)
ii. Sample (b) 75%GL/25%S/1%COMMT – 373% (swelling %)
iii. Sample (c) 75%GL/25%S/3%closite 30B – 636.93% (swelling %)

3] Biodegradability test in soil

i. Sample (a) 75%GL/25%S – total degradation time is 17 days
ii. Sample (b) 75%GL/25%S/3%closite 30B – total degradation time is 27 days
iii. Sample (c) 75%GL/25%S/1%COMMT – total degradation time is 21 days

4] Anti-Bacterial testing

i. Sample (a) 75%GL/25%S – 1200 colonies (control sample)

ii. Sample (b) 75%GL/25%S/1%COMMT – 400 colonies (67% inhibition with respect to control sample)

iii. Sample (c) 75%GL/25%S/3%closite 30B –110 colonies (90.83 % inhibition with respect to control sample)

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