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Efficacy of biogenic film from bioactive waste of Agaricus Bisporous

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ABSTRACT:

This study investigates the development of chitosan/polyvinyl alcohol (PVA) films incorporated with mushroom extract and ascorbic acid (Vitamin C) for enhanced mechanical, antioxidant, and antimicrobial properties. Chitosan, a biodegradable biopolymer, was blended with PVA to form a stable, flexible film matrix, while mushroom extract, rich in bioactive compounds, and ascorbic acid were added to improve the films' functionality. The films were characterized using various techniques, including X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA), to assess their structural, chemical, and thermal properties. XRD analysis revealed the crystalline and amorphous nature of the films, with changes observed upon the incorporation of mushroom extract and ascorbic acid. FTIR spectra confirmed the successful incorporation of the bioactive compounds, with characteristic peaks indicating the interaction between chitosan, PVA, mushroom extract, and ascorbic acid. SEM images showed the surface morphology and homogeneity of the films, revealing a smooth surface and uniform distribution of the additives. TGA analysis provided insight into the thermal stability, demonstrating that the films exhibited improved thermal resistance with the addition of mushroom extract and ascorbic acid. Building on these results, the chitosan/PVA films by varying mushroom extract and ascorbic acid concentrations.

KEYWORDS: Bioactive film, Crosslinking, Thermal stability, Film functionality

INTRODUCTION:

The development of biodegradable polymer films has gained significant attention due to the growing need for sustainable alternatives to conventional materials. A key area of research involves creating polymeric composite films that integrate both natural and synthetic polymers. These composite films are particularly valuable in various industries, offering enhanced mechanical properties, such as increased strength and reduced weight, along with improved functionality. Additionally, they exhibit effective antibacterial activity, which makes them ideal for applications aimed at preserving product integrity and extending shelf life. Among the various polymers utilized for this purpose, Polyvinyl Alcohol (PVA) is a standout material. PVA is a colorless, water-soluble, non-toxic, and biocompatible polymer known for its excellent film-forming properties, high optical transparency, and remarkable thermal stability. As a semi-crystalline material, PVA is easy to process and is biodegradable, making it a prime candidate for environmentally friendly alternatives in a wide range of applications, such as medical devices, agricultural films, and biodegradable coatings.[1]

Chitosan, another key biopolymer, is derived from chitin through deacetylation, which is primarily sourced from the shells of crustaceans such as shrimp, crabs, and lobsters. Chitosan is a polysaccharide that possesses inherent antimicrobial properties, which make it effective in preventing microbial growth. These properties are particularly beneficial in sectors like food preservation, wound care, and drug delivery. The degree of deacetylation and the molecular weight of chitosan influence its characteristics and suitability for various uses. In biomedical and agricultural applications, chitosan-based films are advantageous because they provide antimicrobial activity while also contributing to the mechanical strength and flexibility of the film. However, chitosan alone has limitations, such as water solubility and mechanical weakness. To overcome these drawbacks, it is often combined with other polymers, such as PVA, to create a more durable and functional composite material.[2]

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The main objectives of this research were to explore how the incorporation of ascorbic acid into chitosan/PVA films influences their structural integrity Specifically, the study aimed to:

- Synthesize PVA/chitosan films integrated with ascorbic acid using the solution casting method.
- Investigate the chemical composition of the films by analyzing the functional groups using FTIR spectroscopy.
- Analyze the surface morphology and structural uniformity of the films using SEM.
- The Decomposition of material were done by TGA analysis.

METHODOLOGY:

2.1 MATERIALS:

The materials employed in this study include fungal chitosan, polyvinyl alcohol (PVA), and ascorbic acid, each selected for their specific properties and compatibility in the preparation of mushroom chitosan-based PVA films.

2.1.1 Polyvinyl Alcohol (PVA)

PVA is a synthetic polymer derived from the polymerization of vinyl acetate, which is hydrolyzed to form PVA. The degree of hydrolysis influences its properties, making it a versatile material with a wide range of applications. PVA is water-soluble, non-toxic, and has excellent film-forming, adhesive, and emulsifying properties. It is also resistant to oils, solvents, and grease, making it suitable for preparing hydrophilic membranes with good mechanical properties. Additionally, PVA exhibits transparency, softness, and flexibility, with a high strength-to-weight ratio, which enhances its application in various industries, including as a binding agent in composite films.

2.1.2 Chitin and Chitosan

Chitin is a naturally occurring polysaccharide found abundantly in the exoskeletons of arthropods and in fungal cell walls. Chitosan, derived from chitin through deacetylation, is soluble in acidic solutions and possesses antimicrobial properties. It is biocompatible, biodegradable, and non-toxic, making it ideal for food preservation and medical applications. Chitosan-based films offer low oxygen permeability, which is critical for preserving food, and have moderate water vapor barrier properties. Blending chitosan with other hydrocolloids, such as PVA, improves the mechanical strength and barrier properties of the resulting films.

2.1.3 Ascorbic Acid

Ascorbic acid, commonly known as Vitamin C, is a water-soluble antioxidant that plays an essential role in neutralizing free radicals, which can damage cells. It aids in collagen synthesis, promoting wound healing, and has been shown to have protective effects against oxidative stress. Derived from citrus fruits and various vegetables, ascorbic acid also contributes to food preservation by improving the stability and shelf life of food products. In this study, ascorbic acid is incorporated into the composite films to enhance their antioxidant and antimicrobial properties.[3]

2.2 METHODS:

2.2.1 Preparation of Chitosan:

The materials required for the preparation of mushroom chitosan include mushroom biomass, such as the fruiting bodies or mycelium of edible varieties like *Pleurotus ostreatus* or *Agaricus bisporus*. Sodium hydroxide (NaOH) is used for the deproteinization step, while ascorbic acid or hydrochloric acid (HCl) serves for demineralization to remove inorganic salts. Ethanol and acetone are needed for washing and drying the extracted chitin, and deionized water is used throughout the process for rinsing and purification. Finally, calcium hydroxide ($Ca(OH)_2$) is used in the deacetylation step to convert chitin into chitosan. These materials are essential for efficiently extracting and processing chitosan from mushroom sources.

STEP 1: Collection and Drying: Dry mushrooms (e.g., *Pleurotus ostreatus*, *Agaricus bisporus*) to remove moisture at 50–60°C.

STEP 2: Demineralization: Soak dried mushrooms in 0.1 M HCl or ascorbic acid for 2–3 hours to remove minerals (calcium, magnesium). Rinse with deionized water.

STEP 3: Deproteinization: Treat the demineralized biomass with 5-10% NaOH at 80–90°C for 1–2 hours to remove proteins. Rinse with water.

STEP 4: Chitin Extraction: After demineralization and deproteinization, wash the biomass with ethanol or acetone to purify chitin.

STEP 5: Deacetylation (Chitosan Formation): Immerse chitin in 40–50% $Ca(OH)_2$ at 90°C for 2–4 hours to remove acetyl groups, forming chitosan. Rinse with water.

STEP 6: Drying and Powdering: Dry the chitosan (air, vacuum, or oven at <50°C) and grind into a fine powder.

Chitosan purity can be adjusted based on the deacetylation process.[4]

2.2.2 PVA/ Chitosan/AA Film Preparation:

Chitosan-based PVA films were prepared using a chemical casting method, ideal for producing thin polymer films. To begin, a 1% PVA solution was made by dissolving PVA in deionized water at 90°C with stirring until clear. A 1% chitosan solution was prepared by dissolving chitosan in ascorbic acid at room temperature with constant stirring. The PVA and chitosan solutions were mixed, with ascorbic acid acting as a crosslinker, and stirred for 1 hour to complete the crosslinking reaction.

The resulting viscous solution was cast onto a Petri dish and air-dried, with film thickness controlled by solution volume. After drying, the films were peeled off and preconditioned at a constant temperature for testing. The film's formation involved hydrogen bonding and ionic cross-linking between chitosan's amino groups and PVA's hydroxyl groups.[5]

2.2.3 ASCORBIC ACID:

To prepare ascorbic acid from strawberries using the maceration method, the materials required include fresh, ripe strawberries (preferably organic), distilled water, a blender, a clean glass jar or beaker, and a fine mesh strainer or cheesecloth for filtering. A knife and cutting board are also needed to hull the strawberries, removing the leaves and stems. The process involves blending the strawberries to create a puree, which helps release the ascorbic acid into the solvent (distilled water) during maceration. The mixture is then strained to extract the vitamin C-rich liquid.

STEP 1: Prepare Strawberries: Wash, hull, and remove the stems from the strawberries.

STEP 2: Blend: Puree the strawberries in a blender to increase surface area.

STEP 3: Macerate: Place the pureed strawberries in a glass jar, add enough distilled water to cover them, seal the jar, and let it sit in a cool, dark place for 6–24 hours.

STEP 4: Strain: After maceration, strain the mixture using a fine mesh strainer or cheesecloth to separate the liquid extract.

STEP 5: Store: Store the liquid in an airtight container in the refrigerator to preserve the ascorbic acid (vitamin C).

This method extracts the vitamin C, which is sensitive to heat, light, and air, so proper storage is essential.[6]

2.3 CHARACTERIZATION:

2.3.1 Fourier transform infrared (FTIR) analysis:

Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds and functional groups in a material by measuring its infrared absorption spectrum, where molecules absorb specific wavelengths corresponding to their bond vibrations.

2.3.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) uses a focused electron beam to capture high-resolution images of material surfaces, structure, and composition at the nanometer scale.

2.3.3 X-ray Diffraction (XRD)

X-ray Diffraction (XRD) is a technique used to analyze the structure, phase composition, and properties of crystalline materials by measuring X-ray diffraction patterns. It is widely used in materials science, chemistry, and geology**.**

2.3.3Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) measures mass changes in a sample with temperature or time, under a controlled atmosphere. It is used to study thermal stability, decomposition, moisture content, and material composition**.[9]**

RESULT AND DISCUSSON:

The characterization of mushroom chitosan PVA film was successfully analysed and the result is given below.

3.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS:

FTIR spectroscopy was used to determine the functional groups and also to confirm the successful formation of the PVA film from mushroom chitosan. The observed peaks and their interpretation as follows :

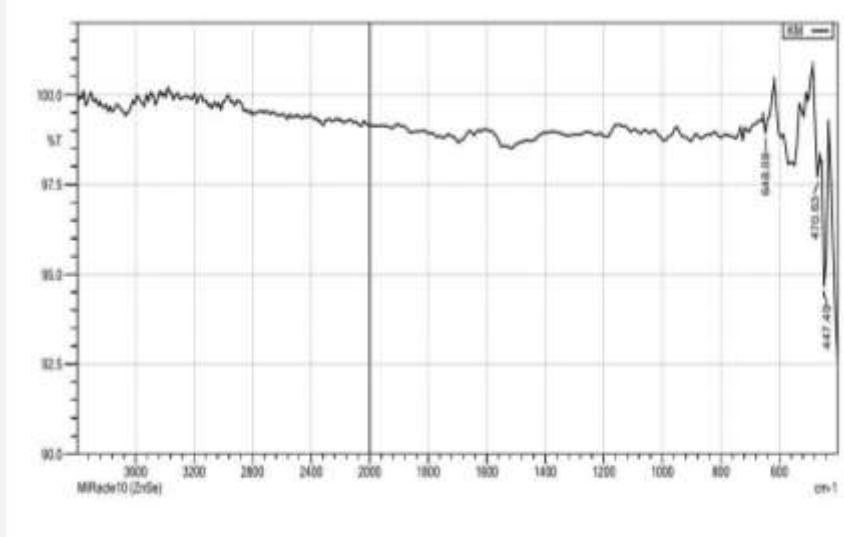


Figure 3.1 FTIR analysis

From the figure 3.1, the peak 648.08 cm^{-1} indicates the C-H bending indicating the presence of aromatic. The peak 470.63 cm^{-1} indicates the metal-halide stretching (M-X), such as C-Cl or C-Br stretching, particularly organometallic compounds or halogenated compounds. The peak 447.49 cm^{-1} indicates M-O stretching (metal-oxygen) commonly seen in inorganic compounds containing metal oxygen bond. These peaks suggest the presence of halogenated organic compounds (like chlorinated compounds) or organometallic compounds, potentially involving M-Cl bonds, or C-H bending in aromatic systems. The FTIR result confirms that the PVA or chitosan chains have aromatic components like contamination or aromatic cross-linkers used in film formation. PVA backbone which also contains alkene-like structures or even any aromatic impurities.

4.2 SCANNING ELECTRON MICROSCOPY (SEM) WITH EDAX:

Scanning Electron Microscopy (SEM) provides high resolution surface image, which also gives information about size, morphology, surface features.

4.2.1 SEM Analysis of Mushroom Chitosan PVA Film:

The chitosan has the network like structure which has a rough surface with micro or nano sized pores, due to its dense network they cause some wrinkling or non-uniformity with PVA. The PVA film is generally smooth and homogeneous at the microlevel which provides an improving mechanical properties. The mushroom derived chitosan has a irregular surface structure influence in textured surface. The cross linking agent ascorbic acid lead to rougher or more rigid surface structure.

4.2.2 EDAX Analysis of Mushroom Chitosan PVA Film:

(i) Composition: The EDAX spectrum confirms the presence of carbon (C) and oxygen (O) as primary element along with nitrogen (N) and sodium (Na) from chitosan.

(ii) Elemental Ratios: The atomic ratios of the elements are given in different peaks

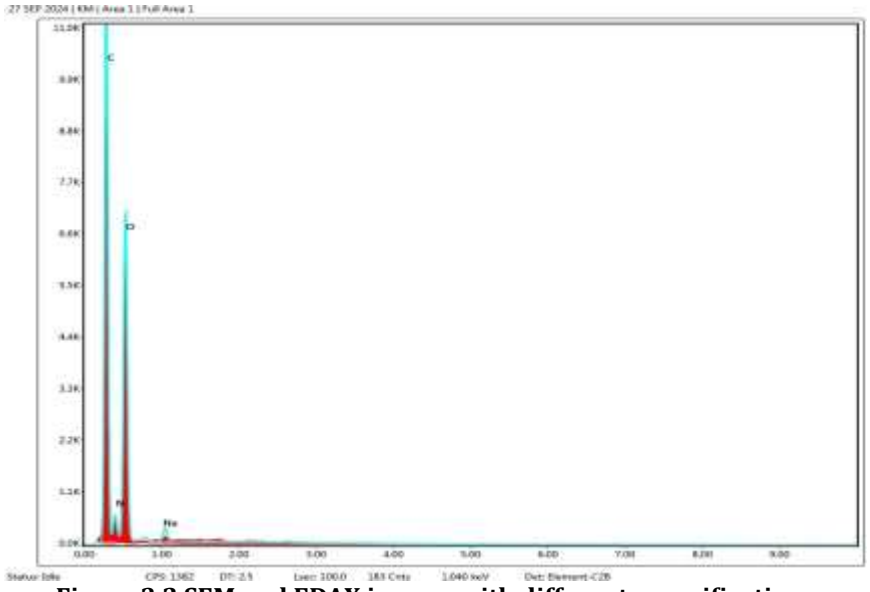
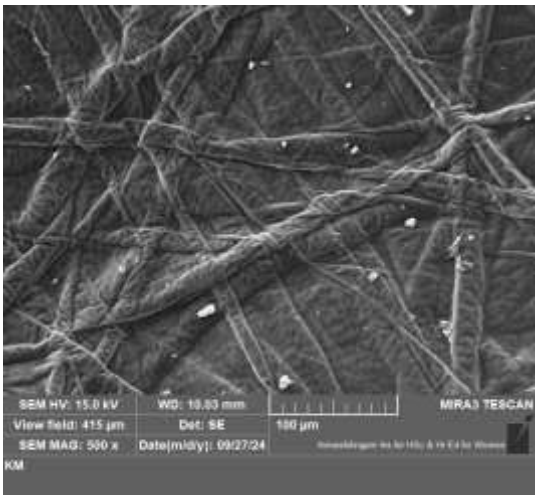
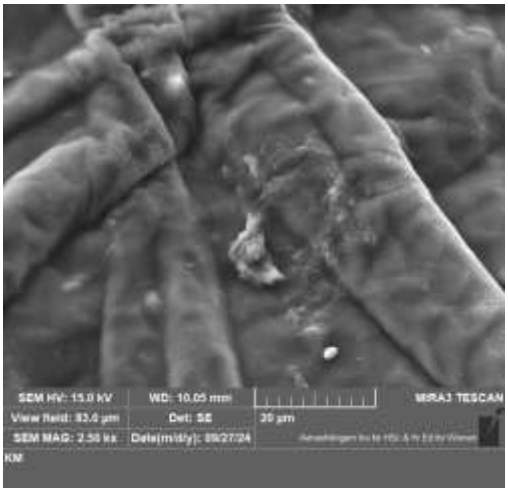
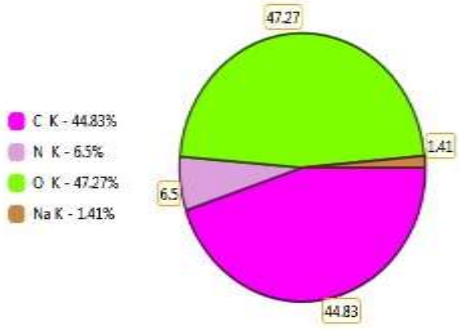


Figure 3.2 SEM and EDAX images with different magnifications





Element	Weight%	Atomic%	Error%
C K	44.83	51.75	5.31
N K	6.50	6.43	16.73
O K	47.27	40.27	9.31
Na K	1.41	0.85	11.18



3.3 X- RAY DIFFRACTION Analysis:

The result for X-Ray Diffraction (XRD) analysis of mushroom chitosan PVA film focuses on the peak identification, crystalline size calculation (Scherrer Equation), Lattice Parameter Calculation, Strain and Defects and crystallinity estimate the degree of crystallinity

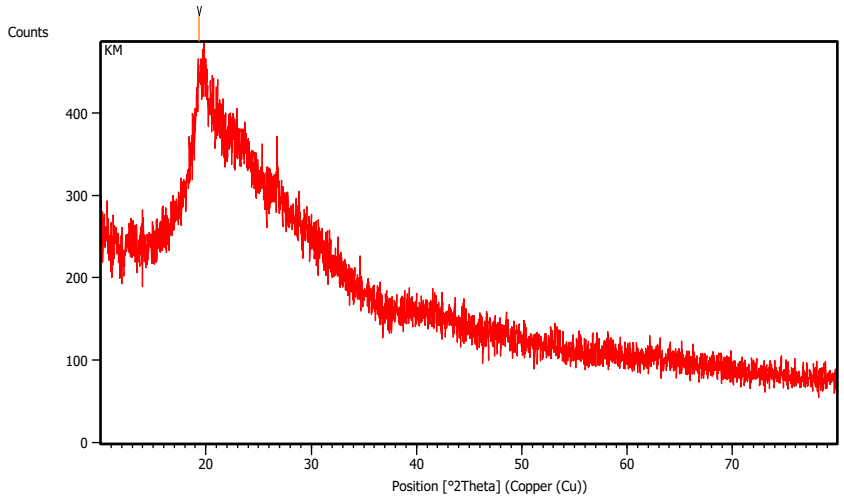


Figure 3.3 XRD Main Graphics, Analyze View

Table 3.1 Calculation of Crystallite Size, Micro strain, Lattice parameters

2θ	FWHM	d-spacing [Å]	D	Delta	Micro strain	Lattice parameter	Relative intensity
19.3275	1.1424	4.58877	70.4	0.00949	0	7.95	100

(i) Peak Position (2θ):
The characterization peak was observed at: 19.3275
(ii) Crystallite Size:
The crystallite size in chitosan is of the range 70.4 nm
(iii) Structure: The peak 19.3275 indicates the semicrystalline structure due to hydrogen bonds between -OH groups of PVA backbone. These peaks were observed in XRD pattern of AA-crosslinked CS/PVA but the intensity of these peaks was decreased because of the reduction in crystallinity because of the reduction of the H-bonding between polymer chain during crosslinking reaction.

4.4 Thermogravimetric (TGA) Analysis:
A TGA curve plots the mass loss of a sample as a function of temperature or time under controlled heating conditions and derive important information about the material's thermal stability, composition, and decomposition behaviour.

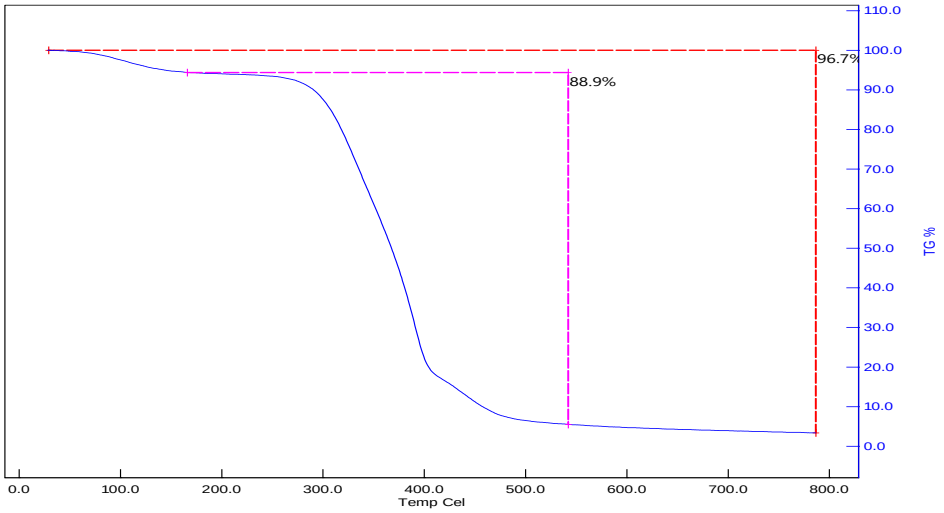


Figure 3.4 : TGA Graph

The material remains stable till 300° C and starts to decomposes above that temperature and TGA percentage was at 96.7% at the initial stage and upon increase in temperature the percentage was lowered to 88.9%.

CONCLUSION:

The PVA/chitosan composite films crosslinked with ascorbic acid demonstrated enhanced mechanical, antimicrobial, and antioxidant properties, making them ideal for sustainable applications. FTIR analysis confirmed successful crosslinking, revealing functional groups indicative of ascorbic acid incorporation. SEM images showed that chitosan provided a rough surface texture, while PVA remained smooth; the addition of ascorbic acid resulted in a more rigid surface, improving mechanical strength. XRD analysis indicated a semi-crystalline structure with a crystallite size of 70.4 nm. The crosslinking process reduced crystallinity by disrupting hydrogen bonding between polymer chains, contributing to greater flexibility. TGA results showed good thermal stability, with minimal mass loss up to 300°C, indicating that the composite films are suitable for use at higher temperatures. These findings suggest that the PVA/chitosan films with ascorbic acid are promising for biodegradable packaging, food preservation, and biomedical applications, offering both mechanical strength and antimicrobial properties. The study underscores the potential of ascorbic acid as an effective crosslinker to improve the functionality and sustainability of composite films, providing an eco-friendly alternative to conventional materials.

REFERENCE:

1. Dr. J. Johncy Caroline, Abinaya. A/Afr.J.Bio.Sc.6(8)(2024) ISSN: 2663-2187 . https:// doi.org/ 10.48047/ AFJBS. 6.8.2024.3186-3196
2. Srivastav, A., Mishra, S. S., Debnath, S., &Datta, D. (2018). Extraction and characterization of chitosan from waste scales of Labeorohita. Journal of Emerging Technologies and Innovative Research (JETIR), 5(6), 540-544.
3. Wu, Z., Wu, J., Peng, T., Li, Y., Lin,D., Xing(2017). Preparation and application of starch/polyvinyl alcohol/citric acid ternary blend antimicrobial functional food packaging films. Polymers, 9(3), 102.
4. Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., & Nava-Saucedo, J. E. (2008). Polymer biodegradation: Mechanisms and estimation techniques–A review. Chemosphere, 73(4), 429-442.
5. Willett, J. L. (1994). Mechanical properties of LDPE/granular starch composites. Journal of applied polymer science, 54(11), 1685-1695.
6. Vroman, I., &Tighzert, L. (2009). Biodegradable polymers. Materials, 2(2), 307-344.