SYNTHESIS AND CHARACTERIZATION OF BIO NANOCOMPOSITE FILMS FOR FOOD PACKAGING APPLICATION

By

Shermeen Tanveer

Zaheer Ahmed

Muhammad Haris Ansar

School of Chemical and Materials Engineering

National University of Sciences and Technology

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By

Leader – 285079- Shermeen Tanveer

Member 1 – 292062-Zaheer Ahmed

Member 2 – 287987- Muhammad Haris Ansar

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Supervisor: Dr. Zakir Hussain

Co-Supervisor: Dr. Usman Liaqat

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CERTIFICATE

This is to certify that Ms. Shermeen Tanveer, Mr. Zaheer Ahmed and Mr. Muhammad Haris Ansar have completed work in this thesis, under the supervision of Dr. Zakir Hussain and Dr.Usman Liaqat at the school of Chemical and Materials Engineering (SCME), National University of Science and Technology, H-12, Islamabad, Pakistan.

Advisor:

Dr. Zakir Hussain
Department of Materials Engineering
School of Chemical and Materials Engineering
National University of Sciences and Technology

Co-Advisor

Dr. Usman Liqat
Department of Materials Engineering
School of Chemical and Materials Engineering
National University of Sciences and Technology

Submitted Through:

15/6/23.

HOD **Dr. Khurram Yaqoob**

Department of Materials Engineering
School of Chemical and Materials Engineering
National University of Sciences and Technology

16-6-2023

Principal/Dean **Dr. Amir Azam Khan**
School of Chemical and Materials Engineering
National University of Sciences and Technology

DEDICATION

With profound reverence,

We dedicate this project to our beloved parents, supportive friends and, most of all, to our Supervisors, Teachers and Lab Engineers, who encouraged us to open our minds to scientific innovation.

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Praise be to Almighty Allah who made us capable to do our best in this research project.

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ABSTRACT

The increasing demand for sustainable and environmentally friendly packaging materials has driven extensive research in the field of biodegradable films for food packaging applications. This thesis presents a comprehensive investigation into the development and characterization of biodegradable food packaging films utilizing a blend of Polyvinyl Alcohol (PVA), Cellulose Nanocrystals (CNC), Zinc Oxide (ZnO), and Whitlockite. The primary objective of this study was to develop a packaging material that not only offers superior mechanical properties and barrier performance but also exhibits enhanced antimicrobial activity and biodegradability. The thesis systematically explores the influence of varying compositions and processing parameters on the physical, mechanical, and thermal properties of the resulting films. To achieve this, PVA was selected as the matrix material due to its excellent film-forming characteristics and biodegradability. The addition of CNC was aimed at improving the tensile strength by 22% and water vapor barrier properties of the films. ZnO nanoparticles were incorporated to enhance the antimicrobial activity and tensile strength up to 32% also ZnO act as a nano fertilizer which produces fertility when degrades, while Whitlockite decreases mechanical strength to 8%.It is a naturally derived substance known for its biodegradability, was used as a compatibilizer to enhance the overall film performance. The thesis presents a detailed investigation of the film fabrication process, including casting, drying, and crosslinking techniques. The resulting films were characterized using various analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Mechanical Testing. Additionally, the antimicrobial efficacy, water vapor transmission rate, and biodegradability of the films were evaluated.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Packaging is an essential part of our modern lifestyle, as it serves a crucial role in protecting, preserving, and storing food products. Packaging also plays a vital role in enhancing the shelf life and quality of food products. However, the use of traditional packaging materials, such as plastics, has raised concerns about environmental pollution and health hazards. It is reported that 40% of the plastic produced is utilized in packaging films. Hence, there is an urgent need to develop sustainable and eco-friendly packaging materials that can replace the conventional packaging materials. In this context, biodegradable and bio-based packaging materials have gained significant attention in recent years due to their environmental compatibility and sustainability.

1.1 BACKGROUND:

Food Packaging films have certain characteristics which enables them to store and preserve the food inside them. These properties can be divided into four categories; Mechanical, Physical, antibacterial, biodegradability and pertaining to fertility.

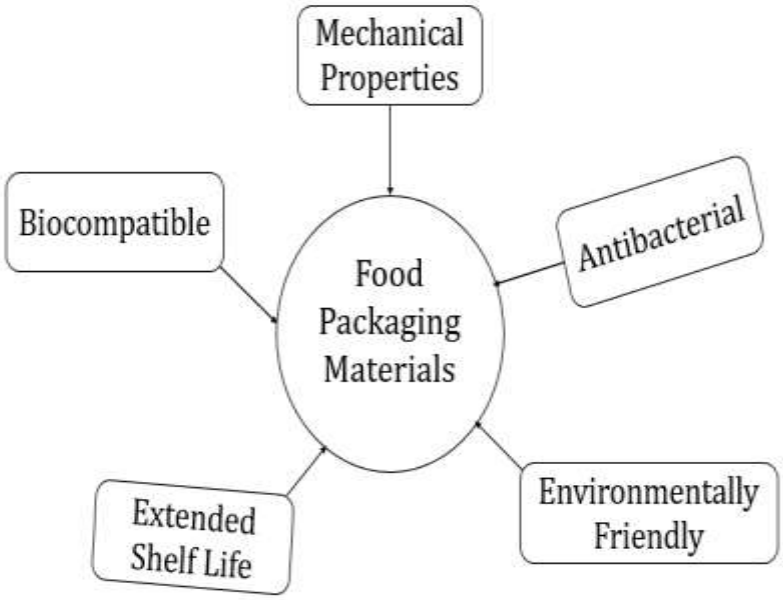

Figure 1: Food Packaging requirements

1.1.1 Mechanical Properties

Food packaging films need to have mechanical properties compare with commercially available films. Mechanical properties include tensile strength, flexural strength, load force and specific elongation. Good mechanical properties are those that ensure that any damage to the film does not expose the food to the external environment and the customer is able to receive a high quality product.

1.1.2 Physical Properties

a. Barrier Properties

These properties indicate how well a film can prevent external substances from entering the food if there is no breakage in the film itself. These properties include oxygen permeability, water permeability etc. Enhanced barrier properties are essential for food packaging films as they increase the shelf life of the food and protect it against faster degradability.

b. Water Uptake and Solubility

If a film is more water soluble, it is more likely to lose its structural integrity and damage its contents. A higher level of water uptake is desirable as that indicates that the film is able to uptake more water from the environments and automatically reduce the amount of water that permeates into the food.

c. Thermal Stability

We prefer that our food packaging films should be thermally stable in local environments e.g. Pakistan's temperatures cross 45 °C. Thermal stability requirements also depend on the application. For instance, if microwave noodles require a packaging, they must be heat resistant and not damage the packaging during heating.

1.1.3 Anti-bacterial Properties

This is an important introduction to food packaging films. Most often, this capability is checked against gram-negative and gram-positive strains of bacteria and it is observed how the bacteria is inhibited on the material. Inhibition of the bacteria can be done through penetration into its outer wall, after which ions from the material may enter and inhibit its growth enzymes. The bacteria's growth may be inhibited in a zone around the packaging material or only the contact points between the bacteria and the material.

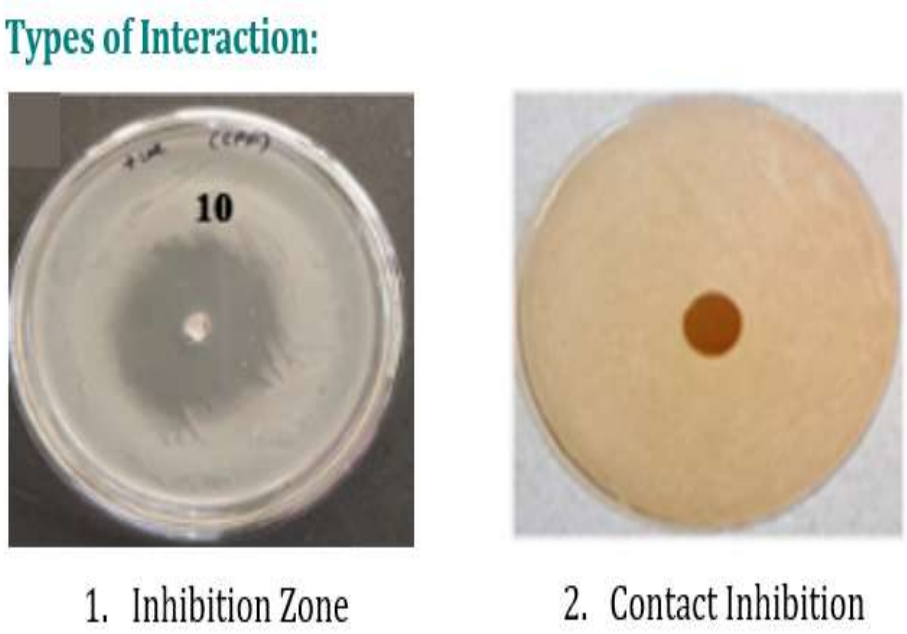

Figure 2: Types of bacterial interaction with anti-bacterial material

1.1.4 Biodegradability

This is an important component of this study. A good measure of biodegradability of packaging films is that they do not degrade while they are still in use and that they must not pollute the environment by sitting there for a longer period of time. These films should degrade once exposed to moisture, oxygen, sunlight and enzymes. And, the by product of this degradation should become part of the earth's natural environment.

1.1.5 Pertaining to Fertility

In recent food packaging films, nano materials are being utilized as fillers to enhance the above stated properties. However, some of these nanomaterials e.g. TiO_2 are imparting harm to the fertility of the soil. Once they become part of the soil, they release ions that inhibit growth in plants. A. Movafeghi et.al. reported that TiO_2 nano-particles were responsible for reducing the frond number of aquatic plants. In this

study, the focus is to introduce materials that can not only prevent harm to plants but, also increase the soil's fertility in their afterlife.

1.2 Problem Statement:

Food packaging plastics possess extraordinary mechanical properties but impart damage to the environment due to lack of biodegradability.

1.3 Objectives:

In this thesis, we aim to develop a bio-nano composite film using ZnO, PVA, CNC, and whitlockite for food packaging applications. The film will be synthesized using a solvent casting method, and its properties will be characterized using various techniques, such as X-ray diffraction (XRD), scanning electron microscopy (SEM) and mechanical and barrier testing. The main objectives of this thesis are:

1. Synthesis of bio-nano composite film using ZnO, PVA, CNC, and whitlockite for food packaging applications.
2. Characterization of the structural and morphological properties of the film using XRD, and SEM
3. Evaluation of the mechanical and barrier properties of the film using tensile testing.
4. Assessment of the antibacterial and antifungal properties of the film using microbiological testing
5. Optimization of the composition of the bio-nano composite film to achieve the desired properties for food packaging applications.

CHAPTER 2

LITERATURE REVIEW

2.1 Requirements for food packaging

The main role of the food packaging is to protect the food against damage and contamination. Along with the preservation and protection, it also reduces contamination and wastage of the food when they are packed. Packing is also done for marketing purposes and traceability of nutrients. Therefore, in order to meet all these requirements several materials such as glasses, metal cans, and non-biodegradable polymer plastics are used for food packaging applications [1]. However, the current materials used in the market are not biodegradable and impart damage to the environment.

In 2014, 63% out of overall 258 million tons of wastes were from the food packaging materials [2]. This shows that more 50% of the total wastes generated are from the food packaging materials. Food packaging materials use a lot of energy along with the emission of green house gases and heavy metal ions, which makes the packaging materials toxic for the environment.

With increasing urbanization of the world population, food packaging is expected to increase up to $3.4 trillion by 2030 from $1.9 trillion in 2020, but the food packaging materials are mainly manufactured for single use and then discarded, which creates environmental problems [3].

2.2 Commonly used packaging materials

Plastic is most commonly used for packaging materials because of its seal ability, mold ability and easy printability. Due to all these qualities, the amount of plastic used for packaging increased to 13 tons annually only in United States [3].

6.3 billion tons of plastics have been produced between 1950 to 2018, among which only 9% have been recycled. The others are discarded into the oceans and landfills which leach out toxic constituents and cause several health issues not only to humans but also to aquatic life [4].

Considering all these factors of non-biodegradability and toxic effects of polymeric plastic food packaging applications, the world is shifting from the common petroleum-based food packaging films to biodegradable food packaging materials which are composed of natural polymers and degrade after disposal.

2.3 Reported Biodegradable food packaging films

Although several biodegradable food packaging materials have already been made and investigated, they all had shortcomings that need to be addressed. J. Zhou et.al produced nanocellulose and TiO_2 food packaging films that excellent mechanical strength along with good antimicrobial and UV-adsorption properties but due to its complex moldability and brittleness, it is unsuitable for a food packaging material [5]. Similarly, PVA/Cellulose Nano-fibril/TiO_2 packaging films have been reported by Z. Yu et.al, but cellulose nanofibrill significantly decreases the degree of crystallinity of the films, which decreases the water vapor barrier of the film [6]. A composition similar to our base composition was developed by S. van Nguyen et.al i.e. polyvinyl alcohol (PVA), cellulose nanocrystals (CNC), and Titanium dioxide (TiO_2). This combination exhibited excellent mechanical, physical and anti-microbial properties [7]. Nano-particles were added because even though CNC added to the mechanical strength, but PVA/CNC composite exhibits poor water resistance and poor water vapor barrier properties due to the high number of hydroxyl groups in both components [18]. However, in a different publication, it was found that TiO_2 is

harmful to aquatic plants upon decomposition of the film, as it reduced their frond number [8].

2.4 The goal pertaining to the Literature

The goal is to fabricate a food packaging film, which is not only biodegradable but also has excellent mechanical and physical properties. At the same time, it should also impart antimicrobial characteristic and induce fertility to the soil in its afterlife.

Keeping these objectives in mind, a food packaging film has been composed. This film contains a polyvinyl alcohol (PVA) and cellulose nanocrystal (CNC) blend as the base composition, and Zinc Oxide (ZnO) nanoparticles, and whitlockite as reinforcement.

The purpose and functionality for using each component of the film is described as below:

2.4.1 Polyvinyl Alcohol (PVA):

Polyvinyl Alcohol (PVA) is used as a matrix material in biodegradable food packaging applications because of its excellent film forming ability, chemical resistance, high crystallinity and non-toxicity [9]. However, PVA alone cannot be used for food packaging applications because of lower mechanical properties and high hydrophilic nature. [10].

2.4.2 Cellulose Nanocrystals (CNC):

Cellulose nanocrystals are inherently biodegradable, and their rod like shape and large surface area increase the strength of the PVA matrix and work as reinforcing agent [11], [12]. CNC is compatible with PVA as hydrogen bonds form between CNC and PVA due to which the mechanical strength of PVA significantly increases with a decrease in hydrophilicity [13], [14].

2.4.3 Zinc Oxide (ZnO) nanoparticles:

Antimicrobial agents incorporated in food packaging materials saves the food from microorganisms and increases its shelf life. Zinc oxide (ZnO) nanoparticles serve as these agents. The mechanism behind the anti-bacterial activity of ZnO nanoparticles is in their interaction with the cell surface. It increases the cell membrane's permeability and permeate into the cell to disturb the oxygen balance in the cell by inducing oxidative stresses, which inhibits the cell growth and causes cell death [15]. ZnO NPs also act as nano-fertilizers, enhancing the overall fertility of the soil [7]. They are used as a nano fertilizer in colloidal solution state, which not only provides nutrients to the plants but also maintains the organic state of the soil by reducing the harmful effect of the chemical fertilizer thus a smaller amount of nano fertilizer can be used as compared to that of chemical fertilizer. [15]. Moreover, Helmiyati Helmiyati et al. discovered in their research that ZnO nanoparticles can reduce the water vapor transmission rate and solubility of the resulting film.

2.4.4 Whitlockite reinforcement

Whitlockite has excellent mechanical strength and is biodegradable. However, along with increasing the strength, it is also capable of heavy ion absorption through exchange between Mg^{+2} and Ca^{+2} ions. This ability of absorbing heavy ions helps protect the food from the heavy ions, which are retained in the packaging material during printing. Moreover, whitlockite remains unaffected by the addition of ZnO. It is also compatible with ZnO, PVA, and CNC [16]. Whitlockite can primarily be used as a non-toxic bio-filler.

2.4.5 Compatibility of the components

All components PVA, CNC, ZnO, and whitlockite are compatible with each other as they form hydrogen bonds with each other, which results in increasing the mechanical strength and decreasing the water absorption properties of the film [17].

The proposed bio-nano composite film has the potential to replace conventional packaging materials, such as plastics, and can provide a sustainable and eco-friendly alternative. Moreover, the antibacterial and antifungal properties of ZnO can improve the shelf life and quality of food products. The use of CNC and whitlockite can also improve the mechanical and barrier properties of the film, making it suitable for food packaging applications. This thesis can contribute to the development of sustainable and eco-friendly packaging materials that can address the current environmental concerns.

Figure 3: Bio-nanocomposite film composition

CHAPTER 3

METHODOLOGY

3.1 Synthesis of Zinc Oxide Nanoparticles

3.1.1 Chemicls and Materials used:

Following chemicals and materials were used in the synthesis of Zinc Oxide (ZnO) nanoparticles.

- Zinc Sulphate ($ZnSO_4$)
- Sodium Hydroxide (NaOH)
- DI water
- Ethanol

Zinc Sulphate ($ZnSO_4$) and Sodium Hydroxide (NaOH) are used as salt precursors while DI water was used as solvent for the salt precursors. Both DI water and Ethanol were used to wash the creamy solution formed after mixing of the precursors solution (Manyasree et al., 2018a).

3.1.2 Apparatus Used:

The Apparatus used in the synthesis of ZnO nanoparticles includes:

- Hot Plates
- Magnetic Stirrers
- Two 100ml Beakers
- Burette
- Iron stand
- Filter Paper
- Vacuum Pump
- Drying Oven
- Muffle Furnace

3.1.3 Procedure

Zinc Oxide (ZnO) nanoparticles were synthesized using co-precipitation method in which 1M solution of Zinc Sulphate ($ZnSO_4$) and 2M solution of Sodium Hydroxide (NaOH) were prepared by stirring each solution separately for 2 hours. After Zinc Sulphate ($ZnSO_4$) and Sodium Hydroxide (NaOH) were completely dissolved in their respective solution. NaOH solution was added to $ZnSO_4$ solution drop wise touching the wall of the beaker (Manyasree et al., 2018b).

After mixing the solution was stirrer for about 2 hours until a white creamy mixture was formed which was washed with ethanol and DI water several times in a vacuum pump. The white precipitate left on the filter paper were then oven dried at 80□ for 2 hours to remove all the moisture.

After moisture removal the product was kept in muffle furnace at 700□ for 3 hours where $Zn(OH)_2$ decomposed into ZnO nanoparticles.

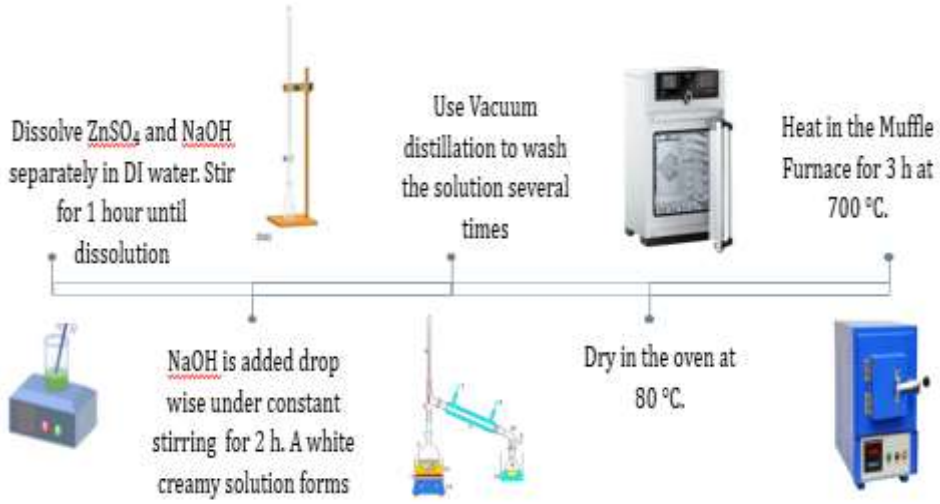

Figure 4: Zinc Oxide Nanoparticle synthesis with co-precipitation method

3.2 Fabrication of Cellulose Nanocrystals (CNC) and Polyvinyl Alcohol (PVA) nanocomposite films

3.2.1 Chemical and Materials used:

- Polyvinyl Alcohol (PVA)
- Cellulose Nanocrystals (CNC)
- DI Water

3.2.2 Apparatus Used:

- Hot Plates
- Magnetic Stirrer
- Beakers
- Petri Dishes
- Sonication Bath

3.2.3 Procedure

A 3wt% PVA solution was prepared by dissolving PVA beads in DI water. The solution was constantly stirred for 2 hours at 90 until no PVA beads were left undissolved.

Similarly Cellulose Nanocrystals (CNC) suspensions of various weight percent (1,3,5, and 7) were prepared by dissolving CNC in DI water and constantly stirred at 1000rpm for 24 hours. After that CNC suspension was sonicated in a sonication bath for about 30 minutes for uniform distribution of CNC suspension.

PVA solution and CNC suspension were then mixed by adding CNC suspension into PVA solution dropwise and the mixture was then stirred for 2 hours and then poured into petri dish and left open for air drying (Van Nguyen & Lee, 2022).

Table 1: PVA/CNC film compositions

Sample	PVA 3wt% (g)	CNC (g)
PVA	1.0	0
PVA/CNC (1wt%)	0.9	0.1
PVA/CNC (3wt%)	0.75	0.45
PVA/CNC (5wt%)	0.6	1
PVA/CNC (7wt%)	0.45	1.75

3.3 Fabrication of PVA/CNC/ZnO nanocomposite film

3.3.1 Chemical and Materials used:

- Polyvinyl Alcohol (PVA)
- Cellulose Nanocrystals (CNC)
- Zinc Oxide (ZnO) Nanoparticles
- DI Water

3.3.2 Apparatus Used:

- Hot Plates
- Magnetic Stirrer
- Beakers
- Petri Dishes
- Sonication Bath

3.3.3 Procedure

A 3wt% PVA solution was prepared by dissolving PVA beads in DI water. The solution was constantly stirred for 2 hours at 90 until no PVA beads were left undissolved.

Similarly Cellulose Nanocrystals (CNC) suspensions of 3wt% (which was optimized to have maximum mechanical strength) weas prepared by dissolving CNC in DI water and constantly stirred at 1000rpm for 24 hours. After that CNC suspension was sonicated in a sonication bath for about 30 minutes for uniform distribution of CNC suspension.

Zinc Oxide Nanoparticles suspension was also prepared by dissolving Zinc oxide in DI water and stirring at 1200 rpm for 24 hours. The suspension was then sonicated for 24 hours for uniform dispersion.

PVA/CNC/ZnO nanocomposite solution was then prepared by adding different weight percentage of ZnO suspension (1, 3, 5, 7, and 10) in PVA/CNC mixture and the overall suspension was stirred for 2 hours and then poured in different petri dishes and left for air drying (Nguyen & Lee, 2022).

Table 2. Composition Table for PVA/CNC/ZnO nanocomposite film

Sample	PVA 3wt% (g)	CNC 3wt% (g)	ZnO (g)
PVA	1.0	0	0
PVA/CNC	0.3	0.15	0
PVA/CNC/ZnO (1wt%)	0.3	0.15	0.0045
PVA/CNC/ZnO (3wt%)	0.3	0.15	0.0135
PVA/CNC/ZnO (5wt%)	0.3	0.15	0.0225
PVA/CNC/ZnO (7wt%)	0.3	0.15	0.0315
PVA/CNC/ZnO (10wt%)	0.3	0.15	0.045

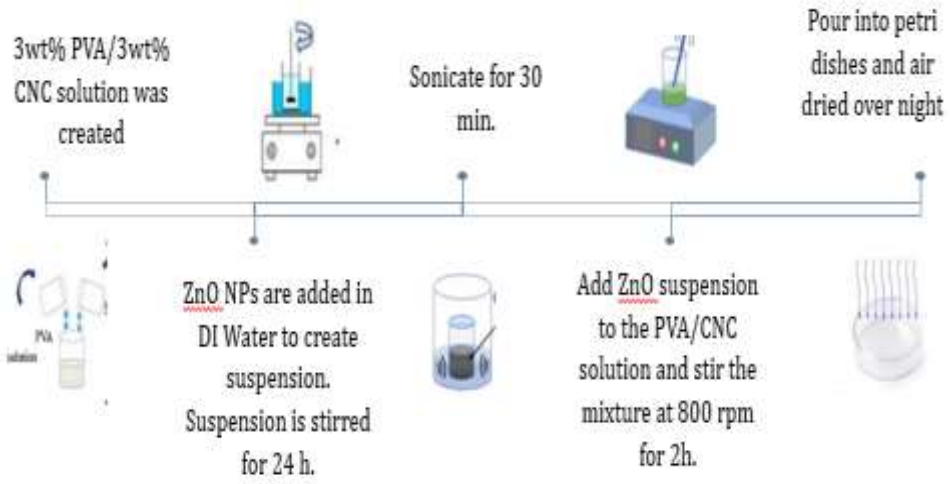

Figure 5: PVA/CNC/ZnO film fabrication

3.4 Fabrication of PVA/CNC/ZnO/Whitlockite nanocomposite film

3.4.1 Chemical and Materials used:

- Polyvinyl Alcohol (PVA)
- Cellulose Nanocrystals (CNC)
- Zinc Oxide (ZnO) Nanoparticles
- Whitlockite Nanoparticles
- DI Water

3.4.2 Apparatus Used:

- Hot Plates
- Magnetic Stirrer
- Beakers
- Petri Dishes
- Sonication Bath

3.4.3 Procedure

PVA/CNC/ZnO nanocomposite mixture with optimized percentage of PVA/CNC/ZnO was then prepared. At the same time Whitlockite suspensions were prepared and stirred at constant rate for 24 hours and then sonicated for 30 minutes for uniform distribution of the nanoparticles.

PVA/CNC/ZnO/Whitlockite nanocomposite suspension of different weight percentage (1,3,5,7, and 10) of whitlockite were prepared and then stirred for 2 hours after which they were poured into petri dishes and left for air drying (Nguyen & Lee, 2022).

Table 3: PVA/CNC/ZnO/WH compositions

Sample	PVA 3wt% (g)	CNC 3wt% (g)	ZnO 5wt% (g)	Whitlockite (g)
PVA	0.3	0	0	0
PVA/CNC	0.24	0.06	0	0
PVA/CNC/ZnO	0.24	0.06	0.015	0
PVA/CNC/ZnO/Whitlockite (1wt%)	0.24	0.06	0.015	0.00315
PVA/CNC/ZnO/Whitlockite (3wt%)	0.24	0.06	0.015	0.00945
PVA/CNC/ZnO/ Whitlockite (5wt%)	0.24	0.06	0.015	0.01575
PVA/CNC/ZnO/ Whitlockite (7wt%)	0.24	0.06	0.015	0.02205
PVA/CNC/ZnO/ Whitlockite (10wt%)	0.24	0.06	0.015	0.0315

CHAPTER 4

CHARACTERIZATION TECHINQUES

4.1 X-Rays Diffraction (XRD)

X-Rays Diffraction (XRD) is a characterization technique used for phase identification of crystalline materials also provide information on the unit cell dimension as well. The XRD pattern of the nanoparticles that were synthesized was analyzed using X-ray diffraction of Cu Kα radiations with wavelength of 1.54 Angstrom.

The XRD pattern was recorded in the 2θ range of 5 to 90 degrees.

4.2 Scanning Electron Microscopy (SEM)

The surface and cross-sectional structure of both PVA/CNC/ZnO and PVA/CNC/ZnO/Whitlockite nanocomposite films were characterized using Scanning Electron Microscope (SEM). For cross sectional analysis the samples were dipped in liquid nitrogen for about 60 seconds and then cut at various points for analysis. The samples were coated with gold and images were taken at 5KeV voltage and various magnifications.

4.3 Mechanical Properties

Tensile test of the PVA/CNC/ZnO and PVA/CNC/ZnO/Whitlockite nanocomposite films was done according to ASTM standard (ASTM-D882). Rectangular samples of 10mm width and 26mm length were prepared cutting 3 samples from different location from each film to analyze the mechanical strength of the film. Three tests per film were performed and the mean value of the strength is reported for each film.

4.4 Water Absorption Test

Water absorption and water solubility test of PVA/CNC/ZnO and PVA/CNC/ZnO/Whitlockite nanocomposite films was performed according to standard ASTM D570-98. Standard size sample were prepared, and oven dried at 50□ for 24 hours after which the samples were cooled weighed and immersed in DI water at room temperature for 24 hours. After 24 hours the samples were taken out and extra water was removed from the surface and weighed. The samples were again reconditioned at 50□ for 24 hours and weighed.

Following equation was used for measuring the water absorption of the films:

$$water\ absorption\ (\%) = \frac{m_{wet} - m_{re-dry}}{m_{dry}} \times 100$$

$$Solubility\ (\%) = \frac{m_{dry} - m_{re-dry}}{m_{dry}} \times 100$$

m_{wet}, m_{dry}, and $m_{re\text{-}dry}$ are the sample mass after drying, wetting, drying again after wetting the samples.

4.5 Antimicrobial Test

Anti-bacterial test was conducted on two types of bacteria colonies: E.coli and S.aureus. Tests were performed on 4 samples against a standard antibacterial test. These samples included PVA/CNC films, PVA/CNC/ZnO Films, PVA/CNC/WH Films and PVA/CNC/ZnO/WH films. All these tests were done in an agar media where bacterial colonies of the bacterias were created. 6mm round samples were placed in the petri dishes against a standard antibacterial test. The test was conducted in triples to ensure accuracy and petri dishes were placed in the incubator to create a conducive environment for the bacteria. Inhibition of the bacteria was noted in the contact area of the films and the surroundings. Tests were also conducted on ZnO and Whitlockite powders, by using agar well method, to see the effect of the nanoparticles on bacterial growth. Sample coding is as follows:

Sample 1: PVA/CNC (3wt%/3wt%)

Sample 2: : PVA/CNC/ZnO (3wt%/3wt%/5wt%))

Sample 3: PVA/CNC/WH (3wt%/3wt%/1wt%)

Sample 4: PVA/CNC/ZnO/WH (3wt%/3wt%/5wt%/5wt%)

Sample 7: ZnO Powder

Sample 8: Whitlockite powder

CHAPTER 5

RESULTS AND DISCUSSION

5.1 X-Rays Diffraction (XRD) analysis

X-Rays Diffraction pattern of Zinc Oxide nanoparticles synthesized show hexagonal phase has been synthesized. The strong and sharp characteristic peaks of Zinc oxide (ZnO) are shown between 2θ range of 30-40 degrees which are associated with (100), (002), and (101) planes of the nanoparticles respectively.

Using Debye Scherrer's equation ($D = 0.9\lambda/\beta\cos\theta$) crystallite size of the nanoparticles was calculated which was 29 nm i.e., 129 Angstroms.

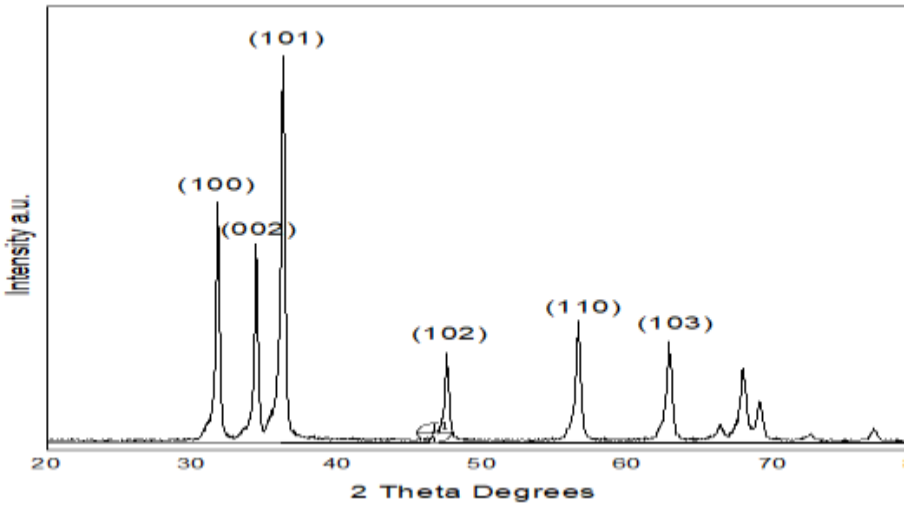

Figure 06: XRD Peak for Zinc Oxide nanoparticles

5.2 Scanning Electron Microscope (SEM) analysis

The Figures below show the image of Zinc Oxide nanoparticles taken at various magnification using Scanning Electron Microscope (SEM). The results show irregular shaped nanoparticles compactly arranged. This result is comparable with

the literature [19]. Some large particles present shows agglomeration of the nanoparticles due to strong interaction between individual particles due to Van Der Waals forces.

The chemical composition of the nanoparticles was confirmed by EDS, which showed Zinc and Oxygen peaks and confirmed their presence. However, some smaller peaks did appear but that were of gold coating and salt precursors that were

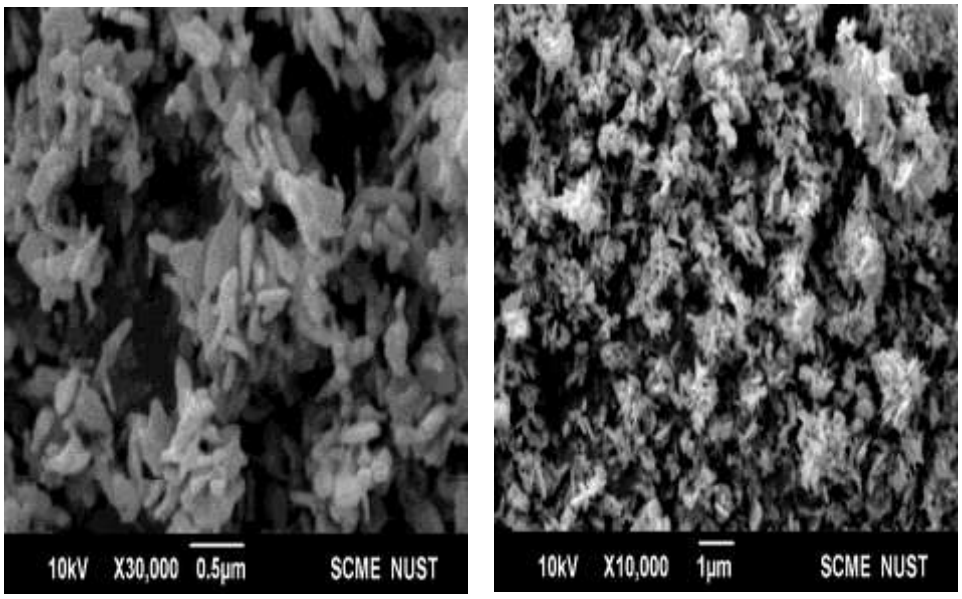

Figure 7: Scanning Electron Microscopy Images of ZnO at 30K and 10K magnification

present.

SEM Analysis of films with compositions mentioned above was done at 5Ekv and at different magnifications. The cross sectional and surface analysis of the different films also supported the results deduced from the mechanical testing of the same films.

First the SEM analysis of PVA/CNC/ZnO Films was conducted at different resolutions and we choose 7,500x to analyse our results. The films had varying compositions of ZnO at 3 wt%, 5wt%, 7wt% and 10wt%, while PVA and CNC were constant at 3wt%. At 3wt% ZnO, there was a smaller amount of ZnO nanoparticles and, as evidenced by the mechanical tests in the next section, it has a smaller tensile

strength. At 5 wt%, the amount of nanoparticles are more but the distribution of NPs have improved. There is only a little agglomeration. However, agglomeration increases in 7 wt% and 10 wt%, while the distribution becomes poor. This also maps onto the mechanical testing results, which show reduced tensile strength in the latter compositions. Cross sectional results of the films were also investigated, which showed a similar trend as above. Big white globules of agglomeration can be visibly seen in 7wt% and 10wt%.

The reason behind this trend was that ZnO NPs improve tensile strenght as they introduce pinning points that can restrict movement of dislocations and prevent deformation. Since 3wt% has a smaller amount of ZnO NPs as compared to 5wt%, the pinning points are fewer so chances of dislocations travelling and causing deformation are higher. In the case of 7 and 10 w%, the amount of ZnO NPs is so high that it forms agglomerates. A large number of dislocations get pinned in the region of agglomeration resulting in stress concentrations at that point. These stress concentrations ultimately lead to failure.

5wt% ZnO came out to be the optimized composition for achieving the highest mechanical properties. The amount was enough to cause dislocation pinning and prevent agglomerates.

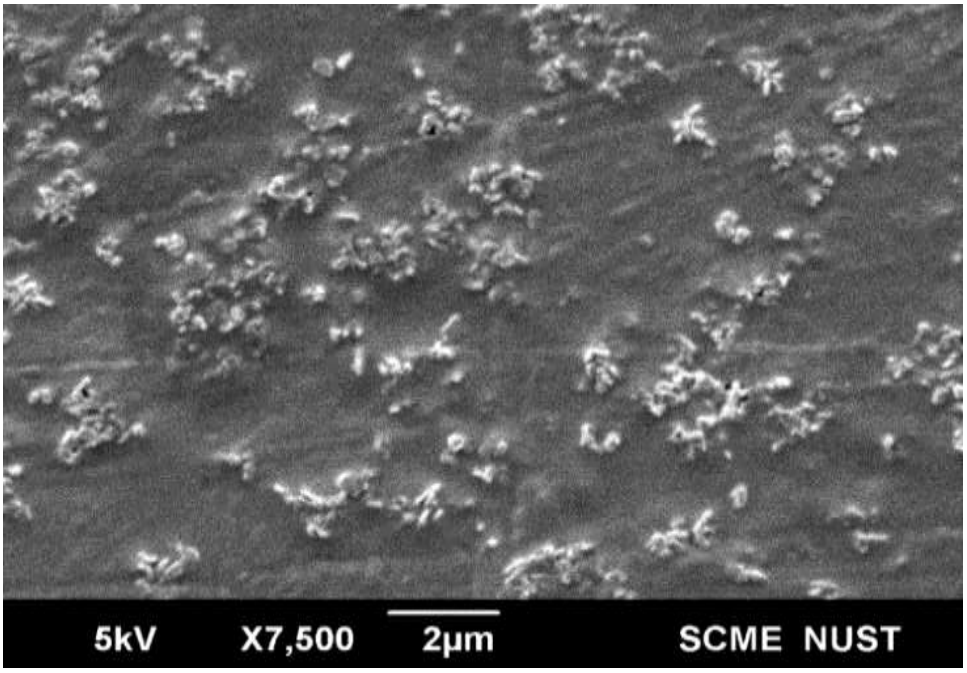

Figure 8: 3wt% ZnO SEM image

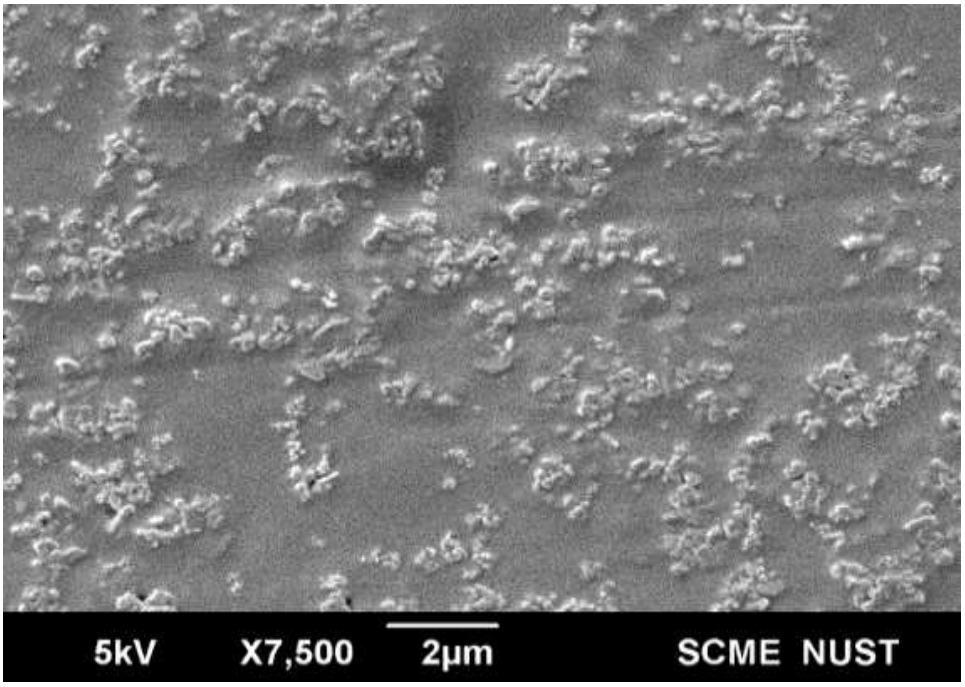

Figure 9: 5wt% ZnO SEM image

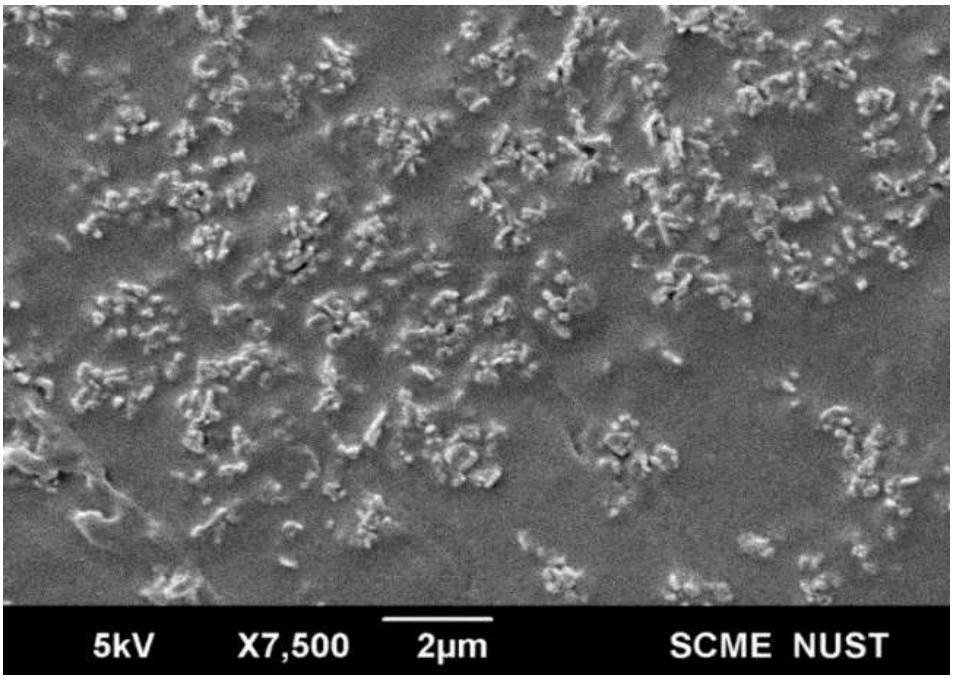

Figure 10: 7 wt% ZnO SEM image

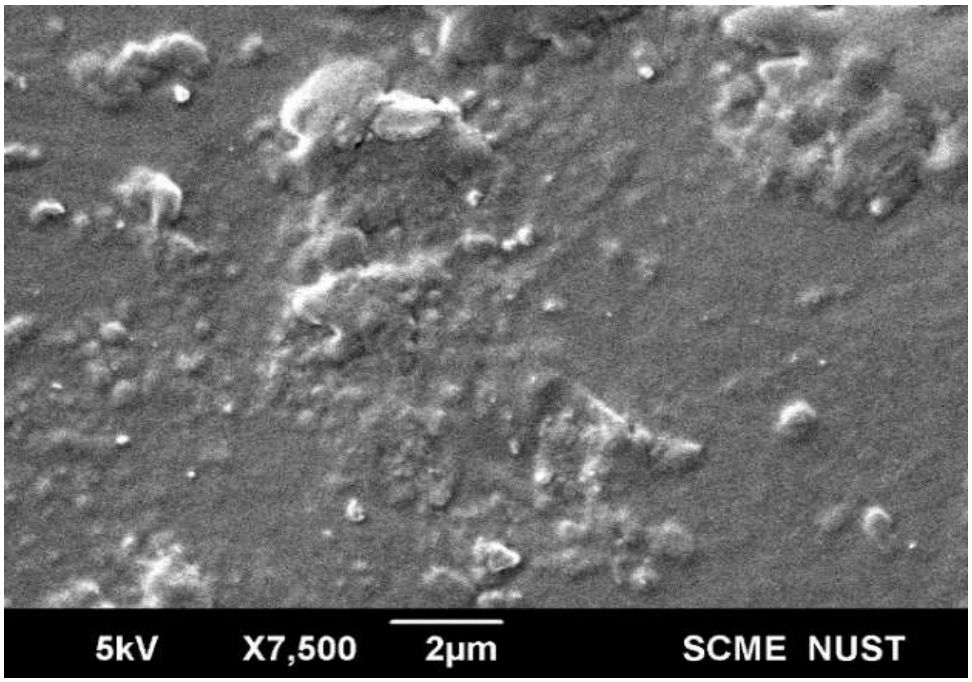

Figure 11: 10 wt% ZnO SEM image

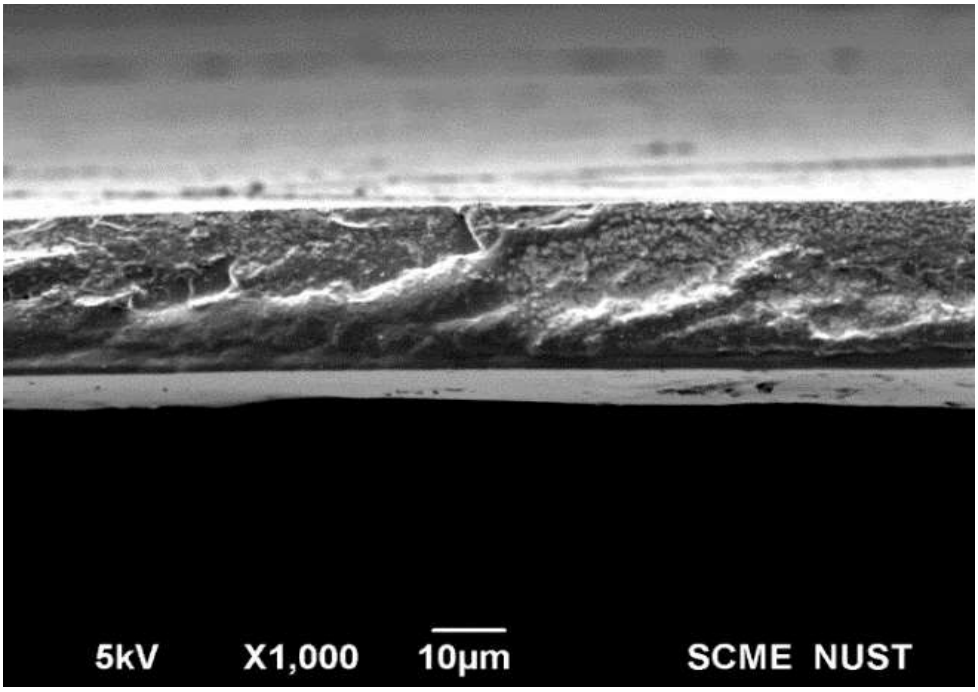

Figure 12: 3 wt% Cross Sectional

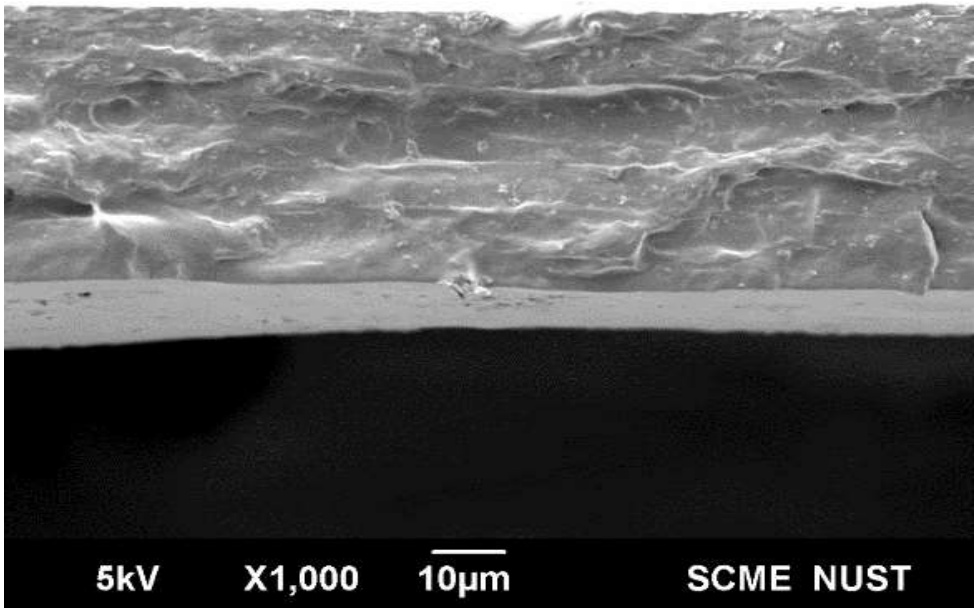

Figure 13: 5 wt% Cross sectional

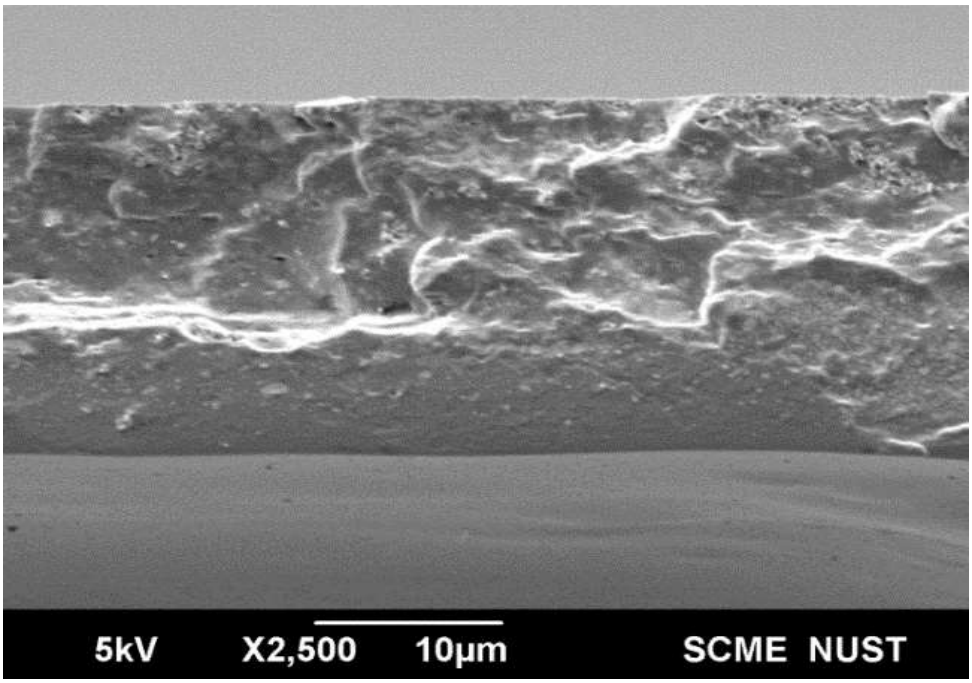

Figure 14: 7wt% Cross Sectional

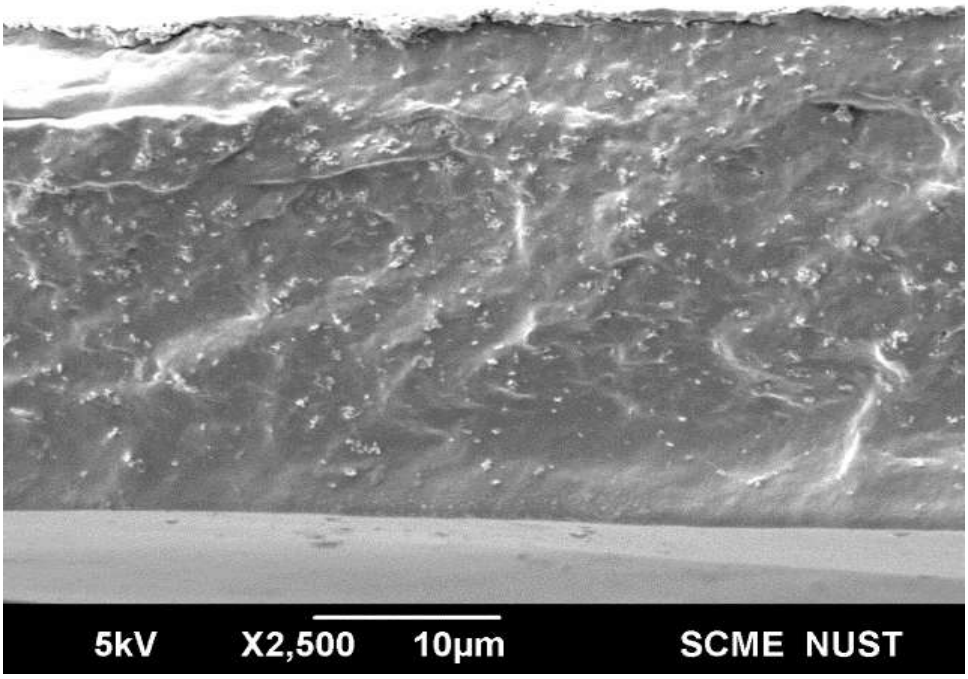

Figure 15: 10wt% Cross Sectional

Finally, SEM analysis of PVA/CNC/ZnO/WH films was conducted in a different SEM set up. Here the composition of PVA/CNC/ZnO remained constant at 3wt%/3wt%/5wt% and only whitlockite amount will vary. Only compositions 1 wt% WH and 3 wt% WH were analysed and their resolution at 500x was used to derive conclusions.

Agglomerates exist in both compositions but there is a higher amount in 3 wt%. the mechanical properties support these results as the tensile strength of 3 wt% WH is lower. We also concluded that there was a higher amount of agglomeration in Whitlockite films, which we believe may be due to increased moisture in the whitlockite powder. The moisture can gather in the sample if it has been stored for longer periods.

1 wt% WH films were the optimized films.

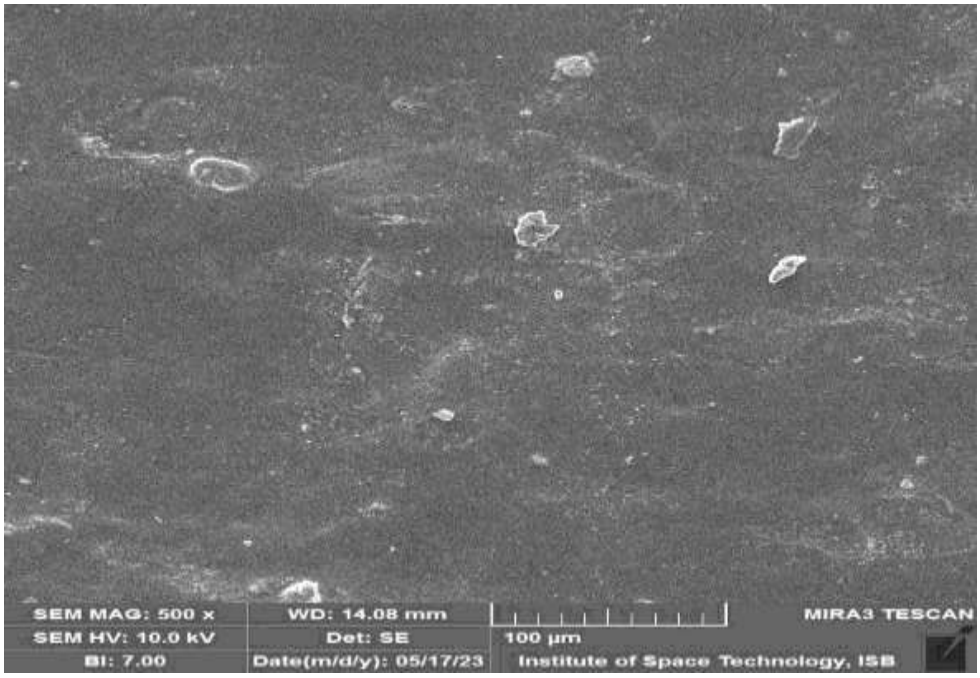

Figure 16: 1 wt% WH SEM image

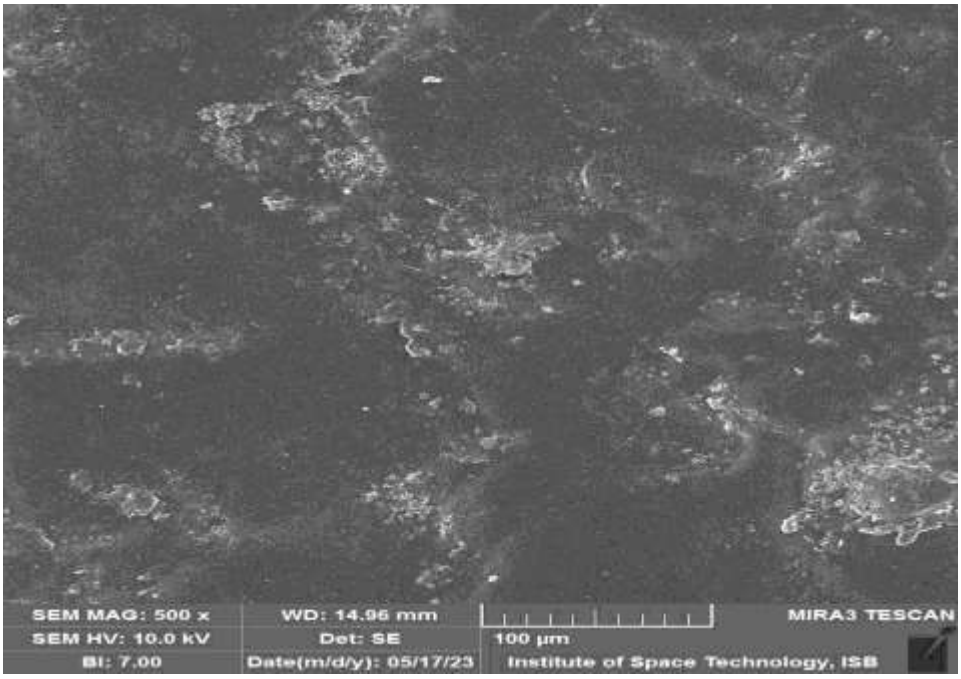

Figure 17: 3 wt% WH SEM image

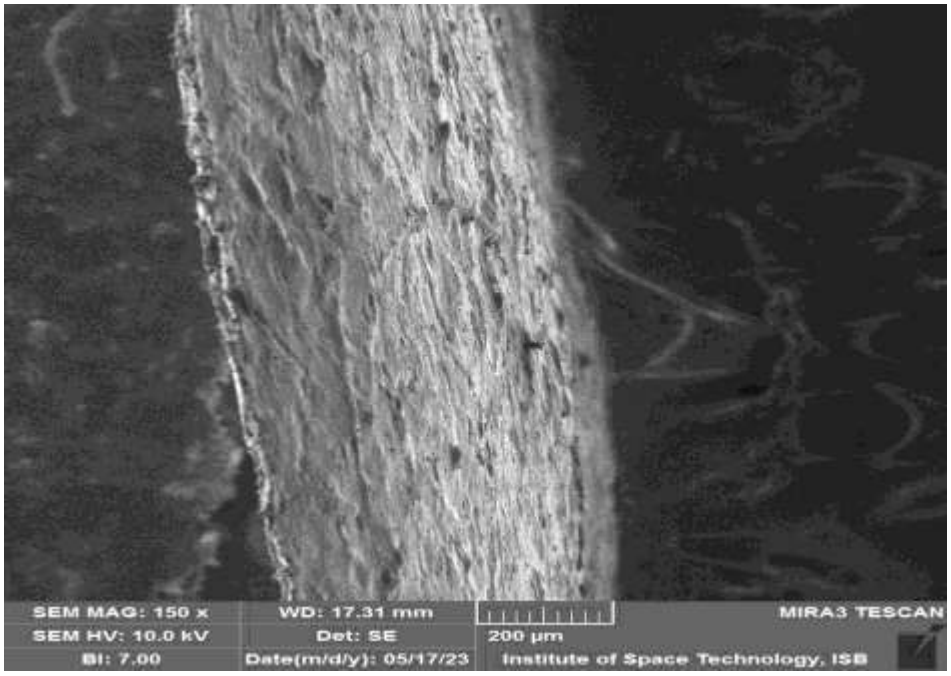

Figure 18: 1wt% WH cross-sectional

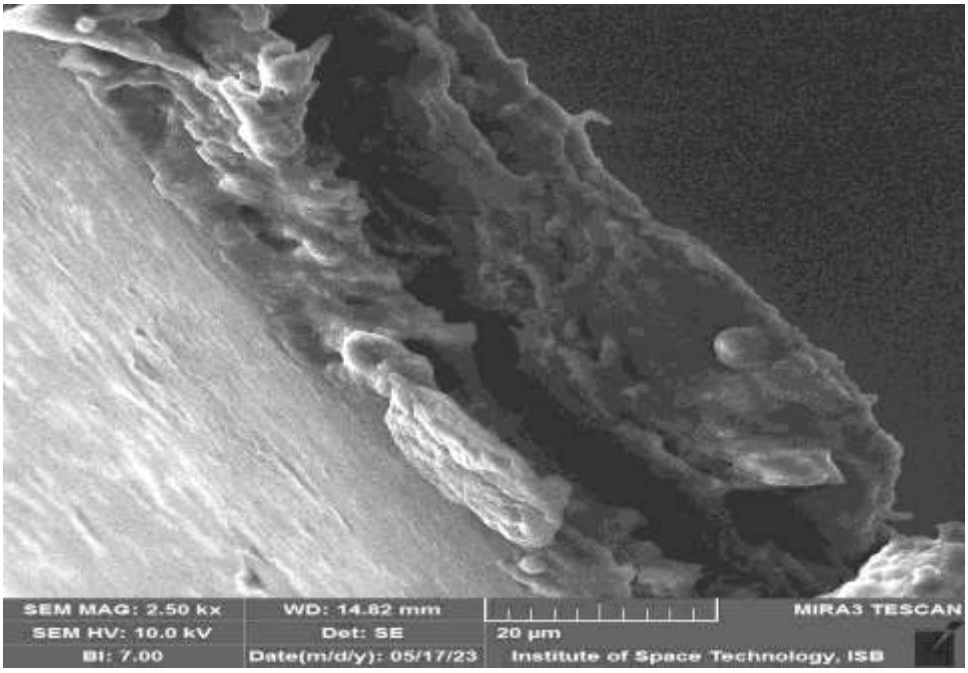

Figure 19: 3wt% WH cross-sectional

5.3 Mechanical Properties

Nanocomposite films of PVA only have high elongation but the tensile strength is relatively low. However, the addition of Cellulose Nanocrystals (CNC) increased the strength by 30%. The results produced are according to the previously reported results of CNC on the mechanical properties of Polyvinyl Alcohol [22,23].

The figure shows the histogram for the tensile strength of PVA/CNC film in which with increase in the weight percentage of CNC the mechanical strength increases and becomes maximum at 3wt% of CNC and then decreases after that. The increase in the mechanical properties is due to the fine dispersion of nanoparticles, which hinders the movement of dislocation. The decrease in mechanical strength after 3wt% is due to the agglomeration when the amount of CNC crystals increase the particles tends to agglomerate due to the greater surface area and Van Der Waal forces and CNC being brittle in nature tend to flow as an individual phase rather

than hindering the movement of dislocations when agglomerated. Overall increase in tensile strength was 22% upon CNC addition.

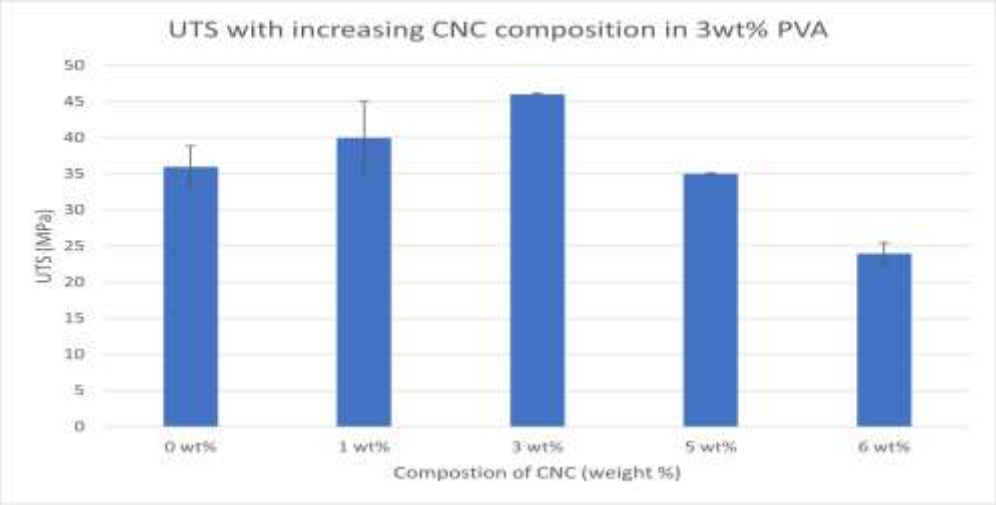

Figure 20: Bar Chart showing the effect of increasing CNC composition on the Tensile Strength of the nanocomposite Film

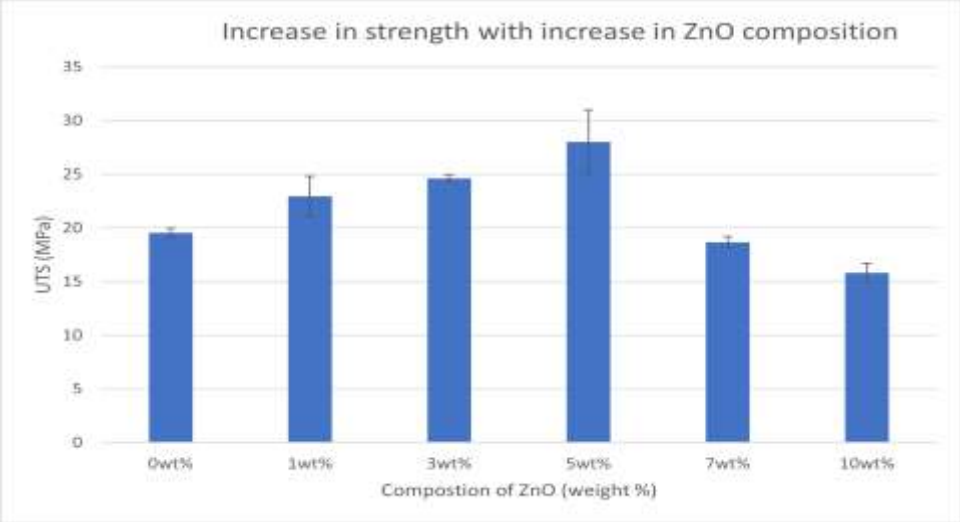

Figure 21: Bar Chart showing the effect of increasing ZnO composition on the Tensile Strength of the nanocomposite Film

After PVA/CNC nanocomposite film being optimized at 3wt% CNC to have highest mechanical properties films with various weight percentage of Zinc Oxide nanoparticles were analyzed. Addition of Zinc Oxide nanoparticles also increased the mechanical properties with increasing number of nanoparticles and became maximum at 5 wt% Zinc Oxide nanoparticles after which agglomeration of nanoparticles starts and mechanical properties tend to decrease. This is shown in the mechanical properties figure given below. Overall increase due to ZnO addition was 32%.

Lastly, PVA/CNC/ZnO/WH films were analyzed. Highest tensile strength was at 1wt% WH. Unlike ZnO and CNC, WH decreased the overall tensile strength by 8%. We believe this is due to an increase in the number of fillers added. As we increase the amount and types of fillers, their interaction with each other greater agglomeration, which lead to stress concentration points and ultimately failure.

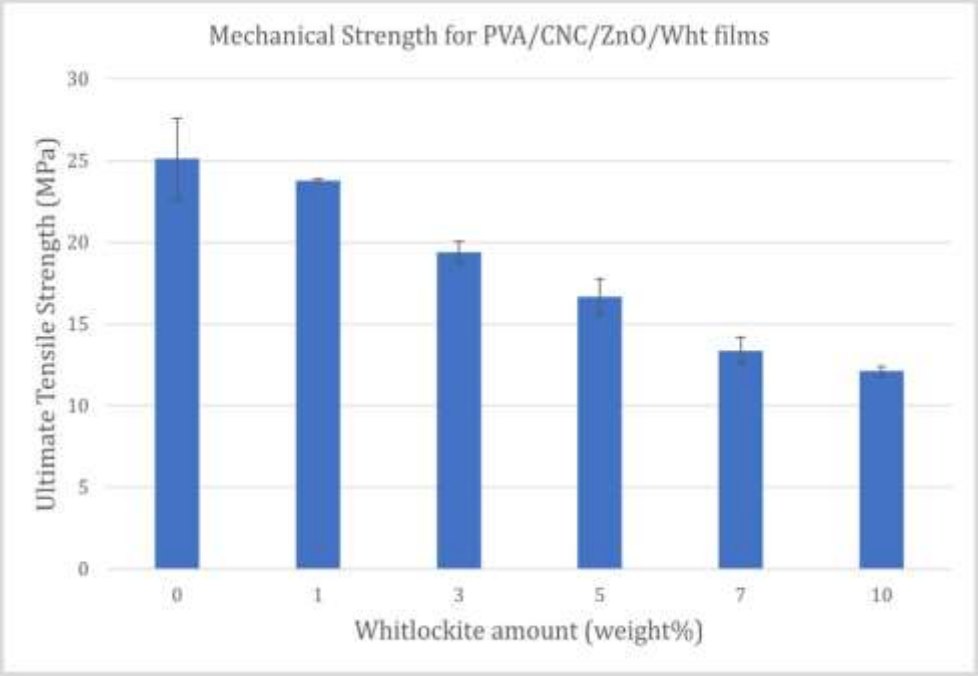

Figure 22: Bar Chart showing the effect of increasing WH composition on the Tensile Strength of the nanocomposite Film

The graph attached below summarizes the mechanical properties of all the optimized compositions; PVA/CNC (3 wt%/3 wt%), PVA/CNC/ZnO (3 wt%/3 wt%/ 5 wt%) and PVA/CNC/ZnO/WH (3 wt%/3 wt%/ 5 wt%/1 wt%)

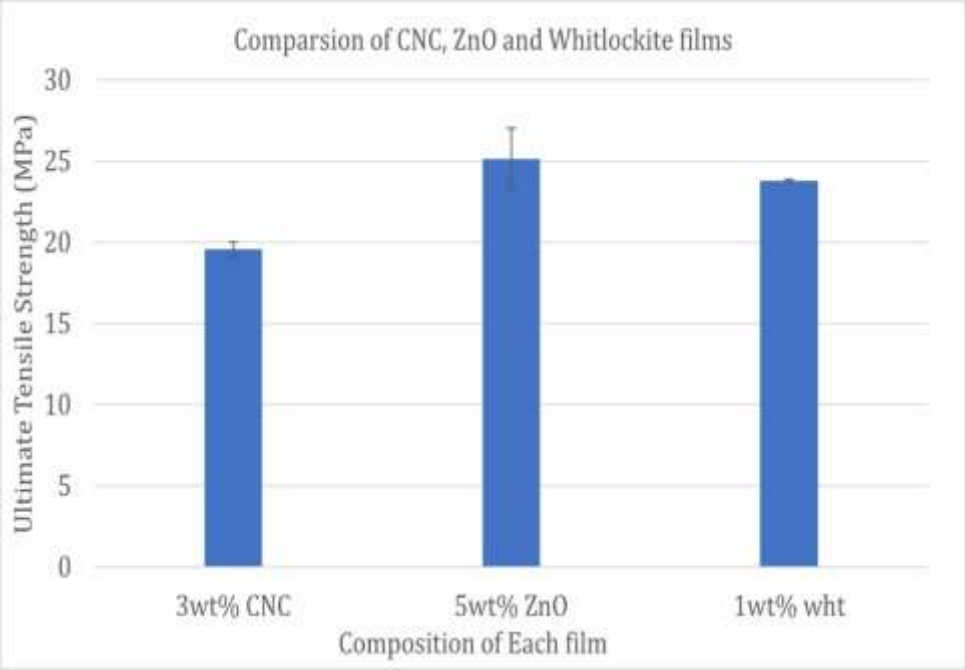

Figure 23: Tensile strengths of all the optimized compositions

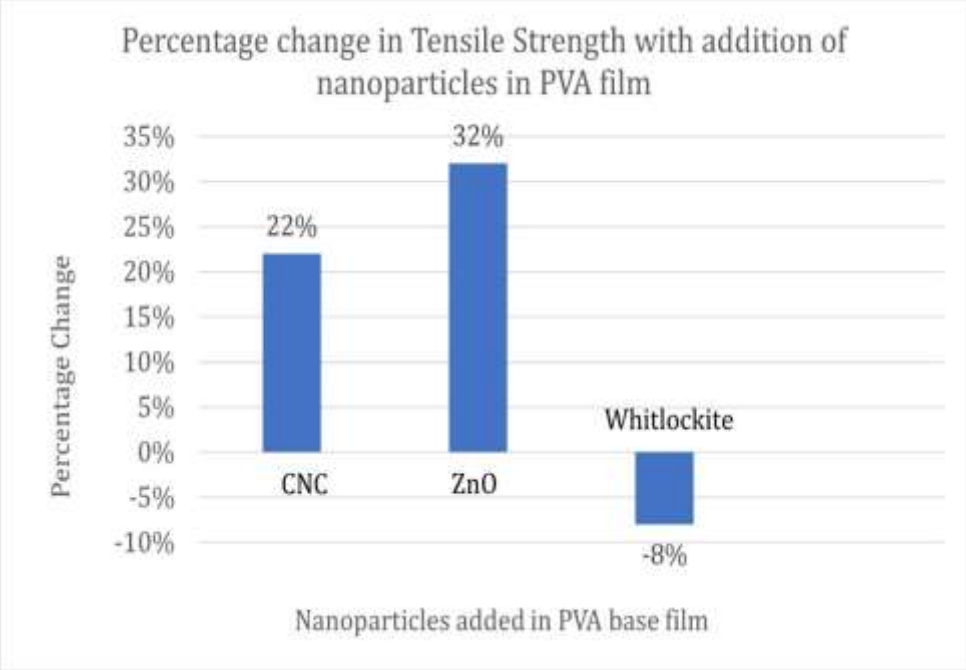

Figure 24: Increase and decrease in tensile strength of optimized films

5.4 Water Uptake and Solubility Tests

These tests were conducted on three samples:

PVA

PVA/CNC/ZnO

PVA/CNC/ZnO/WH

Table 4: Water uptake and solubility percentages of samples

Sample	**Composition**	**Water Uptake**	**Solubility**
PVA/CNC/ZnO	3wt%/0wt%/0wt%	4.9	14.5
PVA/CNC/ZnO	3wt%/3wt%/5wt%	9.4	38.5
PVA/CNC/ZnO/WH	3wt%/3wt%/5wt%/1 wt%	13	56.25

Ideally, we prefer that the film uptakes more water so less of it penetrates into the food. And here water uptake is highest in the film containing Whitlockite due to its hydrophilic nature. ZnO also has hydroxyl groups, which adds to the hydrophilic nature. In the second sample, the water uptake is high due to ZnO's hydrophilic nature.

As for solubility, the WH film may not be suitable as higher solubility results in a film losing its structural integrity in the presence of water. The ZnO film also has a high solubility but the ZnO-CNC bonds reduce solubility while ZnO hydroxyl bonds increase it. This gives us just the optimum solubility level to use PVA/CNC/ZnO films in food packaging applications.

5.5 Antimicrobial Properties

The results from the anti-bacterial tests against E.Coli and S.aureus showed contact inhibition for all films containing ZnO and Whitlockite. ZnO powder created an inhibition zone while whitlockite powder showed contact inhibition.

Fig 25: Sample 1, 2, 3 and 4 results on S.aureus bacteria

Figure 26: Sample 1, 2, 3 and 4 results on E.Coli bacteria

Figure 27: Sample 7 and 8 results on E.Coli and S.Aureus bacteria

Conclusion

In conclusion, the use of biodegradable food packaging films composed of PVA (polyvinyl alcohol), CNC (cellulose nanocrystals), ZnO (zinc oxide), and whitlokite shows great promise in addressing the environmental concerns associated with conventional plastic packaging. These films offer several advantages, including biodegradability, reduced waste, and enhanced barrier properties. They have the potential to significantly reduce pollution and contribute to a more sustainable future in the food packaging industry. However, PVA/CNC/ZnO films showed the most suitable mechanical, physical and anti-bacterial properties for food packaging applications. Further research and development are needed to optimize the performance and cost-effectiveness of these materials to ensure their widespread adoption in the market.

Future Prospects

In the future, different whitlockite morphologies in bio-nanocomposites will be investigated for food packaging application. The goal is to identify and understand the impact of various morphologies on the properties of the composites, such as mechanical strength, barrier properties, and biodegradability.

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