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CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF NATURAL PLANT EXTRACT & NANO LOADED CHITOSAN/POLYVINYL ALCOHOL BASED COMPOSITE FILMS

Sonam Ahuja and Satish M. Padhiyar

Department of Chemistry, Faculty of Applied Sciences, Parul Institute of Applied Science (PIAS), Parul University, Limda, Waghodiya, Vadodara - 391760, Gujarat, India.

Keywords:

Chitosan, Polyvinyl alcohol, NiO-nanoparticles, *Azadirachta indica* [neem], Glutaraldehyde, Antibacterial activity

Correspondence to Author:
Dr. Sonam Ahuja

Assistant Professor,
Department of Chemistry,
Faculty of Applied Sciences,
Parul Institute of Applied Science (PIAS),
Parul University, Limda, Waghodiya,
Vadodara - 391760, Gujarat, India.

E-mail: sonam.ahuja82106@paruluniversity.ac.in

ABSTRACT: We are creating composite films made of chitosan and polyvinyl alcohol that are filled with neem plant extract and NiO nanoparticles. The solution casting technique is used to produce chitosan-polyvinyl alcohol films. When chitosan was combined with polyvinyl alcohol, the water absorption of the film increased, and glutaraldehyde (0.5 ml) became used as the crosslinking agent. The compatibility of these two biopolymers was analyzed by FT-IR and XRD. The drug extract and NiO nanoparticles are characterized by UV analysis. The exact ratio of chitosan and PVA was used. Medicinal plants are widely used in everyday life for various diseases. The extraction of *Azadirachta indica* [neem] and NiO nanoparticles have been used as an antibacterial agent. The composite membrane was found to have the best antibacterial properties, A gelatine swelling assay was also used to evaluate swelling parameters. PVOH/CS films loaded with Neem and NiO nanoparticles were tested for antibacterial activity against Escherichia coli bacteria (*E. coli* bacteria). These results suggest that CS/PVOH bio-composites can be used as special materials in the food packaging and biomedical industries. We analyse various analysis techniques, including SEM and TGA for the properties of the films.

INTRODUCTION: A polymer is a natural or man-made compound comprising macromolecules (large molecules) with many monomers (small chemical units). Polymers, including protein, cellulose and nucleic acids, are found in various living organisms. It is made up of many components present in living organisms. Minerals such as diamond blocks, natural glass, and moonstones, as well as man-made products such as paper, plastic, rubber, *etc*., use it as a substructure. Some common examples of synthetic polymers are polyethylene, polystyrene, neoprene, nylon and many others. Both synthetic and natural polymers are synthesized by polymerization [1].

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Polymers are widespread due to their unique properties, low density, superior thermal/electrical insulation, and low cost. The quality of a polymer can be enhanced for specific use by combining it with other materials, such as composites. They are widely used in packaging applications, pharmaceutical applications, personal hygiene and healthcare, construction and structural applications, paints and lubricants, household appliances, and more. Polymers have high strength; they are resistant to corrosion. There are three types of thermoplastics, thermosetting polymers. An elastomer, polymers are made into useful articles using the following techniques - rolling, extrusion, melding, molding, blowing, foaming, spinning, *etc.* [2].

Chitosan: Chitosan is a linear polysaccharide composed of the amino acids Sugar Substitute and2 – acetamido 2 - deoxyglucose. It is made by using salt, like NaOH, to treat the chitin in the shells of crustaceans and shrimp. The second most prevalent polysaccharide in nature, after cellulose, is chitosan. Due to their bioactivity, biocompatibility, and biodegradability, chitosan (CH) and chitin are highly recommended for use in biomedicine, agriculture, biotechnology, and the food industry [3]. New processing techniques and bio-based polymers are becoming increasingly popular to reduce dependence on crude oil and move to sustainable facilities. It is very popular in food packaging due to its non-toxicity [4]. We considered the qualities of biocompatibility, flexibility, biodegradability and antibacterial properties. In the medical field, chitosan is a substance found in some dressings that help stop bleeding. When the bandage comes in contact with blood, it becomes extremely sticky and this adhesive effect seals the cut. Since it inhibits the growth of germs and fungi, chitosan can be applied to treat burns. In addition, it is used in winemaking as a preservative and smoothing agent. With potential uses in wastewater treatment as a biosorbent, chelating agent, and carrier for other nanomaterials, chitosan has been the subject of many studies [5].

The removal of heavy metals, dyes, organic pollutants, and emerging contaminants is one of the aspects of using chitosan Nanocomposite in wastewater treatment that has been investigated. The solubility of chitosan is affected by several factors, including temperature, pH, the crystallinity of the polymer, degree of acetylation, and molecular weight of the polymer. It is insoluble in water and organic solvents. 1-Macetic acid (10 mg/ml) and dilute acidic aqueous solvents can be used to dissolve turbid solutions [6].

Poly-Ethenol (PVOH): Poly-Ethenol (Polyvinyl alcohol) is a water-soluble artificial polymer. Polyvinyl alcohol is a granular powder that is white or cream in colour, flavorless, and odorless. Other products made with this product include food packaging materials, surgical fibers, and plastics. A thermoplastic polymer that is neither dangerous nor flammable is PVOH. PVOH has a high tensile strength and is flexible. PVOH is used as a biomaterial in medical devices because of its many favorable characteristics, including biocompatibility, non-toxicity, swelling properties, and bio adhesion [7]. It is widely used in medical synthesis, which includes poor protein absorption, bio absorbability, water solubility, and chemical resistance. It is used to manufacture soft contact lenses, eye drops, and embolization beads. PVOH is known for its solubility and biodegradability, which support its little influence on the environment [8, 9].

Azadirachta indica **(Neem Leaf):** Indica is also known as neem, or Indica lilac. Tropical and semi-tropical climates are ideal for its growth. A wide range of nutrients is provided by its fruit and seeds. Neem's function in diabetes. Neem stimulates beta cells and stimulates natural insulin production. The aqueous extract can lower blood sugar by decreasing the activity of acid phosphate and increasing the activity of other compounds. Leaf sap is a tonic to increase appetite and eliminate intestinal worms. The leaves have been used to treat allergic skin reactions, smallpox, and chickenpox. Treatment using the antibacterial properties of leaf extract fights oral infections. In the Ayurvedic health system, the selected herb is also used to treat malaria [10].

Nicaloxid Nanoparticle: Due to its use as catalysts, gas sensors, electrochromic films, fuel cells, magnetic materials, anodes of organic light-emitting diodes, and thermoelectric materials, nanostructured metal oxide materials, such as NiO nanoparticles, used polyvinylpyrrolidone to create well-stay scattered NiO nanoparticles with an average particle diameter of 30 nm. Several methods for producing nickel oxide nanoparticles have recently been devised, including surfactant-interceded methods, basic fluid stage cycles, low-pressure shower pyrolysis, and other processes [11]. Here, NiO nanoparticles were coordinated among several approaches for a regulated union using the co-precipitation synthetic process with an eye toward the arrangement cycle. NiO nanoparticles with 10-15 nm diameters were produced via air-calcination of Ni (OH)2 + NiCO3 + xH_2O [12].

In any application where high surface regions are looked for, like water treatment, and sun-based applications, NiO is particularly beneficial. Because nickel oxide powder has several intriguing electrical, mechanical, and chemical characteristics, it is frequently utilized in additives, electronics, and energy storage applications. The most popular uses for nickel micron powders frequently involve electronics in one way or another. Nickel micron powders are a common component used in the production of several materials, including various ceramics, steels and lubricants due to their special characteristics. Nickel micron particles are extremely beneficial in several industrial applications and for environmental initiatives due to their special characteristics [13].

MATERIALS AND METHODS:

Materials: Chitosan (CS), gelatine powder was purchased from India Mart Pvt. Ltd., Siddhanth Laboratories, Vadodara, Gujarat, Polyvinyl alcohol (PVOH) MW = 85,000-1,25,000 India Mart Pvt. Ltd., Aashish enterprise, Vadodara, Gujarat, acetic acid and methanol were purchased from Siddhanth Laboratories. All chemicals were used as received without further purification. Distilled water (DW) was used throughout the experiment.

Method for Preparing Films: A solution casting technique that is based on Stokes' law was used to create the films [14]. In this process, prepolymer and polymer are uniformly combined and made soluble in the appropriate solution. A 2% (% weight per volume) film-shaping arrangement of chitosan was made in a 2% (volume per volume) acidic corrosive arrangement at room temperature while being consistently mixed with an attractive stirrer (520 to 550 RPM). The mixture was then filtered. On the other hand, at a temperature of 50 °C, a homogeneous fluid 5% PVA (weight per volume) arrangement was created. The Chitosan and Polyvinyl alcohol solutions were first made clear, and then glutaraldehyde (0.5 ml) was added as the crosslinking agent to make a mixed solution[15]. The mixture was then stirred on a magnetic hotplate for 20 minutes at 50°C (500 to 530 RPM). The last combination was then emptied into a glass petri dish in anticipation of film development and dried in a stove at 40 °C. Therefore, the resulting dry film (Chitosan/Polyvinyl alcohol) was separated from the Petri dish and kept at room temperature for 48h overnight. Similarly, pure PVA/CS film with different ratios is also prepared [16].

TABLE 1: CREATION OF DIFFERENT FILM-MOULDED ARRANGEMENTS

Films	% Chitosan solutions	% PVOH solution	Glutaraldehyde	ratio	ml
CS/PVA-1	3% CS (ml)	3% PVOH (ml)	0.5ml	50/50	20-20 ml
CS/PVA-2	2% CS (ml)	5% PVOH (ml)	0.5ml	40/60	25-25 ml
CS/PVA-3	1% CS (ml)	3% PVOH (ml)	0.5ml	25/75	15-15 ml

Extraction of Plant Material: New plant leaves (10 g) of *Azadirachta indica* (neem) are gathered after completely washing the leaves in refined water. The leaves were dried in a broiler at 60 °C for 24 h. The dried leaves were crushed with mortar and pestle and put away in fixed compartments until additional examination.

Neem leaf extract is made by mixing 10 grams of dried leaf powder with 100 ml of methanol and letting it sit for 24 hours. After 24 hours Whatman no. 1 filter paper was used to filter the extract. The colour of the extract is light green [17].

Synthesis of Nickel Oxide Nanoparticles: Nickel Oxide nanoparticles were arranged using NiCl2 6H2O and NaOH as beginning materials and twofold refined water as a dissolvable scattering. To get a predetermined molar fixation at room temperature, 6.01 g of $NiCl_2.6H_2O$ was broken up in 250 ml of twofold refined water as a dissolvable. The arrangement was then attractively mixed for 40 minutes at 50 °C. Then, 10 ml NaOH with a particular molar was added to the arrangement drop by drop until the pH arrived at 8. As a result, the following chemical reaction was used to create NiO nanoparticles [11].

$$NiCl_2.H_2O+H_2O+2NaOH \longrightarrow NiO + 8H_2O+2NaCL$$

Finally, the resultant green gel was cleaned of reaction byproducts with DW and methanol before being dried at 60°C for 14 hours [11]. To acquire Nickel Oxide nanoparticles, dried examples were calcined at 500 °C temperatures for 2 hours. The shade of the example changes from green to dark because of this tempering [11].

Preparation Drug & Nano Loaded Films: The prepared plant extract solution (10ml) and NiO (0.1% weight/weight) nanoparticles solution were then mixed with a 2% CS (20ml) and 5% PVA (20ml) solution. Glutaraldehyde (0.5 mL) as a crosslinking agent. The last blend was then emptied into glass Petri dishes to set up the movies, which were then dried on a stove at 40 degrees Celsius. At last, the dried movies (Chitosan + PVOH + Ext) were eliminated from the Petridishes [15, 16, 18].

Characterization:

Swelling Studies: The composite films' swelling was studied using physiological fluid (PF). This fluid was created by dissolving 8.307 g sodium chloride (NaCl) and 0.367 g calcium chloride (CaCl2) in 1 litter of distilled water. The two were then combined to form the PF solution. Small sections of each ratio film were now cut and weighed separately. The films were immersed in the PF solution and removed at various intervals to be dried with filter paper and weighed. This procedure was repeated several times over 24 hours [19, 26].

FTIR Analysis: FTIR is a technique for determining the composition of a CS/PVA composite film. Films containing 1% of each polymer were dried for 24 h and the spectra ranged from 4000 to 450 cm^{-1}. The FTIR analysis was performed at PNP Analytical Solution, Vadodara taking all necessary precautions [20].

XRD Analysis: XRD is a technique for analyzing a material's crystal structure, chemical constituents, and physiological properties. The XRD analysis was performed at PNP Analytical Solution, Vadodara taking all necessary precautions. They ranged from 5 to 60 degrees. The scan step time was 8.2590 s, and the step size was 0.0090°. Copper K-rays have a wavelength of 1.56070 Å [21].

SEM Analysis: An electron magnifying lens called a filtering electron magnifying instrument examines an example's surface with an engaged light emission to create pictures of the material. As the electrons interact with the sample's atoms, they produce distinct signals that reveal the sample's surface topography and chemical makeup. In order to assess the surface morphology and see how the CS/PVA composite films were dispersed, an SEM examination was done [22].

The SEM analysis was carried out at PNP Analytical Solutions in Vadodara, with all necessary precautions taken. The composite film obtained by grinding was analysed in granular form. The electron beam's accelerating voltage (HV) was set to 5.00 kV, and a working distance (WD) of 6.6 mm was maintained.

UV Analysis: the light-absorbing properties of the developed plant extract of *Azadirachta indica* (neem) and NiO-NPs were analyzed using a UV-1800 series Instrument [23]. The UV-Visible analysis was performed at PNP Analytical Solution, Vadodara, India. The wavelength was set between 200-800 nm. Taking all necessary precautions.

TGA Analysis: TGA is a method of material analysis that assesses the mass of an experiment as a function of time or temperature when controlled heating is present. Temperature and weight loss are noted as the material's constituent parts gradually volatilize over time. Because TGA testing can measure weight loss at very high temperatures, it is useful for evaluating polymers. At about 200°C, polymers normally melt before breaking down, however, some can withstand temperatures of 301°C in air and 505°C in inert gases without degrading. These polymers can be examined by TGA as well [24]. The TGA analysis was performed at PNP Analytical Solution, Vadodara taking all necessary precautions.

Antibacterial Activity: *E. coli* was used to test the antibacterial activity of Neem and NiO Nanoparticles loaded PVA/CS composite film. In a flask, dissolve 2.9 gm of nutrient agar in 100 ml of distilled water. Clean the flask and Petri dish. Allow the media to cool to room temperature before spreading the test organism (*E. coli*) into the solidified agar solution with a spreader. For 24 hours, a piece of the composite film is placed over an agar plate and incubated at 36◦ C. The following day, look for the zone of inhibition [25].

RESULT AND DISCUSSION:

Swelling Studies: The definition of swelling is the increase in solid or gel volume caused by the absorption of liquid or gas. The swelling study was carried out according to the process. A PEF solution is made by mixing 1 litter containing 8.307 g of NaCl and 1 litter containing 0.367 g of $CaCl_2$. A ready-to-use solution for swelling studies [26, 27]. The swelling behaviour of film swelling is defined as a rise in liquid or gas. Swelling is the polymeric network in a polymer chain.

CS/PVA composite film was soaked in pf solution for 30 min and then removed from the PEF solution and dried. And then checked its actual weight. Then start the same process 3 or 4 times [26, 27].

$$SR = (Mt - Mo) / Mo \ g / g,$$

Where Mt = mass at different times and Mo = initial mass

TABLE 2: SWELLING STUDY OF THE RATIO OF ALL FILMS

Time	CH/PVOH 50/50	CH/PVOH 25/75	CH/PVOH 40/60
0	0.8	0.9	0.6
30	1	1.1	0.8
60	1.4	1.3	1.2
90	1.8	1.6	1.5
120	2.1	1.9	1.7
150	2.1	1.9	1.7

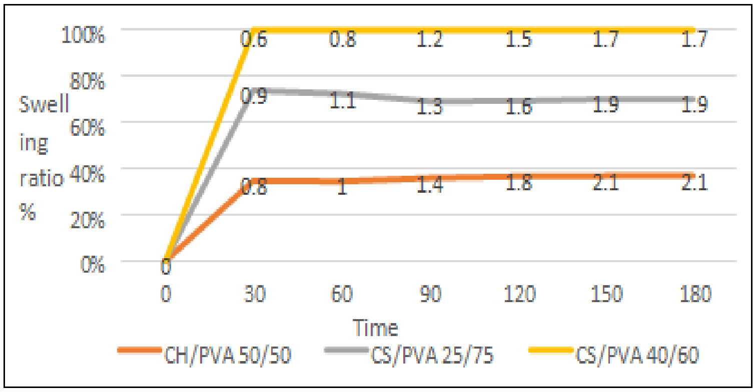

FIG. 1: GRAPH OF SWELLING RATIO

FTIR: FT-IR is a methodology that is utilized to find inorganic and natural parts that could cause item pollution and breakdown. Subsequently, FTIR is frequently used to recognize the introductory phase of any example. As indicated, FTIR spectra of all unique proportion composite movies were obtained. (1) CS/PVOH (25:75) (2) CS/PVOH (50:50), (3) CS/PVOH (40:60). The band recurrence consequences of the three different proportion composite movies were as per the following [28]:

PVA/CS Composite Film: The FTIR spectra of CS/PVOH mixed films with different weight structures. The 3450-3200 cm^{-1} assimilation band in the CS range is allotted to O-H and N-H extending vibrations. The band at 2921.21 cm^{-1} is related to C-H extending. The band at 1647.61 cm^{-1} is ascribed to acetyl bunch C-O extending (amide I). N-H bowing and extending are allotted to the band at 1556.15 cm^{-1} (amide II).

The top at 1288.01 cm^{-1} is related to O-H twisting vibration, while the top at 1007.23 cm^{-1} is related to C-O extending. The saccharine construction is allocated the retention groups at 1141.40 cm1 and 832.10cm^{-1}. The band at 3301 cm1 is ascribed to - Gracious extending vibration in unadulterated PVA, while the top at 1440.33 cm^{-1} is credited to Goodness twisting vibration of the hydroxyl bunch. The vibrational band addresses the topsy-turvy CH2 bunch extending vibration at 2956.03 cm^{1}. The C=C extending vibration is answerable for the top at around 1647.61-1556.15 cm^{-1}.

The C-O extending vibration has a top at roughly 1089 cm1, while the C extending vibration has a band at 832.10 cm^{-1}[29]. As the CS content of the movies expands, the power of the band at around 3301 cm^{-1}. Diminishes this could be expected to the - Gracious extending vibration of PVA with optional - NH gatherings of CS.

The expansion in PVA content in the movies likewise brought about a reduction in the power of the band relating to N-H twisting (amide II) at 1539 cm^{-1} of the CS film. The pinnacle evaporated from the range of the unadulterated PVA film because of the shortfall of the - NH utilitarian gathering.

Moreover, as the PVA content expanded, the force of the ingestion looking at the range of the mixed film to that of unadulterated CS film, the mixed film's retention top at around 1245 cm^{-1}. Disappeared Band relating to the C-H extending vibration expanded at roughly 2925 cm^{-1}[29].

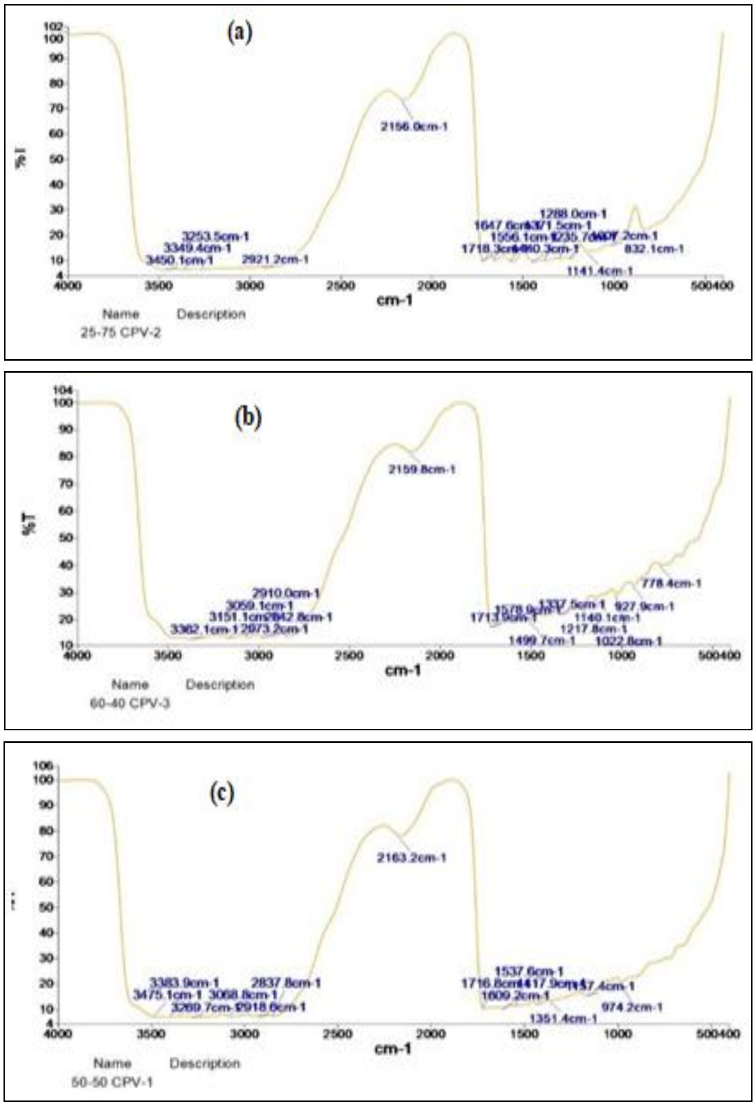

FIG. 2: FTIR SPECTRUM OF CS/PVOH COMPOSITE FILM WITH DIFFERENT WEIGHT RATIOS: (A) CS/PVOH = 25:75; (B) CS/PVOH = 50:50; (C) CS/PVOH = 40:60.

XRD: X-beam diffraction examination is a material science method for deciding the crystallographic construction of a material. This method involves exposing the sample to X-rays and measuring the intensities and scattering of the X-rays as they exit the sample. The XRD spectrum of a PVA/Starch composite film is shown in **Fig. 4**. A hump was observed in the spectrum at 19.5 Å (2Θ = 5.0485), which corresponds to the amorphous nature of the film, as confirmed by an X-ray diffractometer [30].

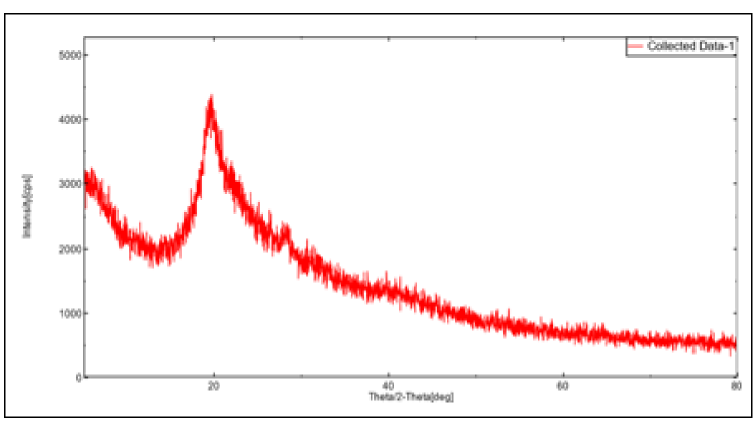

FIG. 3: X-RAY GRAPH OF THE COMPOSITE FILM

SEM: Field outflow checking electron microscopy was utilized to look at the movies' morphology (FESEM). FESEM gives data about the presence of voids, the homogeneity of the composite, the presence of total, the dispersion of nanoparticles inside the consistent framework, and the conceivable direction of nanoparticles. Following the blend, the perceptions were made on the outer layer of the CS/PVOH film [31].

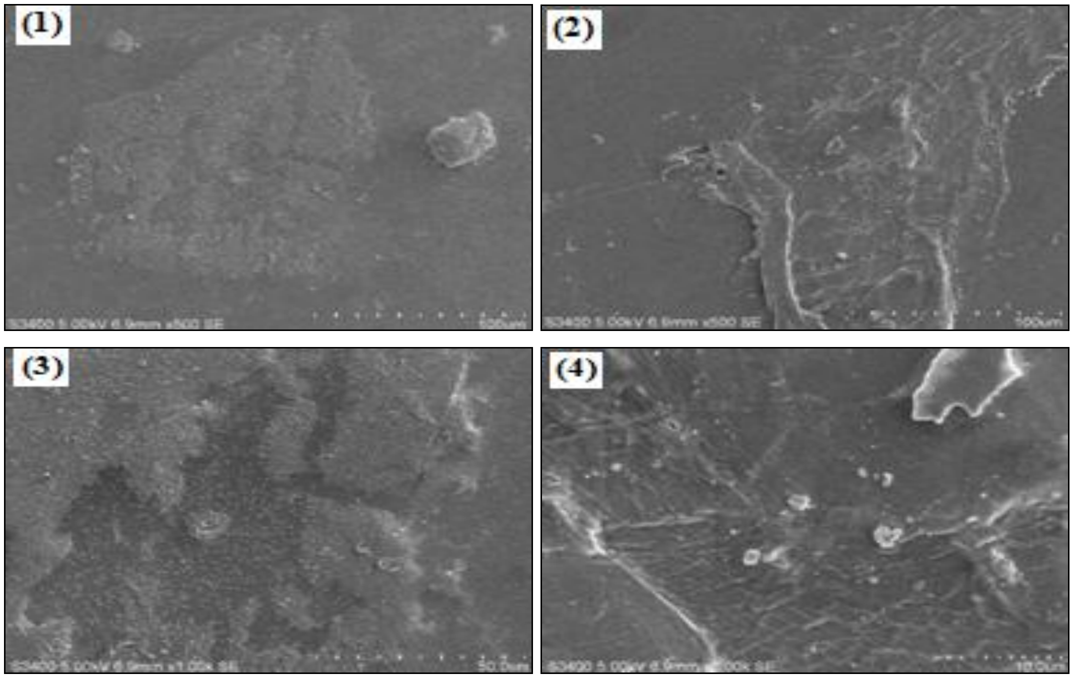

FIG. 4: FESEM PICTURES OF THE OUTER LAYER OF PVOH/CS FILM

UV-Vis Analysis of Neem Extract & Nio Nanoparticles:

Neem Extract: A simple UV spectrophotometric method is developed for determining Azadirachtin, with distilled water having the highest absorbance at 400 nm. Statistical analysis was carried out, with satisfactory results. The wavelength with the lowest absorption is 320 nm[32].

NiO Nanoparticles: UV-vis spectra of NiO nanoparticles after calcination. The assimilation edge is seen in the scope of 280-350 nm this blue shift of the ingestion [11, 12].

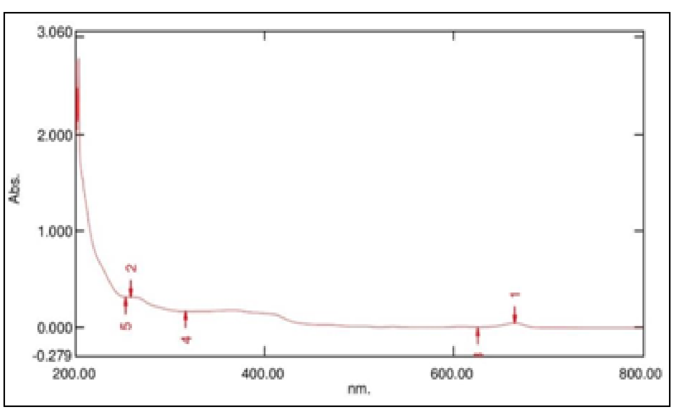

FIG. 5: THE ULTRAVIOLET-VISIBLE SPECTRUM OF NEEM

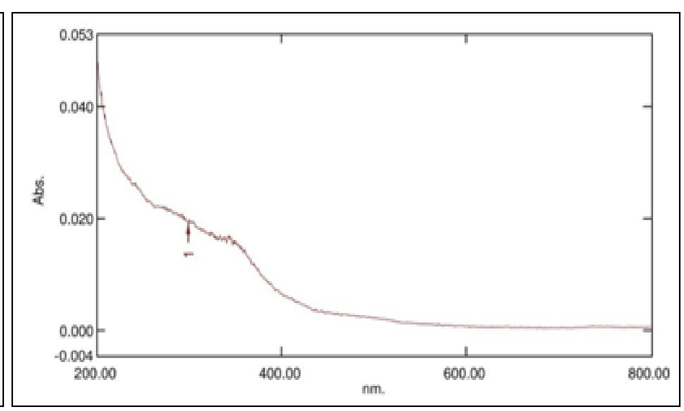

FIG. 6: UV- VIS SPECTRUM OF NIO EXTRACT NANOPARTICLE

TGA: The TGA curves of PVA/CS blended films with varied weight percentages. **Table 2** summarises **Fig. 7** in terms of thermal characteristics such as temperature and weight. It was discovered that the first weight loss occurred at around 100 degrees Celsius due to the evaporation of absorbed water moisture and leftover acetic acid [24].

TABLE 3: TGA ANALYSIS TABLE

Temperature (◦c)	24.88	104.88	204.88	304.88	404.88	504.88	604.88	704.88	734.88
Weight (%)	99.926	95.357	87.384	70.408	37.692	17.192	14.389	11.950	11.140

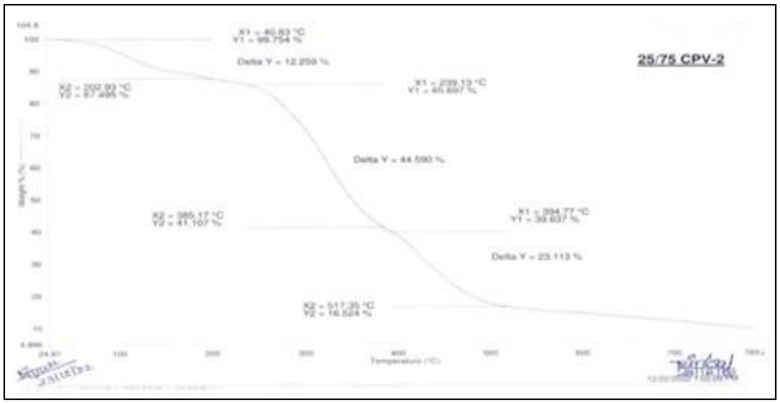

FIG. 7: TGA GRAPH

Antibacterial Activity: *E. coli* was used as the test subject for the antibacterial activity of a film containing NiO nanoparticles and Neem extract. One piece the NiO nanoparticles and Neem extract loaded PVA/CS film (labelled as (A) in the figure) were placed in a petri dish containing an *E. coli* culture. The Characterization section of this study discusses the procedure for creating *E. coli* cultures. The zone of inhibition in the composite film containing Neem extract and NiO nanoparticles was measured at 3.6 cm in diameter, whereas the zone of inhibition in the PVA/CS film was measured at 1.5cm. This indicates the existence of a bioactive component [25].

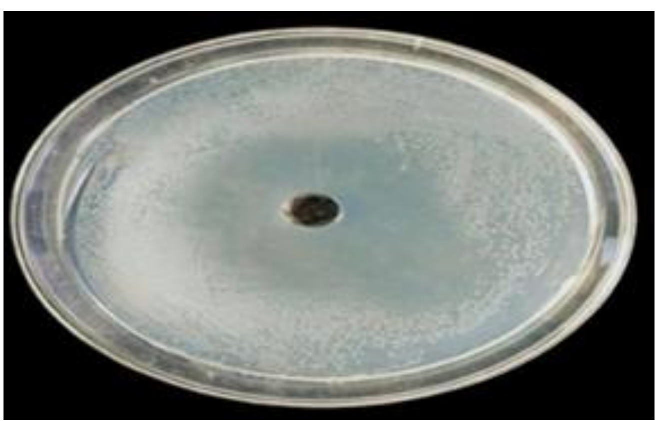

FIG. 8: ANTIBACTERIAL ACTIVITY OF NIO NANOPARTICLES AND NEEM EXTRACT-LOADED PVA/CS FILM AGAINST *E. COLI*

CONCLUSION: In this current review, not many ends can be derived after leading exhaustive portrayal tests. In synopsis, PVOH/CS bio-composite film and NiO nano and neem extricate stacked PVOH/CS film. The arrangements were ready by projecting strategy followed by portrayal tests. This work showed the creation of a PVOH/CS composite film by a dissolvable projecting technique. The composite film was found to contain assimilation properties shown by enlarging study. Besides, attributes like practical gathering and design, and not entirely settled by UV-apparent spectroscopy, Fourier-change Infrared spectroscopy, X-beam diffraction, and Examining electron microscopy. A PVOH/CS composite film loaded with Neem extract and NiO nanoparticles was also made, and its Antibacterial activity was tested using *E. coli* bacteria. The zone of inhibition was larger in the loaded film than in the non-loaded film. At long last, the discoveries will support the improvement of biodegradable and sustainable nanocomposite films that will be very important in different applications.

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CONFLICTS OF INTEREST: Declared None

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