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SYNTHESIS AND CHARACTERIZATION OF STARCH/CASEIN-BASED BIODEGRADABLE NANOCOMPOSITE FILMS INCORPORATED WITH SILVER NANOPARTICLES FOR BIOLOGICAL STUDIES.

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Abstract: In the current scenario many researches are done on polymers and their nanocomposites that help to design and develop new material with distinct properties which can enhance the quality of human life. So such investigations on novel polymers are emerging, since it becomes essential and inevitable in fields of life such as clothing, exterior coatings, food, biotechnology, medicine and so on. Currently need for biodegradable products are increasing day by day. Biodegradable polymers are polymers designed to degrade upon disposal by the action of living organisms into natural bioproducts such as gases, biomass, organic salts and water. Starch and Casein are natural biodegradable polymers with various biological activity. Addition of nano scale fillers allows the modification of physical properties as well as performance of new feature in the polymer matrix. The aim of present study is to prepare starch/casein blend films by solution casting method and its nanocomposite with silver nanoparticles. The prepared polymer and its nanocomposite were characterized by Infrared spectroscopy (FTIR) and UV- Visible spectroscopy. Antibacterial activity of the samples was studied using the agar well/disc diffusion method. Result showed that starch/casein nanocomposite can be used for various biological applications.

Index Terms – **Polymer blend, Ag Nanoparticles, Antibacterial study.**

I. INTRODUCTION

Polymers constitute a fundamental class of materials found extensively in both living organisms and minerals. Naturally occurring polymers such as proteins, cellulose, and nucleic acids are integral to biological functions, while synthetic or inorganic polymers are present in materials like diamond, quartz, and feldspars. Polymers are highly versatile molecules— not limited to repeating monomers of identical chemical composition, molecular weight, or structure—which makes them applicable across a vast range of industries. From basic plastic commodities to specialized components in the aerospace sector, their utility is boundless [24].

Biopolymers, a significant subset of polymers, are complex assemblies that adopt well-defined, three-dimensional structures [2] .These structures confer properties essential in applications ranging from cosmetics to drug delivery systems and from emulsifying agents to adhesives [16] . Most polymers are chemically resistant and serve as excellent thermal and electrical insulators. One important category within polymer science is polymer blends, which, similar to metal alloys, are physical mixtures of two or more polymers to generate new materials with enhanced properties. These blends offer advantages such as increased toughness, elasticity, lightweight characteristics, cost-effectiveness, and ease of processing. [9,10,12,22] For instance, blending biopolymers with synthetic polymers can tailor degradation rates and improve specific physical attributes. [4,17,23]This method is often preferred over copolymerization due to its simplicity and speed. However, a common challenge in polymer blending is immiscibility, which arises due to the low entropy of mixing, necessitating strong intermolecular interactions for miscibility. Still, polymer blends have found critical uses in drug delivery, packaging, film coatings, and controlled-release applications. [14]

Earlier, nanoparticles were synthesized via chemical methods using stabilizing and reducing agents; however, concerns over environmental toxicity have shifted attention to green synthesis approaches. These eco-friendly methods often utilize plant extracts, which contain phytochemicals such as flavonoids, ketones, and terpenoids that can mediate nanoparticle formation. For instance, zinc oxide nanoparticles have been successfully synthesized using extracts from coriander, crown flower, copper leaf, and china rose. [1]

When nanotechnology is integrated with polymer science, it results in the creation of polymer nanocomposites (PNCs)—materials in which nanoscale fillers (10–100 Å) are dispersed within a polymer matrix [20]. These composites combine the advantages of both polymers and nanomaterials, enhancing electrical, thermal, optical, and mechanical properties. Nanoparticles with high surface-to-volume ratios introduce extensive interfaces within the matrix, leading to unique characteristics not found in traditional composites. PNCs demonstrate improved modulus, gas barrier properties, impact resistance, thermal stability, and even antimicrobial activity [17]. Biopolymers like casein and starch serve as sustainable matrices for nanocomposites. Casein, derived from milk, constitutes about 80% of milk protein and shows thermoplastic and film-forming properties. Its resistance to heat and hydrophobicity make it suitable for food packaging and UV protection [18,21].Starch, a biodegradable and renewable carbohydrate from plants, is one of the most abundant natural polymers. Its non-toxic and eco-friendly nature makes it widely applicable in various green technologies [5].

Due to their biodegradability and tunable properties, polymer nanocomposites (PNCs) find wide applications in biology and medicine. In orthopaedics, nanocomposite screws and rods made from Hydroxyapatite (HAP)-based systems are used for internal bone fixation, providing biocompatibility, non-toxicity, and antimicrobial functionality, especially when doped with silver nanoparticles [8,11,13,15]. In drug delivery, PNCs enable controlled and sustained drug release. Silicate-based nanocomposites provide tortuous diffusion paths, improving drug encapsulation and release kinetics. Silver nanoparticles are utilized for their antimicrobial and anti-inflammatory drug delivery capabilities, even in vaccine development [3]. Biosensors made from conductive polymer nanocomposites are used to detect glucose, methanol vapors, proteins, and cancer cells. Their high sensitivity and selectivity make them ideal for real-time monitoring applications [6,7]. In tissue engineering, nanocomposite-based scaffolds facilitate cell growth and tissue regeneration.

II. RESEARCH METHODOLOGY

2.1 Materials

Casein (CAN), Starch, Sodium hydroxide (NaOH) and Silver nitrate ($AgNO_3$) were purchased from Merck and were used as received. All aqueous solution was prepared using distilled water.

2.2 Preparation of CAN/starch solution

CAN (3g) was dissolved in 100 ml of distilled water and the pH of the solution was adjusted to 8 by adding NaOH solution into the mixture. And the mixture was continuously stirred with constant heating. Starch (3g) was dissolved in 100 ml of water and the same procedure was repeated. Blend of CAN/starch in the weight ratio of 30:70 was prepared by solution casting method.

2.3 Preparation of silver nanoparticles

To prepare the aqueous extract of turmeric powder, 6g of organic turmeric or curcumin powder were weighed and mixed with 100 mL of Milli-Q water. Green synthesis of AgNPs was accomplished using the aqueous extract of turmeric powder. The synthesis of silver nanoparticles was carried out using silver nitrate as precursor. A solution of 0.01% of silver nitrate was made up in a 100ml standard flask. 20 mL of the solution silver nitrate was brought to reaction temperature with vigorous stirring. To this add 4ml of turmeric extract. After the reaction, the colloidal solution was stirred to cool at room temperature, transferred to an amber bottle. Appearance of silver nanoparticle is observed by the change in colourless solution to pale yellow color solution.

2.4 Preparation of polymer nanocomposite

Nanocomposite film of the polymer blend of CAN/starch in the weight ratio 30:70 was prepared by solution casting method using silver nanoparticles. CAN and starch was blended using 10 ml of silver nanoparticle solution by dispersing it into the above solution and it was stirred for one hour. This solution of nanocomposites was then poured into a petri dish and the solvent was removed by keeping it in a temperature controlled hot air oven at 40°C. After complete vaporization of solvent for four hours, the films were peeled out from the petri dish.

2.5 Antibacterial studies

Antibacterial of extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc was placed in MHA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc .The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition. Kirby Bauer test or disc diffusion test is for determining the resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds. Muller Hinton agar is the medium for growing pathogenic organism in presence of various antimicrobial impregnated filter paper disks. The ability of the compound to inhibit the organism can be understood by noting the presence or absence of growth around disk.

III. RESULTS AND DISCUSSION

3.1 INFRARED SPECTROSCOPY (ATR- FTIR)

The chemical structure of the CAN/Starch and its nanocomposites were investigated with this spectral study to illustrate the impact on modifying the samples with Ag NPs.Figure:1 clearly shows peak around 2950-2850 cm^{-1} (2925cm^{-1}) corresponding to C-H stretching, broad peak around characteristic peaks at about 3290-3300 cm^{-1} corresponding to the -N-H stretching and out-of-plane bending of amide groups –NH-C=O on the polymer backbone are observed. Peak around 1300-1400 cm^{-1} (1365cm^{-1}) corresponds to $–CH_2$ bending, peak around 1550-1650 cm^{-1} (1644cm^{-1}) corresponds to N-H stretching. .The absorption peaks for –C=C and –C=O can be clearly found at 1533 cm^{-1} and 1641 cm^{-1} respectively. The spectra of proteins generally have characteristic absorption peaks associated with the peptide bonds (-CONH-). In the case of Ag nanocomposites (figures:2) there is very slight shift in the absorption peaks of carbonyl group of amide due to the physical interaction of Ag NPs into the polymer matrix.

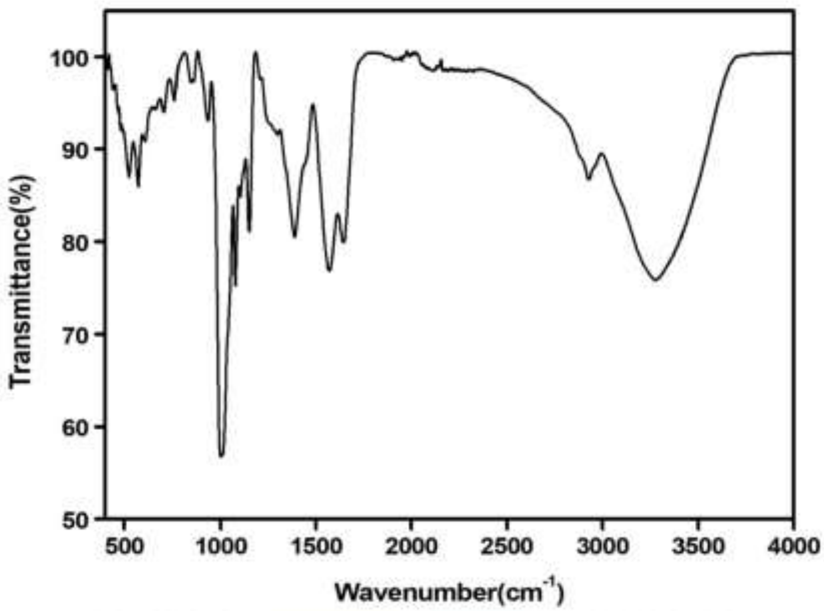

Fig. 1: ATR FTIR spectrum of starch/casein blend

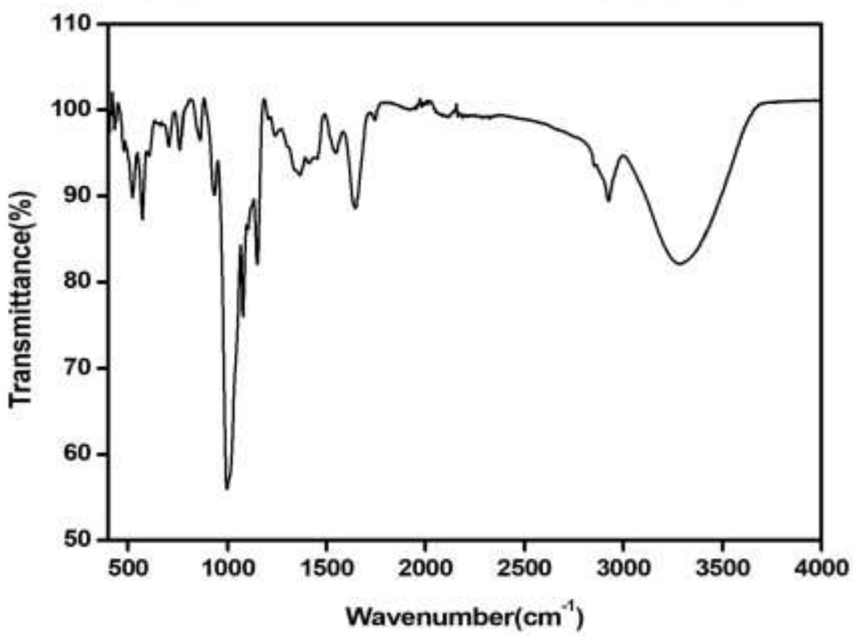

Fig.2: ATR FTIR spectrum of Ag-starch/casein nanocomposite

3.2 UV-VISIBLE SPECTROSCOPY

A strong broad absorption band is observed in the absorption spectra of silver nanoparticles at 420 nm range. This occurs due to the coupling of incident electromagnetic radiation into a surface plasmon at the interface between the particle and the medium surrounding the particle. The resulting energy loss is manifested as an absorbance known as the surface plasmon band. The collective oscillations of the free electrons are sensitive to changes in the size of the particle as it is confined to the surface. As the diameter gets smaller, the energy required to excite the motion of the surface Plasmon electrons increases.

Comparing the spectrum of the polymer blend and polymer nanocomposite incorporated with silver nanoparticles (Figure 3), nanocomposite shows a peak around 410-425 nm which is absent in polymer blend. Thus incorporation of NPs into the polymer matrix is confirmed by the excitation of the typical SPR peak of Ag NPs.

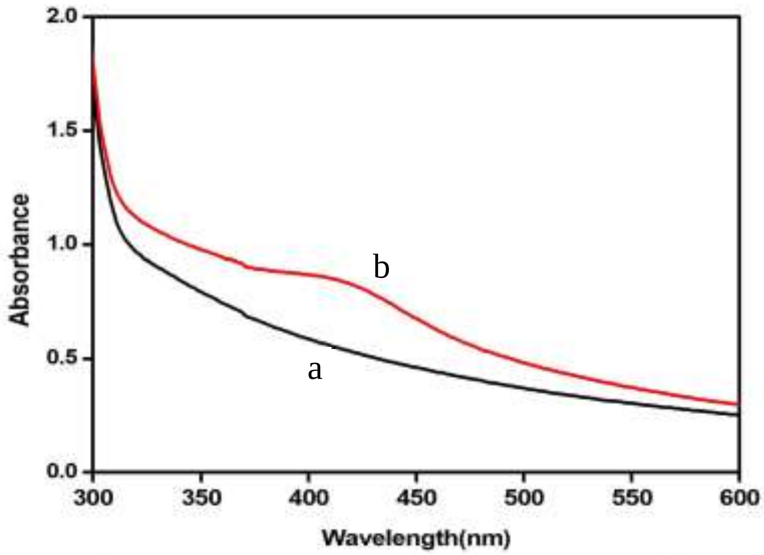

Fig.3: UV-Visible spectra of a) Polymer blend b) Nanocomposite

3.3 ANTIBACTERIAL STUDIES

The antibacterial activity of polymer and polymer nanocomposite were assayed by agar diffusion method. This was studied by using *Staphylococcus aureus*as test organisms. The sensitivity test is mainly based on the size of zone of inhibition. Diameter of the zone of inhibition was measured for sample film.

Sl. No	Sample Name	Zone of Inhibition in mm		
		100 µg/ml	250 µg/ml	500 µg/ml
1.	CAN/Starch	5	5	6
2.	Ag Nanocomposite	8	10	12

Table 1: Zone of inhibition of polymer blend and polymer nanocomposite

Fig. 4: Antibacterial activity of polymer blend.

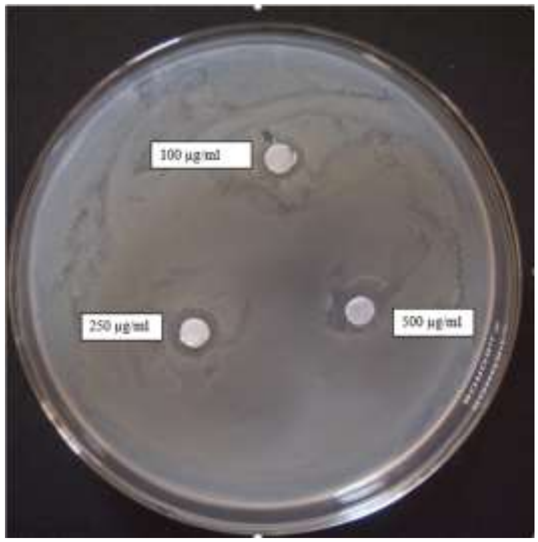

Fig.5: Antibacterial activity of polymer nanocomposites.

IV. SUMMARY AND CONCLUSION

In the present study, we have prepared CAN/Starch blend and its Ag Nanocomposites.Ag nanoparticles were prepared by using turmeric extractas a reducing agent and protecting agent. Prepared polymer nanocomposites were characterized by using UV-Visible absorption spectroscopy (UV) and Infra Red spectroscopy (FTIR)The antibacterial activity of the Ag nanocomposites was studied using the disk / well diffusion method. Microorganisms (Gram positive) was tested for their sensitivity to the polymer nanocomposites. Samples showed antimicrobial activity against the tested organisms. Results showed that the prepared nanocomposites can be used for the application of food packaging.

V. ACKNOWLEDGMENT

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