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SYNTHESIS, CHARACTERIZATION AND BIO-MEDICAL APPLICATION OF ESSENTIAL OIL LOADED CHITOSAN-GELATIN COMPOSITE FILM

AHUJA S*, PATEL M AND TAILOR K

Department of Chemistry, Parul Institute of Applied Sciences, Parul University, Limbda-391760, Waghodiya, Vadodara, Gujarat, India

***Corresponding Author: Dr. Sonam Ahuja: E Mail: sonam.ahuja82106@paruluniversiry.ac.in**

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ABSTRACT

In biomedical field, a bio adhesive wound-dressing material based on a correct ratio of gelatin (g) and chitosan (cs) was created and successfully applied. A chitosan – gelatin film was created using a solution casting process. When chitosan was combined with gelatin, the amount of water takes up by the film increased. Compared to the chitosan film. In all formulations, glycerol (1 ml) was utilised as a plasticizer. FTIR and XRD were used to test the compatibility of these two biopolymers. For the best wound-healing action, the right ratio of chitosan and gelatin was used. In everyday life, medicinal plants are used to treat a variety of ailments. Extraction of black pepper were used for antibacterial. The best antibacterial properties were found in composite film. As a result of these findings, Chitosan-gelatin bio composites can be employed as a replacement for traditional plastics in the packaging and bio-medical industries.

Keywords: Chitosan, gelatine, composite film, black pepper, wound healing, antibacterial

1. INTRODUCTION:

Any of a class of natural or manmade substances made up of very big molecules known as micro molecules, which are made up of many smaller chemical units known as monomers. Polymer may be naturally found in plants and animals (natural polymer) or manmade (synthetic polymers) units called monomers. Natural polymers are made from renewable resources in big quantities, whereas synthetic polymers are made from non renewable petroleum resources. Biodegradable polymers are renewable resources. Packaging, agriculture, health, and other industries employ biodegradable materials. Biopolymers are biodegradable polymers that are found in nature. Proteins and other natural polymers can be used to create biodegradable products. Collagen is the most abundant protein in animal connective tissues **[1]**. Gelatine is a high molecular weight polypeptide generated by denaturation or physical – chemical degradation of collagen **[2]**. Gelatin, a transparent, colourless, brittle, and tasteless protein generated from collagen, enhances general health. It has a film-forming property and is well-known for its wound healing properties **[3]**. Gelatin is water soluble. Glycine, proline, and hydroxyproline are the most abundant amino acids in hydrolyzed collagen, accounting for around half of the total amino acid content. The barrier and mechanical characteristics of these films are dependent on the gelatine's physical and chemical properties, particularly the gelatine's ability to absorb water. Gelatine films have mechanical and water vapour barrier characteristics **[4]**. Gelatin a food gelling agent that is profitable dispersion systems, drug encapsulation, and other related topics biodegradable film. Stand-alone gelatin films, on the other hand, are brittle. Due of the polymer's brittleness, it is prone to cracking. Cohesive energy density that is robust furthermore, these film sare thermally unstable and provide insufficient protection because of their hydrophilic nature, they act as a water barrier, and mechanical qualities are unsatisfactory as a result, crosslinking agents and plasticizers are used.

Figure 1: Gelatin

Figure 2: Chitosan

Natural polymer as polysaccharides. Polysaccharides obtained from seaweed. Chitin is the most prevalent biopolymer in nature. It's a 1,4-linked linear copolymer of n-acetyl glucosamine and n-glucosamine. Depending on the biopolymer processing procedure, these units are dispersed randomly or in blocks across the biopolymer chain. Crabs, shrimp, crawfish, and insects all have chitin in their shells. Give an alternate chitin source **[5]**. It describes the distribution of chitin and chitosan in nature, as well as how bacteria can biosynthesise chitin and chitosan. Chitosan is produced via partial alkaline n-deacetylation of chitin. Glucosamine units are the most abundant in chitosan. The crystallinity, surface energy, and degradation rate of chitosan are all affected by this approach. Water and alkaline media do not dissolve chitosan. This is owing to its crystalline structure, which is stiff and compact. Strong intramolecular and intermolecular hydrogen bonding chitosan is only soluble in a few types of water. In acidic solutions, chitosan dissolves. Biodegradable films chitosan is degraded by enzymes like chitosanase and lysozymes. Because chitin and chitosan are insoluble in most solvents, their applications are limited **[6]**. Chemical changes can be carried out on chitosan since it contains amino and hydroxyl reactive groups. Prepared for use in cosmetics and wound care. Drug delivery, cell delivery systems, orthopaedics, wound healing, ophthalmology, and bone healing all use chitosan **[7]**. Chitosan has antimicrobial properties antibacterial action **[8]**. Chitosan is non-toxic, biocompatible, degradable, bacteriostatic, and has good film-forming capabilities **[9]**. Chitosan has good wound healing properties **[3]**. The gelatin-chitosan blended film is an example of a protein-polysaccharide combination **[10]**. Both chitosan and gelatin have wound-healing and other health-promoting characteristics, so it's expected that the two will work well together.

Figure 3: black pepper

Black pepper used as a natural drug in chitosan-gelatin composite film. Piperine is found in the piperaceae family of plants, such as *piper nigrum* l, often known as black pepper **[11]**. It is the most abundant alkaloid ingredient in the fruit's skin and seeds. It is a solid material that is essentially water insoluble **[13, 14]**. Piperine and other alkaloids have been studied for their biological and antimicrobial properties **[15]**. Black pepper has a wide range of health benefits **[12]**. Black pepper can be used for a variety of things, including human diets, medication, preservatives, and bio control agents **[16, 17]**. It includes the primary pungent alkaloid piperine (1-peperoyl piperidine), which has a variety of pharmacological effects **[18]**. Since ancient times, *P. Nigrum* (*piper nigrum*) has been utilised for medical purposes in various regions of the world. Antibacterial properties of p. Nigrum have been used in medicine.

2. MATERIAL AND METHODS:

2.1 Material:

Chitosan, gelatin was obtained from (parul university), glycerine and distilled water.

2.2 Method of film preparation:

Chitosan solution: 2% chitosan solution was prepared by 0.5 gm chitosan is dissolve in 2% 25ml acetic acid with constant stirring by a magnetic stirrer at 50 °C for 30 min.

Gelatin solution: 10% gelatin solution were prepared by 5gm gelatine is dissolve in 50ml distilled water with constant stirring by a magnetic stirrer at 80 °C for 40 min.

Composite preparation: chitosan-gelatin composite were prepared by blending of 2% chitosan with 10% gelatin in a hot plate with constant stirring by magnetic stirrer at 50 °C for 30 min. For casting, the resulting solution was deposited in a thin plate. Plate was dried in oven at 90 °C for 24 h.

Figure 4: Chitosan-gelatin composite film

2.3 Extraction method:

Material: the seed of spice black pepper used for the present study were collect from the local market of Halol, Vadodara (India). Black pepper, methanol, conical flask, whattman filter paper 1, funnel.

2.4 Extraction of black pepper

5 gm of black pepper powder were placed in a conical flask containing 50ml of methanol and were kept for a period of 72 hours. After that, whattman filter paper no.1 was used to filter the contents.

2.5 Essential oil loaded polymer film:

20 ml 2% chitosan- 10% gelatine and 5 ml of black pepper extract were blend and cast in petri dish. Petri dish was dried in oven at 90°C for 24 h.

Figure 5: Essential oil loaded chitosan-gelatin composite film

3. RESULT AND DISCUSSION

3.1 FTIR spectroscopy:

FTIR (Fourier transform infrared spectroscopy) is highly versatile materials analysis technologies that can assist identify organic and inorganic contaminants that could cause product contamination or malfunction. As a result, we frequently offer FTIR as the initial stage in the testing procedure to our clients. The Fourier transform infrared spectroscopy (FTIR) analysis of this sample films was performed using a FTIR spectrum 400 (Parul University). The analysis was carried out in range from 400 to 4000 cm^{-1} with a 4 cm^{-1} resolution of scans. The FTIR spectra were recorded in transmittance mode. We have prepared film a dried and then crushed than prepared powder than analysis of FTIR. FTIR spectrum of chitosan-gelatin composite film is characterized by absorption peaks of chitosan and gelatin (3200-3450 cm^{-1} O-H & N-H stretching), 1030-1100 cm^{-1}(C-O stretching), 1640 cm^{-1}(C=O stretching), 1530-1580 cm^{-1} (amide ii). C–H stretching vibrations of methylene in chitosan and alkyl groups in gelatin are responsible for the absorptions at 2,920-2950 (CH_2) and 2,850-2870 cm^{-1} (CH_3). The absorptions in the range 1,550–1,500 cm^{-1} are due to N-H bending motions **(Table 1, Figure 6)**.

3.2 XRD Sprectra:

X-Ray patterns of chitosan/gelatine composite film were analyzed using an x-ray diffractometer (d2 phaser, bruker). When an x-ray passes through a crystal, it diffracts in a pattern that is unique to that crystal's structure. The diffraction pattern is obtained from a powder of the material rather than an individual crystal in powder x-ray diffraction. Because powder diffraction does not require an individual crystals, it is generally easier and more convenient than the single crystal diffraction. The intensity of a diffraction pattern is plotted versus the detector's angle, 2θ. The resulting image is known as a diffractogram **(Figure 7)**.

3.3 Swelling study:

Chitosan-gelatin composite films were soaked in pf solution for 0.5h and then take out from pf solution and dried it. And then checked its actual weight. Then start same process for the period of 1,2 & 3 hour. The ability of the films to swell is critical when creating a suitable drug carrier. As a result, swelling studies were conducted. Carried out in the case of polymer films. The swelling ratio percentage was calculated using the equation **(Table 2, Figure 8)**.

$$SR\% = \{(Mt-Mo)/Mo\} \times 100$$

SR = swelling ratio

Where Mt and Mo are the weights (g) of the swollen and dry polymer films respectively.

3.4 Antibacterial activity:

The antibacterial activity test result of film by disc method it was found that the black pepper extract was performed against *E.coli*. The media used for the growth of bacteria was nutrient agar and agar agar. The bactericidal activity of methanol extract was tested against zones of inhibition of 12mm. The presence of a bioactive component in the extract of medicinal plants was suggested by the antibacterial activity results. The methanol extract was maximum activity against *E.coli* which was shown **Figure 9**.

Table 1: (FTIR)

Peak frequency(cm^{-1}) for chitosan-gelatin composite film	
frequency range(cm^{-1})	**Functional group**
3413	**O-H stretching**
1622.92	**C=O stretching**
1546.08	**N-H bending**
1425.39	**O-H bending**
1099.22	**C-O stretching**
779.31	**C-H bending**

Figure 6: FTIR Spectra of Chitosan-Ggelatin Composite Film

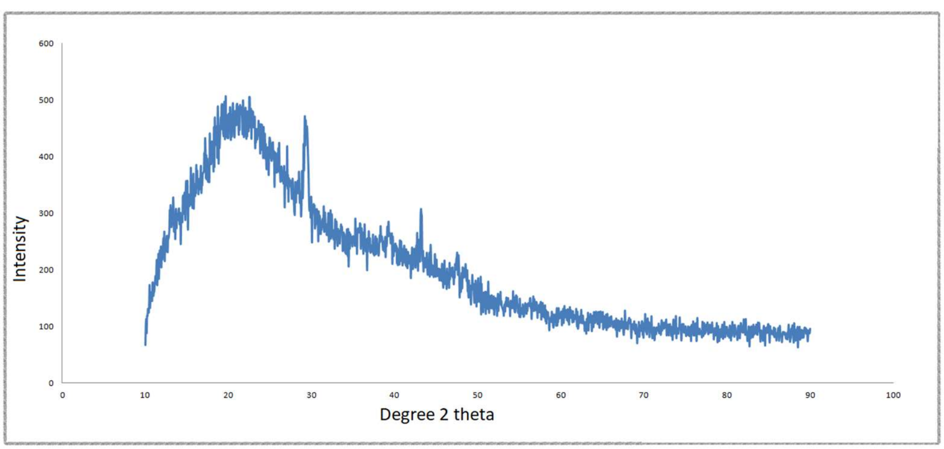

Figure 7: XRD spectra of Chitosan-Ggelatin Composite Film

Table 2: Swelling

TIME (min)	1%CS-10%G (SR%)	2%CS-10%G (SR%)	1.5%CS-10%G (SR%)
30	152	87	132.3
60	222.2	129	175
90	230	145	198
120	236	158	225
150	241	167	228

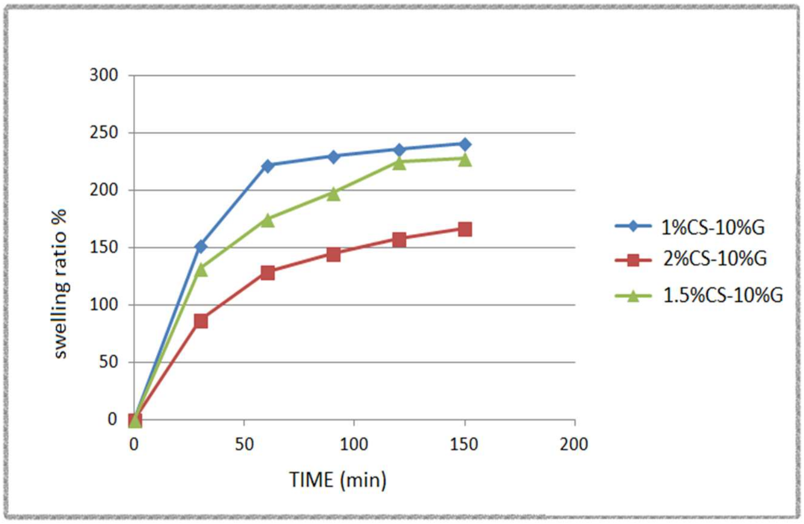

Figure 8: Swelling Study of Chitosan-Ggelatin Composite Film

Figure 9: Antibacterial Activity Of Essential Oil Loaded CS-G Composite Film

4. CONCLUSION:

The findings of this investigation demonstrated that chitosan might be advantageously added to gelatin for the production of a soft and elastic complex with good tissue scaffolding properties. Intermolecular interactions were discovered using FTIR research. Gelatin and chitosan, and these interactions resulted in the excellent interoperability of the composites. However, the release rate was improved with higher chitosan content in the film at later stages (after 3 h), which was attributable to the film's increased swellability in the pf solution. The composite films exhibit an antibacterial effect, according to the antimicrobial sensitivity research.

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