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GELATIN/PECTIN COMPOSITE FILM WITH CHARACTERIZATION AND APPLICATION

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ABSTRACT: Due to its similarity to the cell membrane seen in living organisms, gelatin is a natural biopolymer commonly used in tissue engineering applications. However, the rheological properties of gelatin formulations preclude their use in extrusion-based bioprinting. Using pectin as a viscosities modifier and formaldehyde for gelatin as a gelatin-pectin crosslinking agent, we offer a technique in the current work to improve the gelatin bioprinting capabilities. The primary goal of creating the gelatin-pectin formulation is to create a homogenous gelatin-pectin composite. Through defrost, microporous gelatin-pectin-formaldehyde sponges are produced, and their inborn characteristics, such as porosity, particle shape, degree of swelling, flexural modulus, and cell attachment examined. This is so that the crosslinking reaction may be completed when using formaldehyde, which calls for a drying phase. Rheological studies were conducted after that.

INTRODUCTION: Examination of current research in polymeric materials that can break down into environmentally safe molecules while exposed environmental factors. to Ιt demonstrated that the fabrication of composites made of synthetic and natural polymers is the most effective technique for producing such materials. Direct fabrication of layered composite films is possible by alternately depositing the combined dispersions of the two constituents. Biodegradable polymers are a subset of a family of polymer materials that have found use in various industries, including packaging and film production and medicinal applications like tissue engineering, wound care, medication delivery, and orthopedic implants.



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Pectin: Fruits provide a carbohydrate known as pectin, notably abundant in citrus fruit and apple rind ⁵. Pectin was first introduced as a homeostatic agent in 1935, promising to reduce bleeding under several circumstances. Pectin polymer is made from natural sources and has several benefits; thus, the pharmaceutical and biotechnology industries are using it more and more frequently. Pectin is stable and is employed in medication delivery systems under acidic conditions. The non-toxicity of pectin has long been recognized.

Gelatin: It is a natural polymer formed from the hydrolytic destruction of collagen protein, and its particular amino acid makeup provides various medical benefits. Gelatin is made composed of 18 distinct types of complex amino, with glycine, pralines, and hydroxyproline accounting for around 57% of the total, and the other 43% made up of other well-known amino acid families containing alanine, arginine, aspartic acid, and glutamic acid. 25.2% oxygen, 6.8% hydrogen, 50.5% carbon, and 17% nitrogen make up gelatin, while gelatin is composed of a combination of these components ¹.

Cinnamon: Cinnamon acts as a coagulant, preventing bleeding. Cinnamon also improves uterine blood circulation and promotes tissue regeneration. This plant is valuable as a spice, but its essential oils and other compounds have antibacterial, antifungal, antioxidant, and antidiabetic properties.

Cinnamon possesses anti-inflammatory, antiemetic, antimitotic, and anticancer properties. Cinnamon has also been utilized previously to treat headaches, dental disorders, oral microflora, and foul breath.

Use of Composite: Film's application in biomaterials, medical dressings, and skin tissue engineering, wound dressing

Dressing: Today's moist interactive dressings function on the same basis. They promote healing by producing a moist environment and relaxing nerve endings, eliminating wound discomfort and allowing healing to develop more naturally.

Skin Tissue Engineering: Though bioprocess for skin is a relatively recent multidisciplinary area, it has its roots in the sciences of polymer chemistry as well as cell culture ⁹. The goal of bioprocess for skin is to regenerate the natural architecture and physiology of native skin.

Autologous keratinocytes have been utilized in therapeutic settings for over 18 years. However, standard plastic surgery treatments, such as autologous full-thickness skin grafts, pedicle or free flaps, are the only way to achieve the aforementioned goal.

Cultured skin substitutes have only lately begun to fully replicate the structures and biological factors of the skin ⁹. As a result, it may be argued that the entire potential of engineering skin tissue has not yet been

MATERIALS AND METHOD:

Materials: Pectin (Hemadri chemicals mulund west, Mumbai, India). Gelatin (Eklayya biotech private limited Ghatkopar East, Mumbai, India). Formaldehyde (Suvidhi Industries GIDC, Vapi Dist, Valsad) Cinnamon (buy from local market).

Method:

Preparation of Film: Combining two different types of biomolecules is a common step in

producing biodegradable films or composites. Films were produced with a gelatin-pectin emulsion by casting two solutions with various gelatin-to-pectin ratios, the mix films were created. Gelatin was dissolved in 40 cc of distilled water, and the mixture was continually swirled as it was heated to 60 °C for 30 minutes. After that, the pectin was magnetically stirred into 40 mL of10 -15 minutes of heating distilled water to 68 °C. Combine both of the prepared solutions next. The emulsion solutions were continuously stirred with an ultrasonic homogenizer to ensure good dispersion.

Preparation of Cinnamon Extract: Take 100ml water and boil it for 15min and, then add 30gm cinnamon and boil more 30 min then cool it and Filter the solution.

Preparation of Drug Loaded Film: The creation of biodegradable films or composites often involves combining two different types of biomolecules ⁴. Films made from an emulsified gelatin-pectin combination. The blend films were made by casting two solutions with different gelatin-to-pectin ratios. Gelatin was dispersed in 40 ml distilled water and heated to 60 °C for 30 minutes while being stirred continuously until gelatin dissolved. The pectin was then dissolved in 40 mL of distilled water with magnetic stirring and heated for 20 minutes at 70 °C. Then, combine both prepared solutions. The emulsion solutions were continually agitated with an ultrasonic homogenizer to achieve excellent dispersion. Finally, 1 ml of formaldehyde was added to the solution as a plasticizer and mixed gently with a low stirring pace for 20 minutes to remove the air bubbles. The film-forming solution was then cast in 50 ml, then add 5.0ml of cinnamon extract and stir it more for 5 minutes. Take 10.0ml solution in Petridis. The composite film's equivalent titles are PE/GE-1, PE/GE-2, and PE/GE-3³.

Chemical Analysis:

Physiological Fluid: The sample's water solubility (WS) was evaluated using the Gunter et al. method. Before determining the initial solids content, three films (2 2 cm) were baked in a dry oven at room temperature for 24 hours ². Next, The samples were combined in a 50 ml beaker containing 30 ml of the PF solution ⁶. And submerge it in the movie. After

24 hours of drying, the films underwent another 24 hours of drying in a dry oven to determine the solid content. Check every 30 minutes, and take one reading ⁶ Data below in **Table 1.**

TABLE 1: SWELLING STUDY

| Test | Time | PEC:GEL | PEC:GEL | PEC:GEL |
|--------|-------|---------|---------|---------|
| Number | | 70/30 | 60/40 | 50/50 |
| 0 | 09:52 | 0.612 | 0.0137 | 0.260 |
| 1 | 10:22 | 0.359 | 0.354 | 0.714 |
| 2 | 10:52 | 0.389 | 0.36 | 0.822 |
| 3 | 11:22 | 0.390 | 0.345 | 0.925 |
| 4 | 11:52 | 0.404 | 0.41 | 0.966 |
| 5 | 12:22 | 0.393 | 0.348 | 1.000 |
| 6 | 12:52 | 0.395 | 0.394 | 0.963 |
| 7 | 01:22 | 0.400 | 0.346 | 0.955 |
| 8 | 01:52 | 0.368 | 0.345 | 0.938 |

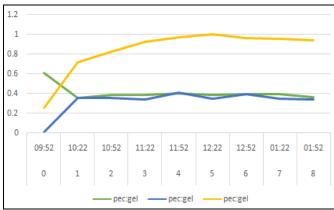


FIG. 1: PHYSIOLOGICAL FLUID TEST

Characterization:

Scanning Electron Microscopy (Sem): A scanning electron microscope was used to analyze the surface and reformat of the films (S-2400, Hitachi, Japan) ² ⁶. To account for conductivity. Before the testing, the samples were 14 nm thickly sputter-coated with gold (K-550, Emitech, England) ⁶.

Ftir-Atr Spectroscopy: Energy Dispersive Reflection (ATIR) FTIR spectrum of materials and PE-GE films were obtained using an FTIR spectroscope in the 4,000-600 cm range. In all cases. Using software from the (NEXUS-870 Thermo Nicolet Corporation), a constant resolution of 2 cm was kept.

Thermo Gravimetric Analysis (TGA): Both pectin & gelatin membrane's thermal properties were assessed using NETZSC H TG 209 F1 at a heating rate of 10 °C/min and a nitrogen flush rate of 100 ml/min ^{3, 7}. A JEOL JSM-670 IF Field

Emission Scanning Electron microscope (FESEM) was used to examine the pectin/gelatin composite surface morphology ¹⁰. Before being stored in a deep freezer, all of the composite film were given the chance to expand in artificial intestinal fluid. 80°C of freezing for two days ³. The materials were then freeze dry using a LABCONCO (United states) froze drying device for three days at 49 °C. The samples were kept under vacuum prior to the platinum sputtering procedure ³.

X-Ray Diffraction Study: A Phillips X-Pert diffractometer was used to obtain the X-ray diffraction patterns of the Composite film and natural resources. That worked at 40 kV and 25 mA and employed a Cu-Ka-radiation source ³. Plus, X-pert Highest Score. The diffractometer was controlled by software on a computer ³. The scan rate for X-ray diffraction patterns was 4.2 degrees per minute ³.

Antibacterial Test: The extracts' antibacterial activity was evaluated using seven pathogenic bacterial strains. Too with four additional Grampositive and Gram-negative microbes, *Mycobacterium luteus* (ATCC 4698), Bacillus cereus (ATCC 11778), *Enterococcus faecalis*, and Enterobacter sp. were assessed¹⁸.

Agar-Diffusion Method: The Berghe and Vlietinck technique was used to perform the antibacterial activity assay (1991). On the surface of Luria-Bertani (LB) agar medium, microorganism culture suspensions (200 L) containing 106 colony forming units (CFU/mL) of bacterium cells were smeared. Then, 60 mL of film were put into wells that had been punched in the agar layer, along with 5 mg/mL of BOPP-dissolved water.

To allow the extract to diffuse, all plates were kept at 4°C in the dark before to incubation. Antibacterial activity were discovered at the conclusion of the incubation period thanks to the appearance of quantifiable unambiguous inhibitory zones.

Sterilized water was used to prepare the negative controls. Over the past 50 years, the agar diffusion test has become vital for determining bacteria sensitivity to antibiotics ⁵ **Fig. 2.**



FIG. 2: ZONE OF INHIBITION 18MM

RESULT: Scanning Electron Microscopy (Sem):

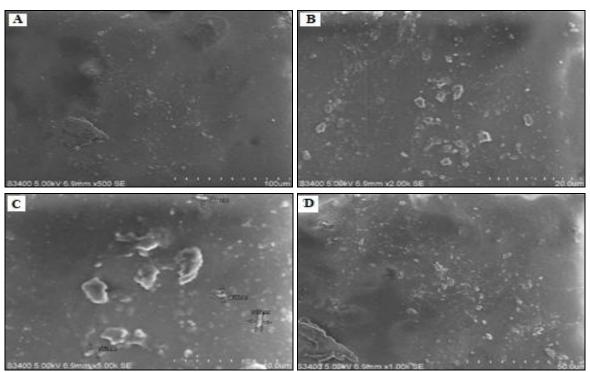


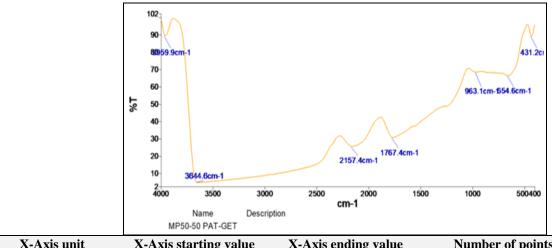
FIG. 3: SEM OF THE FILM MADE OF PECTIN AND GELATIN. (A) SURFACE (3400); (B) CROSS-SECTION (3400); (C) SURFACE (3400); (D) SURFACE (3400)

Similar repeating units throughout the polypeptide chains are absent in proteins, which is one of the key distinctions between them and synthetic polymers ³. Due to this irregularity, the protein chains are less likely to consolidate ³. It is quite likely that this characteristic helps explain the proteins' quick biodegradability.

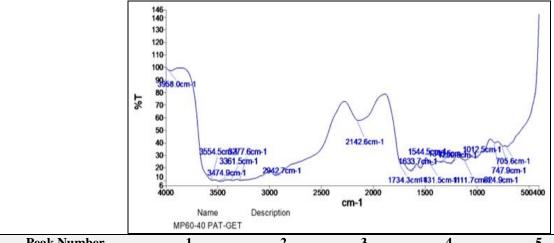
Contrarily, synthetic polymers often contain brief pieces that recur and this regularity promotes crystallization, blocking enzyme access to the hydrolysable groups ^{2, 3}. In conclusion, the automation characteristics mixed on pectin and gelatin composite film are discussed above. According to the findings of the current inquiry, the combination of irradiation and the use of agricultural by-products in manufacturing packing

materials may be utilized to improve the mechanical performance (Fig. 3).

FTIR-ATR Spectroscopy: By contrasting the FTIR spectra of the monomer and polymer samples, the grafting of the monomer onto the gelatin backbone polymer was verified. The bands of reactive functional groups were FTIR to determine the grafting of the compound. FT-IR was used to capture the IR spectra of the crossing and control groups at a resolution of 4 cm⁻¹ and in the 400–4000 cm⁻¹ range. In the IR spectra demonstrated the efficacy of the grafting process. Bands emerged to show the new ties created. FTIR to determine the grafting of pectin, gelatin Fig. 4(A), (B), (C), (D).



| X-Axis unit | X-Axis starting value | X-Axis ending value | Number of points | Y-Axis unit |
|-------------|-----------------------|---------------------|------------------|-------------|
| cm-1 | 4000 | 400 | 3601 | %T |



| Peak Number | 1 | 2 | 3 | 4 | 5 | 6 | |
|-------------|-------|---------|---------|---------|---------|---------|--|
| X(cm-1) | 3958 | 3554.53 | 3474.94 | 3361.46 | 3277.62 | 2942.71 | |
| Y(%T) | 97.59 | 10.12 | 9.13 | 9.82 | 9.88 | 13.94 | |

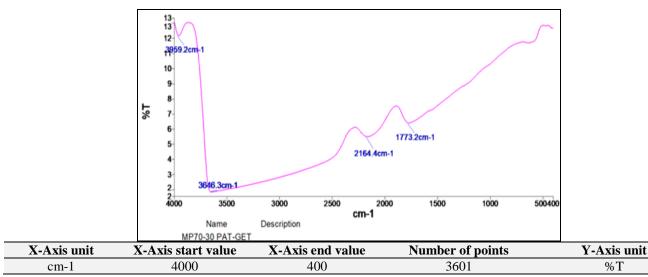


FIG. 4: (A), (B), (C), (D) FTIR SPECTRA OF DIFFERENT FILM SAMPLE

Thermo Gravimetric Analysis (TGA): Using a thermo gravimeter analyser, the samples thermo stability was accomplished. A 10 mm film sample

was subjected to temperature changes that ranged in diffuse to 736°C at a continuous rate of 10°C ¹. **Fig. 5.**

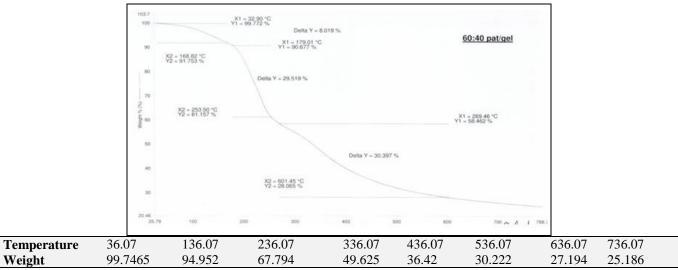


FIG. 5: TGA ANALYSIS GRAF

X-Ray Diffraction Study: The pure pectin X-ray diffractogram is displayed in Figure. The pectin

diffractogram clearly displays crystalline peaks that support the crystalline nature of the material **Fig. 6.**

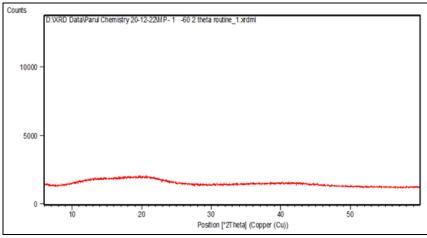


FIG. 6: XRD OF FILM SAMPLE

CONCLUSION: Solution casting was used to pectin/gelatin-based create film membranes. Investigation of the film using FTIR spectroscopy, SEM, XRD, and TGA reveals. The pectin and gelatin's interparticle interactions. According to the film spectroscopy, composite XRD crystallinity reduces as the gelatin volume in the resultant film increases ³. Gelatin concentration in the membranes was shown to boost the film's mechanical characteristics initially but eventually diminish them. In comparison to pectin, the film was shown to be more thermally stable. The results of the film's swelling research show that all of the films' swelling percentages were more than 100%, confirming that the film is naturally superabsorbent. A good application of the mix membrane might result in wet wound dressings.

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CONFLICTS OF INTEREST: Declared None REFERENCES:

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