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**CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF
NATURAL PLANT EXTRACT-LOADED PECTIN/SODIUM
ALGINATE-BASED POLYMERIC FILM**

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ABSTRACT

We developing a two-component, Pectin and polyvinyl Sodium alginate (NaAlg) based composite film loaded with natural plant extract of Sunflower seed. The solution casting technique is used to produce pectin and sodium alginate films. When Pectin was combined with Sodium alginate, the water absorption of the film increased, and glutaraldehyde (0.5 ml) was used as the crosslinking agent. The compatibility of these two biopolymers was determined by FT-IR and XRD. The exact ratio of Pectin and Sodium alginate was used. Medicinal plants are widely used in everyday life for various diseases. The extraction of Sunflower seed has 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. Pectin/Sodium alginate films loaded with Sunflower seed were tested for antibacterial activity against Escherichia coli bacteria (E. Coli bacteria). These results suggest that Pectin/Sodium alginate bio-composites can be used as special materials in the food packaging and biomedical industries. We analysed various analysis techniques, including SEM, and TGA to examine the properties of the films.

**Keywords: Pectin/Sodium alginate (NaAlg), Sunflower seed, Antibacterial& SEM,
TGA, FTIR, XRD**

1. INTRODUCTION:

The development of edible and biodegradable packaging films utilizing natural polymers is gaining popularity. Pectin and sodium alginate (NaAlg) are two common polysaccharides used in food, pharmaceuticals, and industrial applications. These components are utilized in cuisine as a thickening and gelling agent, imitation cherry, and other soft fruits (water-soluble pouches, bags for washing linens, and nonwoven fabric). These polysaccharides are used to make films, although they have a poor high humidity creating a water barrier. They are also less flexible than industrial synthetic packaging films that are used to extend the shelf life of food products.

1.1 Pectin Polymer:

Pectin is a complex polysaccharide that mostly consists of esterified D-galacturonic acid residues in an α -(1-4) chain. These residues are broken up by short Rhamnose inserts, which throw off the chain-helix structure. Other side chains include a variety of neutral sugars. These side chains give the molecule the appearance of sections that are smooth (have no side chains) and "Hairy" (have side chains). The methyl esterification of the galacturonate residue varies in degree. Pectin is crystallized and has a crystallinity of around 6% in citrus pectin, according to X-ray and electron microscopic investigations. Fibrous crystals can be seen

in naturally occurring pectin. from a variety of research [1-5].

1.2 sodium alginate:

Brown algae are the source of sodium alginate, which is a linear polymer with an anionic nature. Their two main components are mannuronic acid and uronic acid [6]. it is accountable for its acts. Alginates are natural substances that keep the wound wet for better healing and are biodegradable [7]. For wounds with considerable exudate production, alginates are frequently used excessively [8].

1.3 Antibacterial drug:

Sunflower (*Helianthus annuus* L.) is the world's second-largest oilseed crop in terms of total oil production, just after soybean. Antibacterial, anti-inflammatory, wound-healing, cardiovascular, and antioxidant benefits can all be derived from sunflower seeds. Oil has been produced from sunflower, because it has a high linoleic acid concentration, including meal and many other items. The sunflower has a wide range of uses in medicinal sciences due to its high oleic acid content. Sunflower seeds' powerful antibacterial capabilities enable the treatment of many infectious disorders with fewer drugs. Alkaloids, tannins, saponins, flavonoids, terpenoids, 44–72% linoleic acid, 11.77% oleic acid, and alkaloids are all present in sunflowers. *Staphylococcus aureus*, *pseudomonas*

aeruginosa, *Escherichia coli*, and *Bacillus subtilis* may all be successfully treated with sunflower seeds.

MATERIALS:

Pectin is purchased from HI Media Laboratories Pvt. Ltd.- Mumbai (INDIA). Sodium alginate is bought via Loba Chem. Pvt. Ltd. in Mumbai (INDI). Antibacterial drug (Sunflower) is purchased from the general pharmaceutical shop in the market which is mfg. by Cedilla Pharmaceuticals- Ahmedabad (INDIA).

METHODS:

1. Preparation of Pectin & sodium alginate Film: For the preparation of the film, take 50 ml of distilled water in a beaker, placed it on a magnetic stirrer, and stir for 15 to 20 minutes at 90 to 100 degrees Celsius and 500 to 520 RPM. 20 minutes later, add 1 g of Pectin and mix until the Pectin has melted. After that 3 g sodium alginate add for improved Film stability. And then

glutaraldehyde was added as a crosslinking agent to make a mixed solution. After that Preparation of Pectin & sodium alginate Film For the preparation of the film, take 50 ml of distilled water in a beaker, placed it on a magnetic stirrer, and stir for 15 to 20 minutes at 90 to 100 degrees Celsius and 500 to 520 RPM. 20 minutes later, add 2 g of Pectin and mix until the Pectin has melted. After that 2 g sodium alginate add for improved Film stability. and then glutaraldehyde was added as a crosslinking agent to make a mixed solution. The final mixture was then decanted into a glass petri dish in preparation for film formation and dried in an oven at 40 °C. Therefore, the resulting dry film (pectin/NaAlg) was separated from the petri dish and kept at room temperature for 48h overnight. Similarly pure Pectin/NaAlg film with different ratios is also prepared [1].

Table 1: Composition of different film-forming solutions

Samples	% Pectin solutions	% NaAlg solution	Glutaraldehyde	ratio	MI
Pectin/NaAlg 1	1% pectin (ml)	3% NaAlg (ml)	0.5ml	20/20	20-20 ml
Pectin/NaAlg 2	2% Pectin (ml)	2 % NaAlg (ml)	0.5ml	25/25	25-25 ml

1. **Extraction of essence from the sunflower seeds:** sunflower seeds put in the Reagent bottle and add 50 ml of Distilled water and heat for 2 hours. After that use this solution as an antibacterial drug.

2. **Preparation of drug-loaded antibacterial film:** Prepare the film by placing 50 ml of distilled water in a beaker and stirring it with a magnetic stirrer, and stir for 15 to 20 minutes at 90 to 100 degrees Celsius and 500 to 520 RPM. 20 minutes

later, add 1 g of pectin and mix until the pectin has melted. after that add 3 g sodium alginate and add a 4-5ml mixture of the drug which is made by adding 50 ml of pure Distilled water.

Characterization

1. Gelatine Expansion study: Using the variation in diameter of a circular film sample in a 10% gelatine solution, the expansion of the wound dressing film on the wound surface was examined. In a nutshell, 100 ml of warmed distilled water was used to dissolve 10 g of gelatine powder while stirring continuously until a clear solution was achieved. All ratio films with known diameters were then submerged in the gelatine solution in a petri dish, and the diameter change was continuously observed until the sample's diameter stabilized. The following expression was used to calculate the Expansion ratio (ER);

$ER = Dt/Do$, Where Dt is the diameter at time t and Do is the initial diameter

2. FTIR analysis: FTIR or Fourier transform infrared spectroscopy, is a method for figuring out the makeup of a composite film made of Pectin and Na Alg. After being dried in an oven for 24 hours with 1% of each polymer present, the spectra varied from 4000 to 400 cm^{-1} . PNP Analytical Solution in Vadodara performed the FTIR analysis while following all essential safety procedures.

3. XRD analysis: A method for analysing a material's crystal structure, chemical composition, and physiological characteristics is called X-ray diffraction (XRD). At PNP Analytical Solution in Vadodara, the XRD analysis was carried out with all relevant safety measures. from 5 to 60 degrees, the. The step size was 0.0090° , and the scan step duration was 8.2590 s. The wavelength of copper K-rays is 1.56070 \AA .

[12]

4. TGA analysis: TGA analysis measures the mass of an experiment as a function of time or temperature when controlled heating is present. TGA is a method of material analysis. Temperature and weight loss are seen when the material's constituent elements gradually volatilize over time. TGA testing is a helpful method for assessing polymers because it can track weight loss at extremely high temperatures. Polymers typically melt at temperatures of about 200°C before decaying, however, some can sustain temperatures of 301°C in air and 505°C in inert gases without degrading. TGA may also be used to study these polymers. At PNP Analytical Solution in Vadodara, the TGA analysis was carried out with the utmost caution [14].

5. SEM analysis: The surface of a sample is scanned using an electron microscope called a scanning electron microscope (SEM), which uses a focused electron beam to take pictures of the sample. The numerous

signals generated by the interactions of the electrons with the sample's atoms disclose the surface topography and chemical composition of the sample. SEM investigation was carried out to evaluate the surface morphology and determine how the Pectin/NaAlg composite films were disseminated. PNP Analytical Solutions in Vadodara conducted the SEM analysis while taking all required safety procedures. The ground-up composite film was examined in granular form. The working distance (WD) of the electron beam was kept at 6.6 mm, the electron beam's accelerating voltage (HV) was adjusted at 5.00 kV, and a working distance (WD) OF 6.6 mm was maintained [13].

RESULT AND DISCUSSION

1. Gelatine Expansion: Expansion studies are performed to assess the growth in diameter of polymer films. The following expression was used to determine the Expansion ratio (ER):

$ER = Dt/Do$, where Dt is the diameter at time t and do is the starting diameter (**Table 2, Figure 1**).

2. FTIR

Chemical and inorganic contaminants that potentially taint or harm items are found using the Fourier transform infrared spectroscopy (FT-IR) technique. As a result, the first stage of any sample is frequently detected using FTIR. This led to the acquisition of FTIR spectra for all varied

ratio composite films Pectin/NaAlg (1:3), and Pectin/NaAlg (2:2), The two various ratio composite films' band frequency findings were as follows: [11]

Pectin/NaAlg composite film: Pectin and sodium alginate's Fourier transform infrared spectroscopy spectra show distinctive absorption peaks. At the infrared spectrum, the hydroxyl group of sodium alginate may be seen at 3444.09 cm^{-1} . The existence of water and the alginate's carboxyl group was verified by peaks at 3323.58 cm^{-1} and 3221.75 cm^{-1} , whereas the peak at 3111.69 cm^{-1} was caused by -OH bending vibration [9]. Pectin's spectra revealed two peaks: the peak at 2141.69 cm^{-1} suggested ester carbonyl group (C=O) stretching, and the peak at 2932.25 cm^{-1} indicated a -OH stretching vibration, which can establish inter- and intramolecular hydrogen bonds [10]. As a result of the carboxylate anion's C=O stretching, another band can be seen at 1624.56 cm^{-1} . Raft displayed a peak at 1393.89 cm^{-1} , which is the typical peak of pectin's -OH stretching vibration. The raft's spectrum shows distinctive peaks for water and the carboxyl group of sodium alginate at 1124.83 cm^{-1} and 759.68 cm^{-1} , respectively. Raft confirmed the sodium alginate's -OH bending vibration with prominent peaks at 1027.74 cm^{-1} . The spectra of the formulation for the raft of APR15 showed that sodium alginate, pectin, and other excipients did not interact chemically with one another. FTIR

spectra thus support the raft's stability [11] (Figure 2).

3. XRD: A method used in material science to ascertain the crystallographic structure of a material is X-ray diffraction analysis. By exposing the sample to X-rays, this technique analyses the intensity and scattering of the rays as they leave the material. Figure 3 depicts the XRD spectrum of a composite Pectin/NaAlg film. An X-ray diffractometer showed a hump at $33.5(2\theta = 5.0485)$ in the spectrum, which is consistent with the film's amorphous nature [12].

4. SEM: We investigated the morphology of the films using field emission scanning electron microscopy (SEM). SEM gives data on the distribution of nanoparticles within the continuous matrix, the homogeneity of the composite, the existence of aggregate, the presence of voids, and the potential orientation of nanoparticles. On the Pectin/NaAlg film's surface, observations were taken after the synthesis [13].

The films' morphology was investigated by field outflow checking electron microscopy. (SEM). SEM provides information on the presence of voids, the homogeneity of the composite, the presence of total, the dispersion of nanoparticles within the

consistent framework, and the possible orientation of nanoparticles. Following the mix, perceptions were produced on the outer layer of the PC/NaAlg film [13] (Figure 4).

5. TGA: The graph and table of TGA shown above show how stable the gel can be up to what temperature, with the final measurement at $784.97\text{ }^{\circ}\text{C}$, its weight remaining only %. The 27.699 % addition of the more stable second component slowed the pyrolysis of the above-shown Gelatine-based formulations. These results could be attributed to the amount of Gelatine dispersion in the blend. As a result, the more stable component should act as a shield, preventing the less thermally stable component from disintegrating [14] (Figure 5).

6. Antibacterial Activity: The antibacterial effectiveness of loaded Pectin/NaAlg composite film was tested using sunflower seeds. 100 ml of distilled water should be used to dissolve 2.9 g of nutritional agar in a flask. The flask and Petri dish must be cleaned. Before adding the test organism (*E. coli*) to the solidified agar solution with a spreader, let the media cool to room temperature. 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 (Figure 6).

Table 2: Gelatine Expansion study table

Time	PC/NaAlg (1:3)	PC/NaAlg (2:2)
0 min	1	1
15 min	1.5	1.6
30 min	1.11	1.13
45 min	1.17	1.19
60 min	2.22	2.25

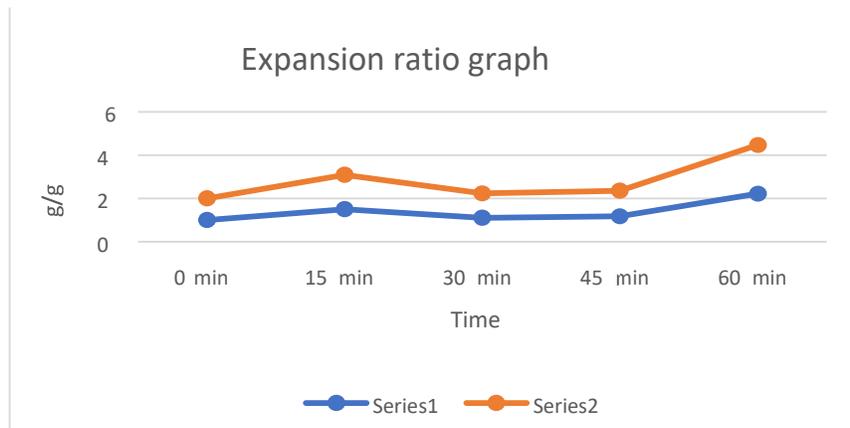
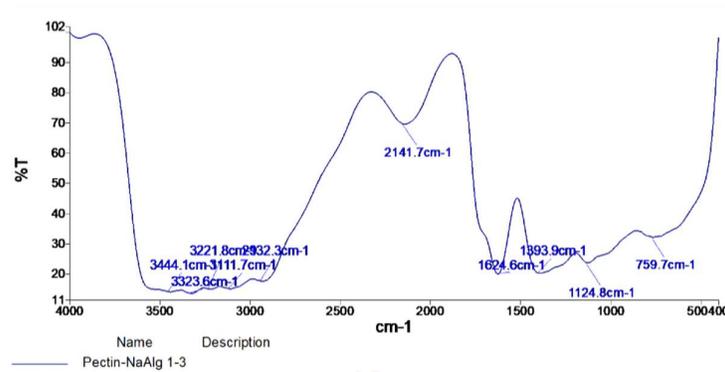
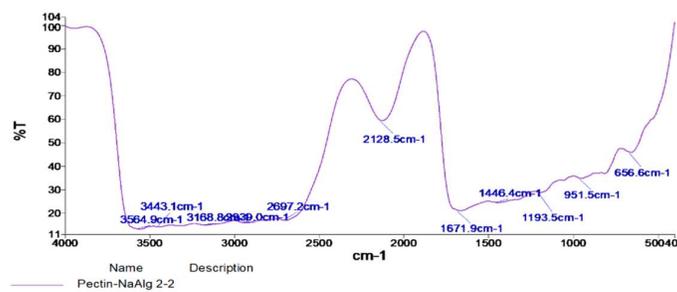


Figure 1: Graph of swelling ratio



(a)



(b)

Figure 2: FTIR spectrum of Pectin/NaAlg composite film with different weight ratios: (a) Pectin/NaAlg = 1:3; (b) Pectin/NaAlg =2:2

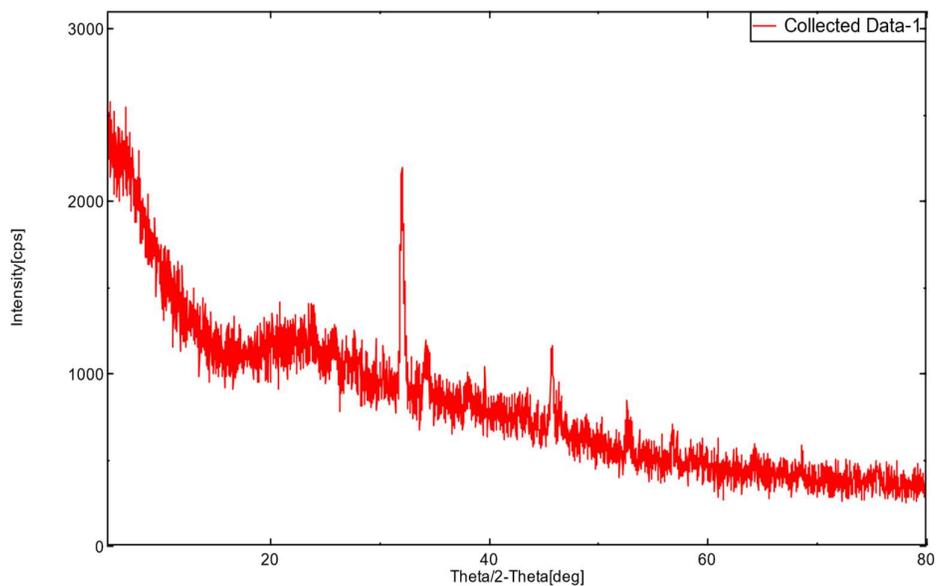


Figure 3: XRD graph of the composite film

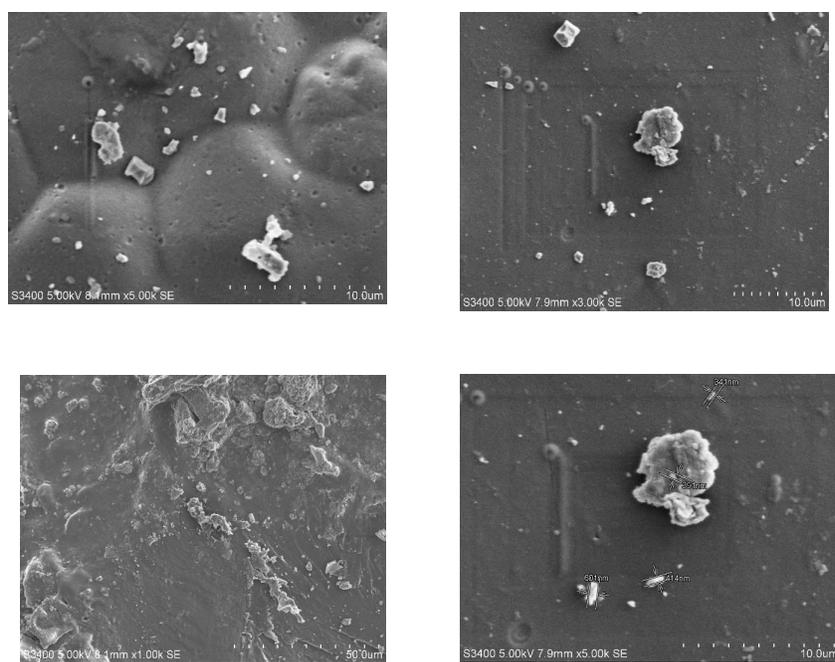


Figure 4: FESEM images of the surface of Pectin/NaAlg film

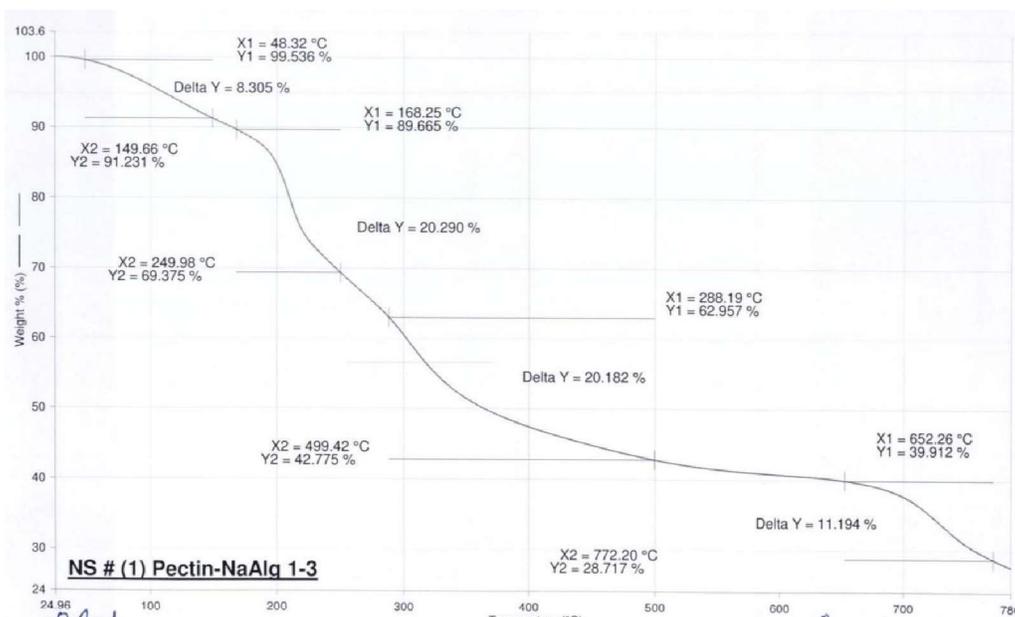


Figure 5: TGA Analysis graph

Table 3: TGA Analysis

temperature	24.97	104.97	204.97	304.97	404.97	504.97	604.97	704.97	734.97
weight	99.973	95.410	82.674	59.279	47.124	42.598	40.688	37.208	32.965



Figure 6: Antibacterial Activity

CONCLUSION:

The work produced stable hydrocolloid films containing cefazolin nanoparticles for topical application to wounds using sodium alginate and pectin. The 0.5% cross-linked films outperformed the 1% cross-linked films in terms of breaking elongation, water absorption, water vapor transfer, wetting ratio, and release profile. In vivo study is

necessary, however, before it can be concluded that cross-linked films and nanoparticles significantly affect how rapidly wounds mend.

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