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Development and characterization of pectin and chitosan based biocomposite material for bio-medical application

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Abstract

Pectin and Chitosan are the naturally occurring polymers which possess various beneficial properties. Pectin and Chitosan are abundantly available versatile polysaccharides with wide range of applications. The inherent properties of Pectin and Chitosan could be exploited to develop a biocomposite material which can be used as wound dressing material. Pectin is generously available in plant materials mainly citrus fruit peel. In th[e present study, Pectin was extracted from orange peels by Citric Acid and Alcohol Precipitation method as this method retains the Pectin properties and increases the yield of Pectin extraction. Characterization of Pectin extract by solubility tests and SEM analysis revealed the presence of Pectin. The extracted Pectin is sufficient enough to enhance the gelling ability of the biocomposite material. The extracted Pectin along with Chitosan extra pure was used for development of Pectin Chitosan biocomposite materials with the help of suitable solvents. The biocomposite material was prepared by using lactic acid or glycerol by solvent casting method. The biocomposite material was further characterized by SEM analysis which revealed that the surface of the material was smooth and heterogeneous. Also, antibacterial test against Bacillus subtilis confirms that the Pectin and Chitosan retains its antibacterial property in biocomposite material [1]. The raw materials and the manufacturing processes are cost effective and sustainable. Henceforth, this method leads to a development of promising product for commercialization in the arena of medical applications. Chitosan-based materials not only exhibit the excellent activities of chitosan but also show other appealing performance of combined materials, even give the good synergistic properties of chitosan and its composite materials. Further studies are needed to define the ideal physicochemical properties of chitosan for each type of biomedical applications. The development of various functional chitosan-based materials for biological applications will be an important field of research, and this kind of material has important commercial value.

Keywords

Pectin • chitosan • biocomposite • citric acid

Introduction

Pectin is a natural polymer and it is one of the main components of plant cell wall. Pectin is chemically constituted by α- 1, 4 galacturonic acid. Pectinprovides the suitable polymeric matrix for the preparation of biocomposite material. One of the major advantages of Pectin is that it can be easily extracted from the fruits peel. Pectin also possesses antimicrobial properties and cytocompatible gelling mechanism which can be exploited for medical applications.

Chitosan is the biopolymer produced by deacetylation of chitin. It is a polycationic polymer constituted by poly-b-1, 4-linked glucosamine. Chitosan issoluble in diluted acids andit also has an ability to interact with other anionic polymers. Chitosan possesses several beneficial properties such as anti-inflammatory properties, broad antimicrobial propertiesand its permeability to oxygen along with its adhesive property which can be used for dermal applications [2]. Hence, it is a potential candidate for pharmaceutical applications.

Pectin-Chitosan biocomposite material is adhesive material which is focused on biologically derived compounds[9]. Blending of Pectin and Chitosan forms a polyelectrolyte complex at low pH values in the range of 3–6. In addition tothis, Pectin and Chitosan also interact by forming hydrogen bonding at low pH values[3,4]. As at these pH values Pectin is in unionized state electrostatic interaction is supressed and hence, the interaction between Chitosan and Pectin will probably by hydrogen bonding.

The Biocomposite material requires minimal processing and is biologically safe. Chitosan present in the film provides a non-protein matrix with a 3D tissue growth capable for tissue engineering. Lactic acid and acetic acid solvents used for biocomposite material preparation improves film flexibility and obtain high water vapour

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permeability. Pectin-Chitosan film retains its antimicrobial microbial properties even after processing. Hence it is capable of accelerating the healing process at molecular, cellular and systemic levels [5].

MATERIALS AND METHODS

Materials

Lime Pectin was extracted from the under-utilized waste such as orange peels. Chitosan was obtained from SRL Company. Glycerol was of ACS reagent grade and distilled water was used for the preparation of all solutions.

Extraction of Pectin from Orange peels

Orange peels were collected from juice centre near RV College of Engineering, Bengaluru, Karnataka. Pectin was extracted from orange peels by employing direct boiling and water based extraction method. Small pieces of orange peels were soaked in boiling water for 5 minutes to remove the dirt. Later, the peels were dipped in ethanol for 30 minutes to remove the oil content. The peels were washed with distilled water and air dried which were later grinded to fine powder [6].

20 g of orange peel powder was taken in conical flask containing 350 ml of distilled water. The pH was adjusted to 3.5 using citric acid and the mixture was boiled in water bath at 60 °C and continuously stirred for 1 hour. The hot mixture was filtered using muslin cloth, extract was obtained after the filtration. Absolute ethanol was added in the ratio 1:1 to the extract and refrigerated for 3 h at 4 ° C. The crude Pectin was coagulated and was filtered with muslin cloth and it was washed 2-3 time with 85 % ethanol to remove further impurities. The Pectin pellets obtained were air dried and powdered.

Characterization of crude Pectin

The extracted material was characterized to confirm the presence of Pectin. The properties of Pectin were studied by conducting the following tests.

Solubility of Dry PectinIn Water

0.25 % of the Pectin samples were separately placed in a conical flask with 10 ml of 95 % ethanol added followed by 50 ml distilled water. The mixture was shaken vigorously to form a suspension which was then heated at 85-95°C for 15 min.

Solubility of PectinIn Saturated Sugar Solution

0.5 g of Pectin was dissolved in 10 ml of saturated sugar solution and the observation was recorded.

Yield of Pectin

The yield of crude Pectin for 20 g of orange peel powder was calculated by using the amount of pectin extracted in gram.

Preparation of Pectin Chitosan biocomposite material

Biocomposite material was prepared by solvent casting method. Initially, 5% (w/v) of Pectin solution was prepared by dissolving Pectin powder in deionised water and simultaneously 1% Chitosan solution was prepared, by dissolving Chitosan powder in deionised water containing 0.3 % (w/v) acetic acid.

100 ml of Chitosan solution was added to 100 ml of Pectin solution and beaten and 1000 rpm. Mixture was placed at room temperature for 48 hours and 1:1 mass ratio of PectinChitosan solution was obtained[7].

The mixture was centrifuged and pellet was collected, the pellet was washed with deionised water. 2% of glycerol was added to the obtained PectinChitosan complex and mixed thoroughly. Mixture was poured on acrylic glass plate and dried at 50 ℃ in hot air oven for 12 hours.

After 12 hours the thin layer of biocomposite material was formed on a glass plate and it was removed gently using forceps.

Characterization of Pectin and biocomposite material

SEM was carried out to study the morphology of pectin and synthesized biocomposite material at an operating voltage of 20 kV was used. The SEM analysis helped in charecterizing the Pectin and biocomposite material based on surface morphology.

Test for antibacterial properties of Pectin-Chitosan biocomposite material

Nutrient agar media was used to test antibacterial property of both Pectin and biocomposite material against Bacillus subtilis organism

RESULT AND DISCUSSION

Pectin has been extracted from orange peel waste following the protocol for water based method. The crude Pectin extract.

Pectin Extraction from Orange Peel Waste by Water Based Method

Pectin was extracted from direct boiling and water based method. The obtained Pectinwas stored in air tight container for furthercharacterization.

CHARACTERIZATION OF PECTIN

Following test were performed for confirmation of Pectin.

Solubility of Dry Pectin in Water

It was observed that the extracted Pectin was dissolved in water as shown in Fig 9 (a).

Solubility of Pectin In Saturated SugarSolution

It was observed that the extracted Pectin was soluble in saturated sugar solution and clear solution was obtained as shown in 9 (b)

Figure 1. (a) Pectin powder dissolved in distilled water.

Figure 2. (b) Pectin dissolved in 0.1 N NaOH solution.

Yield of Pectin

The yield of crude Pectin for 20 g of orange peel powder was calculated using the followingequation, Where y pec (%): is the extracted Pectin yield in percent (%), P: is the amount ofextracted Pectin in grams and, Bi: is the initial amount of orange peel powder weighed.

$$Y(pec)\ \% = (P/Bi)*100$$

$$Y(pec)\ \% =(4.2/20)*100$$

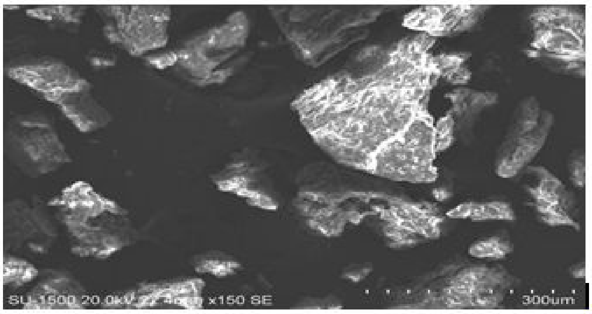
$$Y(pec)\ \% = 21\%$$

Scanning Electron Microscope (SEM) Analysis For Pectin

This technique is used to inspect in a relatively easy way the morphological features of the membranes, especially since this technique is very suitable to evaluate if the film is dense or porous and the pore size distribution and elemental analysis (EDS) are a standard procedure to identify and quantify the elemental composition of sample areas of up to several cubic micrometers. The sample material is bombarded with electrons in a scanning electron microscope, and the X-rays produced are measured with an X-ray spectroscope. Each element has a characteristic wavelength through which it can be identified.

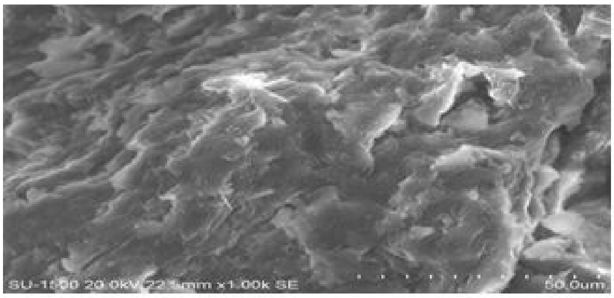
The images of an electron microscope are obtained by the detection, processing, and visualization of the signals resulting from the interactions between a high-energy electron beam and matter. These interactions can provide information on topography, composition, and crystallographic structure.

SEM analysis was performed for both extracted Pectin and standard Pectin for comparison between their morphological surface structures.



(a)

Figure 4. (a) SEM image of extracted Pectin



(b)

Figure 5. (b) SEM image of control i.e, standard Pectin at 20 µm

Two films were prepared by following two different protocols. The resultant film developed from following each protocol is shown in the fig 8(a) and fig 8(b).

Figure 6: Pectin-Chitosan film developed from protocol 2 (FilmB)

CHARACTERIZATION OF BIOCOMPOSITEMATERIAL

The antibacterial property of the biocomposite material was tested against Bacillus subtilisThe SEM analysis was performed to analyse the surface morphology of the biocomposite material

Antibacterial Test ForPectin And BiocompositeMaterial

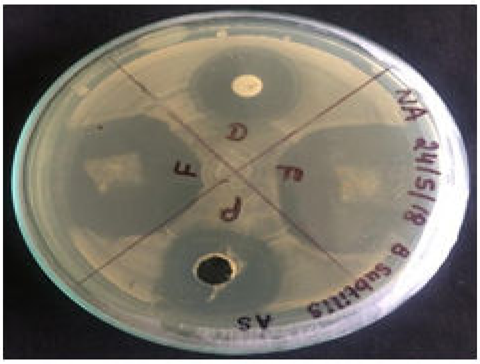
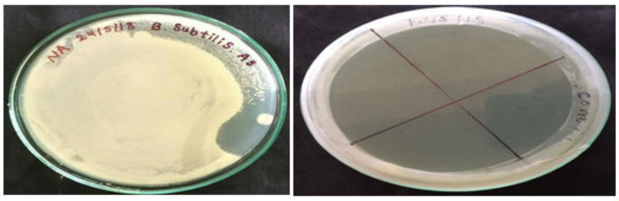


Figure 7: (a) Antibacterial activity of Pectin and Pectin-Chitosan film against Bacillus subtilis bacteria. D- chloramphenicol disc, P- 5 % Pectin solution, F-Pectin-Chitosan film.

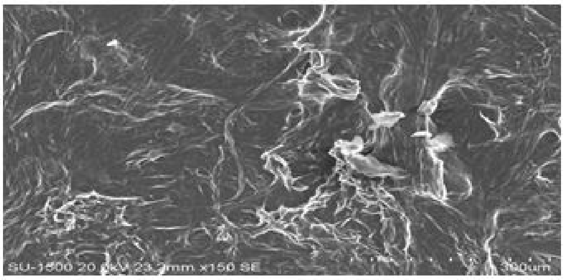


(b) (a)

Figure 8: (a) positive control for Bacillus subtilisbacteria. (b) Negative control for Bacillus subtilisbacteria.

Scanning Electron Microscope (SEM) Analysis for Biofilm

Hitachi SU-1500 SEM was used to study the morphology of synthesized biocomposite material at an operating voltage of 20 kV was used. The SEM images helped in characterizing the film based on surface morphology.



(a)

Figure 9: (a) are the SEM images of film. SEM results signify the arrangement of Pectin-Chitosanbiocomposite material.

Selection of solvent plays an important role in development of biofilms. Lactic acid has been used as the solvent for dissolving Chitosan powder as it also improves film flexibility. Lactic is Generally RecognisedAs Safe (GRAS) by US Food and Drug Administration. This organic acid is accurate to prepare bio-adhesive films useful for dermal applications because it is biological safe, is a natural constituent of the human body and is proven to be non-irritant to the skin ofrabbits.

This biocomposite material becomes hard as plastic and can take the shape of the mould in which it has been placed, when it is autoclaved at 121 °C at 15 psi for 20 min. this property can be exploited for development of novelbioplastic.

CONCLUSION

Pectin is one of the biopolymers which is abundantly available in the nature. It was extracted from orange peels by employing direct boiling and water based method. The crude extract was characterized for the presence of Pectin. SEM analysis of the crude Pectin extract signified the morphological structure. The inbuilt antibacterial property of Pectin was substantiated against Bacillus subtilis. Another biopolymer, Chitosan, was used to prepare a biocomposite material. Pectin-Chitosan biocomposite material was prepared using solvent casting method by employing lactic acid and glycerol. The biocomposite material was subjected to characterization by evaluating its antibacterial property against Bacillus subtilis and also studied the morphological structure via SEM analysis. Hence, Pectin-Chitosan biocomposite material is a potential candidate to be employed for various medical applications.

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