

Bioprocessing and Characterization of Polyhydroxyalkanoate blends from Cassia seed

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Abstract - Blending PHB with another bio-based and/or biologically degradable material. Poly-hydroxybutyrate (PHB) is blended with another biodegradable polymer called Galactomannan. Preparation of copolymers of PHB with other natural polymers (viz., Cassia gum, Tamarind seed, Xanthan gum, Alginates). Copolymerization technique was also employed as a modification technique in addition to blending and both the techniques were compared. Analyze the physico-mechanical properties of copolymers using various analysis and tests. The blends were solvent casted into films of different proportions. Blend films were evaluated and characterized by Fourier transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Moisture absorptivity studies were also carried out for the various blend films. This to, be a good option to develop PHB blends maintaining its biodegradability and bioreabsorption properties.

Keywords: Blending, Galactomannan, Fourier transform infrared spectroscopy, Scanning Electron Microscopy, Biodegradability.

1. INTRODUCTION

During the past few decades, biopolymers have attracted much attention for industrial, biomedical and pharmaceutical applications. In the field of polymers for industrial applications, more than 80% of plastic production is based on polyolefins (PP, PVC, PE, PS etc.) which are mostly produced from fossil fuels, consumed & discarded into the environment, ending up spontaneously as nonbiodegradable wastes which means that 80% of plastic refuse is practically eternal and is becoming a global environmental problem. For this reason microbial plastic polyhydroxyalkanoates (PHA) have gained importance since it can easily be dismantled in nature. Their life span is longer than several hundred years. After the end of their use, the polymers end up either in landfills or littering the environment [1]. Landfilling is becoming increasingly prohibitive, due to increasing costs, scarcity of land and other health and environmental considerations, such as ground water contamination. Biopolymers are promising candidates to replace conventional polymers, because of their biodegradable nature and they can be made from renewable resources as raw material. They can be categorized as a) bio resourced, b) biodegradable and c) bio resourced and biodegradable [2]. To utilize their potential and penetrate new markets, the improved properties of

biopolymers must be increased considerably. Consequently, the modification of these materials is in the focus of scientific research. In order to adjust the properties of biopolymers to the intended application, wide varieties of approaches are used for their modification, like plasticization, the incorporation of fillers and reinforcements, blending and impact modification [3, 4]. In contrast to the development of novel polymeric materials and new polymerization routes, the preparation of heterogeneous polymeric systems is a relatively cheap and fast method to conform the properties of plastics. As a result, this approach may play a crucial role in increasing the competitiveness of biopolymers [5]. The purpose of this research work is to obtain blends of PHB and Galactomannan with several compositions, using an internal mixer, and to study the miscibility, morphology and physical-chemical properties of these systems.

2. MATERIALS AND METHODS

2.1. Preparation of GM-PHB blend films

1 g of cassia gum was dissolved in 100 ml of distilled water and heated for 1 h at 60°C. PEG was added to this solution in different percentages. The solutions were blended with 1% PHB-Chloroform solution in different ratios by magnetic stirring overnight to obtain a uniform dispersion. The viscous mixture was centrifuged at 10,000 rpm for 10 mins and the clarified supernatant solution was then casted into petridishes having area of 63 cm² and 1.5 cm wall height. Petridishes were incubate in hot air oven for 8 hours at 50° C. After drying films were removed with the help of a sharp blade and kept in desiccator for 24 hrs. Films with air bubbles, cuts or imperfections were excluded from the study. Selected films were subjected for different evaluation parameters were represented in previous manuscript.

2.2 Characterization studies on Copolymer films

2.2.1 Thickness of the films

The thickness of the patch was measured using micrometer with a least count of 0.01 mm at different spots of the film. The thickness was measured at three different spots of the patch and average was taken.

2.2.2 Folding endurance

The folding endurance was measured manually. The films were conditioned at 55% relative humidity at 25-30°C for 24 h before testing. A strip of film (3 x 3 cm) was cut evenly and repeatedly folded at the same place until it breaks. The number of times counted until the film could be folded at the same place without breaking, the actual value of folding endurance was calculated.

2.2.3 Tensile strength

Tensile testing was conducted using Universal Testing Machine INSTRON 3369 at room temperature with a strain rate of 15 mm/min. The film was cut into 30 x 20 mm strips. Tensile tests were performed according to ASTM International Test Method for Thin Plastic Sheeting (D 882-02). Each test strip was placed in tensile grips on the testing machine. Initial grip separation was 50 mm and crosshead speed was 1 inch/min. The test was considered concluded when the film breaks.

2.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

The presence of different functional groups in the PHB, GM and PHB-co-GM was confirmed using FTIR analysis. FTIR spectra of polymer were recorded on a Nicolet Magana, 750. Transmission method of FTIR spectrometer was at room temperature in the range from 4000 to 500 cm⁻¹ at a resolution of 4 cm⁻¹. The samples were mixed with KBr and were pelletized [6].

2.2.5 Viscometric studies

The molecular weight of PHB and PHB-co-GM was determined with a capillary glass type Ostwald dilution viscometer. Intrinsic viscosity was measured at 30±0.5 °C. All the measurements were carried out in chloroform solution. Initially, a 1% solution of the polymer in chloroform was prepared. At least four dilutions viz 0.2%, 0.4%, 0.6% and 0.8% of polymer stock solutions were prepared before carrying out the actual measurement. Dilution was done using the volumetric expression, V₁N₁=V₂N₂. For the measurement 25 ml of the solvent was poured into the viscometer. By keeping the upper mark of small reservoir of the viscometer parallel to the eyes, the pure solvent was allowed to flow down to the lower mark and the efflux time in seconds was noted using a stop watch. The same procedure was repeated for at least twice to get the average efflux time (t₀) for pure solvent. Similarly, the efflux time of various concentrations of polymer-chloroform system (i.e., t) was noted adopting the same procedure. Specific viscosity was calculated as

$$\eta_{\text{spec}} = (t - t_0) / t_0$$

Molecular weight was calculated from the Mark-Houwink equation with the following coefficients:

$$[\eta] \text{ In dl/g} = 1.18 \times 10^{-4} \times M^{0.78}$$

To determine the intrinsic viscosity $[\eta]$, the experimental reduced viscosity values (η_{spec}/C) were plotted against the polymer solution concentration, C

2.2.6 Scanning Electron Microscopy (SEM)

The polymer blends were subjected to SEM analysis in order to study surface morphology. The surface morphology blend samples were studied on JOEL 100 Vegas 3 TESCAN SEM (USA). Before observation, they were coated with a thin conductive layer of gold.

3. RESULTS AND DISCUSSIONS

3.1 Evaluation of Copolymer films

The copolymer films produced using different combinations of carbon substrates were evaluated for their Physico-Mechanical properties represented in the previous manuscript.

3.2 Molecular weight determination

Each solvent has its own viscosity. Addition of a polymer to a solvent of low viscosity results in sharp increase in its viscosity. The extent of increase in its viscosity depends on the molecular weight and amount of the polymer added. This principle was exploited to determine the molecular weight of the polymer by using the Mark Houwink empirical equation. In polymeric materials, the rheological behavior and industrial process ability is strongly dependent on the molecular weight and structure, and hence examination of molecular weight vs viscosity is necessary. The Fig 1 and Fig 2 shows the plots for determination of intrinsic viscosities for PHB and PHB-co-GM respectively for molecular weight calculation.

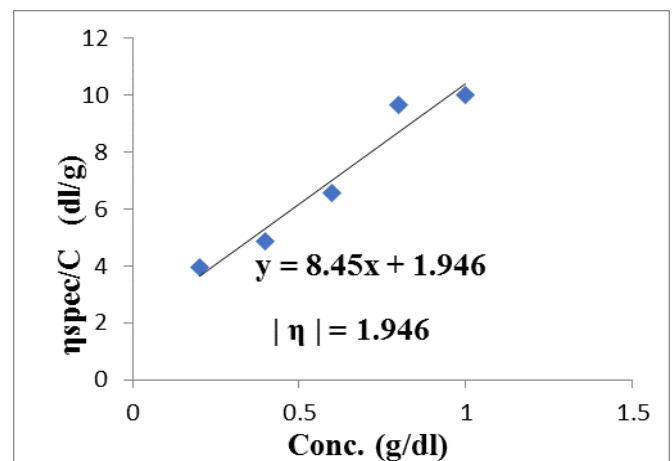


Fig. 1 Determination of intrinsic viscosity of PHB in chloroform at room temperature

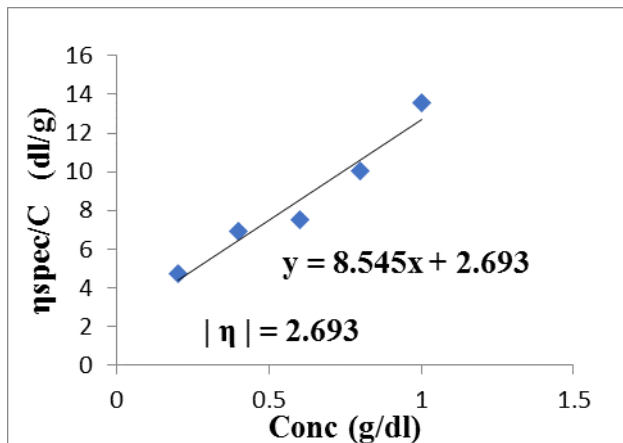


Fig. 2 Determination of intrinsic viscosity for PHB-co-GM copolymer in chloroform at room temperature

From the above two graphs, the molecular weight of the polymer sample was calculated and it was compared with literature values which are listed in Table 1.

Table 1. Compare the molecular weights of extracted polymer, copolymer and other samples with literature values

Polymer	(M _w) (x 10 ⁵ Da)	Reference
PHB	1.94	This work
PHB-co-GM	3.86	This work
PHB	1.1	[7]
PHB	1.7	[8]
PHB-co-PHV	4.7	[9]

The differences in the molecular weight values was attributed to the fact that molecular weight of PHB depends on the organism, substrate used, growth conditions and isolation techniques. The molecular weight of PHB extracted was found to be higher compared to other studies and higher molecular weight attributes the enhanced properties like lower crystallinity and exceeding process ability for packaging applications.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

The chemical structure and functional groups of the extracted PHB, cassia gum and prepared PHB-co-GM films were investigated by means of FTIR analysis as shown in FIG 3.

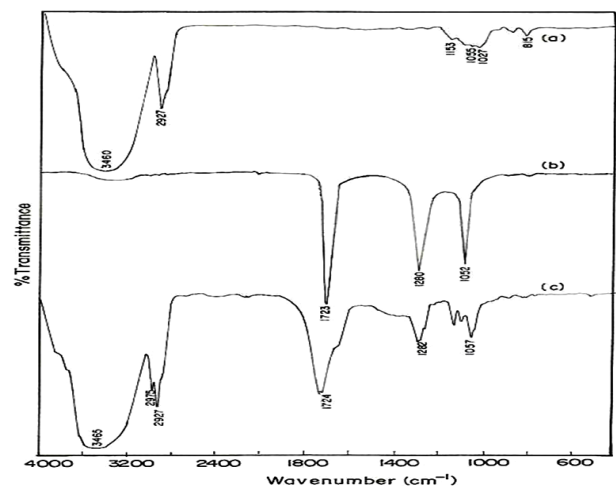


FIG. 3. FTIR spectrum of (a) Cassia gum, (b) PHB and (c) PHB-co-GM

The spectra 'a' depicts the functional groups of cassia gum with broad bands ranging between 2800–3000 and 3100–3600 cm⁻¹ attributed to C–H stretching and to O–H stretching vibration of cassia respectively. The spectra 'b' depicts PHB showing peaks at 1723.3 cm⁻¹ and 1280.6 cm⁻¹ corresponding the distinct rotations around the carbon atoms of the exact functional groups. The spectra 'c' shows that the extracted product by bacterial metabolism is a PHB-GM copolymer incorporating the functional groups of both PHB and GM [10].

3.3 Scanning Electron Microscopy

SEM observation on the surface morphology of GM-PHB is shown in FIG 4.

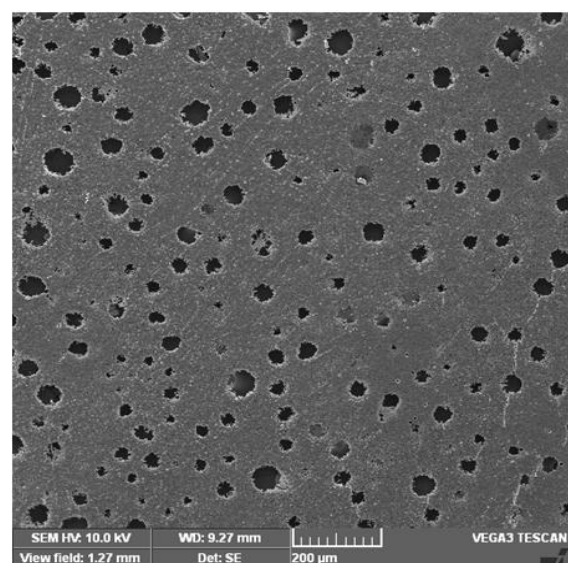


FIG.4. SEM image of 80:20 GM-PHB blend film

It can be found that the 80:20 GM-PHB blend showed some unique characteristics, which might be the effect of a stronger plasticizing interaction and more perfect film formed. And the image of this blend film resulted in a more unique homogenous and patterned structure without any phase separation was observed. In GM-PHB blend image it could be observed that small crystal phases of PHB were well dispersed in the continuous amorphous GM phase. The patterned surface should be very useful for packaging applications.

4. Conclusions

The PHB was blended with GM to produce films of various proportions which was characterized by using SEM. SEM image of 80:20 ratio of GM: PHB blend film showed that the film was smooth, uniform with well dispersed small crystals of PHB in the amorphous GM phase. Also copolymerization technique was also employed as a modification technique in addition to blending and both the techniques were compared. The PHB-co-GM copolymer films was better compared to films obtained by blending and also other copolymer films of PHB with XG, xanthan gum, alginate etc. FTIR analysis of the copolymer film replicates the peaks of both cassia and PHB indicating the presence of good synergy of both compounds in the blend. Besides PHB-co-GM showed higher folding endurance of 27, higher tensile strength of 4.7 MPa and lower moisture uptake of 7.1 %.

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