CHAPTER: III

MATERIALS AND METHODOLOGY

This chapter outlines the materials utilized for the fabrication of composite, nanocomposite and nanofiber films and the methodology employed to characterize it.

3.1 Materials

Polyvinyl alcohol (PVA) was purchased from Research-lab Fine Chem Industry, India (viscosity, degree of polymerization, pH value of water solution, volatiles, ash, and purity of 26 cp, 1757, 6.1, 3.6%, 0.6% and > 95%, respectively). Carboxymethyl cellulose (CMC) was supplied by Merck, India (Na; on dried substrate, pH value, viscosity, Chloride, Arsenic, heavy metals as Pb of 6.5-10.8%, 6-8, 1100-1900cPs, ≤ 0.25%, ≤ 0.0001%, ≤ 0.002, respectively). Glycerol (molecular weight: 92.10 g/mol) and calcium chloride (molecular weight: 110.98 g/mol) were obtained from Hi Media Laboratories, India. Graphene oxide was sourced from AD-nano Technologies, India (thickness, lateral dimension, purity, oxygen content, surface area, bulk density is 0.8-2 nm, 5-10 µm, 99%, 30%, 120m^2/g, 0.8-1g/cm^3, respectively).

3.2 Experimental techniques/methodology for analysing film properties

Different characterization and analytical techniques are utilized to assess the properties of polymeric films. The following provides a detailed summary of the methodologies and standards employed in assessing the film's properties.

3.2.1 Film thickness

The thickness of the developed films was measured by a micrometre (Alton M820-25, China) with a least count of 0.01 mm. To determine the average thickness of a film, measurement was taken from ten different sections of the film.

3.2.2 Mechanical properties

The tensile properties, i.e., elongation at break (EAB) and tensile strength (TS) of the prepared films have been evaluated according to D882-02 ASTM standard, using a computer- based Texture Analyzer (TA-HD Plus, Stable Microsystems, UK) [1]. Each prepared film was used to create a set of rectangular test samples with dimensions of (60 mm ×10 mm) and a Tensile Grip (A/MTG) was used to hold the samples, with a 5 kg load cell. The parameters for the tensile test are as follows: test speed = 3 mm/s, pre-test speed = 2 mm/s, post-test speed = 10 mm/s, trigger force = 10 g, and distance = 250 mm. The developed film's TS and EAB have been evaluated using Equation 1, and 2 [2].

$$Tensile\ strength\ = \frac{Force}{Thickness \times Width\ of\ sample} \times 100 \quad (1)$$

$$\%\ Elongation\ = \frac{Lenght\ of\ film\ after\ elongation - Actual\ length}{Actual\ length} \times 100 \quad (2)$$

3.2.3 Morphology

Surface characteristics were examined utilizing a Field Emission Scanning Electron Microscope (FESEM) (SIGMA VP, Carl Zeiss, Germany) and the procedure involved in FESEM is entitled in reference [2]. ImageJ software is used to measure the diameter of nanofibers. Transmission Electron Microscopy (TEM) analysis was also performed to investigate the morphology of films using Tecnai G2 20 S-Twin (200 KV), FEI company, USA instrument.

3.2.4 Water contact angle

The assessment of wettability of nanofiber mats was conducted utilizing the contact angle measurement system (Data Physics OCA 20, Germany) employing the sessile drop method as described in the provided reference [3].

3.2.5 Conductivity and viscosity

The solution conductivity and viscosity were measured in Eutech CON 700 and DV-79-Digital Viscometer with F-type probe respectively [4].

3.2.6 Rheology

Rheology analysis of the polymer solutions was determined in (Physical MCR 72; Anton

Paar, Austria) employing a parallel plate geometry (PP50). Steady shear tests were carried out at 25°C with shear rates varying from 10 to 100 s^{-1}, while dynamic frequency sweep tests were performed across a range of 0.1 to 100 rad/s with a consistent strain of 1% [5].

3.2.7 Water vapor permeability

The water vapor permeability **(**WVP) of developed films was carried out by using a glass cup filled with dried anhydrous calcium sulphate (Ca_2SO_4) to attain a relative humidity (RH) approximate to 0% [2]. The cups were individually covered with each prepared film before being placed in the desiccator. Within the desiccator, a saturated potassium sulphate (K_2SO_4) solution was used to maintain relative humidity (RH) of more than 90% throughout the films. The RH within the cup remained constantly lower than the surrounding air, and the rate of water vapor transmission (WVTR) was calculated as a function of the amount of weight gained by the cup when it was in a steady state. The variation in cup weight was recorded for ten days at every 24 h interval and plotted graphs with respect to time. Linear regression was used to determine the gradient of each line, and WVTR was evaluated by dividing the straight-line slope by the test area. Equation 3 is used to determine the WVP (g/m.hr.Pa) of the developed films [6].

$$WVP = \frac{WVTR \times X}{P \times (R_1 - R_2)} \quad (3)$$

Where, P, R_1, R_2, and X represent the vapor pressure of water in pascal (Pa) at 25 ℃, relative humidity within the desiccator, relative humidity within the cup, and film thickness (m) respectively. The driving force that was considered by [$P\ (R_1 - R_2)$] under the mentioned condition is 3073.93 Pa.

3.2.8 Moisture retention capacity, water solubility, and swelling ratio

To determine the moisture retention capacity (MRC) of the developed films, rectangular dimension (20 mm×20 mm) samples were taken and initial weight (W_i) is measured. The samples were then dried in a vacuum oven at 40 ℃ for 6 h to achieve a constant weight before the final weight measurement (W_f) [7]. The % MRC of the films was evaluated using Equation 4. The water solubility and swelling ratio of the films were assessed in accordance with the methods described in the literature [2].

$$\% MRC = \frac{W_f}{W_i} \times 100 \quad (4)$$

3.2.9 Color, light absorbance, and film opacity

To determine the color parameter of the developed films, Hunter Lab colorimeter (Ultrascan VIS, Hunter Lab, Inc., USA) was used. The light barrier properties of the fabricated films were investigated in UV-Vis spectrophotometer (Spectronic 20D+, Thermo Scientific, USA) using air as a standard reference and wavelengths between 200 and 800 nm[6]. Rectangular dimension (40 mm ×10 mm) samples have been cut off from the developed films and placed inside the spectrophotometer cell. Equation 5 is used to evaluate the opacity of the films [6, 7].

$$Opacity = \frac{Absorbance}{Film\ thickness} \quad (5)$$

3.2.10 Thermal properties

Differential scanning calorimetry (DSC) was conducted utilising the NETZSCH 5 equipment. Approximately 5±3 mg of the film's sample was enclosed within aluminium pans, and the experiment was conducted under nitrogen atmosphere. The samples were heated at a rate of 20 °C/min from 30 to 400 °C [2,6]. Equation 6 is used to calculate the crystallinity degree (X_C) of the film [6].

$$X_C = \frac{\Delta H_m}{\Delta H°_m} \quad (6)$$

Thermogravimetric analysis of the samples was performed to determine their thermal stability, and degradation temperature using Thermal Analyzer (TGA-50, Shimadzu instrument). The sample weight and heat flow rate were considered to be 4–5 mg and 10 °C /min respectively at 30 ℃-600 ℃ in a nitrogen atmosphere of 30 ml/min flow rate [8].

3.2.11 Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) spectrometer (PerkinElmer, USA, Spectrum 100) was used to obtain infrared spectra of the developed films within a scanning range of 4000–4500 cm^{-1} [9].

3.2.12 Antimicrobial

The antimicrobial activity of the developed films was investigated by disc diffusion method [7]. Two different food pathogens i.e. *S. aureus* gram-positive and *E. coli* gram-negative bacteria were selected for the analysis. The pathogens were cultured at a temperature of 37 ℃ for a duration of 16 h and diluted in stages by adding sterile saline solution to the culture broth before being spread out on agar substrate. Each film sample measuring 6 ± 0.5 mm was placed on the surface of agar media and incubated at a temperature of 37 °C for a duration of 24 h. After incubation period, a clear inhibition zone was formed around the film samples which was then measured.

3.2.13 Biodegradation analysis

For the present study two different types of biodegradability analysis were performed for the developed films.

(a) Biodegradability test using modified ASTM G21- 70

Two bacterial strains *Bacillus subtilis (B. subtilis) and Pseudomonas putida (P. putida)* were used to perform the experiment and the procedure used for the analysis were referred from [10,11]. Briefly, 5 ml of overnight grown cultures were centrifuged at 7000 rpm for 10 min. The supernatant was discarded and the pellet was suspended in Phosphate Buffer Saline (pH 7.4) for washing the pellet. The suspension was centrifuged again at 7000 rpm for 10 min and the step was repeated one more time. Then the pellet was resuspended in PBS and a final OD of 0.4 was set at 600 nm in UV-Vis spectrophotometer. ASTM G21-70 method was used to evaluate qualitative biodegradability test with few modifications. Briefly, bioplastic and synthetic plastic (negative control) each 2 cm × 2 cm were placed directly on M9- agar plates. Then, 100 µL of above prepared cultures (0.4 OD) was put on each sample. The plates were incubated for 5 days at 30 °C and biodegradability was determined [12].

(b) Soil burial test

The soil biodegradation analysis of the developed film was conducted in an indoor environment i.e. 35.49 ± 5 °C, 85.49 ± 10.5% relative humidity for 20 days [13]. Initially, the soil was manually inspected to remove the undesirable materials such as stone fragments, brick pieces, clay chunks, plant matter, paper, plastics, and other similar items. After the screening process, the remaining small lumps were ground to powder manually. The soil was thereafter subjected to a drying process in a shaded area for a duration of 24

h in order to achieve a free-flowing consistency. To promote biodegradation, the prepared soil was then moistened by sprinkling water (approximately 20–25%) and one-third of the container was filled with it. The films were cut into an average dimension (3 cm×3 cm), which was thereafter placed on the soil. Finally, additional soil was added to the container and levelled, allowing the film segments to be buried to a depth of around 10 cm. On alternate days, the soil was kept moist by sprinkling 30-40 ml of water. The entire experimental arrangement was subjected to incubation at ambient temperature for a duration of 20 days, with ten-day sampling intervals. Eventually, on day 0, the weight of each film was measured. After 10 days, all the films in the pots were recovered, brushed to eliminate attached soil, and weight was measured. The weight loss of the films was determined using Equation 7 [12].

$$\%\ Degradation = \frac{(W_{t-}\ W_o)}{W_o} \times 100 \qquad (7)$$

where W_t is the initial weight of the sample at day 0, and W_o is the weight of the sample after removal from soil.

3.2.14 Statistical analysis

All the experiments were conducted in triplicates, and the evaluated data were shown as mean ± standard deviation (SD). A significant difference between the the test samples was measured at 95% confidence ($p < 0.05$) and these differences in means were determined by Duncan's multiple range tests, using SPSS software (Version 20.0; SPSS Inc., Chicago, IL, USA) [4].

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