CHAPTER 6

Mesua ferrea L. Seed Oil Based HBPE/MWCNTs Nanocomposites

6.1. Introduction

Nowadays with the hasty advancement of nanoscience and technology, extensive research and development are implemented on high performance polymeric nanomaterials for targeted applications in various fields. The amalgamation of nanoscale reinforcements into the polymer matrix is also an endeavor to develop polymer nanocomposites in different scientific and industrial fields. After the first report by Iijima in 1991, the carbon nanotubes (CNTs), due to their excellent potentiality in unique nanostructure with inimitable physical characteristics, remarkable electrical and mechanical properties, and high aspect ratios, are ideal advanced reinforcing agents for polymer nanocomposites. The reinforcing effects of CNTs in polymer nanocomposites are generally optimized by the degree of dispersion, their alignment and the interfacial adhesion between the CNTs and the polymer matrix. However, due to high aspect ratio and strong van der Waals attractions, CNTs are often tend to bundle together leading to some agglomeration which result an inhomogeneous dispersion in polymer matrices. Also the surface of CNTs provides poor interfacial adhesion to the matrix, which limits effective load transfer from the matrix to the nanotubes. Therefore, to improve the dispersion and interfacial adhesion between CNTs and the polymer matrix, functionalization of CNTs has been done in most of the cases via oxidative method by using surfactant and ultrasonication.

In recent days, the development of biodegradable polymeric materials with desired material’s properties is an issue of active research interest to the academia and industrialists. This is due to the concerns over the persistence of polymers in the environment, shortage of landfill space, emissions during incineration, and negative impact on wildlife through ingestion and entrapment. Its pertinent to mention that biodegradable polyesters synthesized from diol and dicarboxylic acid through polycondensation reaction are commercially promising high performance materials complemented by low-cost inputs and easy production-processibility. The biodegrad

This work is communicated.
-ability of these polymers is due to the hydrolyzable ester bond present in their main chain, which is susceptible to hydrolysis and microbial attack, and leads to the formation of low molecular weight and eventually to fine chemicals.\textsuperscript{10} Other factors revealing the biodegradation include molecular weight, hydrophobic/hydrophilic properties, degree of crystallinity and morphology.\textsuperscript{10} Again, the tailor made properties and ease of fabrication make polymers excellent biomaterials. Desirable material properties, biocompatibility and time-frame dependent biodegradability are indispensable requisites for a polymeric biomaterial for specific application in the biomedical niche. In this context, development of CNTs-reinforced biodegradable polymeric biomaterial seems to be an interesting proposition.

These days, "going green" has become the steering guideline in various research domains including material science. This is of significance in the context of dwindling natural resources, global economic set-backs, and the intertwined problems of pollution and biological niche degradation. Exploiting plant based raw materials contributes to global sustainability without diminution of scarce resources.\textsuperscript{11} In this perspective \textit{Mesua ferrea} L. seed oil based HBPE is used as the matrix for preparation of modified MWCNTs nanocomposites. The potentiality of this oil based materials in different fields has already been reflected from the earlier reports.\textsuperscript{12-15} The unique architecture, large surface functionalities and low viscosity of the highly branched polymer facilitate the dispersion of CNTs in the matrix through different physico-chemical interactions with the nanofillers.\textsuperscript{16} To validate the prospects of using the reported nanocomposite as biomaterial with desired material properties, assessments on biodegradability and cytocompatibility are also reported. Thus, the present study stands at the intersection between nanotechnology and biology to develop environmentally benign application-oriented CNTs based polymer nanocomposites.

Therefore, in this chapter the preparation, characterization and properties of the \textit{Mesua ferrea} L. seed oil based HBPE/MWCNTs nanocomposites are described. The effect of MWCNTs on various properties like mechanical, thermal, biodegradation and cytocompatibility of the HBPE matrix are also discussed. Thus the potentiality of HBPE/MWCNTs nanocomposites as biomaterial is primarily focused here.

\textbf{6.2. Experimental}

\textbf{6.2.1. Materials}
Multiwalled carbon nanotubes (MWCNTs) with diameter 10-20 nm and length of about 20 μm were obtained from Iiljin Nanotech, Korea. The details about the MWCNTs were described in Chapter 1, section 1.3.1.2.

Potassium permanganate (KMnO₄) was obtained from S.D. Fine Chemical Ltd., Mumbai. It appears as purplish-bronze-gray needles. Its m.p. is 240 °C, Mₜ is 158.03 g/mol and density is 2.73 g/cm³. It was used as an oxidizing agent to modify the MWCNTs.

Cetyltrimethylammonium bromide (CTAB) was purchased from Merck, India. Its melting range is 237-243 °C and Mₜ is 364.45 g/mol. It was used as a surfactant in the modification of MWCNTs.

Acetic acid was obtained from Merck, India. It is a colorless liquid with Mₜ 60.05 g/mol and density 1.049 g/cm³. Its m.p. is 16.5 °C and b.p. is 118.1 °C.

The other required chemicals and solvents such as Mesua ferrea L. seed oil, glycerol, PA, MA, bis-MPA, lead mono oxide, DMF, xylene, epoxy resin, poly(amido amine) etc. were of same specifications as described in Chapter 2, section 2.2.1. The necessary minerals used for preparation of bacterial broth culture to study the biodegradation were of same specifications as described in Chapter 4, section 4.2.1. The Pseudomonas aeruginosa bacteria with strains PN8Al and vsl; and Bacillus subtilis with strain MTCC73 used for biodegradation study of the nanocomposites, along with all other items used for the RBC hemolysis protection assay were taken from the Department of Molecular Biology and Biotechnology (Department of Biotechnology, DBT Centre, Government of India), Tezpur University. All other reagents employed in the study were of reagent grade and used without further purification.

6.2.2. Instruments and Methods

The FTIR and TGA analyses were carried out using the same instruments and conditions as mentioned in Chapter 2, section 2.2.2. and Chapter 3, section 3.2.2. respectively. The XRD, SEM and TEM were carried out using the same instruments and conditions as mentioned in Chapter 4, section 4.2.2. The measurements of gloss, impact resistance, scratch hardness and chemical resistance were performed according to the standard methods as described earlier in Chapter 2, section 2.2.2. The mechanical properties were evaluated by the similar methods as mentioned in the Chapter 2, section 2.2.2. Sonication was done with the same instrument as mentioned in Chapter 3, section...
3.2.2. at fixed amplitude and a half cycle for proper dispersion of the nanotubes in the HBPE matrix.

*Mesua ferrea* L. seed oil was extracted from the matured seeds by the same method as described in Chapter 2, section 2.2.2. The percent of swelling of the nanocomposites films was determined using the same method as discussed in Chapter 2, section 2.2.2.

6.2.2.1. Purification and Modification of MWCNTs

The MWCNTs were purified by thermal annealing at 500 °C for 1 h in muffle furnace followed by their functionalization by the similar method as reported by Zhang et al.17 Briefly, the modification method was as follows. A mixture of 0.12 g of nanotubes and 1.0 g of CTAB in 25 mL of dichloromethane was sonicated for 30 min. 5 g of KMnO₄ powder was then added in small portions over a period of 2 h followed by addition of 5 mL of acetic acid. The whole mixture was then stirred mechanically for 48 h at room temperature. The color of the mixture changed from dark purple to dark brown due to the reduction of Mn⁺⁷ to Mn⁺. The final material was then obtained by post filtration of the mixture through Teflon membrane sheet followed by different washing steps with conc. HCl, water and finally acetone. The purified nanotubes were then dried under vacuum oven at 40-50 °C for 24 h before use.

6.2.2.2. Preparation of HBPE/MWCNTs Nanocomposites

The *Mesua ferrea* L. seed oil based HBPE was prepared by using the same method as described in the experimental section of Chapter 2, sections 2.2.2.1. Prior to the preparation of nanocomposites the HBPE was placed in a vacuum oven at 50 °C to remove entrapped volatiles. HBPE was then mechanically mixed with desired dose of modified MWCNTs for 30 min followed by ultrasonication for 30 min. After sonication, calculated amount of bisphenol-A based epoxy resin (polyester: epoxy = 60: 40 in weight ratio) and poly(amide amine) (25% with respect to epoxy resin) hardener were added and stirred mechanically for 30 min. The prepared nanocomposites were coded as PCNTC0.5, PCNTC1 and PCNTC2 corresponding to the amount of 0.5, 1 and 2 wt.% MWCNTs.

6.2.2.3. Fabrication of Nanocomposites into Thin Films and Their Curing
The prepared nanocomposite was degassed for 30 min under vacuum to make it completely bubble free. Thin films of the nanocomposites were then prepared by drawing the homogeneous mixture on mild steel plates (150 mm × 50 mm × 1.60 mm) and glass plates (75 mm × 25 mm × 1.75 mm) using a micro adjustable thickness gauge under ambient conditions. After removal of sufficient amount of solvent under atmospheric conditions, the coated strips were degassed under vacuum at (45 ± 5) °C for 45 min and kept overnight in a vacuum dessicator. Then the coated nanocomposite films were cured by heating at 120 °C in an oven for a specified time period. The cured nanocomposite films were kept under ambient conditions for 24 h for further processing. Then the dried films were peeled off from the glass plates by immersing them in warm water. The films were kept in a dessicator under vacuum and stored for 7 days prior to testing. The thickness of the completely dried nanocomposite films was measured by the same instrument as described in Chapter 2 and was found to be in the range of 25-35 μm. The curing time was determined similarly as discussed in the Chapter 2.

6.2.2.4. Preparation of Bacterial Culture Media
The mineral salt medium for biodegradation study was prepared with the following composition. 2.0 g of (NH₄)₂SO₄, 2.0 g of Na₂HPO₄, 4.75 g of KH₂PO₄, 1.2 g of MgSO₄·7H₂O, 0.5 mg of CaCl₂·2H₂O, 100 mg of MnSO₄·5H₂O, 70 mg of ZnSO₄·7H₂O, 10 mg of H₃BO₃·5H₂O, 100 mg of CuSO₄·7H₂O, 1 mg of FeSO₄·7H₂O and 10 mg of MoO₃ using the similar method as described in Chapter 4, section 4.2.2.7.

6.2.2.5. Bacterial Strains
Pure cultures of the bacterial strains were grown in nutrient broth by the same method as discussed in the Chapter 4, section 4.2.2.8. The growth of the tested bacterial strains was measured spectrophotometrically using the method as described in the Chapter 4, section 4.2.2.8.

6.2.2.6. Cytotoxicity by Hemolytic Activity Assay
Fresh goat blood was collected from the slaughter house. Sodium citrate, dissolved in phosphate buffer saline (PBS), pH 7.4 was used as anticoagulant. The blood samples were immediately placed in an ice bath. Red Blood Cells (RBCs) were separated by centrifugation (3000 g for 10 min at 25 °C) and re-suspended in PBS. This procedure
was repeated until the supernatant became colorless. The packed cells were suspended in 4 volume of PBS to perform the experiment. The experimental nanocomposite films with uniform thickness were cut to obtain 10 mg sample for each. The films were surface sterilized under ultraviolet light for 30 min in a laminar air flow hood. The experiments were performed according to protocols described by Dutta et al.\textsuperscript{18} and Hassan et al.\textsuperscript{19} after slight modifications. 2 mL of erythrocyte suspension was treated with 10 mg of each type of film. $\text{H}_2\text{O}_2$ solution with concentration of 100 $\mu$M and PBS (pH 7.4) was taken as positive and negative control respectively. The RBC suspensions were incubated for 30 min at 37 °C and then 200 $\mu$L of the suspensions was diluted to eight times using PBS solution. The samples were then centrifuged at 3000 g for 5 min at 25 °C and the absorption of the supernatant in each case was measured at a wavelength of 540 nm against PBS as the blank solution.

6.3. Results and Discussion

6.3.1. Purification and Modification of MWCNTs

Most of the production processes of CNTs, generate a range of carbonaceous particles such as amorphous carbon, fullerenes, nanocrystalline graphites, transition metal catalysts etc. Hence, it is indispensable to eliminate these effluents before using CNTs as nanofillers. Otherwise proper dispersion as well as interfacial adhesion of the nanotubes with the polymer matrix will not be proper. So, to remove these unwanted by-products thermal annealing in air or oxygen was done at 500 °C in furnace for selective etching of the amorphous carbons. Besides this other techniques like mechanical centrifugal separation, size exclusion chromatography and microfiltration may also be applied as well.

Functionalization of the thermally annealed MWCNTs, as described in the experimental section, generates carboxylic or hydroxyl groups on the sidewalls of the nanotubes.\textsuperscript{17} These reactive groups facilitate the dispersion as well as interfacial adhesion of MWCNTs with the polyester matrix. CTAB acts as phase transferring agent which extracts the $\text{KMnO}_4$ into the organic medium (dichloromethane) containing the suspended nanotubes. The modification was done at ambient temperature as CTAB substantially decreases the activation energy and hence increases the rate of reaction. The acetic acid used in the reaction neutralizes the hydroxide ions and hence promotes the reaction further.\textsuperscript{20}
6.3.2. Preparation of HBPE/MWCNTs Nanocomposites

Relatively lower stiffness and strength of polymers as compared to ceramics or metals limit the former’s engineering or industrial applications. Impregnation with nanoscale materials is viewed as one of the many routes to overcome the challenge of poor mechanical attributes of vegetable oil based polyester resin. For this the modified MWCNTs were incorporated into HBPE matrix by ex-situ technique by intense mechanical mixing and ultrasonication. The presence of carboxyl/hydroxyl groups on the modified MWCNTs facilitates the dispersion of the nanotubes. The unique architecture and high surface functionality of the matrix along with its low viscosity help to enhance the interfacial adhesion between the ester groups of the matrix and the carboxyl/hydroxyl groups of MWCNTs through H-bonding. The use of high speed mechanical stirring and ultrasonication also aids in the de-agglomeration and proper dispersion of MWCNTs in the polyester matrix.21,22

6.3.3. Solvent Dispersibility

The modified MWCNTs were dispersed in different organic solvents such as ethanol, methanol, DMF, toluene, dichloromethane etc. The nanotubes tend to agglomerate due to their nanoscale dimensions, high surface energy and strong van der Waals interactions.23 However, the functionalization of the MWCNTs and the formation of acidic groups dramatically improve the dispersibility in various solvents. Fig. 6.1 shows the dispersibility of neat-MWCNTs, modified MWCNTs and HBPE/MWCNTs nanocomposites in DMF post 15 days storage. In DMF, neat-MWCNTs agglomerated and sedimented at the bottom of the container, but the modified MWCNTs as well as the HBPE/MWCNTs were well-dispersed. The improved dispersion of HBPE/MWCNTs nanocomposites is due to the strong interactions of

Fig. 6.1: Stability of neat MWCNTs in (a) DMF (b) HBPE, and modified MWCNTs in (c) DMF (d) HBPE nanocomposites
carboxylic/hydroxyl groups attached on the MWCNTs with the HBPE matrix through H-bonding (Scheme 6.1).

### 6.3.4. Characterization of Nanocomposites

#### 6.3.4.1. FTIR Study

FTIR spectra of neat and modified MWCNTs are shown in Fig. 6.2. For the neat MWCNTs, the band at 3400 cm\(^{-1}\) is attributed to the presence of the hydroxyl groups (–OH) on the surface of the MWCNTs. This could have appeared either from ambient moisture bound to the MWCNTs or during the purification of the raw material.\(^{24}\) For modified MWCNTs, the characteristic band observed at 1720 cm\(^{-1}\) corresponds to the stretching vibrations of carboxylic acid groups indicating the effective modification. The relative increase in intensity of the band at around 3400 cm\(^{-1}\) suggests the presence

\[
\text{HOOC} - \overset{\text{H-bonding}}{\equiv} \text{OOH}
\]

\[
\text{HOOC} \quad \overset{\text{H-bonding}}{\equiv} \text{OOH} \quad > \quad \text{MWCNT}_{\text{mod}} \quad \text{MWCNT}_{\text{mod}}
\]

\[\text{Scheme 6.1: Possible interactions of MWCNTs with polyester, epoxy and hardener system}\]

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of more -OH groups on the surface of the MWCNTs after oxidation and water treatment. 25

Fig. 6.3 shows the FTIR spectra of the nanocomposites. The carboxylic groups on the MWCNTs are expected to enhance the interfacial interactions between the HBPE and MWCNTs. The broad band at 3430 cm\(^{-1}\) is the result of overlapping of hydrogen bonded -OH stretching of polyester and -OH stretching of modified MWCNTs. Further, with the increase of MWCNTs loading the shifting of C=O band from 1730 to 1710 cm\(^{-1}\) was observed. This is attributed to the interaction between the host polymer and nanofillers.

![Fig. 6.2: FTIR spectra of (a) MWCNTs and (b) modified MWCNTs](image)

![Fig. 6.3: FTIR spectra of (a) HBPE, (b) PCNT0.5, (c) PCNT1 and (d) PCNTC2](image)

6.3.4.2. XRD Study
Fig. 6.4 illustrates the XRD patterns of modified MWCNTs, HBPE and its nanocomposites with the addition of 0.5, 1 and 2 wt.% of MWCNTs. The pure HBPE showed a characteristic broad peak at around $2\theta = 20^\circ$ which confirmed the amorphous nature of the polymer. The X-ray diffraction pattern of the MWCNTs revealed the presence of two crystalline peaks at $2\theta = 25.9^\circ$ and $43.1^\circ$, corresponding to the interlayer spacing of 0.34 nm and 0.21 nm attributed to (002) and (100) planes of the carbon atom, respectively. It was noticed that incorporation of MWCNTs does not significantly affect the structure of HBPE, as supported by the XRD pattern of the HBPE/MWCNTs nanocomposites. In this study, as low amounts of MWCNTs (up to 2 wt.%) were used to prepare the nanocomposites, so no noticeable X-ray diffraction peak appeared for MWCNTs in the nanocomposites (Fig. 6.4). The nanocomposites with high MWCNTs content may show significant diffraction peaks due to the presence of MWCNTs.

![XRD diffractograms](image)

**Fig. 6.4**: XRD diffractograms for (a) HBPE, (b) PCNTC0.5, (c) PCNTC1, (d) PCNTC2 and (e) MWCNTs

6.3.4.3. SEM Study

The surface morphology and distribution of the modified MWCNTs in the HBPE matrix can be visualised in the SEM micrographs (Fig. 6.5). For the nanocomposites with 0.5-2 wt.% MWCNTs, the individual MWCNTs are well dispersed throughout the polyester matrix. Its pertinent to mention that chemical functionalization of the MWCNTs assists good wetting by the matrix and enhanced interfacial adhesion between them through interactions of -OH/-COOH groups of MWCNTs with the ester groups of polyester by H-bonding or other polar-polar interactions (Scheme 6.1).
6.3.4.4. TEM Study

The representative TEM images of the nanocomposites are shown in Fig. 6.6. The TEM images of the nanocomposites were illustrative to the degree of intercalation, exfoliation and aggregation of the individual MWCNTs. The TEM micrographs showed the disintegration or exfoliation and homogeneous dispersion of the individual MWCNTs with average outer diameter in the range of 15-20 nm. The reason for this excellent distribution and exfoliation is again due to strong interactions between the polar carboxylic ester groups of the polymer and the -OH/-COOH groups of modified MWCNTs attached on the sidewalls (Scheme 6.1).

![TEM micrographs for A. PCNTCl and B. PCNTC2](image1)

![SEM micrographs for A. HBPE, B. PCNTC0.5, C. PCNTC1 and D. PCNTC2](image2)

**Fig. 6.5:** SEM micrographs for A. HBPE, B. PCNTC0.5, C. PCNTC1 and D. PCNTC2

**Fig. 6.6:** TEM micrographs for A. PCNTC1 and B. PCNTC2

6.3.5. Performance Characteristics of the Nanocomposites
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The different performance characteristics of HBPE/MWCNTs nanocomposites are shown in Table 6.1. The mechanical properties of a nanofiller-reinforced polymer mainly depend on the state of dispersion, distribution and orientation, high aspect ratio of the nanofillers, size and shape of domain and degree of compatibility. Good interfacial adhesion and high load transferring efficiency between the MWCNTs and polymer matrix are the key factors for improvements of mechanical properties of the nanocomposites. The tensile strength of HBPE/MWCNTs nanocomposites enhanced significantly from 2.68 to 27.78 N/mm\(^2\) with the increase of MWCNTs loading from 0.5 to 2 wt.%. This is due to the nano-reinforcing effect of the MWCNTs with high aspect ratio. After functionalization of the MWCNTs the carboxylic acid groups are attached on the defect sites on sidewalls of MWCNTs. As illustrated in Scheme 6.1, possible interactions between the carboxylic acid groups of the modified MWCNTs and the ester groups in polyester macromolecular chains through hydrogen bonding result in enhance interfacial adhesion between them. This also leads to good dispersion of the nanotubes in the HBPE matrix. This improved dispersion of MWCNTs in the HBPE matrix was supported by the SEM and TEM images. This favors effective load transfer from the polymer matrix to the nanotubes that leads to substantial improvement in tensile strength of the nanocomposites.\(^{26}\) Again, the elongation at break of the nanocomposites increased at 0.5 wt.% loading. However, the value decreased at high loading of MWCNTs. This may be attributed to the formation of more stiff nanocomposites by the MWCNTs and the generation of micro-voids around the nanotubes.\(^{27}\) However, the value was still higher than pristine matrix system.

It was observed that the curing time (Table 6.1) of the nanocomposites decreased with the increase of MWCNTs loading from 0.5 to 2 wt.%. The interaction of the homogeneously dispersed MWCNTs in the matrix restricts the mobility of the polymer segments. The high aspect ratio of MWCNTs favors the H-bonding mediated crosslinking reaction between -OH or -COOH groups of MWCNTs and hydroxyl/carboxyl groups of polyester along with the epoxy group of the epoxy resin (curing agent) and amine groups of the poly(amide amine) hardener (Scheme 6.1).

Smoothness or surface texture influences the gloss of coated material. The gloss values of the nanocomposites (Table 6.1) improve with the increase in loading of MWCNTs, indicating the improved compatibility of the various components in the system. The crosslinking density increases as the amount of MWCNTs increases as
supported by swelling value (Table 6.1). The smoothened surface augments the gloss value of the nanocomposites with MWCNTs loading from 0.5-2 wt.%.

Scratch hardness represents the response of the material under serious dynamic surface deformation. A significant improvement in scratch hardness was observed in the nanocomposites reinforced with 0.5-2 wt.% MWCNTs (Table 6.1). The inclusion of MWCNTs with uniform dissemination enhances the crosslinking reaction resulting in an effective network formation. This prevents the indentation and enhances the scratch hardness.

Addition of MWCNTs with loadings from 0.5 to 2 wt.% significantly improves the impact resistance of the nanocomposites (Table 6.1). The nanofillers act as crack stoppers. The homogeneous dispersion of the MWCNTs and good crosslinking play vital role for this improvement in impact resistance.

Table 6.1: Performance characteristics of HBPE/MWCNTs nanocomposites

<table>
<thead>
<tr>
<th>Property</th>
<th>HBPE</th>
<th>PCNTC0.5</th>
<th>PCNTC1</th>
<th>PCNTC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gloss at 60°</td>
<td>98</td>
<td>102</td>
<td>106</td>
<td>110</td>
</tr>
<tr>
<td>Scratch hardness (kg)</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Impact resistance (cm)</td>
<td>80</td>
<td>85</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>Tensile strength (N/mm²)</td>
<td>2.68</td>
<td>13.84</td>
<td>16.24</td>
<td>27.78</td>
</tr>
<tr>
<td>Elongation at break (%)</td>
<td>24</td>
<td>129.26</td>
<td>92.16</td>
<td>65.95</td>
</tr>
<tr>
<td>Curing time (h at 120 °C)</td>
<td>3</td>
<td>2</td>
<td>1.75</td>
<td>1.5</td>
</tr>
<tr>
<td>Swelling in xylene (%)</td>
<td>32</td>
<td>30</td>
<td>28</td>
<td>27.6</td>
</tr>
</tbody>
</table>

6.3.6. Thermal Properties

The thermal degradation behavior of the nanocomposites can provide useful information to determine the optimum processing conditions and can identify the potential applications of the final products. Thermal degradation behavior of the HBPE/MWCNTs was investigated by employing thermogravimetric analysis (TGA) under the nitrogen atmosphere. TGA thermograms of the nanocomposites are presented in Fig. 6.7. The pristine MWCNTs have no weight loss below 600 °C, as shown in Fig. 6.7(e). From the TGA thermographs of the nanocomposites it was observed that even very low amount of nanofiller contributed considerably to the increase in initial thermal degradation temperature (Tₐ). The addition of 0.5 wt.% of MWCNTs into the HBPE matrix increases the Tₐ of the nanocomposites by 14 °C in comparison to the pristine HBPE. The Tₐ increases further with the increase of MWCNTs amount and
enhancement of 44 °C at 2 wt.% loading. The well-dispersed MWCNTs hinder the flux of degradation product and thereby delay the onset of degradation.\textsuperscript{3,29} The presence of carboxylic groups on the nanotubes surface leads to the strong interfacial interactions between the polymer matrix and the nanotubes in the nanocomposites. Since thermal degradation of a polymer starts with chain cleavage and radical formation, the modified MWCNTs in the nanocomposite might act as radical scavengers to delay the onset of thermal degradation and hence improved the thermal stability of polyester. Another reason of this improved thermal stability is the high thermal conductivity of the nanotubes that facilitates heat dissipation within the nanocomposite.\textsuperscript{29}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6.7.png}
\caption{TGA thermograms for (a) HBPE, (b) PCNTC0.5, (c) PCNTC1, (d) PCNTC2 and (e) modified MWCNTs}
\end{figure}

\textbf{6.3.7. Biodegradation Study}

Biodegradation of the HBPE/MWCNTs nanocomposite films was assessed by their direct exposure to the microbial attack by different bacterial strains. McFarland turbidity method\textsuperscript{18} was used to monitor the growth of the bacterial strains. The degradation of the nanocomposites as well as neat polyester was not so noticeable upto 2 weeks. However, the bacterial growth started to increase from the 3\textsuperscript{rd} week onward [Fig. 6.8 (a), (b), (c)] implying a direct correlation of time with the gradual increase in the extent of biodegradation. The degradation of the nanocomposites was more than that of the pristine system which can be evaluated from the time-course dependent optical density of the culture media containing the nanocomposites. The degradation was found to increase with the increase of MWCNTs concentration which may due to
the formation of exfoliated structure. The growth of bacteria in the nanocomposites film was also observed in the SEM micrographs [Fig. 6.9 (d), (e), (f)].

Degradation of the nanocomposites occurs by the ester hydrolysis of the crosslinked polyester resin with generation of fragments and respective monomeric carboxylic acids and diols. Similar to degradation of polymers by enzyme, the bacterial degradation takes place chiefly in two stages. In the first stage, polyester chains with high molecular weight are hydrolyzed to form oligomers. An acid, base, or moisture can accelerate the reaction. The micro-organisms then consume the low molecular weight oligomers in the last stage to produce CO₂, H₂O and humus. The absorption of water by the polar ester groups of the matrix along with the -COOH/-OH groups of the MWCNTs is the rate determining step for biodegradation.

![Graph showing bacterial growth](image)

**Fig. 6.8:** Bacterial growth of *Pseudomonas aeruginosa* with strains (a) PN8A1 and (b) vs1, and *Bacillus subtilis* with strain (c) MTCC73 on nanocomposites
6.3.8. Cytotoxicity Study

Evaluating the events at the bio-nano interface is indispensable. Although CNTs are becoming increasingly studied new materials of emerging technology particularly for controlled drug delivery, there is paucity of information on their toxicological attributes. In parallel lines, establishing the biocompatibility of a biodegradable polymeric material under a specified environment is pre-requisite.

In the present report, the cytotoxicity of the HBPE/MWCNTs nanocomposites was studied by RBC hemolysis protection assay. Fig. 6.10 shows the non-cytotoxic nature of the nanocomposites films. The hemolytic activity of the nanocomposites was checked by measuring absorptions of the suspensions containing the RBC at 540 nm against 100 μM H2O2 as the positive control and PBS as the negative control. The absorption value (OD at 540 nm) was found almost constant with MWCNTs loading from 0.5 to 2 wt.% in the HBPE matrix.

Hemolysis assay indicates the nature of particle/membrane interactions and which detects direct damaging interactions between particle surfaces and membranes, which can be driven by oxidative stress for some particles. By hemolysis assay we can also measure the direct membranolytic or membrane-perturbing action of any substance.30 As hemolysis was found to be negligible in case HBPE/MWCNTs
nanocomposites, it may be concluded that the nanocomposites were almost non-toxic in nature.

![Bar diagram showing cytotoxicity test results for the nanocomposites](image)

**Fig. 6.10: Bar diagram showing cytotoxicity test results for the nanocomposites**

### 6.4. Conclusions

Renewable resource based HBPE/MWCNTs nanocomposites were prepared by *ex-situ* technique with high shearing force and ultrasonication. The HBPE displayed suitable matrix properties for dispersion of the modified MWCNTs as evident from the XRD, SEM and TEM results of the nanocomposites. The mechanical and thermal properties of the nanocomposites unveiled that a small amount of MWCNTs loading can substantially reinforce the HBPE matrix. The incorporation of the modified MWCNTs improved the biodegradability of the nanocomposites to a great extent. Assessment of the toxicity at the cellular level revealed the cytocompatible attribute of the prepared nanocomposite material. Thus, the *Mesua ferrea* L. seed oil based HBPE/MWCNTs nanocomposites have a good prospect of serving as polymeric biomaterial for applications in the biomedical niche.

### References


