Different Amounts of Chitosan on the Morphological, Thermal and Antibacterial Properties of Poly(L-lactic acid)/Microcrystalline Cellulose Biopolymer Composites

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Abstract: The objective of this research is to develop PLLA based bio-composites with extended physical and antibacterial properties to broaden the applications in medical and ecological fields. Different amounts of chitosan (0, 5, 10, 20, 40 wt%) were blended with the composites of poly(L-lactic acid) (PLLA)/microcrystalline cellulose (MCC) with a fixed ratio (3%) of MCC. Here MCC was extracted from jute fiber by conventional acid hydrolysis method with 65% H2SO4, and chitosan was extracted from shrimp shell by the subsequent treatment with HCl and NaOH. Composites were prepared by solution casting method using a suitable solvent for each. Prepared samples were characterized by FTIR, WAXD, TGA, DTG, DTA, SEM, and antibacterial properties. FTIR analysis showed that except for hydrogen bonding, there are no new characteristic absorption peaks observed in the spectrum of composites and PLLA, chitosan, and MCC may be compatible through intermolecular hydrogen bonding. SEM analysis found that MCC and Chitosan were dispersed homogeneously into PLLA matrix with 10 wt% chitosan. However, there is some groove structure with 20 wt% chitosan. We may conclude that up to 10 wt% chitosan incorporation improves morphological and thermal properties, increasing more percentages improve more antimicrobial activity to inhibit the growth of *Escherichia coli*.

Keywords: Poly(L-lactic acid); Chitosan; Microcrystalline Cellulose; Blends; Thermal Stability; Antibacterial Activity

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1. Introduction

Poly(L-lactic acid) (PLLA) is a biodegradable, biocompatible, compostable, and non-toxic polyester derived from renewable resources such as starch of crops. It has biomedical applications that are the most promising substitute for petroleum-based polymers in a wide range of commodity and engineering applications [1-4]. During the last three decades, enormous research on PLLAs was carried...
out to develop the following properties: low melt strength, slow crystallization rate, poor processability, high brittleness, low toughness, and low service temperature. Due to the excellent biocompatibility of PLLA it has been extensively used in food packaging, tissue engineering, degradable sutures, drug-releasing particle, and porous scaffolds for human body cells [5]. To broaden the applications in food packaging and the medical sector, it should have better mechanical properties and thermal resistivity. Natural fibers or cellulose are used as a reinforcing agent in PLLA based composites, and modified cellulose has better compatibility with PLLA matrix, which provides excellent mechanical performance and thermal resistivity [6-8]. Microcrystalline cellulose (MCC) shows a better surface morphology, thermal and mechanical properties in blends due to its short length, flexibility, strong particle-particle interactions through hydrogen bonds [9, 10]. PLLA/MCC composites are being used in food packaging and medical science for tissue engineering fields. However, a more extensive application of PLLA/MCC blends is being prohibited to its relatively poor antibacterial, thermal, and mechanical properties. It must have antimicrobial or antifungal properties during implanted into tissue engineering to remove infection hazard [11]. Several studies have already been executed for improving the antibacterial activity of PLLA by blending with antibacterial agents like nano-silver [12]. To experience detrimental effects for using synthetic antimicrobial agents, researchers are encouraged to accept natural products such as chitosan. Chitosan is an interesting form of biopolymer and comes from fully or partially deacetylation of chitin, which performs some excellent biological properties such as good biocompatibility, biodegradation, and various functionalities such as homeostatic activity, antithrombogenic, immunity enhancing and wound healing [13-15]. It also has the capability to inhibit the microbial growth of some bacteria, such as Escherichia coli and Staphylococcus aureus [16].

In this research, we investigated the effects of different amounts of chitosan on the morphology, crystalline, thermal and antibacterial properties of PLLA/MCC/Chitosan composites. Here we use 3% MCC in all samples for acting as a nucleating agent to improve crystallization properties as well as thermal properties that support reported results [11, 17-19]. To the best of our knowledge, the effects of chitosan on the properties of PLLA/MCC/Chitosan biopolymer composites have not been reported so far. The objective of this study is to develop PLLA based composites with extended physical and antibacterial properties in order to broaden the applications in medical and ecological fields.

2. Materials and Methods

PLLA was collected from Mitsubishi Chemical Corporation, UNITIKA Plastics Division, Japan. Micro Crystalline Cellulose (MCC) was isolated from Tossa Jute (Corchorus olitorius) fiber by conventional acid hydrolysis [20]. Chitosan was derived from the partial deacetylation of chitin extracted from the chemical treatment of shrimp shell [21, 22]. Tossa jute (Corchorus olitorius) and shrimp (Penaeus monodon) shell were collected from the local market of the southern part of Bangladesh.

2.1. Extraction of Micro Crystalline Cellulose from Jute Fiber

Washed and dried raw jute fibers were cut into small length (approximately 2 cm) and bleached with 0.08 M sodium chlorite (NaClO2) solution along with 2M CH3COOH, at pH 4 [23]. Bleaching was conducted at temperature 85ºC - 90ºC for 90 mins with a ratio 1g:80ml (w:v). Before bleach, the buffer solution was prepared by acetic acid and sodium acetate to maintain constant pH. After washing and drying at 105ºC for 24 hours, the bleached fiber was treated with 17.5% NaOH for removing of β and γ cellulose [24]. The treated fiber was washed several times with distilled H2O for removing NaOH from α-cellulose and dried at 75ºC for 48 hours using a vacuum drier. From previously prepared α- cellulose, MCC was prepared by acid hydrolysis process using 65 wt% H2SO4 for 25–30 mins at 35ºC-40ºC in a ratio 1g:10ml (w:v) with constant stirring, which resulted in breaking down glycoside bonds of the cellulose polymeric chain [25]. The reaction was immediately stopped by quenching with cold ice water to obtain gel-type MCC [26]. H2SO4 was completely removed by washing with distilled H2O. MCC was stored in acetone, as dispersion, by replacing H2O by centrifugation.
2.2. Extraction of Chitosan from Shrimp Shell

By using the general methods of demineralization, decolorization, and deacetylation, chitosan was extracted from shrimp shell. Raw shrimp shells were washed, dried, and was crushed to a small size (approximately 0.5cm×0.5cm). It was then dipped into 1.57 M HCl solution in 1gm: 10ml (w/v) ratio for 6–8 hours for demineralization at ambient temperature under constant agitation [27]. Demineralized shells were then kept in 0.5% KMnO4 followed by the keeping of aq. H2C2O4 for 2–3 hours at 60–70°C with a ratio 1g:15ml (w/v), to remove the color from the experimental shell [28]. After decolorization, it was treated with 1.25 M NaOH 100°C for 30 mins resulted in chitin as the product which was further carried out with 50% NaOH at 100°C for 4–5 hours at a ratio between shell & solution is 1g:20ml (w/v) for the process of deacetylation resulting in chitosan as the end product [29, 30].

2.3. Composites Preparation

Samples with different compositions were prepared by a well-known solution casting method. Chloroform was used to prepare PLLA solution with continuous stirring [31]. Because of –OH group, hydrophilic property and reactivity of MCC can be increased. Therefore, MCC was dispersed in non-aqueous solvent acetone with 90 mins sonication [32]. Chitosan was dissolved with 1–2% acetic acid as a solvent [33]. To obtain the samples, the calculated amounts of these three solutions were cast on Petri dishes. Prior to cast, it was blended with vigorously stirring with a magnetic stirrer until homogenous solutions were obtained [34]. Petri dishes were evaporated at room temperature for 3–4 days under vacuum to have dry samples [35]. Sample codes were designed as LMCC-X, where L, MC, and C-X mean PLLA, MCC, and chitosan with X, stand for percentages of chitosan in the composites, respectively.

2.4. Characterization

Shimadzu IR Prestige-21 Fourier Transform Infrared (FTIR) Spectroscopy, Japan, was used to conduct FTIR analysis at a resolution of 4 cm-1. KBr powder was used to make the disk by compressing with samples. X-ray diffraction analysis of different blends and composites were performed at 25°C with a D ADVANCE BRUKER diffractometer operating at 40 kV and 30 mA, using the radiation (λ= 0.1546 nm). The percentage of the crystalline structure of the samples was determined as the ratio of the areas of crystalline reflections to the whole area (after subtraction of background) in the 2θ range [36, 37], and can be expressed as follows:

For thermal properties measurement, Thermogravimetric analysis (TGA) and Differential thermal analysis. (DTA) were conducted in a TG/DTA (SII-6300 analyzer). 10 mg (approximately) of each sample was heated from 20ºC-580°C at 15°C min⁻¹ under N2 gas supply. The percentage of weight change and its derivative (DTG) was recorded as a function of temperature.

To investigate the surface morphology and distribution of particles, scanning electron microscope (SEM) micrograms were obtained from SEM Leica AS-360FE-SEM instrument, with magnification ranging from 20X to approximately 30,000X, the spatial resolution of 50 to 100 nm.

2.5. Antibacterial Analysis [38-40]

After washing and sterilization at 121°C, the glassware and other accessories were dried by laminar airflow hood at room temperature to avoid further contamination by bacteria. 1.5L media was prepared by taking 19.5 gm of nutrient broth in a volumetric flask and stirred well until a clear solution was obtained. It was sterilized in an autoclave for an hour at 121°C, cooled to the room temperature through the laminar air flow hood, and was kept in the refrigerator for further use. 10 ml 1% and 5% solution of each composites i.e. LMCC-0, LMCC-5, LMCC-10, LMCC-20 and LMCC-40 were prepared. 250 ml 10 conical flasks were taken in a laminar hood. Then, 100 ml 0.05% solution of each sample was prepared by taking 1 ml 5% sample solution in a conical flask and then adding a media solution to the sample solution up to the mark. Thus, 100 ml 0.005% solution was prepared from 1% sample solution by taking 0.5 ml sample solution and adding the required amount of media solution. pH of each solution was determined with a modern pH metre, and the pH value was noted. 200 microliter media solution was taken in an appendrop tube. A healthy colony of cultured bacteria was selected from a culture medium and taken to the solution with a sterilized platinum loop. The colony was rinsed well to form a uniform bacterial solution. Then, 20 microliter of the bacterial solution was taken and added to the final solution in the conical flask. The flask was shake a bit. After the addition of a bacterial solution to the
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...solution. Thus, several readings were recorded at an interval of 4 hours for 20 hours of observation keeping the solution in a shaking incubator.

3. Results and Discussion

3.1. FTIR Spectroscopy

FTIR spectroscopy technique is a widely used and well-known method to investigate the intermolecular and intramolecular hydrogen bonding and phase behavior between the polymers. FTIR spectrum of PLLA, chitosan, MCC, LMCC-0, and LMCC-10 were analyzed to explore the interaction between them are shown in Figure 1, and their characteristic absorption bands are shown in Table 1.

![Figure 1. FTIR spectra of pure components and composites.](image)

Table 1. Peaks of PLLA, MCC, Chitosan, LMCC-0, and LMCC-10 were obtained from their FTIR spectra and possible bond for the absorption.

<table>
<thead>
<tr>
<th>PLLA cm⁻¹</th>
<th>MCC cm⁻¹</th>
<th>Chitosan cm⁻¹</th>
<th>LMCC-0 cm⁻¹</th>
<th>LMCC-10 cm⁻¹</th>
<th>Accounted Bond for the absorption cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>3447.82</td>
<td>3383.14</td>
<td>-</td>
<td>-</td>
<td>3653.96</td>
<td>-OH stretching overlapping with N-H stretching</td>
</tr>
<tr>
<td>3000.32</td>
<td>-</td>
<td>3024.58</td>
<td>3022.45</td>
<td>3018.60</td>
<td>-CH stretching (Asymmetric)</td>
</tr>
<tr>
<td>2951.14</td>
<td>2885.51</td>
<td>2883.58</td>
<td>2899.72</td>
<td>2879.72</td>
<td>-CH stretching (Symmetric)</td>
</tr>
<tr>
<td>1759.11</td>
<td>1732.06</td>
<td>1735.93</td>
<td>1735.93</td>
<td>1735.97</td>
<td>-C=O stretching</td>
</tr>
<tr>
<td>1383.70</td>
<td>1365.60</td>
<td>1355.60</td>
<td>1365.50</td>
<td>-CH bending</td>
<td></td>
</tr>
<tr>
<td>1132.23</td>
<td>1220.94</td>
<td>1220.94</td>
<td>1219.01</td>
<td>1219.01</td>
<td>-C-O- stretching for Acid</td>
</tr>
<tr>
<td>1093.68</td>
<td>1024.20</td>
<td>1035.77</td>
<td>1099.43</td>
<td>1065.92</td>
<td>-C-O- stretching for Alcohol</td>
</tr>
</tbody>
</table>

The FTIR spectrum of the neat PLLA (Figure 1) clearly showed the characteristic absorption bands at 3447.82 cm⁻¹, 3000.32 cm⁻¹, and 1759.11 cm⁻¹ due to O–H bending and stretching vibration, C–H asymmetric stretching vibration, and C=O stretching vibration, respectively [8,17, 47]. The FTIR spectra of PLLA also showed the presence of bands at 3837.70 cm⁻¹ for C–H bending in the CH₃ and 1132.23 cm⁻¹ and 1093.68 cm⁻¹ for –C=O– stretching for acid and alcohol, respectively. In LMCC-10, a small peak was observed at 3653.14 cm⁻¹ and 3018.60 cm⁻¹ can be originated from overlapping of OH stretching overlapping with N–H stretching and –CH stretching (symmetric) vibration has shifted towards higher wavenumbers with narrowband intensity upon the incorporation of chitosan. From Figure-1 also found that at 2951.14 cm⁻¹ and 1759.11 cm⁻¹ the observable band intensity of PLLA has significantly decreased by the addition of MCC and chitosan. The C–H stretching peak of LMCC-0 (3000.32 cm⁻¹) shifted to a higher wavenumber (3022.45 cm⁻¹) in LMCC-10; this may occur due to the presence of chitosan in LMCC-10. C=O stretching in PLLA shifted from high region 1739.11 cm⁻¹ to lower 1735.93 cm⁻¹ in LMCC-0 and 1735.97 cm⁻¹ in LMCC-10. It indicates that MCC and chitosan produce intermolecular hydrogen bonds between O–H and C=O group to weaken the C=O in the ester group in PLLA. On the contrary, the FTIR spectra of LMCC-0 and LMCC-10 represented the PLLA/MCC and PLLA/MCC/Chitosan blends respectively was found that the characteristics absorption band of LCMC-0 in at 3022.45 cm⁻¹, 2883.58 cm⁻¹, and 1735.93 cm⁻¹ due to -CH stretching (Asymmetric), -CH stretching (symmetric) and C=O vibration had shifted towards lower wavenumbers when chitosan was added into PLLA/MCC matrix. Similarly, other bending peaks shifted to the lower wavenumber for the intermolecular hydrogen bonding and supported our previous result [47]. Most of the characteristic absorption peaks of the PLLA/MCC/Chitosan blend shifted from the characteristic absorption peaks of PLLA/MCC blend. Therefore, it can be understood that MCC and chitosan adequately dispersed in the PLA...
matrix with some levels of interaction between them, forming the PLLA/MCC/chitosan composites. A similar result has also been reported by other researchers [41].

3.2. Wide Angle X-ray Diffraction

The WAXD pattern of the PLLA/MCC/chitosan blends materials comparing to the PLLA/MCC blend as diffractograms are presented in Figure 2. Here two sharp peaks at \(2\theta = 16.9^\circ\) and \(19.2^\circ\) are the characteristic peaks for the homo crystalline structure of PLLA [42].

![WAXD profiles of composites](image1)

**Figure 2.** WAXS profiles of composites with different percentages of chitosan (a) and their crystallinity as a function of chitosan percentages (b).

The crystalline region of PCM-0 was increased after the addition of MMC is caused MCC contains nano-sized particles that act as a nucleating agent and developed the crystal formation of PLLA analogous to the previous research outcome [6]. However, the degree of crystallinity of chitosan decreases after dissolving in chloroform and blended with PLLA due to the semi-crystalline nature of chitosan. The arrangement of molecules can affect by the interaction between two polymeric chains of PLLA and Chitosan and decrease the degree of crystallinity of both PLLA and Chitosan. With the addition of chitosan, the change in the crystalline structure of different blends has been shown in Fig. 2. 5% chitosan reduced the crystallinity of PLLA/MCC blends from nearly 25.75% to 20.34%. Because 5% can also interact with PLLA by the hydrogen bonding and increase the randomness in the chain arrangement of PLLA analogous to the previous research outcome [6]. But in the case of PCM-10, chitosan contains finer particles that can act as a nucleating agent and develop the crystal formation. That is why the crystallinity of PLLA was being increased with the increase of chitosan amount up to 10%, and LMCC-10 showed the maximum crystal structure among those tri-blends [43]. Furthermore, with an increase in the percentage of the chitosan in PLLA/MCC blends (LMCC-20, LMCC-40), the crystalline structure of the PLLA has been strongly inhibited by chitosan.

3.3. Thermal Stability Analysis

Figure 3 shows the TGA profiles of PLLA/MCC/Chitosan composites and 50% weight loss as a function of chitosan percentages in the composites.

![TGA profiles of composites](image2)

**Figure 3.** TGA profiles of composites (a) and 50% weight loss as a function of chitosan percentages in the composites (b).

It was observed that the desorption of gases and the first weight loss appeared at around 90°C-180°C due to the evaporation of absorbed water moisture and residual acetic acid. At around 280°C – 390°C, major decomposition was started in the PLLA/MCC/Chitosan blends was attributed to the rapid decomposition of polymer segments of PLLA and chitosan. TGA analysis shows a sharp increase
in initial degradation from LMCC-0 to LMCC-10. In the case of LMCC-20 each degradation point decreased with the increase in the percentage of chitosan. Initial degradation point shifted from 309°C to 317.7°C, 306.3°C, and 311.2°C for LMCC-5, LMCC-10, LMCC-20, and LMCC-40, respectively. Chitosan can act as a reinforcing agent in the PLLA/MCC matrix and decrease flexibility and mobility of the polymeric chain by holding them tightly, which results in a more compact composite structure with better heat resistivity. In the case of composites with higher chitosan percentages (LMCC-20 and LMCC-40), chitosan has a tendency to accumulate by hydrogen bonding in a higher percentage and caused a sudden drop in heat degradation profiles. Half degradation point as a function of chitosan percentages are shown in Figure 4(b). In addition, the initial degradation (T_{onset}) and final degradation (T_{max}) of the blends change slightly with the different weight percentage composition. However, the T_{onset} and T_{max} of the blends lie between chitosan, MCC, and PLLA, which results deduced that there three polymers are well blended together. From the WAXRD and TGA, it was observed that the crystallinity and thermal stability of the PLLA/MCC/Chitosan increased with the increase of the chitosan percentage, which can be concluded that thermal stability is correlated to the crystalline properties.

DTG profiles of composites, maximum degradation rate, and maximum degradation peak as a function of chitosan percentages of composites are shown in Figure 4. The maximum degradation rate and peak point were determined by DTG analysis to have shown in Figure 4(b and c). The maximum degradation rate decrease with increasing chitosan percentages. The maximum degradation point increased similarly up to LMCC-10 and decreased for LMCC-20 and LMCC-40. For LMCC-0, LMCC-5 and LMCC-10, the T_{max} are 329.1°C, 327.0°C, 371.4°C respectively, and for LMCC-20 and LMCC-40 the T_{max} are placed on 365.8°C and 362.8°C respectively.

Figure 5 shows DTA profiles of composites and 1st and 2nd melting peak as a function chitosan percentages of composites. Depending upon the DTA curves, the melting point (Tm) change for different composition of blends. The addition of chitosan DTA data shows a similar trend in the shifting of Tm. Tm for LMCC-0 is 154.9°C shifted gradually up to 165.3°C in LMCC-10 and then similarly dropped to 164.1°C in LMCC-20. The incorporation of chitosan Tm has been positively changed can be derived based on their intermolecular attraction between PLLA, MCC, and chitosan. The high Tm caused due to the formation of hydrogen bonding among PLLA, MCC & chitosan. The distance between molecular decreases alternatively increases the molecular attraction. However, the low Tm of LMCC-20 may be due to the amorphous region increasing provided by a chitosan or an intermolecular bond formation between consecutive chitosan molecules.

Figure 4. DTG profiles of composites (a), maximum degradation rate (b), and maximum degradation peak (c) as a function of chitosan percentages in the composites.
3.4. Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) analysis was attempted in order to obtain a clear view of the surface morphology. SEM micrographs of different composites LCMC-0, LMCC-10, and LMCC-20 are shown in Figure 6. A large number of microvoids are present in the LMCC-0 resulted in a relatively low melting point and heat degradation point. Besides, in LMCC-10 showed smooth and homogeneous surface upon insertion of 10% chitosan in the composites. It can also be seen that the fibrils and micro-holes are substantially reduced after the incorporation of chitosan. Moreover, the surface of LMCC-10 seems to have no or fewer voids and more homogeneous compared with LMCC-20. This result indicates that PLLA and chitosan were blended in the matrix, and the interaction between PLLA and chitosan was strong, which confirmed that PLLA and chitosan at this concentration (10%) are compatible. Though with the increase of chitosan in LMCC-20, the surface was compacted but can be ascribed as the accumulation of chitosan can make that groove structures and results in the low heat degradation temperature and low melting point.

3.5. Antibacterial Analysis

Figure 7 shows the anti-bacterial activity of nutrient broth (control), LMCC-10 (87% PLLA, 3% Cellulose and 10% Chitosan), LMCC-20 (77% PLLA, 3% Cellulose and 20% Chitosan) and LMCC-40 (57% PLLA, 3% Cellulose and 40% Chitosan), respectively.
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the period from 4 to 16 hours, the growth of bacteria increases gradually, but the rate of enhancement is lower than the initial period in all cases. In the case of control, this can be attributed to the reduction of nutrients and the accumulation of metabolic wastes. The lowering of the rate of bacterial growth for LMCC-10, LMCC-20, and LMCC-40 is most probably due to the antibacterial activity of chitosan [46]. From the figure, it is seen that the rate of inhibition increases with the increase in the percentage of chitosan in the composites. Moreover, after 16 hours, the bacterial growth remains constant for control and LMCC-40, but the rate of inhibition increases slightly for LMCC-10, and LMCC-20 shows more inhibition than even in the case of LMCC-40, which may be due to the formation of L-lactic acid, which itself has a bit antibacterial activity in this period. The hypothesis is also supported by the lowering of the pH of the sample after 16 hours and is shown in Table 2.

Table 2. Initial pH and after 20 hours pH of the samples during the observation of antibacterial properties.

<table>
<thead>
<tr>
<th>Name of sample</th>
<th>Initial pH</th>
<th>pH after 20 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.81</td>
<td>7.82</td>
</tr>
<tr>
<td>LMCC-10</td>
<td>6.79</td>
<td>7.60</td>
</tr>
<tr>
<td>LMCC-20</td>
<td>6.76</td>
<td>7.50</td>
</tr>
<tr>
<td>LMCC-30</td>
<td>6.18</td>
<td>7.15</td>
</tr>
</tbody>
</table>

4. Conclusions

Chitosan with MCC was efficiently blended with PLLA matrices by the solution casting method. FTIR analysis indicated some level of the attractive interaction between chitosan, MCC, and PLLA, it is hereby proposed that chitosan can also be considered as reinforcing filler for the PLLA/MCC/Chitosan composites. The presence of chitosan particles increased the crystallinity of up to 10 wt% of the composites and decreased with increasing the percentages of chitosan. Thermal stability analysis also supports this. Microstructural studies showed good attractive interaction among the composites’ components with visible agglomeration at higher than 10 wt% chitosan loading. In the case of antibacterial analysis, the rate of inhibition increases with the increase in the percentage of chitosan in the composites, even up to 40 wt% chitosan loading. A lower amount of chitosan in composites (10 wt%) will be better for more thermally stable applications, and a higher amount of chitosan (40 wt%) will be better for the applications where antibacterial properties are essential.

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Conflicts of Interest

The authors declare no conflict of interest.

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