Improvement of thermal properties of polyhydroxybutyrate by grafted chemicals

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Abstract: The crystallization and thermal degradation behaviors of polyhydroxybutyrate (PHB) grafted with maleic acid (MAc) and exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (ETA) are analyzed with differential scanning calorimetry (DSC), dynamic thermogravimetric analysis (TGA), and gel permeation chromatography (GPC). It is obtained that the PHB grafted with MAc or ETA has a faster crystallization rate, larger crystallinity, and better thermal stability than the as-received PHB. The thermal stability of specimens are in the order of the as-received PHB < PHB grafted with ETA < PHB grafted with MAc. The grafting ratio of MAc is larger than that of ETA. The thermal stability of PHB can be greatly improved by grafting a small amount of MAc. The activation energy of the PHB degradation reaction are little affected by the grafted chemicals, nonetheless, the reaction order and frequency factor are significantly affected by the grafted MAc.

Keywords: polyhydroxybutyrate, exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride, grafting, maleic acid, degradation, crystallization.

Introduction

The biosynthesized polyhydroxyalkanoates (PHAs) attract increasing attention because their biosynthesis nature can alleviate problems resulting from the uses of oil-based synthetic polymers. Among all different PHAs, the properties of the earliest biosynthesized polyhydroxybutyrate (PHB) are mostly studied since its early appearance in the 1920’s [1-8]. The unique biodegradation/biocompatibility properties of PHB have led to a lot of potential applications of PHB in the medical field; however, the wide acceptance of PHB in the industry would be unlikely unless its inherent inferior properties, i. e., brittleness and bad thermal stability, can be improved.

In general, the brittleness of PHB is resulted from its great crystallinity and post crystallization happening after the processing, which cause the formation of irregular pores on the surface and limit the flexibility of amorphous chains in PHB. The methods such as annealing molded PHB at a high temperature, cold-drawing of PHB, blending PHB with other thermoplastics, adding low molecular weight plasticizers or nucleating agents into PHB, and copolymerizing PHB with different flexible constituents (e.g. 3-hydroxyvalerate (HV) and 3-hydroxyhexanoate (HHx)) are proved to be effective on improving the flexibility and toughness of PHB [9-15].

In these toughening methods, some can simultaneously enhance the thermal stability of PHB, e. g., blending PHB with more stable thermoplastics or adding the HV and HHx to form copolymers of PHBHv and PHBHHx. The PHBHv and PHBHHx...
prepared have lower melting temperatures than PHB because of the smaller crystallinity and the incorporation of flexible chains. Henceforth, the copolymerization broadens the processing window of PHB during processing due to the use of a low molding temperature and then causes less severe degradation. The uses of thermoplastics and plasticizers have a similar effect on lowering the processing temperature of PHB. In addition to these, the thermal degradation of PHB can also be improved by grafting various unsaturated chemicals, such as acrylics (methyl methacrylate, 2-hydroxyethyl methacrylate, and acrylic acid), maleic anhydride (MA), and styrene, mostly because of the hindrance of the degradation reaction by the grafted chemicals [16-21].

As indicated, the thermal stability of PHB can be enhanced by adding suitable constituents to lower the melting (or processing) temperature of PHB and by grafting suitable monomers onto PHB chains. In general, these are practical routes to improve the thermal properties of PHB because the thermal degradation of PHB can not be effectively retarded by adding the conventional stabilizers or antioxidants that are usually used in stabilizing commodity thermoplastics. It is attributed to the fact that the thermal degradation reaction of PHB is a non-radical, random chain scission, and cis-elimination reaction of β-CH including a six-membered ring transition [22, 23]. The thermal degradation of PHB could start near the melting temperature and form olefinic and carboxylic acid compounds at a degradation temperature below 300 °C [22-24].

In the previous studies, the thermal stability of PHB is shown to be significantly improved by grafting MA to PHB by different methods [20, 21]. The use of a mechanical grafting method results in the best improvement in the crystallization and degradation properties of PHB due to less severe shear and thermal conditions applied during the grafting reaction. In this study, it is shown that the grafting of two different unsaturated monomers, one with the similar structure as MA and one with a bulky main chain, with PHB can result in different changes of the crystallization and degradation behaviors in PHB.

Results and discussion

DSC results

The DSC characteristics obtained from the first heating scan of different PHB specimens are presented in Table 1 for comparison purposes. The DSC results obtained previously from the as-received PHB are used as references [20]. As shown in Table 1, the as-received PHB and grafted PHB have melting temperatures in the range of 165.3 ~ 170.8 °C and with the melting heat of 81.5 ~ 101.7 J/g. The differences obtained among specimens resulted from different thermal histories and preparation steps of the specimens. The as-received PHB is aged and crystallized in the room temperature for a long time while the grafted PHB are additionally processed under a shear and thermal condition for hours with the decrease of molecular weight (explained later); henceforth different endothermic results from the DSC first heating scan are obtained.

The differences among specimens can be objectively compared from the cooling thermograms taken after the first heating scan and five-minute isothermal stay at 185 °C (used for erasing different thermal histories of specimens). As shown in Figure 1A,
the as-received PHB reference has a quoted small broad exothermic peak with a low peak crystallization temperature (Tc) near 41.6 °C and exothermic heat about 30.4 J/g (ΔHc) (see Table 1) [20]. The obtained small crystallinity and slow crystallization rate are mainly due to the serious degradation in the as-received PHB, which is confirmed in Table 2 that the Mw of which greatly decreases to 3878 g/mol (PDI=1.76) after only 5 minutes stay at 185 °C [20]. As indicated previously, this serious thermal degradation is mainly attributed to catalytic impurities in the as-received PHB, i.e., long chain hydrocarbon compounds and residual Ca2+ (702 ppm) and Mg2+ (94 ppm) [20-28]. The great decrease of Mw causes a low nucleation density, slow crystallization speed, and small crystallinity in PHB [29].

Tab. 1. The characteristic data obtained from DSC thermograms.

<table>
<thead>
<tr>
<th>DSC Scan</th>
<th>1st Heating Scan</th>
<th>Cooling Scan</th>
<th>2nd Heating Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tm* (°C)</td>
<td>ΔHm# (J/g)</td>
<td>Tc (°C)</td>
</tr>
<tr>
<td>PHB Specimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(As-received)**</td>
<td>(170.8)</td>
<td>(81.5)</td>
<td>(41.6)</td>
</tr>
<tr>
<td>PHB - 5 MAc</td>
<td>167.3</td>
<td>96.4</td>
<td>105.3</td>
</tr>
<tr>
<td>PHB - 10 MAc</td>
<td>165.3</td>
<td>86.5</td>
<td>108.5</td>
</tr>
<tr>
<td>PHB - 5 ETA</td>
<td>169.0</td>
<td>101.7</td>
<td>107.0</td>
</tr>
<tr>
<td>PHB - 10 ETA</td>
<td>166.8</td>
<td>95.1</td>
<td>108.1</td>
</tr>
</tbody>
</table>

* The standard deviation (σ) is smaller than 2 °C.
# The standard deviation is smaller than 3 J/g.
**The data quoted from the reference [20].

Fig. 1. The representative DSC cooling thermograms obtained from the (A) as-received PHB [20], (B) PHB-5MAc, (C) PHB-10MAc, (D) PHB-5ETA, and (E) PHB-10ETA.

The degradation and slow crystallization rate of the as-received PHB can be improved by grafting MAc onto PHB molecules. The cooling thermograms of the MAc
grafted PHB are shown in Figures 1B and 1C. As shown, a specimen prepared from the PHB reacted with 5 parts MAc (referred to as PHB-5MAc) has a large exothermic crystallization peak at 105.3 °C with crystallization heat of 83.3 J/g, which are all much greater than those from the as-received PHB during cooling (see Figure 1A and Table 1). It is obvious that the PHB-5MAc has a much faster crystallization rate than the as-received PHB, which also leads to significantly larger crystallinity.

With regard to the specimen prepared from the PHB reacted with 10 parts MAc (referred to as PHB-10MAc), a similar enhancing effect on crystallization is observed. It is shown in Figure 1C and Table 1 that the PHB-10MAc has a great exothermic crystallization peak at 108.5 °C with crystallization heat of 84.5 J/g during the cooling scan. As expected, a significant increase of the crystallization rate and crystallinity is also obtained in the PHB-10MAc.

The effect of grafted MAc on affecting the crystallization of PHB is mostly due to the fact that the MAc modified PHB degrades less. It is shown in Table 2 that the PHB-5MAc has the M_w near 79000 g/mol with PDI=1.9 and the PHB-10MAc has the M_w near 36000 g/mol with PDI=2.0, which are all much greater than the as-received PHB after the high temperature stay. It is well known that the polymer with a molecular weight too low, e. g., the as-received PHB after the thermal stay, would be difficult to crystallize and consequently have a small crystallinity because a short chain polymer is too mobile to accommodate the crystalline structure (the formation of large negative entropy). As a result, the increase of the M_w of the MAc grafted PHB resulted from the improvement of thermal stability which causes a significant increase in the crystallization rate and crystallinity.

It is shown previously that the inferior thermal stability of PHB could be improved by grafting maleic anhydride (MA) onto PHB molecules mainly due to the inhibition of the formation of six-member cyclic rings by the steric hindrance from attached bulky maleic anhydride molecules [18-20]. The DSC and GPC results indicate that the maleic acid grafted would also have a similar effect on enhancing the thermal stability of PHB. In this study, the MAc grafting ratios of the PHB-5MAc and PHB-10MAc are 0.62 and 0.87%, respectively, which increase with the amount of maleic acid added in the grafting process. The grafting reaction of the MAc is also confirmed by 13C-NMR analyses. Similar to the grafting reaction of MA, the maleic acid is attached onto PHB main chains through subtracting tertiary hydrogens from PHB molecules and then the steric hindrance to the formation of degraded products is obtained [18, 20, 30, 31]. The small MAc grafting ratios indicate that a small amount of the grafted maleic acid can effectively decrease the thermal degradation of PHB.

However, factors other than the M_w could also affect the crystallization. As shown in Tables 1 and 2, although the PHB-10MAc has a lower M_w and larger PDI after the first DSC heating scan, it has a similar crystallization rate and crystallinity (both within statistical errors) than the PHB-5MAc. In addition to the effect of M_w mentioned previously, the crystallization behavior obtained from the grafted PHB is affected by interactions among the grafted amount of the MAc, M_w, and M_w distribution. In general, a crystalline polymer with a narrower M_w distribution in a proper range, i. e., without the presence of extra larger (too viscous) or smaller (too mobile) molecules, would be easier to crystallize. The amount of the MAc grafted would also affect the crystallization because the grafted MAc could introduce irregularity into molecular chains and add hindrance to retard the crystallization, and which effects are similar to
those found in the maleic anhydride grafted PHB [16]. The negligible differences in crystallization behaviors obtained between the PHB-5MAc and PHB-10MAc are results of these mutual effects.

Tab. 2. The molecular weights of PHBs after different treatments.

<table>
<thead>
<tr>
<th>PHB Specimens</th>
<th>As-prepared</th>
<th>After DSC 1st Heating Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mw</td>
<td>PDI</td>
</tr>
<tr>
<td>(As-received)**</td>
<td>(605000)</td>
<td>(1.5)</td>
</tr>
<tr>
<td>PHB - 5 MAc</td>
<td>210000</td>
<td>3.8</td>
</tr>
<tr>
<td>PHB - 10 MAc</td>
<td>170000</td>
<td>3.2</td>
</tr>
<tr>
<td>PHB - 5 ETA</td>
<td>300000</td>
<td>4.5</td>
</tr>
<tr>
<td>PHB - 10 ETA</td>
<td>330000</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Mw: weight-average molecular weight
**The reference data quoted from the reference [20].

It is also clear that the difference in Mw between the grafted PHB after the thermal exposure is also related to changes in the Mw after the grafting reaction. As shown in Table 2, the PHB-5MAc has the Mw near 210000 g/mol with PDI=3.8 and the PHB-10MAc has the Mw near 170000 g/mol with PDI=3.2 after the grafting process. The long time mechanical grinding condition used for grafting would generate heat and shear force and then result in the decrease of Mw. The PHB-10MAc has a higher grafting ratio but a lower Mw than the PHB-5MAc after the grafting reaction, and which is attributed to the catalytic effect of the maleic acid and the different reaction efficiency between the two grafted PHB. Consequently, this also leads to the result that the PHB-10MAc has a smaller Mw than PHB-5MAc after the DSC thermal exposure. It is confirmed by DSC analyses that the PHB-10MAc specimen without acetone rinsing after the grafting reaction, i.e., the PHB-10MAc specimen containing a noticeable amount of the residual MAc, would have a much lower Tc near 50.1 °C and a much smaller crystallization heat of 52.4 J/g because of the accelerated degradation during the first heating scan.

As regards the PHB grafted with a bulky ETA, a similar DSC cooling result can be obtained. It is shown in Figures 1D and 1E that an exothermic peak near 107.0 °C with enthalpy about 79.6 J/g is obtained from the PHB grafted with 5 parts ETA (referred to as PHB-5ETA), and an exothermic peak near 108.1 °C with enthalpy about 81.0 J/g is obtained for the PHB grafted with 10 parts ETA (referred to as PHB-10ETA) (also see Table 1). Comparing to those from the as-received PHB, the result indicates that the both ETA grafted PHB also have much faster crystallization rates and higher crystallinity than the as-received PHB. The enhancement of the crystallization is also attributed to the improvement of thermal stability by the grafted ETA since it is confirmed in Table 2 that the Mw of the PHB-5ETA and PHB-10ETA are 98000 g/mol (with PDI=2.1) and 86000 g/mol (with PDI=1.2) after the first DSC heating scan, respectively, which are much higher than that of the as-received PHB.

The results of GC analyses indicate that the grafting ratios of ETA from the PHB-5ETA and PHB-10ETA are 0.39% and 0.53%, respectively. The ETA grafted onto PHB similar to that of MA is also confirmed in the study by NMR analyses. As that from the MAc grafted PHB, the higher grafting ratio is obtained by using a greater amount of ETA in the grafting reaction. The presence of a small amount of the
grafted bulky ETA molecules can also block the formation of six-member cyclic rings, and then improve the thermal stability and crystallization of PHB.

Comparing to those obtained from the MAc grafted PHB, the two PHB grafted with ETA have lower grafting ratios but much larger M\textsubscript{w} left after the grafting reaction. This indicates that MAc and ETA have different reactivities with the as-received PHB and which affects the amount of reactants reacted and the M\textsubscript{w} of PHB after the grafting reaction. Surprisingly, regardless of the differences obtained in M\textsubscript{w} and grafting ratios, the PHB grafted with MAc and ETA have very similar T\textsubscript{c} and \(\Delta H\textsubscript{c}\) (within statistical errors) and which implying that all the grafted PHB have similar crystallization behaviors despite the use of different grafted chemicals and the different grafting ratios obtained. This can also be confirmed from the melting endotherms shown below.

The DSC endotherms of crystallized specimens obtained from the second heating scan are shown in Figure 2. The melting endotherm from the as-received PHB is also included for comparison [20]. It is clear that the endotherms of all four grafted PHB are similar but significantly different from that of the as-received PHB. In the case of the as-received PHB, the large decrease of M\textsubscript{w} causes a slow crystallization rate, low crystallinity, and the formation of small and various imperfect crystallites, which result in a small exothermic cold crystallization peak observed around 40.5 °C (\(\Delta H\textsubscript{c} = 16.1\) J/g) because of the crystallization of under-crystallized PHB and a broad/small endothermic peak with a low melting peak temperature (T\textsubscript{m}) shown at 122.9°C (\(\Delta H\textsubscript{m} = 48.9\) J/g) in Figure 2A [20]. None of the cold crystallization, low T\textsubscript{m}, broad
endothemic peak, and low $\Delta H_m$ is observed in the endotherms of the four grafted PHB.

As shown in Figure 2B-2E, only one narrow large endothermic peak with almost the same $T_m$ (in the small range of 164.9 to 166.7 °C), which is much greater than that of the as-received PHB, is obtained from the grafted PHB. As indicated previously, the PHB grafted with either MAc or ETA has a much faster crystallization rate and larger crystallinity at the cooling scan than the as-received PHB because of less degradation during the thermal exposure. As a result, the grafted PHB all have better crystals formed with a thicker lamellar thickness, consequently, a sharper and higher $T_m$ than the ungrafted PHB. In addition, it is also expected that the four grafted PHB have small differences in $\Delta H_m$ due to similar $T_c$ and $\Delta H_c$ obtained during cooling (see Table 1).

It is also interesting to note that secondary crystallization happens during the second heating scan and which results in a larger $\Delta H_m$ than $\Delta H_c$ in the grafted PHB. From Table 1, the differences between $\Delta H_m$ and $\Delta H_c$ from the four grafted PHB are in the narrow range about 9.5~11.7 J/g and which also implies that the extent of the cold crystallization of them are similar. However, this secondary crystallization effect is not observed in the as-received PHB because of the severe decrease of $M_w$.

**TGA analyses**

The increase of thermal stability by the grafted MAc and ETA is further confirmed by TGA analyses. As shown in Figure 3, the representative TGA thermograms obtained at a scanning rate of 10 °C/min, the onset temperatures ($T_i$) of weight losses from the PHB-5MAc, PHB-10MAc, PHB-5ETA, and PHB-10ETA are at 201.7, 235.4, 212.0, and 223.4 °C, respectively, which are all significantly greater than 189.8 °C from the as-received PHB (Table 3) [20]. The results of $T_i$ indicate that the thermal stability of the four grafted PHB are obviously better than the as-received PHB and follow the order of the PHB-5MAc < PHB-5ETA < PHB-10ETA < PHB-10MAc. A drastic delay in the starting temperature of the weight loss process to more than 40 °C is obtained from the PHB-10MAc with the MAc grafting ratio of 0.87%.

As expected, the peak temperatures of the weight losses ($T_p$: the temperature at the maximal weight loss rate) shown in Figure 3 also follow the same order of the PHB-5MAc (235.6 °C) < PHB-5ETA (238.7 °C) < PHB-10ETA (245.2 °C) < PHB-10MAc (270.3 °C) (see Table 3). The results imply that the grafted PHB specimens have different degradation kinetics since the difference in peak temperatures is usually attributed to the difference in the kinetic parameters of the degradation reaction. This is confirmed later by dynamic TGA analyses.

It is interesting to note that the trend of the thermal stability obtained from $T_i$ is different from that obtained from $M_w$ after DSC thermal exposure which is in the order of the PHB-10MAc < PHB-5MAc < PHB-10ETA < PHB-5ETA. Regardless of the small differences obtained among PDI, this trend is unexpected since the specimen with the greatest molecular weight left after the DSC isothermal exposure should also have the best TGA thermal stability. In general, the $M_w$ should decrease to a certain low value before the loss of volatile degraded products and large molecules usually need more time or a higher temperature to downsize into a certain short chain length in order to evaporate. However, the PHB-10MAc which has the lowest $M_w$ after the DSC test still has the best thermal stability in the TGA analyses. It is believed that the
complex interactions among the grafted amount, type of grafted chemicals, and $M_w$ left after the grafting reaction on the degradation reaction could result in this unexpected result of TGA.

**Fig. 3.** The representative TGA thermograms obtained from the (A) as-received PHB [20], (B) PHB-5MAc, (C) PHB-10MAc, (D) PHB-5ETA, and (E) PHB-10ETA.

**Tab. 3.** The Degradation Temperatures Obtained from TGA Thermograms of PHBs with a scanning rate of 10 °C/min.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>$T_i$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>PHB -5 MAc</th>
<th>PHB -10 MAc</th>
<th>PHB -5 ETA</th>
<th>PHB -10 ETA</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-received**</td>
<td>(189.8)</td>
<td>(239.7)</td>
<td>201.7</td>
<td>235.6</td>
<td>235.4</td>
<td>270.3</td>
</tr>
</tbody>
</table>

* The standard deviation is smaller than 2 °C.
**The data quoted from the reference [20].

As shown in Table 3, the differences obtained between $T_p$ and $T_i$ from the grafted specimens are in the order of the PHB-10MAc (34.9 °C) ~ PHB-5MAc (33.9 °C) > PHB-5ETA (26.7 °C) > PHB-10ETA (21.8 °C). Consequently, the average weight loss rates of these specimens during the TGA test would follow a reverse order due to a similar total weight loss percentage during the degradation. The two PHB grafted with MAc have longer thermal degradation time but smaller degradation rates during this period than the specimens grafted with ETA. These changes indicate that the grafted PHB have different degradation kinetics and can be significantly affected by the type and amount of the grafted chemical (also influenced by $M_w$ (PDI) obtained after the grafting process).

The changes of degradation kinetics of various grafted specimens are also confirmed by using a TGA dynamic scanning method. As shown in Table 4 and Figure 4, $T_p$
obtained from all three representative specimens increase with increasing the TGA scanning rate due to the kinetic effect and follow the same order of the as-received PHB < PHB-10ETA < PHB-10MAC as that obtained in Table 3.

**Fig. 4.** The representative dynamic TGA thermograms obtained from the as-received PHB at different heating rates.

**Tab. 4.** $T_p$ (°C) obtained from TGA Thermograms at different scanning rates.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Scanning rate (°C/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>(As-received)</td>
<td>209.3</td>
</tr>
<tr>
<td>PHB - 10 MAC</td>
<td>240.2</td>
</tr>
<tr>
<td>PHB - 10 ETA</td>
<td>215.6</td>
</tr>
</tbody>
</table>

* The standard deviation is smaller than 2 °C.

By using the isoconversion plot as proposed in the Flynn-Wall-Ozawa method, the activation energies at different conversions, reaction order, and frequency factor of the degradation process can be obtained [32, 33]. The calculated isoconversion TGA plots of the three representative specimens are shown in Figures 5~7. It is interesting to note that four isoconversion lines in three figures are all nearly parallel. This indicates that the two grafted and the as-received PHBs have almost unchanged activation energies during the degradation process (at 20, 40, 60, and 80% conversions). It is confirmed in Table 5 that the activation energies calculated from the three specimens slightly decrease with increasing the conversion but within experimental errors. As a result, all three specimens have very similar activation energies at the same conversion. The results here indicate that the grafted chemicals
have few effects on changing the main degradation mechanism of PHB, i. e., the chain scission after the formation of the six-member ring structure.

Fig. 5. The representative isoconversion curves calculated from the dynamic TGA thermograms of the as-received PHB at different conversions ($\beta$: the heating rate).

Fig. 6. The representative isoconversion curves calculated from the dynamic TGA thermograms of the PHB-10MAc at different conversions ($\beta$: the heating rate).
Nonetheless, the possibility of the ring formation during the high temperature exposure can be decreased by the grafted chemicals. It is shown in Table 5 that the frequency factors and reaction orders of three representative specimens follow the order of the as-received PHB $\geq$ PHB-10ETA $>>$ PHB-10MAc.

![Graph showing isoconversion curves](image)

**Fig. 7.** The representative isoconversion curves calculated from the dynamic TGA thermograms of the PHB-10ETA at different conversions ($\beta$: the heating rate).

**Tab. 5.** Thermal degradation characteristics calculated from TGA thermograms.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Activation energy (kJ/mol) at different conversions (%)</th>
<th>n</th>
<th>lnA (1/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>As-received PHB</td>
<td>107.7</td>
<td>108.3</td>
<td>105.3</td>
</tr>
<tr>
<td>PHB - 10 MAc</td>
<td>104.4</td>
<td>102.3</td>
<td>101.1</td>
</tr>
<tr>
<td>PHB - 10 ETA</td>
<td>110.4</td>
<td>107.7</td>
<td>104.6</td>
</tr>
</tbody>
</table>

The specimen with the best thermal stability also has relatively lowest reaction order and frequency factor. This is consistent with the previous result that the grafted MAc can effectively inhibit the formation of six-member cyclic rings by offering the steric hindrance through the attached bulky molecules and consequently decrease the frequency of the ring formation. Although the differences obtained between the as-received PHB and PHB-10ETA are small, the result here still implies that the grafted ETA can also improve the thermal stability of PHB with a smaller extent mainly because of a smaller molar grafting amount than that of the grafted MAc.

**Conclusions**

The crystallization and thermal degradation properties of polyhydroxybutyrate (PHB) grafted with maleic acid (MAc) and exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic
anhydride (ETA) by the mechanical method are analyzed. The crystallization rate, crystallinity, and thermal stability of grafted PHB could be greatly affected by the grafted chemicals. All grafted PHB have the better thermal stability than the as-received PHB mostly due to the steric hindrance from the grafted chemicals. The best thermal stability is obtained from the PHB grafted with MAc. A limited amount of MAc grafted could significantly improve the thermal stability of PHB. The reaction order and frequency factor of the PHB degradation reaction are significantly affected by the grafted MAc while the activation energies are little affected by the two grafted chemicals.

**Experimental:**

**Grafting methods**

The as-received PHB was biosynthesized from *E. coli* with crude glucose as the medium (98% pure obtained from Nan-Tien company with the weight-average molecular weight ($M_w$) = 605000 and polydispersive index (PDI) = 1.56, with residual Ca$^{2+}$ (702 ppm) and Mg$^{2+}$ (94 ppm), stored in the open air at the room temperature) and used without further purification. Maleic acid (MAc), exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride (ETA), and benzoyl peroxide (BPO) initiator were purchased from Aldrich and used as-received. The mechanical grafting method used in this study was done by grinding PHB, MAc (or ETA), and BPO in a ball mill (Restch type 51) at 300 rpm for 18 hours at the room temperature. Different parts of MAc (or ETA) and BPO were reacted together with 100 parts PHB, but only the results from the representative grafted specimens (5 and 10 parts monomers with 2 parts BPO) are shown here. The unreacted residual MAc (or ETA) and BPO were removed by rinsing the reacted PHB with acetone. The unreacted MAc (or ETA) in the rinsing acetone solution was analyzed by gas chromatography (GC), and from which the amounts of unreacted MAc (or ETA) were determined by the GC calibration curves obtained from the acetone solutions with different concentrations of MAc (or ETA). The grafting ratio is defined as the ratio of the weight of MAc (or ETA) reacted to the weight of PHB.

The molecular weight distributions of prepared specimens were measured by using gel permeation chromatography (GPC) at 25 °C. GPC is consisted of PLgel 5 micrometers 10000A, 5 micrometers Guard, and 5 micrometers Mixed-C columns (from Polymer Lab.), RI detector (L-2490 from Hitachi), and PU-980 Intelligent HPLC pump (from Jusco). Chloroform was used as eluent at a flow rate of 1 ml/min and with PHB concentration of 20.0 mg/ml for GPC analysis. The polystyrene standards (PS, with different molecular weights of 580 ~ 841700 g/mole) were used to calibrate GPC results. The molecular weights obtained using PS were used for qualitative comparison purposes only due to the inherent uncertainty involved in the GPC measurement by using the standards of different chemical compositions. The prepared films were stored in a refrigerator below 0 °C before analysis to avoid post crystallization during storage.

**DSC analyses**

A Perkin-Elmer DSC-7 is used to measure the endotherms and exotherms of different specimens. The thermogram was measured at a scanning rate of 10 °C/min from -40 °C to 185 °C, stayed at 185 °C for 5 minutes to erase the thermal history,
subsequently cooled to -40 °C at a cooling rate of 10 °C/min to observe the crystallization (cooling scan), and then reheated to 185 °C at a scanning rate of 10 °C/min (heating scan), under the nitrogen environment with a nitrogen flow rate of 40 ml/min. Three specimens prepared from hermetic aluminum (Al) pans were tested for each DSC measurement. The thermograms obtained were calibrated by the baseline obtained from the empty Al pan and the indium standard.

**TGA analyses**

Different specimens were further analyzed with a Perkin-Elmer Thermogravimetric Analyzer (TGA) Pyris-1. The weight-loss behaviors of the specimens were measured with a dynamic scanning mode. The specimen was scanned from the room temperature to 300 °C at the heating rates of 2.5, 5, 10, and 20 °C/min with a nitrogen flow rate of 40 ml/min. The peak temperatures of derivative weight-loss curves, weight-loss percentages, and thermal degradation characteristics were obtained from TGA thermograms.

**References**