Reactions of Palm Oil-Based mcl-PHAs with Epoxidized Natural Rubber

Siang Yin Lee,[1] Aziz Hassan,[1] Irene K. P. Tan,[2] Katsumi Terakawa,[3] Naoya Ichikawa,[3] Seng Neon Gan[1]

[1]Department of Chemistry, University of Malaya, Kuala Lumpur 50603, Malaysia
[2]Institute of Biology, University of Malaya, Kuala Lumpur 50603, Malaysia
[3]Material and Process Technology Research Department, SRI R&D LTD, 2-1-1 Tsutsui-Cho, Chuou-ku, Kobe 651-0071, Japan

ABSTRACT: A palm oil-based medium-chain-length polyhydroxyalkanoate (mcl-PHA) was allowed to react with epoxidized natural rubber (ENR). There was no noticeable reaction at ambient temperature for short reaction times. However, after 30 min at 170°C, the mcl-PHA underwent thermal degradation to generate carboxylic terminal groups that attacked the epoxy groups of the ENR. Evidence of the ring-opening reaction was provided by both FTIR and ^{1}H-NMR. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 2039–2043, 2010

Key words: biopolymer; blending; degradation; FTIR; NMR

INTRODUCTION

Polyhydroxyalkanoates are bacterial polyesters that are produced in nature by a wide variety of Gram-negative bacteria, mainly the *Pseudomonas*. Medium-chain-length polyhydroxyalkanoates (mcl-PHAs) consist of repeating units having a chain length of typically C6 to C12. They have attracted research and commercial interests because of their chirality, biodegradability, and elastomeric properties and have the potential for applications as environmentally biodegradable polymers and functional biomaterials for medical and industrial applications.[1,2] Different hydroxyalkanoate units[3,4] have been identified as constituents of biosynthetic PHA. Of the microorganisms capable of producing mcl-PHAs, *Pseudomonas oleovorans* has been investigated most extensively.[5,6] *P. putida* has also been reported for the production of mcl-PHAs.[7] Recent results in our laboratory have demonstrated that *P. putida* can produce mcl-PHAs from palm oil derivatives.[8]

On the other hand, epoxidized natural rubber (ENR) is produced from the chemical modification of natural rubber (NR), where a portion of the carbon–carbon double bonds have been converted to epoxy groups. This has led to improvements of certain properties such as oil resistance, low gas permeability, good wet grip, and higher damping characteristics.[9] Both the epoxy and unsaturated sites in ENR could be utilized for chemical modifications, where the double bonds can be involved in addition reactions such as vulcanization by sulfur and peroxide, while the epoxy groups provide alternative sites for interaction with other functional groups.[10–13]

Polymer blends containing PHA and rubbers have been reported recently, for example, the mixing of poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) with ENR[14] and the blending of mcl-PHA with different rubbers, including natural, nitrile, and butadiene rubbers.[15] However, there was no detailed explanation about the reactions between these rubbers and mcl-PHA.

To replace some of the toxic petroleum-based additives in tire compounds with materials that are more environmental friendly, we had investigated the incorporation of low levels of palm oil-based mcl-PHA into ENR rubber compounds which were cured at 170°C for 3 to 40 min. At 2 to 10 parts of PHA per hundred parts ENR, the PHA could improve the vulcanization efficiency of ENR compound and significantly suppress reversion of the vulcanized rubber.

Hence, the aim of this work was to investigate the reaction of similar level of the palm oil-based mcl-PHA with ENR at conditions comparable to that of the rubber vulcanization, i.e., at 170°C for 30 min.

EXPERIMENTAL

Materials

Epoxidized natural rubber with a 50% epoxidation level, commercially available as "Epoxyprene 50," was obtained from the Rubber Research Institute of Malaysia. The mcl-PHA was synthesized at the Biotechnology Laboratory at the Institute of Advanced Studies, University Malaya, by process that has been reported earlier.[8] This mcl-PHA was derived from oleic acid ($C_{18 : 1}$) and it was a copolymer consisting mainly of n-alkanoate monomers ranging from C_6 to C_{14}, with C_8 as the predominant component. Toluene, chloroform, ethanol, and methanol are reagent grade solvents and were used as supplied.

Preparation of ENR solution

ENR was mechanically masticated using laboratory two rolls mill to homogenize the sample, reduce the molecular weight, and increase its solubility in toluene. About 10 g of the sample was milled at nip setting of 4 mm for 46 passes. The rubber was then dissolved in toluene to produce an ENR solution of 11% w/v.

Reactions of ENR and mcl-PHA

One milliliter of mcl-PHA solution (containing 41% mcl-PHA w/v in toluene) was introduced into 35 mL of ENR solution in toluene in a conical flask, bubbled with nitrogen for 2 min before being closed tightly with stopper. The mixture was stirred with a magnetic stirrer.

The reaction was investigated at two different temperatures. The first mixture was stirred at an ambient temperature of 27–29°C for 30 min, and was then poured into 500 mL of methanol to precipitate the reacted ENR, which was isolated by filtration and dried in a vacuum oven overnight. The second mixture was heated in an oil bath at 170°C for 30 min. The mixture was then poured into 500 mL of methanol to precipitate the reacted rubber, which was isolated by filtration and the rubber dried as before. ENR/PHA denotes the reaction product of ENR with mcl-PHA. The mcl-PHA was soluble in excess methanol, thus any unreacted mcl-PHA would have been separated during the precipitation and filtration process.

NMR

Samples were dissolved in deuterated chloroform to form a solution of ∼ 4% w/v. ^{1}H-NMR spectra were recorded at ambient temperature using a JEOL JNM-LA 400 FTNMR spectrometer.

Determination of acid number

The acid numbers of mcl-PHA samples were determined by dissolving a known weight of the samples in a mixture of ethanol and toluene (1 : 2) and titrated with standardized potassium hydroxide solution, a procedure adapted from ASTM D 1980-87.

Determination of intrinsic viscosity

An Ubbelohde type of viscometer was used in determining the intrinsic viscosities of ENR. A known concentration of the diluted polymer solution in toluene was prepared and the flow time of the solution through a capillary between two designated marks on the viscometer was measured. All measurements were conducted in a water bath maintained at 30.0°C ± 0.1°C, a procedure adapted from ASTM D 446-93.

FTIR

Samples were cast from solution as thin films on clean glass plates and dried under vacuum. FTIR spectra were recorded with a Perkin Elmer FTIR 1600 spectrometer at room temperature, with 16 scans from 4000 to 600 cm^{-1}.

RESULTS AND DISCUSSION

Thermal degradation of ENR

ENR50 contains equimolar amounts of epoxy and cis-1,4-polyisoprene units, as shown below, randomly distributed in the backbone.
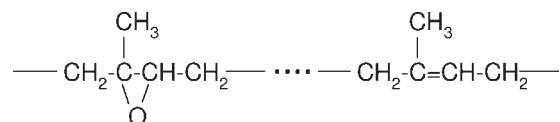
Upon heating at elevated temperature, any thermal degradation of ENR would lead to a decrease in molecular weight, which could be seen from the intrinsic viscosity measurement. The relation between intrinsic viscosity and molecular weight of a polymer is described by the Mark-Houwink equation, $[\eta] = K M_v^{a}$, where η is the intrinsic viscosity, M_v is the viscosity average molecular weight, K and a are constants for a specific polymer and are dependent on the temperature and solvent.

The viscosity average molecular weight of ENR could be approximated by taking $K = 5.02 \times 10^{-4}$ dL g^{-1} and $a = 0.667$, assuming the constants for ENR is approximately the same as that of natural rubber[16,17] in toluene at 30°C.

As shown in Table I, the slight decrease in M_v is more in line with thermal degradation of ENR through the main chain scission at the —C=C— bonds. Degradation involving epoxy units would

inevitably lead to a decrease in the epoxy content; ^{1}H-NMR spectroscopy has shown that the epoxy content was unchanged after heating ENR at 170°C for 30 min.

Thermal degradation of PHA

The mcl-PHA used in this study was a biopolymer produced by *P. putida* using oleic acid ($C_{18 : 1}$) as the carbon substrate. Figure 1 shows the ^{1}H-NMR spectrum of this mcl-PHA. It can be represented by the following structure, where each molecule of the mcl-PHA has one hydroxyl and one carboxylic terminal group.

$$H-\left[O-\overset{\overset{\displaystyle R}{|}}{C}H-CH_2-CO\right]_n OH$$
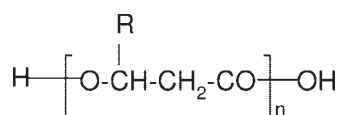
where R stands for an aliphatic group with 3, 5, 7, 9, or 11 carbons.

Morikawa and Marchessault[18] have observed that most biodegradable polyesters tend to degrade above its melting point to shorter chains. Thermal
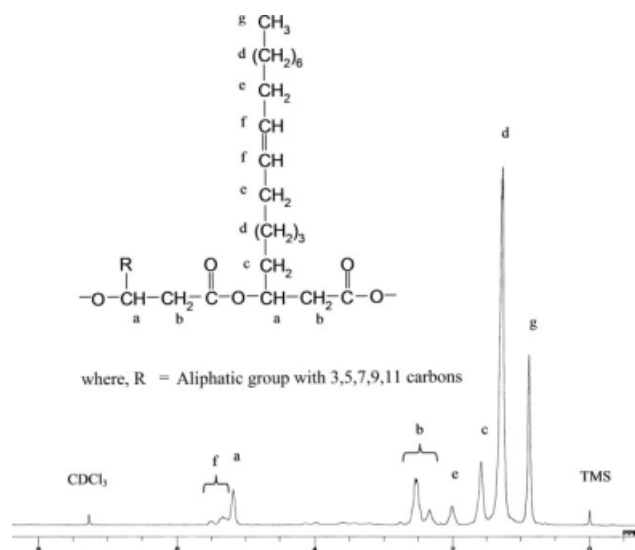
Figure 1 ^{1}H-NMR spectrum of mcl-PHA derived from oleic acid.

TABLE II
Property Changes in mcl-PHA Before and After Heating
at 170°C

Property	Heating at 170°C	
	Before	After 30 min
Acid number, mg KOH/g resin	22.6	46.6
—COOH terminal concentration, mol g^{-1}	4.03×10^{-4}	8.31×10^{-4}
Number average molecular weight, M_n	2.48×10^3	1.20×10^3

degradation of PHA at high temperature has been reported.[19,20]

In this article, the number-average molecular weight of mcl-PHA was determined from the end group analysis. Titration of mcl-PHA with standardized potassium hydroxide solution showed that the initial mcl-PHA contained 4.03×10^{-4} mol g^{-1} of —COOH groups, giving an acid number of 22.6 mg KOH g^{-1}. Since one mcl-PHA chain has one carboxylic group, the number-average molecular weight, M_n, of the mcl-PHA is $1/(4.03 \times 10^{-4}$ mol $g^{-1})$, which is 2.48×10^3 g mol^{-1}. The acid number of mcl-PHA increased after it was heated at 170°C for 30 min. The resulting mcl-PHA has 8.31×10^{-4} mol g^{-1} of —COOH group and the number-average molecular weight of 1.20×10^3 g mol^{-1}.

Table II shows the acid number, —COOH terminal concentration, and number-average molecular weight of the mcl-PHA before and after being heated at 170°C for 30 min.

Reactions of ENR and mcl-PHA

Figure 2 shows the overlay FTIR spectra of ENR, PHA, and ENR/PHA. Spectra (C) and (A) were almost identical except for the small peak at 1741 cm^{-1} which could be attributed to the carbonyl of the free carboxylic group of the PHA. This was consistent with the fact that the ENR/PHA sample contained only $\sim$ 10 parts PHA to 100 parts ENR, thus the weaker peaks of PHA had been overshadowed by the stronger peaks of ENR, except for the carbonyl peak at 1741 cm^{-1} which was due to —COOH, that was exclusive to the PHA. Spectrum (D) showed that after the reaction at 170°C for 30 min, the carbonyl peak had shifted to 1737 cm^{-1}. As the epoxy groups were in great excess over the —COOH groups, it was expected that all the —COOH would have reacted with epoxy to form ester.

^{1}H-NMR spectroscopy

Figure 3 showed the ^{1}H-NMR spectra of NR and ENR50. The two peaks at 2.7 and 5.1 ppm
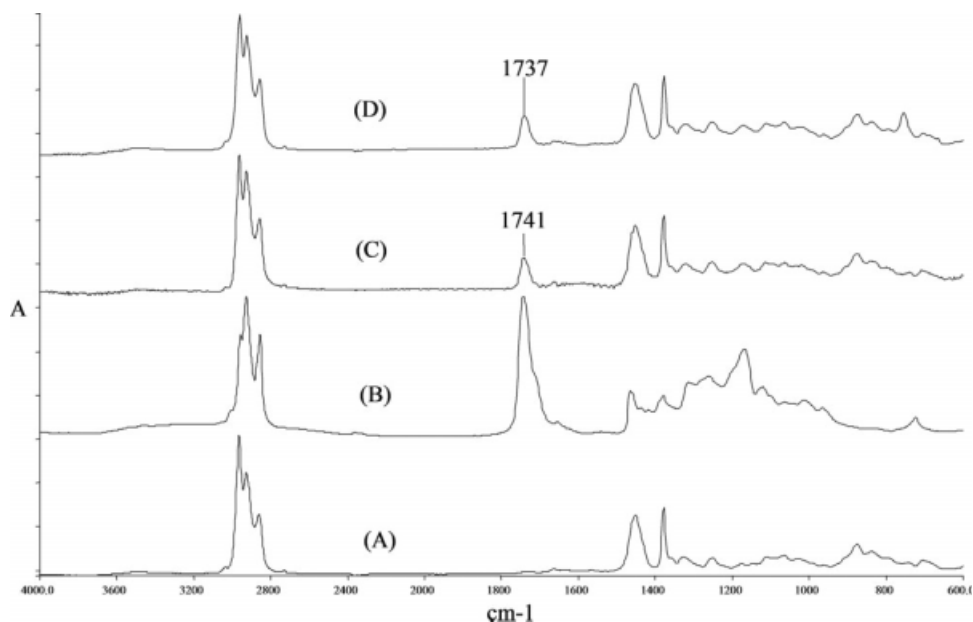
Figure 2 FTIR spectra of ENR (A), PHA (B), ENR/PHA blend at initial conditions (C), and after reacting at 170°C for 30 min (D).

were due to the tertiary proton on the epoxy ring and the vinylic proton of the isoprene unit, respectively.

In this study, the ENR/PHA was prepared from 1 mL of mcl-PHA solution (41% w/v in toluene), which contained ~ 0.41 g mcl-PHA, mixed with
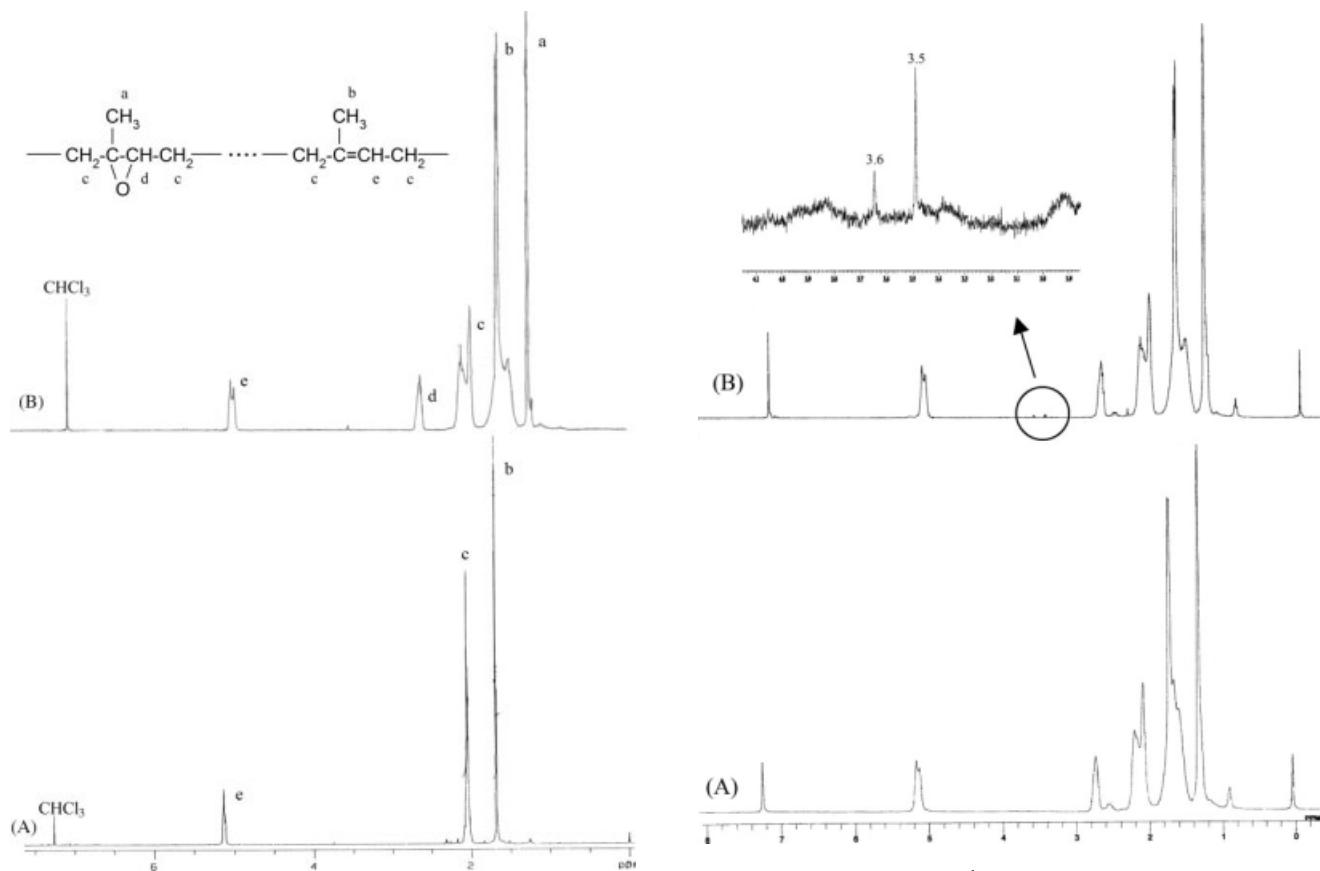
Figure 3 The overlay ^{1}H-NMR spectra of NR (A) and ENR 50 (B).

Figure 4 The overlay ^{1}H-NMR spectra of ENR/PHA blends, reacted for 30 min at ambient temperature (A) and 170°C (B).

Figure 5 A plausible reaction mechanism between ENR and PHA.

35 mL of ENR solution (11% w/v in toluene), which contained 3.85 g of ENR. In the initial mixture, the 0.41 g of mcl-PHA would contain 0.41 g $\times$ 4.03 $\times$ 10^{-4} mol g^{-1} of —COOH group (i.e., 1.65 $\times$ 10^{-4} mol), and 3.85 g of ENR 50 would contain 2.53 $\times$ 10^{-2} mol of epoxy group. The initial amount of —COOH group is less than 1% of the epoxy group. After the specified reaction time, the ENR was precipitated with methanol and the sample was analyzed by ^{1}H-NMR. Figure 4 shows the overlay ^{1}H-NMR spectra for the ENR/PHA before and after reaction for 30 min at 170°C, respectively.

The two NMR spectra have very similar appearances due to the very low level of reaction of PHA with ENR. However, the expanded region at 2.0–4.0 ppm of spectrum (B) showed two distinct peaks at 3.5 and 3.6 ppm, which could be attributed to the hydroxyl protons of the epoxy ring-opened structures as shown in Figure 5.

CONCLUSION

Heating at 170°C for 30 min has caused a slight reduction in the molecular weight of ENR, which has undergone some thermal degradation through chain scission via the —C=C— bond of the isoprenyl unit, as ^{1}H-NMR spectroscopy did not detect any change in epoxy content. On the other hand, when the mcl-PHA was heated under the same conditions, the M_n was reduced by half, and the amount of —COOH group has doubled. When about 10 parts

of mcl-PHA was mixed with 100 parts of ENR at 170°C, there was a readily observable reaction. FTIR has provided evidence of the conversion of free carboxylic acid to ester group with the shift of carbonyl peak from 1741 to 1737 cm^{-1}. ^{1}H-NMR results further confirmed the ring-opening of the epoxy group by the carboxylic terminal of the PHA, as shown in Figure 5.

References

1. De Koning, G. J. M.; van Bilsen, H. M. M.; Lemstra, P. J.; Hazenberg, W.; Witholt, B.; Preusting, H.; van der Galiën, J. G.; Schirmer, A.; Jendrossek, D. Polymer 1994, 35, 2090.
2. Madison, L. L.; Huisman, G. W. Microbiol Mol Biol Rev 1999, 63, 21.
3. Steinbüchel, A.; Valentin, H. E. FEMS Microbiol Lett 1995, 128, 219.
4. Witholt, B.; Kessler, B. Curr Opin Biotechnol 1999, 10, 279.
5. Fritzsche, K.; Lenz, R. W.; Fuller, R. C. Int J Biol Macromol 1990, 2, 85.
6. Kim, D. Y.; Kim, Y. B.; Rhee, Y. H. Macromolecules 1998, 31, 4760.
7. Song, J. J.; Yoon, S. C. Appl Environ Microbiol 1996, 62, 536.
8. Tan, I. K. P.; Kumar, K. S.; Theanmalar, M.; Gan, S. N. Appl Microbiol Biotechnol 1997, 47, 207.
9. Gelling, I. R. J Nat Rubber Res 1991, 6, 184.
10. Baker, C. S. L.; Gelling, I. R.; Azemi, S. J. J Nat Rubber Res 1986, 1, 135.
11. Loo, C. T. In Proceedings of International Rubber Conference; Rubber Research Institute of Malaysia: Kuala Lumpur, 1985; p 368.
12. Gan, S. N.; Burfield, D. R. Polymer 1989, 60, 1903.
13. Hashim, A. S.; Kohjiya, S. J Polym Sci Part A: Polym Chem 1994, 32, 1149.
14. Chan, C. H.; Ismail, J.; Kammer, H. W. Polym Degrad Stab 2004, 85, 947.
15. Bhatt, R.; Shah, D.; Patel, K. C.; Trivedi, U. Bioresour Technol 2008, 99, 4615.
16. Kurata, M.; Iwama, M.; Kamada, K. In Polymer Handbook; Bandrup, J., Immergut, E. H., Eds.; Wiley: New York, 1975; Chapter IV.
17. Burfield, D. R.; Gan, S. N. Polymer 1977, 18, 607.
18. Morikawa, H.; Marchessault, R. H. Can J Chem 1981, 59, 2306.
19. Grassie, N.; Murray, E. J.; Holmes, P. A. Polym Degrad Stab 1984, 6, 127.
20. Doi, Y. Microbial Polyester; VCH: New York, 1990; Chapter 7.