BIOBASED NANOCOMPOSITES FROM TOUGHENED BACTERIAL BIOPLASTIC AND TITANATE MODIFED LAYERED SILICATE: POTENTIAL REPLACEMENT FOR REINFORCED TPO

Yashodhan S. Parulekar and Amar K. Mohanty*
The School of Packaging, 130 Packaging Building,
Michigan State University
East Lansing, Michigan 48824
* E-mail: mohantya@msu.edu

Abstract

Biobased 'green' nanocomposites are the materials for the 21st century. Polyhydroxybutyrate (PHB), a bacterial bioplastic is recently highlighted because of its renewable resource based origin and its potential to replace/substitute petroleum derived non-biodegradable plastic like polypropylene (PP). The major drawback of PHB is its brittleness. This work investigates toughening mechanisms for PHB via incorporation of elastomeric components. Maleated polybutadiene with high grafting and low molecular weight was identified as the compatibilizer. The toughened PHB was characterized through their thermo-mechanical, rheological and morphological analysis. The resulting toughened PHB showed ~440% improvement in impact strength over pure PHB with only 50% loss in modulus. The loss of modulus was recovered to permissible extent through incorporation of titanate modified montmorillonite clay. The hydrophilic clay was modified by titanate-based treatment to make it organophilic and compatible with the polymer matrix. The toughened PHB on reinforcement with 5 wt.% titanate based modified clay gave ~400% improvement in impact properties and 40% reduction in modulus over virgin PHB. The novel toughened bioplastic nanocomposites show potential as a green replacement/substitute of specific TPO for use in structural applications.

Introduction

Recent developments in biobased materials are raising the prospects that naturally derived resources can be used for commercial products and develop as strategic options to meet the growing need for sustainable materials for the next century. The exponential growth of the use of polymeric materials in everyday life has led to the accumulation of huge amounts of non-degradable waste materials across our planet. This growing threat to the environment has led many countries to promote special programs directed towards the replacement of non-degradable, commonly used materials by biodegradable alternatives [1]. Polyhydroxyalkanoates (PHAs) are a class of renewable resource based biodegradable polymers synthesized by bacteria. PHAs were discovered at the Pasteur Institute with the first PHA identified as polyhydroxybutyrate (PHB) [2]. PHB is an enantiomerically pure polymer with a methyl substituent present regularly along the backbone adjacent to the methylene group. This structure is comparable with isotactic polypropylene (PP) and hence it has many similar properties with that of PP. The isotacticity combined with the linear nature of the chain results in a highly crystalline material that exhibits attractive strength at room temperature and under moderate rates of deformation but becomes brittle under severe conditions of deformation [2]. Because of this poor performance at extreme conditions there has been considerable commercial and scientific interest in the toughening of PHB.

The incorporation of dispersed rubber particles into a brittle thermoplastic matrix is known to improve the impact properties and the toughness of the polymer, at the cost of modulus. Under proper conditions and using appropriate compatibilizers, synergistic effects arise to create high impact toughened blends. The effectiveness of this toughening mechanism highly depends on the mechanical properties of the matrix, the elastomeric modifier, the dispersion of the modifier, and the interfacial adhesion among the different phases [3].

The dominant properties of such blends are dictated by the continuous phase and properties of the continuous phase. The plastic and the elastomeric phases are vastly different in their rheological properties. Hence for the elastomeric phase to be continuous it either has to be added in large amounts or a compatibilizer needs to be introduced to increase interfacial adhesion. The compatibilizer also reduces the interfacial tension that is responsible for phase separation. Studies on blends of scrap rubber and linear low-density polyethylene (LLDPE) reported the use of maleated LLDPE and epoxidized natural rubber (ENR) as dual compatibilizer which vastly improved the interfacial adhesion thus justifying the use of a maleated compatibilizer in this study [4]. Toughened blend of polylactic acid and rubber has also been reported to be successfully compatibilized by using block copolymers [5].

In this study, various toughening mechanisms for PHB were developed. The optimal toughness was achieved by incorporation of functionalized elastomeric components into the PHB matrix. A compatibilizer was also investigated to improve the interfacial adhesion between the incompatible elastomer and plastic phases.

The impact modification resulted in significant improvement in toughness but the stiffness of the PHB was sacrificed. Hence nanoclay platelets were introduced into the toughened PHB matrix to regain the modulus. This nano-reinforcement of biobased polymers with organo-clay can create new value-added applications in the 21st century materials world. Development of polymer-clay nanocomposites is one of the latest evolutionary steps of the polymer technology. Dispersion of clay in organic matrices and polymers has been well established in the early 1950's however it was only after the publication of research by researchers from Toyota on polyamide/montmorillonite composites with impressive materials properties in the early 90's and work by Giannelis et al. that reported melt mixing of polymers with clays without the use of organic solvents that these polymer nanocomposites attracted strong interest [6-8]. Since these breakthroughs, the hunger for industrial applications has motivated vigorous research, which yielded nanocomposites with improvement of many properties when compared with virgin polymers or conventional composites. Nanocomposites also exhibited flame-retardancy and dramatic improvements in barrier properties that could not be realized by conventional fillers [9].

Pristine clay is hydrophilic by nature and is difficult to disperse into the organic polymer matrix. Hence the surface of the clay needs to be modified to make it organophilic and compatible with the polymer[10]. In this work we report the chemical modification of pristine montmorillonite with an alkyl-titanate complex. The hydroxyl functionality on the surface of the clay platelet is substituted by alkyl-titanate group from the titianate modifier making the surface organophilic. These titanate coupling agents form chemical bonds between inorganic and organic species via proton coordination and form an atomic layer on the surface of the clay by chemical modification [11-15]. The large alkyl group also will increase the inter-clay spacing and hence can facilitate intercalation and exfoliation.

Laboratory-scale melt processing through extrusion followed by injection molding was adopted in fabricating the nanocomposites. By adding the organically modified montmorillonite clays into the toughened PHB matrix during melt extrusion with high shear force, we hope to get exfoliated and/or intercalated clays inside the continuous matrix. Thermal (dynamic mechanical analysis), morphological (transmission electron microscope (TEM), Atomic force microscopy

(AFM) and Environmental Scanning electron microscopy (ESEM)), and mechanical (impact) properties were investigated in this study.

Experimental Procedure

Polyhydroxybutyrate (PHB) used in this study is a plasticized PHB (Biomer P226) (Biomer, Germany) with their proprietary plasticizer. The rubber used as impact modifier was a latex grade natural rubber (Standard Malaysian Rubber SMRCV60, Centrotrade, OH). Epoxidized natural rubber (ENR), a chemically modified form of natural rubber with epoxide rings on the chain is used as functionalized elastomer. This ENR had 25% epoxidation and was obtained from the Malaysian rubber board as research samples. Polybutadiene grafted with maleic anhydride was used as the compatibilizer system provided by Sartomer (Sartomer Inc., PA) as Ricon series Maleated PB (RI130MA20). Pristine montmorillonite clay was purchased from Nanocor (Nanocor, IL). The specific clay used in the study is called PGV with a specific gravity of 2.6, cation exchange capacity of 145 meq/100 g and aspect ratio of 150-200. The surface modifier used is *neopentyl (diallyl)oxy tri(dioctyl) pyrophosphato titanate* which was provided by Kenrich Petrochemicals, Inc. as Ken-react® LICA-38 (Bayonne, NJ) and was used as received. Toluene, hexane and deionized water of scientific grade were obtained from Aldrich and used as received. Thermoplastic polyolefin (TPO) was obtained from Basell Polyolefins as research sample.

The materials were analyzed prior to processing by thermal instruments to obtain the thermal transitions. A differential scanning calorimeter (DSC Q100, TA instruments, DE) was used to determine the glass transition and the melting temperatures of the materials. These experiments were done at a ramp rate of 10°C/min from – 40°C to 200°C.

The surface modification reaction of clay was carried out in toluene. The proposed reaction of the hydroxyl group from the clay with the titanate-coupling agent is represented in Scheme 1 [11-15]. Similar type of mechanism for the reaction of titanate coupling agent with inorganic
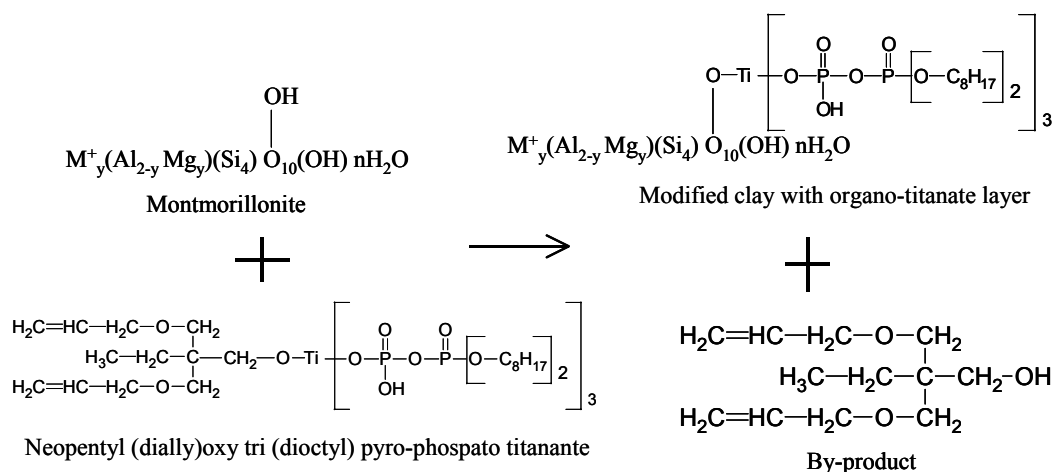
Scheme 1: Titanate based modification mechanism

substrate has also been reported by Monte [11].

PGV monmorillonite clay (50g) was first suspended in the organic medium (300 ml), and the calculated amount of neopentyl (diallyl)oxy tri(dioctyl) pyrophosphato titanate was introduced in the reaction vessel and stirred for 2 hours at ambient temperature. Two levels of surface treatment were achieved by varying the amount of modifier; modified clay MC-1 having titanate modifier weight corresponding to 3.8 % of clay weight and MC-2 having titanate modifier weight corresponding to 11.4 % of clay weight. The modified clay was washed thrice with toluene in order to remove the modifier in excess and byproducts and dried at 55 °C in a vacuum oven for 5 hours before use.

Melt compounding of PHB with impact modifiers was carried out in a micro twin screw extruder with injection molder system (TS/I-02, DSM, Netherlands). The mini extruder is equipped with co-rotating screws having length 150 mm, with L/D 18 and net capacity 15 cc and an attached injection molding unit capable of 120 psi injection force. PHB and elastomer were weighed as per calculated compositions and mixed together and fed to the extruder barrel. The materials were melt compounded for specific processing conditions and after extrusion the melted materials were transferred through a preheated cylinder to the mini injection molder (pre-set at mold temperature of 60 °C) to obtain the desired specimen samples for various measurements and analysis. The compatibilizer system was injected inside the barrel exactly midway through the experimental residence time. The optimized processing conditions (speed, temperature and residence time) were determined from analysis of extensive experiments with variations in parameters.

Nanocomposites were also melt compounded in the micro-compounding-molding instrument. The nanocomposites were processed for 3 minutes at 165 °C at 200 rpm. After extrusion, the molten materials were transferred through a preheated cylinder to the mini injection molder (pre-set at mold temperature of 60 °C) to obtain the desired specimen samples for various measurements and analysis.

Surface elemental analysis was performed on the pristine and modified clays on an X-ray photoelectron spectrometer (XPS). XPS measurements were performed using a Physical Electronics PHI-5400 ESCA workstation. X-Ray photons were generated from a polychromatic Magnesium anode (1254 eV). The analyzer was operated in the fixed energy mode employing a pass energy of 89.45 eV for survey scans and 17.9 eV for utility scans. Clay samples were affixed to the specimen holder with double-sided tape. Semi-quantitative information was obtained by measuring the C 1s and O 1s peak areas and applying the appropriate sensitivity factors[16]. The C 1s spectral envelope was fitted using a non-linear least-squares curve fitting routine. Goodness of fit was tested with a simple materials balance by comparing the O/C atomic ratio estimated from the deconvolution to the actual value. The amount of surface modifier grafted onto the clay surface (expressed in mequiv of grafted titanate per g of montmorillonite) was determined from the difference ΔC (wt %) of carbon content after and before modification using equation (1), [17, 18].

$$\text{Grafted amount (mequiv/g)} = \frac{10^3 \Delta C}{1200 \, N_c - \Delta C (M - 1)} \tag{1}$$

where N_c and M(g/mol) designate the number of carbon atoms and the molecular weight of the grafted titanate molecule, respectively (N_c = 48 and M = 1273). The difference of carbon content was obtained from the XPS analysis.

X-ray powder diffraction patterns were obtained using a Rigaku 200B X-ray diffractometer (45 kV, 100 mA) equipped with CuKα radiation (λ= 0.1541 nm) and a curved graphite crystal

monochromator at a scanning rate of 0.5 °/min. The d_{001} basal spacings were calculated from the 2θ values. The water contact angles for the clays were measured on a CAHN 322 microbalance (ThermoCahn, WI) in the capillary wicking mode using a modified Washburn equation [19,20].

Izod notched impact properties of the materials were measured on an Izod impact tester (TMI Model 43-02, TMI, NY) as per ASTM D256 for notched Izod impact testing with a 5 lb-f pendulum. The samples were notched up to prescribed depth using a mechanical notcher (TMI Model 22-05, TMI, NY) and then conditioned for 48 hours at 50% RH and 23°C. For each sample material 10 specimens were tested. Modulus of the materials was measured using a Dynamic mechanical analyzer (2980 DMA, TA instruments, DE) over a temperature range of – 50°C to 150°C at a frequency of 1 Hz.

Morphology analysis was done using Environmental Scanning Microscopy (ESEM), Transmission Electron Microscopy (TEM) and Atomic force microscopy (AFM). For ESEM studies, samples were fractured by Izod impact and the fracture surface was observed in the ESEM microscope. AFM studies of polished samples were done using an AFM microscope (Digital Instrument MultiMode SPM with Nanoscope IV controller, Digital Instruments, NY) in the force modulation mode. A transmission electron microscope (TEM) (Jeol 100 CX) was used to analyze the morphology of nanocomposites at an acceleration voltage of 100 kV. Cryogenically microtomed ultra thin film specimens with thickness of 100 nm were used for TEM observation. The microtoming was carried out at –130°C using diamond blade.

Results and Discussion

Semi-crystalline polymers and natural rubber form an immiscible blend and this has been widely reported in literature [21-25]. PHB is a linear polymer with no side-chains and hence
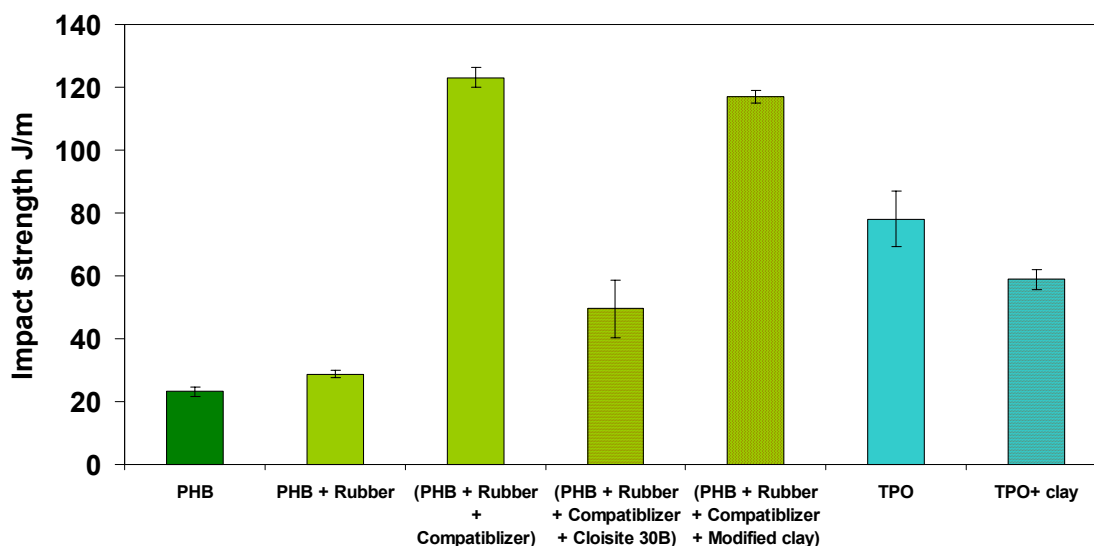

Figure 1: Notched Izod Impact strength of PHB and toughened PHB and its nanocomposites. (Note: Rubber: Epoxidized natural rubber, Compatibilizer: Maleated rubber and Modified clay: Cloisite Na$^+$ with 11.3 wt. % titanate modifier.)

there is minimal entanglement and this causes the chains to slip and move among themselves easily at elevated temperatures [26]. Because of this effect, PHB exhibits a drastic drop in viscosity with increase in temperature very much like a pseudoplastic material. The viscosity of rubber is a function of its purity and quality and the qualitatively best rubber has been reported to have the highest initial viscosity [27]. Our study used a high purity grade rubber and hence the viscosity values were a magnitude of 50 higher than those of PHB. In order for the toughness of the blend to be close to the toughness of rubber, the rubber phase has to be continuous. But the high viscosity of natural rubber in comparison with PHB makes it almost impossible for this to happen. Hence phase inversion can occur only by either using large quantities of rubber in relation to PHB or by using a low molecular weight rubber which will give lower viscosity or by using compatibilizers. The first two methods are not feasible for obvious reasons and hence the compatibilizer technique was selected to improve the blend properties.
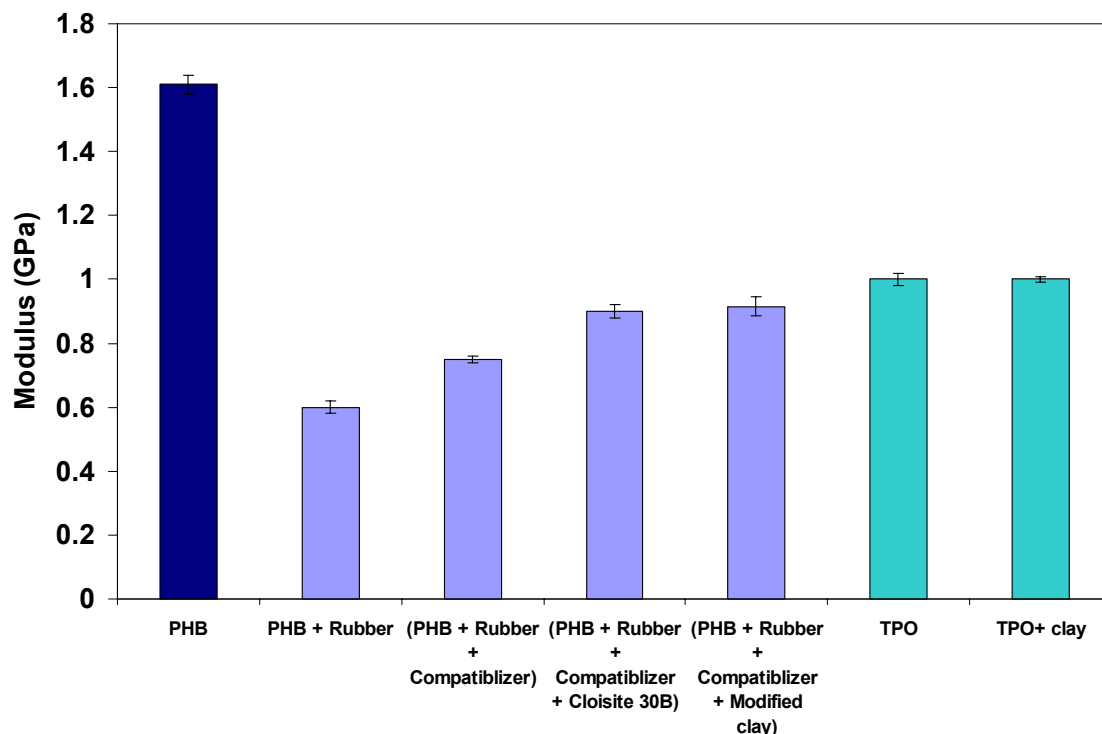

Figure 2: Storage Modulus from DMA for PHB and toughened PHB and its nanocomposites (Note: Rubber: Epoxidized natural rubber, Compatibilizer: Maleated rubber and Modified clay: Cloisite Na+ with 11.3 wt. % titanate modifier.)

Maleated polybutadiene with reactive maleic anhydride groups was used as the compatibilizer in the blend. The compatibilizer system was observed to significantly improve the toughness of the PHB-ENR as evidenced by the 440% increase in impact strength (Figure 1). The modulus of PHB reduced by 63% by addition of ENR and by 56% when MR and ENR were added together (Figure 2). Morphology analysis of the blends validated these observations and the ESEM micrographs (Figure 3C) of the compatibilized system showed good dispersion and evidence of droplet morphology and a rough fracture surface unlike a clean fracture surface for the PHB-ENR system without compatibilizer (Figure 3B) and for pure PHB (Figure 3A). These interfacial adhesions led to the dramatic improvement in toughness yet with acceptable loss in modulus.

This loss in modulus needed to be overcome to use the material for structural applications and hence we introduced nanoclay platelets to regain the stiffness. Optimum clay exfoliation and clay surface chemical modification gives nanocomposites with enhanced properties and has been widely reported in literature [9, 28]. To gain more insight into the modification process, elemental (XPS) analysis was used to determine surface composition of the clay and the amount of alkyl-titanate molecules chemically anchored on the clay after extensive washing of the un-reacted coupling agent. X-ray photoelectron spectroscopy utilizes emitted photoelectrons from a sample by photo-ionization and energy-dispersive analysis to study the composition of the sample surface. The XPS analysis of pristine montmorillonite clay and the two modified clays gave the atomic concentration profiles of the surfaces (Table I). Pristine clay shows the presence of silicon and aluminum atoms that are integral to the clay structure. The high oxygen atom concentration on the surface is attributed to the hydroxyl groups on the hydrophilic surface. These hydroxyl groups are targeted to be exchanged with alkyl-titanate complexes from the surface modifier in the modification reaction. The XPS spectra of the modified clays show significant reduction in the atomic concentration of oxygen thus justifying the modification mechanism. The titanium and phosphorous atoms in the alkyl-titanate complex of the surface modifier that has reacted onto the clay surface are also evident in the atomic profile. The increase in carbon content is due to the alkyl chains and this increases corresponding to the amount of surface modifier added to the clay. The carbon content for MC-1 (with 3.8 wt. % titanate-modifier loading) is 33.07% and this increases to 35.53% for MC-2 clay (with 11.4 wt. % titanate-modifier loading). Another important observation is the reduction in the silicon and aluminum atomic concentrations due to the modifier monolayer covering the surface of the clay. Equation 1 quantifies the amount of surface modifier grafted onto the clay surface (expressed in mequiv of grafted titanate per gram of montmorillonite). For MC-1, the grafted amount is calculated to be 0.29 mequiv/g, and for MC-2, the grafted amount is 0.40 mequiv/g thus increasing proportionate to the amount of modifier added to the clay.
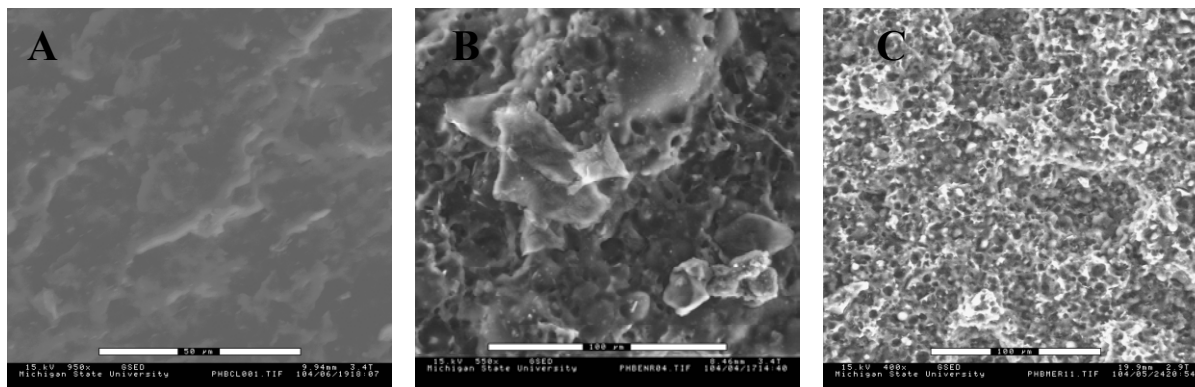

Figure 3: ESEM micrographs of fracture surfaces of pure PHB and its toughened blends. A) PHB (scale bar: 50μm), B) PHB + 40% ENR (scale bar: 100μm), C) PHB + 10% MR + 30% ENR (scale bar: 100μm)

Table I: Elemental concentrations from XPS spectra of the pristine and surface treated clay

	Pristine clay	Modified clay-1	Modified clay-2
Carbon	20.59	33.07	35.53
Nitrogen	0.77	1.05	1.1
Oxygen	53.24	46.33	43.74
Sodium	1.64	0.82	0.87
Aluminum	6.47	5.45	4.52
Silicon	17.29	11.56	11.5
Phosporous	0	1.3	2.03
Titanium	0	0.42	0.71

Table II provides the contact angle for water calculated by wicking experiments and the basal spacing as calculated from the X-ray diffraction (XRD) spectra of raw and functionalized montmorillonite clays. The initial basal spacing of 9.81 Å corresponding to pristine montmorillonite increased to 12.55 Å for the MC-1 titanate-grafted clay, while the XRD patterns of the MC-2 clay powder indicate nearly same increase of the spacing. The increase in the basal spacings suggests that polycondensates are formed in the interlaminate space. Similar results have been reported in the literature for the modification of layered polysilicates using various alkyl-silanes [9].

Table II: Basal spacings and Contact angles for pristine and modified montmorillonite clays

Material	2θ °	d spacing (A°)	Contact angle (Degrees)
Pristine clay	8.98	9.81	6.0
MC-1	6.96	12.70	31.9
MC-2	7.04	12.55	44.2

The contact angle for pristine clay is close to zero as is expected for the hydrophilic surface with ample hydroxyl groups capable of interacting with water. For the modified clay, the organic groups in the alkyl-titanate complex increase the surface energy of the clay surface. This increase in the surface energy reflects in decrease in wettability and thus the contact angle increases. The increase in contact angle was seen to be in agreement with the increase in organic modifier content on the clay surface as previously determined from the XPS. Thus the clay surface has successfully been modified to make it organophilic and thus ideal for organic matrices.

Addition of clay not only acts as reinforcement but also leads to breakup of rubber particles due to the increasing melt viscosity. The accompanying chemical modifiers of the clay also act as interfacial agents and reduce the interfacial tension leading to reduction in the particle size [29]. The addition of a filler reduces the impact strength of the material if the filler does not bond with the matrix. But in case of the nanocomposites with 5 wt. % novel titanate modified clay, the impact properties were significantly retained (Figure 1). This can be attributed to the surface treatment of the clay which has created bonding between the clay and the matrix.

The modulus of the toughened PHB reduced to 0.7 GPa from 1.6 GPa of virgin PHB because of the impact modifier (Figure 2). The titanate-modified clay nanocomposites regained
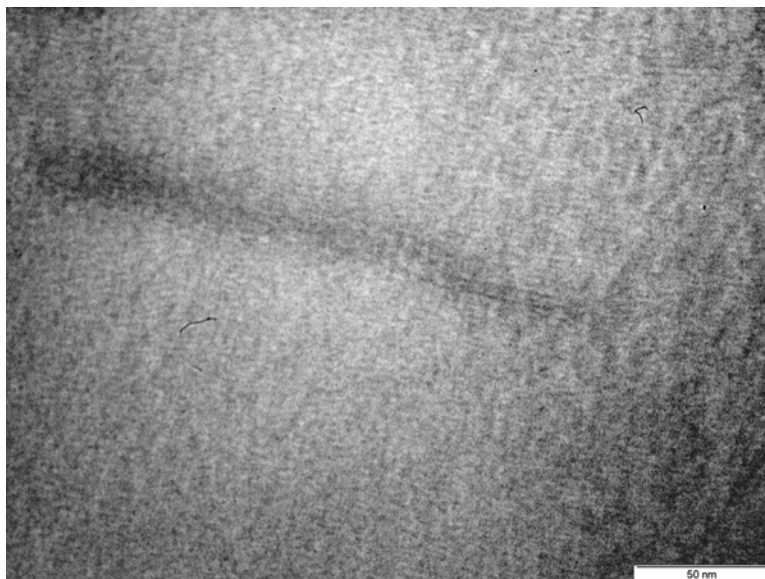
Figure 4: TEM micrograph showing exfoliated platelets of clay in toughened PHB. Scale bar: 50nm.

the modulus to 0.9 GPa. This improvement in modulus from the toughened PHB gives a material with the required stiffness for structural applications as well as retains the impact strength.

The TEM micrograph (Figure 4) of the titanate-modified clay shows morphology of the clay platelets. We have observed clusters of platelets that suggest incomplete exfoliation. Further process and material optimization studies are in progress to find more improved performance of these developed toughened PHB based nanocomposites.

Conclusions

Toughening of polyhydroxybutyrate, PHB, by functionalized elastomer and compatibilizer resulted in enhanced impact properties. The toughening reduced the modulus and this was regained to the permissible extent by the addition of novel modified nanoclay under the present experimental conditions. Elemental analysis and contact angle measurements validated the successful surface modification of pristine montmorillonite clay using a titanate coupling agent. Nanocomposites with this modified clay showed more than 400% improvement in impact properties and around 40% reduction in modulus in comparison with virgin PHB. These experiments were done on a laboratory scale and further experiments using pilot-scale equipment are in progress. Optimized processing needs to be researched which may yield enhanced properties through exfoliation of clay platelets. The target is to achieve improved toughness without sacrificing the stiffness of the resulting green nanocomposites.

Acknowledgements

References

1. Duncan, M., 2003, Journal of Industrial Ecology, 7, 3-4, p. 193-201
2. Fiechter, A., Plastics from Bacteria and for Bacteria: Poly (B-Hydroxyalkanoates) as Natural, Biocompatible, and Biodegradable Polyesters, Springer-Verlag, New York, 1990, p. 77 - 93.
3. Bartczak,Z., Argon, A. S., Cohen, R. E. and Weinberg, M., Polymer, 1999, 40, 2331–2346
4. Guo, B., Cao, Y., Jia, D. and Qiu, Q., Macromol. Mater. Eng., 2004, 289, 360–367.
5. Grijpma, D. W., Van Hofslot, R. D. A., Supèr, H., Nijenhuis, A. J., Pennings, A. J., Poly Eng Sci, 1994, 34, 22, 1674-1684.
6. Okada, O., Kawasumi, M., Usuki, A., Kurauchi, T., Kamigaito, O., 1990, Material Research Society Symposium Proceedings, 171, 45.
7. Alexandre, M., Dubois, P. ,2000, Mater. Sci. Eng. R: Reports, 28, 2, 1-63.
8. Giannelis, E.P., Krishnamoorti, R., Manias, E., 1998, Adv. Polym. Sci., 138, 107-147
9. Gilman, J.W., Jackson, C.L., Morgan, A.B., Harris, R., Manias, E., Giannelis, E.P., Wuthenow, M. , Hilton, D. and Philips, S.H., 2000, Chem. Mater. , 12, 1866-1873,
10. Vanoss, C.J. and Giese, R. F., 2003, J. Dispersion Sci. Technol., 24, 3, 363-376.
11. Monte, S. J., 2002, Polymers and Polymer Composites, 10, 1, 1-32.
12. Kenrich Chemicals Reference manual and product literature, www.4kenrich.com
13. Parulekar, Y.S. and Mohanty, A.K., J. Nanoscience Nanotech., Accepted May 2005.
14. Parulekar, Y.S. and Mohanty, A.K., Paper no. 102078, SPE ANTEC 2005, Boston, May1-5, 2005.
15. Mohanty A.K. and Parulekar, Y.S., US Provisional Patent application, April 29, 2005, MSU 4.1-755 ID05-009, Serial no. 60/676,204.
16. Sherwood, P. M. A., 1990, *Practical Surface Analysis by Auger and X-ray Photoelectron Spectroscopy* 2nd Ed., edited by D. Briggs, and M.P. Seah, Wiley.
17. Herrera, N.N., Letoffe, J., Putaux, J., David L. and Bourgeat-Lami, E., 2004, Langmuir, 20, 1564-1571.
18. Berendsen, G. E. and De Galan, R. J., 1978, J. Liq. Chromatogr., 1, 561,
19. Ayala, R., Casassa, E. and Parfitt, 1987, G., Powder Technol., 51, 3,
20. Washburn, E., 1921, Phys. Rev., 17, 3, 273-283
21. van der Wal, A., Nijhof R. and Gaymans, R. J., 1998, Polymer, 39, 26, 6781-6787
22. van der Wal, A., Nijhof R. and Gaymans, R. J., 1999, Polymer, 40, 22, 6031-6065
23. Wang, Y., Cai, Z. and Sheng, J., 2004, Journal of Macromolecular Science, Physics, 5, 1075-1093
24. Aravind, I., Albert, P., Ranganathaiah, C., Kurian, J. V. and Thomas, S., 2004, Polymer, 45,14, 4925-4937.
25. Hess, W. M., Herd, C. R., Vegvari, P. C., 1993, Rubber Chemistry and Technology, 66, 3, 329-346
26. Cartier, H. and Hu, G. H., 2001, Polymer, 42, 21, 8807-8816.
27. Levin, N., 1996, Natuurrubber 5
28. Cho, J. W. and Paul, D. R., 2001, Polymer, 42, 3, 1083
29. Mehta, S., Mirabella,F. M., Rufener, K. and Bafna, A., 2004, J App Poly Sci, 92, 928–936