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Bionanocomposites based on 3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) and cellulose nanowhiskers (CNWs)

TREBALL FINAL DE GRAU

Grau en Enginyeria dels Sistemes Biològics

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RESUM

En la darrera dècada, el biopolímers, sobretot en el negoci de l'empaquetatge, han adquirit una major importància, degut al consum massiu de derivats del petroli i la creixent preocupació per temes com el medi ambient i la sostenibilitat. La urgent neccessitat de tenir polímers d'alta eficiència, renovables i de base natural, s'està expandint mundialment. Al mateix temps, el preu d'aquests polímers augmenta proporcionalment al nombre d'estudis que hi ha sobre aquests. Per aquesta raó, molts investigadors s'han centrat en la combinació de polímers amb materials naturals com fibres de cel·lulosa, per aix íreduir el seu cost, mantenint, fins i tot millorant les seves propietats. En aquest projecte, reproduïm un sistema d'obtenció de CNWs per a millorar el comportament mecànic i la comprensió de les interaccions entre CNWs i PHBV. Dividim el treball en dos sub-objectius: la manufacturació de CNWs i el compòsit amb PHBV, i l'a avaluació de les propietats mecàniques i tèrmiques del còmposit.

RESUMEN

En la última década, bio-polímeros, sobretodo en embalajes, han adquirido mayor importancia, debido al sobre consumo de los derivados del petróleo y la creciente preocupación por temas como el medio ambiente y la sostenibilidad. La urgente necesidad de tener polímeros de alta eficiencia, renovables y de base natural, está expandiéndose mundialmente. Al mismo tiempo, el precio de esos polímeros aumenta proporcionalmente al número de estudios sobre estos. Por esa razón, muchos investigadores se han centrado en la combinación de polímeros con materiales naturales como fibras de celulosa, para así reducir su coste, manteniendo o incluso mejorando sus propiedades. En este proyecto, reproducimos un sistema de obtención de CNWs para mejora de comportamiento mecánico y la comprensión de las interacciones entre CNWs y PHBV. Dividimos el trabajo en dos sub-objetivos: la manufacturación de CNWs i el composit con PHBV, y la evaluación de las propiedades mecánicas y términcas del composit.

ABSTRACT

Over the past decade, bio-based polymers have gained a bigger role, especially in packaging, due to the over consuming of petrol derivates and the gradually growing concerns related to environment and sustainability issues. The urgent need to have high-performance renewable and natural-base polymers is increasing world-wide. At the same time, the price of those polymers is rising in direct proportion to the number of studies. Therefore, many researchers have focused on the combination of these polymers with other natural materials such as cellulose fibers, in order to reduce their cost while maintaining or enhancing their properties. Here, we reproduce a system to obtain the functional reinforcement cellulose nanowhiskers (CNWs) for enhancing mechanical behavior and understanding the interaction between the CNWs and PHBV. We divide the main objective in two parts: manufacturing the CNWs and the composite with PHBV of it in the lab, and an evaluation of the mechanical and thermal properties of the composite. The result shows that the CNWs can be obtained by acid hydrolysis in lab and can enhance part of the physical properties of PHBV.

1) INTRODUCTION

1.1 background of the study

Poly (3-hydroxybutyrate-co-3-hydroxyvalerate), known as PHBV, is one of the copolymers of PHA (Polyhydroxyalkanoates). PHA is considered environmentally friendly and sustainable since it can be biosynthesized by chemical means or by biological approaches. Additionally, the PHA production is conducted by microorganisms (in overall eukaryotes, such as bacteria and archaea) grown in aqueous solution and can take sustainable resources as nutrients. In many cases these microorganisms produce short-chain-length (scl) PHA copolymers, including PHBV, consuming of C_3 and C_5. The copolymers of scl PHA possess useful mechanical properties which is similar to petroleum-based polymer. Thus, they have been preferred materials for further development (Chen, 2010). However, during a melting process the copolymer PHBV exhibits reduced strength and stiffness and enhances its ductility by increasing the content of (R)-3-hydroxyval-erate (3-HV) (Holmes, P.A., 1988). This unstable mechanical-thermical property prevents PHBV from being used as packing material. In order to solve this problem, it is necessary to combine PHBV with others components, such as a reinforcing agent to boost its mechanical properties under a change of temperature.

From the beginning of life, far from our appearance on earth, nanomaterials have been employed by nature. The inorganic part of the soil is a nanomaterial that is able to filter the particles on a Nano level. Our bones contain plate-like crystals as reinforcement materials. Allowing nature to be a source of ideas, the world turned their sight to nanomaterials. Much of the renewed interest in introducing nanomaterials to the other materials in order to obtain synthetic stronger nanocomposites is the direct reason of the big bang of related researches in the last decade. Among them, one that has gained most prominence is the cellulose nanowhiskers (CNWs). The use of CNWs increases the interface area between the reinforcement agent and the matrix thus promoting good interaction of two components, which makes them suitable candidate for being use as polymer reinforcement agent.

Cellulose nanowhiskers are rod like elementary crystallites. According to some research (M.M.S. Lima.et.al., 2004), the charged nanowhiskers are a good model for the study of electrolytic properties of rod-shaped polymers. In most cases the CNWs product are exacted from cellulose material using chemical treatment. As cellulose based material, the CNWs can be obtained abundantly from plants that grow in a short period under a variety of weather conditions (bamboo, jute…), which means an economic and ecological advantage. Among these plants, cotton offers the most advantages due to its high cellulose content that results in a high yield even without any intensive purification process.

The CNWs have been used to reinforce not only biodegradable polymers, but also non-biodegradable ones. Some polymer matrix materials in which the CNWs successfully improved the mechanical-thermical properties of the resulting composite are include polyurethanes (Li, Y., & Ragauskas, A., 2011), polylactide-based polymer (Goffin, A. L.,et.al., 2011), polyvinyl acetate (Garcia de Rodriguez, N. L.,et.al., 2006), pea starch (Chen, Y.,2009) and PHBV (Ten, E., 2010).

1.2 State of Art

1.2.1 Introduction to PHA and PHBV

Polyhydroxyalkanoates (PHA) are a family of biodegradable polymers. PHAs were first discovered by Maurice Lemoigne in 1923 when cultures of *Bacillus subtilis* lysed in distilled water, decreasing the pH of the water and producing poly-(3-hydroxybutyric acid) (which is the most common group of PHA). That is to say, the PHA naturally exist in the *Bacillus subtilis* when there are limited nutrient sources (Khanna, S., et.al., 2005). PHBV is a common copolymers of the PHA family.
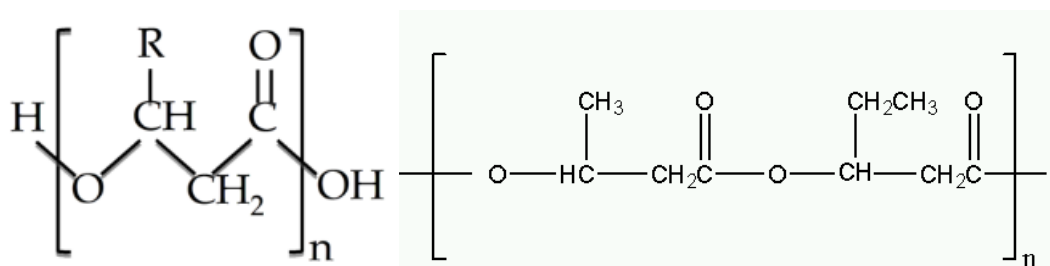

Figure1. The Chemical structure of PHA (left) and PHBV (right)

1.2.2 Mechanical properties of PHBVa

Poly (3-hydroxybutyrate) (PHB) is a highly crystalline polymer and has a melting point, strength, and modulus comparable to those of isotactic polypropylene (Holmes, P.A., 1988).

As a thermoplastic polymer, PHBV suffer from an excessively stiff and brittle nature arising from the relatively high crystallinity of the material. The mechanical properties of PHB and PHBV are said to be similar to others polymers of the PHA family, but the lower ductility and impact strength prevent PHB and PHBV from effectively be replaced by other thermal plastic products (Bledzki, A. K., et.al., 2010). Compared to PHB, PHBV possesses higher ductility which means it can be deformed even at lower temperature but exhibits slow crystallization rate and lower impact strength. By adding reinforcement agents, the crystallization rates of PHBV can be significantly decreased (Qian, J., et.al., 2007).

1.2.3 Type of additives for polymer products

The commercial plastic products usually are polymers and additives. Typically, only polymer may not provide some requirements of final product such as mechanical properties, resistance of degradation and appearance. Thus, different types of additives are incorporated into the polymer products in order to archive a final product with enhanced properties and reduced fabrication cost. Table1 below shows some types of additives and their functions in polymer products.

Table 1: Effects of additives on polymer properties (Tobergte, D. R., et.al., 2013)

Additives	Common materials	Effects of polymer properties
Reinforcing fibers	Carbon, fibrous minerals, glass, Kevlar	• Increase flexural modulus. • Increase heat-deflection temperature (HDT). • Resist shrinkage and warpage.
Conductive fillers	Aluminum powders, carbon fiber, graphite	• Improves electrical and thermal conductivity.
Coupling agents	Silanes, titanates	• Improve interface bonding between polymer matrix and fibers.
Flame retardants	Chlorine, bromine, phosphorus, metallic salts	• Reduce the occurrence and spread of combustion.
Extender fillers	Calcium carbonate, silica, clay	• Reduces material cost.
Plasticizers	Monomeric liquids, low-molecular weight materials	• Improves melt flow properties. • Enhances flexibility.
Colorants (pigments and dyes)	Metal oxides, chromates, carbon blacks	• Provide colorfastness. • Protects from thermal and UV degradation (with carbon blacks).
Blowing agents	Gas, azo compounds, hydrazine derivatives	• Generate a cellular form to obtain a low-density material.

1.2.4 Introduction to CNWs

Cellulose is considered as one of the world's most abundant and renewable natural biopolymers. It is the main component of plant cell walls, and the basic building block of many textiles and of paper which give them remarkable strength. Although cellulose is classified as a carbohydrate, unlike others organic components which belong to this term, it can be either synthesized from, or hydrolyzed to monosaccharides.

Cellulose is made by cellulose microfibril during biosynthesis as shown in Figure 2. The fibril, which is a long string-like bundle of molecules is formed by aggregation of poly-β-(1→4)-D-glucosyl and linked by intermolecular hydrogen bonds (Andresen, M., et. al., 2006). Single cellulose microfibril has diameters in the range of 2 nm to 20 nm and consists of a thread of cellulose crystals bonded along the microfibril axis by disordered amorphous regions.
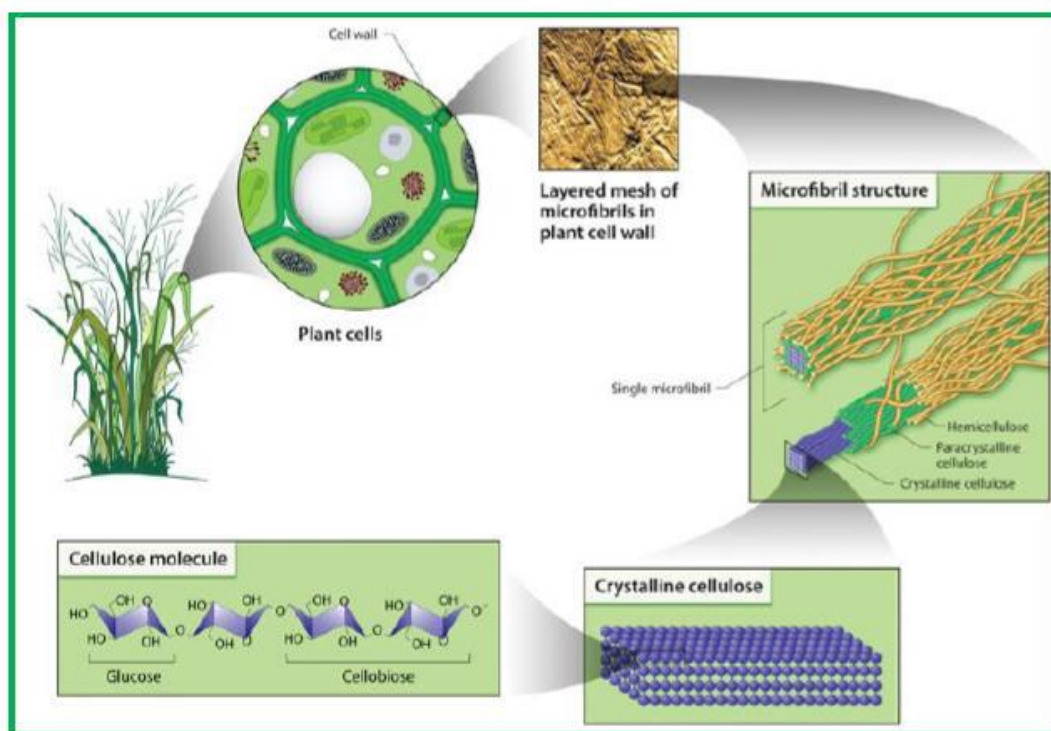
Figure2. Extraction of Microfibrils from Cellulose Cell Wall (Siqueira.G., et.al., 2010).

Cellulose is a linear homopolysaccharide made up of β-glucopyranose units bonded together by β-1-4-linkages. Figure3 below shows the basic structure of cellulose. Each monomer has three hydroxyl groups. These hydroxyl groups can form hydrogen bonds and therefore contribute to crystalline order and also control the physical properties of cellulose.
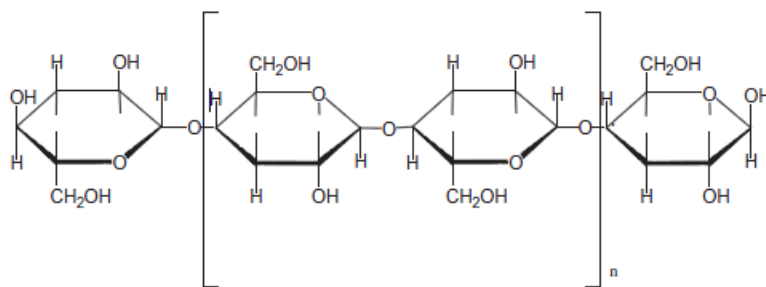
Figure3. Basic Chemical Structure of Cellulose (Akil.H, et. al., 2011)

Because of the equatorial positions of the hydroxyls on the cellulose chain, they protrude laterally along the extended molecule. This positioning makes them readily available for hydrogen bonding. These hydrogen bonds cause the chains to group together in highly ordered (crystal-like) structures. Since the chains are usually longer than the crystalline regions, they are thought to pass through several different crystalline regions, with areas of disorder in between and form amorphous region (the fringed-micelle model as shown in figure4).
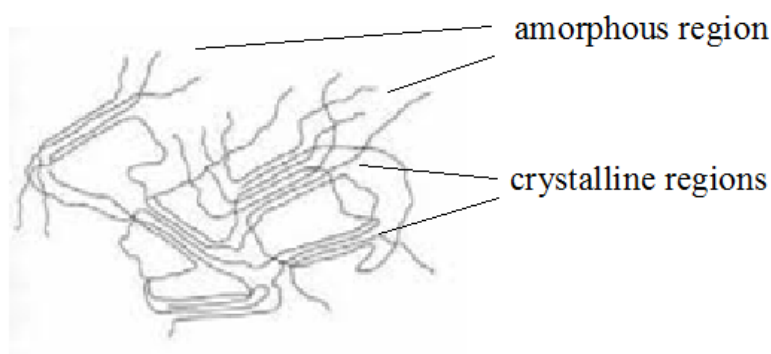

Figure4. The fringed-micelle model of polymer crystallites

The inter-chain hydrogen bonds in the crystalline regions are strong, giving the resultant fibers good strength and insolubility in most solvents. They also can prevent cellulose from melting. In the less ordered regions, the chains are further apart and more available for hydrogen bonding to other molecules, such as water. Thus, cellulose swells, but does not dissolve, in water. In the crystalline regions, cellulose chains are closely packed together by a strong and highly intricate intra/ intermolecular hydrogen-bond network while the amorphous domains are regularly distributed along the micro fibrils. When lignocellulosic biomass is subjected to pure mechanical shearing, and a combination of chemical, mechanical and/or enzymatic treatment, the amorphous regions of cellulose microfibrils are selectively hydrolyzed under certain conditions because they are more susceptible to be attacked in contrast to crystalline domains. Consequently, these microfibrils break down into shorter crystalline parts with high crystalline degree, which are generally referred to as cellulose nanowhiskers (CNWs).
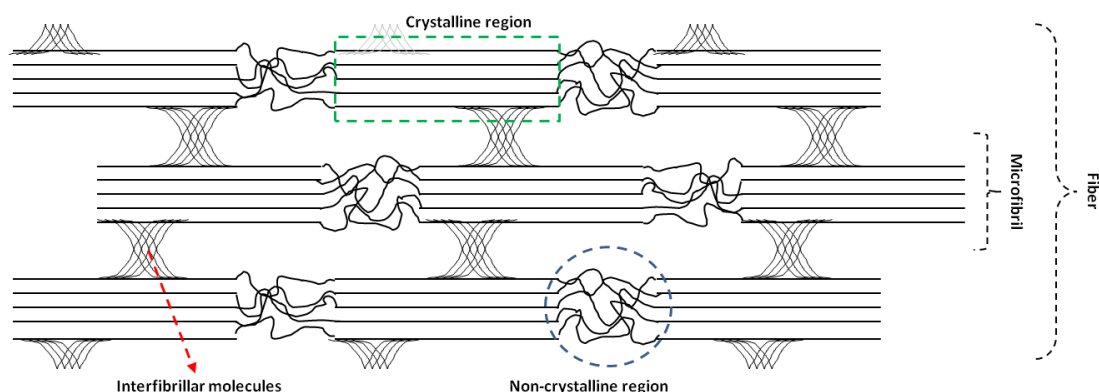
1.2.5 Pretreatment and acid hydrolysis of cellulose

Figure5. Microfibrils formed by crystalline and non-crystalline regions (Börjesson,M., et.al., 2015)

Figure5 shows the crystalline regions and non-crystalline region of cellulose fibres. It can be observe that among the microfibrils, the crystalline regions connecting with each other by some interfibrillar molecules. Meanwhile, the non-crystalline regions exist between the crystalline regions which are in the same microfibril. Mechanical pretreatment will break the interfibrillar bond of cellulose and cut the cellulose fibers into micrometer sizes. After that, acid hydrolysis can remove the non-crystalline regions and crystallized nanoparticles of cellulose will remain. In the early stage of the hydrolysis the acid diffuses into the non-crystalline parts of the cellulose fiber and hydrolyzes the glycosidic bonds. Therefore, the acid can easily access and break the glycosidic bonds in the polymer. Finally hydrolysis occurs at the reducing end group and at the surface of the crystalline regions and causes the rod-shaped nanocrystals. When a 64 wt% sulphuric acid solution, sulfate groups will be attached to the surface of the nanocrystal and charge them (Beck-Candanedo, S., et. al., 2005). As a result, the acid-treated CNWs will form colloidal dispersion when diluted in water.

1.2.6 Basic properties of CNWs

CNWs are rigid rod-like crystals with diameter under 10nm and lengths in the range of 200-400 mm (Bondeson,D., et al., 2006). With a Young's modulus of over 100 GPa and a surface area of several hundred m^2/g (Sturcova, A., et.al., 2005), CNWs are considered as one of the ideal nano-reinforcements for polymer matrices (including both water-soluble and water-insoluble polymers) and have already been incorporated into many polymer matrices to produce reinforced composites. In addition, high aspect ratio, low density, low energy consumption, inherent renewability, biodegradability and biocompatibility are also advantages of the environmentally-friendly CNWs. Because of the growing interest in the bioconversion of renewable lignocellulosic biomass and the unsurpassed quintessential physical and chemical properties of CNWs mentioned above, substantial academic and industrial interests have been directed toward the potential applications of CNWs in polymer-based nanocomposites for various fields, such as high performance materials, electronics, catalysis, biomedical, and energy.

1.2.7 Surface modification of CNWs

According to its structure, CNWs possesses an abundance of hydroxyl groups on the surface, where chemical reactions can be conducted. Among the three kinds of hydroxyl groups, the OH group on the sixth position acts as a primary alcohol, where most of the modification predominantly occurs. In order to develop composites with better mechanical properties and environmental performance, it becomes necessary to increase hydrophobicity of the cellulose fibers and to improve the interface between matrix and fibers in order to remove the problems such as lack of good interfacial adhesion, low melting point, and poor resistance towards moisture. Pretreatments of the cellulose fiber can clean the fiber surface, chemically modify the surface, stop the moisture absorption process, and increase the surface roughness. (Kalia, S., et.al., 2008)

Due to its specific structure, various methods have been used to modify the surficial properties of CNWs in many recent studies. Among them, the best methods are listed below because all materials that applied to them are environmental-friendly:

- The Silylation using Silane-coupling agents to increase the cross-link degree in the matrix-cellulose material interface (R. Agrawal, et.al., 2000).

- Mercerization increases the aspect ratio and reduces cellulose fiber diameter. It leads to the development the roughness surface which results in better fiber-matrix interface adhesion and an increase in mechanical properties (K. Joseph, et.al., 2000).

- Peroxide treatment: Applying organic peroxides that tend to decompose easily to free radicals, which further react with the hydrogen group of the matrix and cellulose materials (M.S. Sreekala, et.al., 2000).

- Benzoylation treatment: benzoyl group in the fiber is responsible for the decreased hydrophilic nature of the treated fiber (B. S. Kaith, et.al 2005).

- Polymer grafting: directly changing the surface of interaction (B. S. Kaith, et.al 2008).

- Bacterial modification: Form a coating of bacterial onto the cellulose to control the interaction between cellulose material and polymer matrix (M. Shoda, et.al 2005).

2) OBJECTIVE

The objective of this study is to obtain the functional reinforcement cellulose nanowhiskers (CNWs) and enhance the mechanical and thermal characteristics of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with it. To achieve this main objective, the following sub-objectives are identified:

- To manufacture the CNWs and the composite CNWs/PHBV material in the lab.
- To evaluate the mechanical and thermal properties of the composite.

3) EXPERIMENTAL SECTION

As it is mentioned in the objectives, this study seeks to obtain and analyze biocomposite of CNWs and PHBV. To archive this goal, the experimental section has been divided into four sections: preparation of the CNWs from commercial microcrystalline cellulose product; confirming the existence of CNWs using atomic-force microscope; combining the extracted pure PHBV with the obtained CNWs from the previous steps into biocomposites and determination of mechanical/thermal properties of the composite.

3.1 Materials

The final biocomposite consist of these materials below:
o CNWs powder obtained from cotton originated microcrystalline cellulose (MCCs) provided by Sigma-Aldrich®
o PHBV product ENMAT® Y1000P provided by TIANAN

3.2 Preparation of CNWs

The goal of this process is to isolate the crystalline region (the CNWs) from the microfiber cellulose by acid hydrolysis using 96% solution of sulfuric acid. As mentioned in the part of introduction, this treatment method is one of the most efficient and easiest accessible ways to obtain the CNWs samples. Next, the acid should be washed by centrifugation and dialysis bags in order to have the CNWs observed under a microscope. Finally the CNWs will go through a freeze-drying process to eliminate the water for further use and conservation.

3.2.1 Acid hydrolysis and Dialysis

The acid hydrolysis procedure is listed as below:

- 5g of MCCs powder is mixed with deionized water. This suspension will be separate easily into solid and liquid layer if you let it stand for 1 min even after fully mixed.
- Put the suspension in an ice bath and stir it with a glass rod while adding the concentrated sulfuric acid drop by drop until the acid concentration is 63.5 wt% in the whole suspension.
- Heat the suspension at 45-50 °C for 120min while stirring. The best choice of hitting is to emerge the Erlenmeyer flask that contains the suspension in a thermos water bath (higher than 50 °C) because of the potential danger of hitting a strong acidic solution directly.
- Wash the obtained suspension with deionized water under centrifuge cycles 5-6 times (10 min at 4000rpm for each cycle) until the supernatant become turbid.

- Dialysis the suspension against deionized water until the wash water maintained nearly neutral PH.
- The fully washed and sonicated (by ultrasonic processor) suspension with proportion of appox. 1g CNWs: 100ml distillated water will be in a colloid form.

3.2.2 Freeze-Drying

In order to facilitate process of combining the CNWs and organic dissolvent, it is necessary to eliminate the humidity of the CNWs sample. To archive this goal the freeze-drying technology is applied to this procedure.

Accordingly, there are two stages in a freeze drying process. The wet sample is frozen by refrigeration first. The humidity of CNWs will turn into ice crystals and will be completely separated from the sample under reducing pressure. The fully dried CNWs samples are white powders. There is a possibility that the powders can form fragmented plate-like material that caused by the accumulation of whiskers (Peng, Y., et. al 2012).

3.3 Characterization of CNWs by atomic-force microscope (AFM)

Morphology of the CNWs was investigated by an AFM. In an AFM, images are obtained by sensing the sample surface by a nanometer probe tip that connected to the end of a fixed resilient cantilever. When the probe tip touches and scans the sample surface, the repulsive force between the probe and the atoms of the surface will slightly deform the cantilever. Therefore, the deformation of the cantilever can be used as a direct measure of the repulsive force. AFM use a laser beam deflection system where is reflected from the back of the AFM lever and project the image onto a photodetector. We can take photos and measurements from the image that has been delivered to the computer program.

The AFM Veeco® Dimension 3100 provided by CCIT, UB was applied to this test. Before applied to the AFM, the CNWs sample needs to be sonicated in water bath and diluted into 1:10000 in deionized water for a better dispersion of whiskers. The solution should be dried by evaporation at ambient condition before scanning. The individual CNW is scanned by a Si tips with a resonance frequency of ca. 783 kHz.

3.4 Preparation of composite of PHBV and CNWs

Once its morphology is confirmed, the CNWs can be used as a reinforcement material in the preparation of biocomposite. In order to understand how the concentration of the CNWs affects the proprieties of composite, 4 different samples are designed as table2 shows. The PHBV product can be easily dissolved by chloroform at 80°C or N, N-Dimethylformamide （DMF）at 100°C. However, the CNWs can only form colloidal suspension in DMF after ultrasonicating process. Moreover, before adding the CNWs into the matrix, the PHBV product contains additives and need to be extracted. In this

case, the double solvent-casting method is used to archive a pure PHBV/CNWs composite. The detailed procedure shows below:

Caution: Due to the toxicity of the solvent, it is recommended to realize this procedure under a fume hood.

- Dissolve the PHBV grain in Chloroform at 80 ℃ with 45 min of continued stirring. The completely dissolved pure PHBV-chloroform solution should be a transparent viscous liquid. However, this PHBV product contains mineral impurity substances and it is necessary to precipitate them by leaving the whole solution standing still for one night.
- Separate the solution layer and the precipitation layer and eliminate the impurity of the liquid by centrifugation (only one cycle with rpm less than 1000).
- Cast the solution onto a clean, smooth surface of glass and evaporate the chloroform in the fume hood under the room temperature.
- Collect the formed film of pure PHBV and cut them into pieces for the compositing step.
- Add the CNWs powder in 100 ml N, N-Dimethylformamide (DMF) and mix the solution with the ultrasonic liquid Processors until the liquid become a cloud-like colloid. The formulation of percentage of CNWs is listed in the table2.
- Quickly put 0.5g PHBV film into the CNWs-DMF solution and heat the mixture to 100 ℃ under the continued mechanical stirring until the film is completely dissolved.
- Heat the glass surface with boundary to 50 ℃ and cast the CNWs-PHBV composed solution onto it. It will cost more than 6 hours to fully evaporate the DMF of solution of 1mm depth while maintaining the heating temperature.
- The film obtained from previous step has irregular thickness. It needs to be remelted in a steel mold which will define the sample of a thickness of 0.5mm approx. under the temperature of 180 ℃ and a high pressure.

Sample name	extracted pure PHBV	CNWs	% of CNWs contents in PHBV
CNW-PHBV-0		0g	0%
CNW-PHBV-1	0.5g	0,005g	1%
CNW-PHBV-3		0,015g	3%
CNW-PHBV-5		0,025g	5%

Table 2. Formation of the sample

3.5 Characteristization of PHBV/CNWs composite

3.5.1 Cutting samples for tensile test and Dynamic Mechanical Analysis (DMA)

The samples have been cut into bone shape in order to fit the standard and function of the tensile test. The ideal dimension of the sample is shown in figure 7. Although the samples applied in the test contain variable errors in dimensions. Before applied to the tensile and DMA test, the true dimension of every sample should be introduced to the measurement program so that the data of every single test can be automatically acquired and calculated.
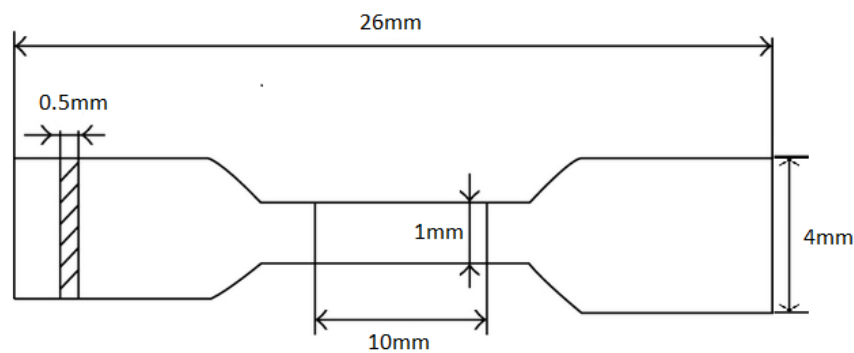

Figure6. Bone shape sample for tensile test and DMA test.

3.5.2 Tensile Test

Generally, before a new material is brought in to operation of engineering application, there are several tensile properties to be determined in order to ensure quality. Among them, the strength is always the primary concern. The strength of a material is a concept that can be measured by knowing the maximum stress that it can support (Tensile/fracture stress). At the same time, the strength applied to a material will cause appreciable plastic deformation (Ductility/Strain). (ASM International.2004) To measure these two properties of a material, the most common type of test is the Tensile Test. These properties which can be determined from the test can provide a series of information about the sample:

- The yield and fracture stress/strength
- The elastic modulus

Generally speaking, more strength can support the material, more elastic modulus it has, and tougher it can be.

3.5.3   Dynamic Mechanical Analysis (DMA) of the composite

The Dynamic Mechanical Analysis (DMA) is widely used in the field of thermoplastic and other composite materials. The principle of DMA is applying a known variation amplitude and frequency to the sample material under a controlled escalated temperature and measuring the loss factor (Tanδ) of the sample material as a function of temperature and frequency. All material will change their physical properties when they are affected by three environmental parameters: force, frequency and temperature. The material properties will vary when strengths are applied to different sizes of it. The material shows a more rigid response under high-frequency probes while low-frequency applied forces allow the material to relax, thus showing a relatively more soften response. In contrast, the change in temperature is more apparent, since all materials have viscosity and elastic characters. Therefore, the DMA apply force on the sample and the sample response delay, which defines the phase angle difference (δ) and the loss modulus (E "). From those two data, the storage modulus (E') can be calculated. But it also can be calculated by knowing the stress and strain which change recurrently by the oscillation. The materials that are high in Tanδ behave higher viscous. In the other hand, low Tanδ materials represent elastic behavior more obvious. By the data obtained above, the strength of the material varies with temperature, viscosity, elasticity, shock effect and material mixing effect…etc. can be calculated. That is to say, properly used DMA is able to replace most of the instruments that measure physical properties.

In this experiment, the DMA Q800 provided by TA instrument® is used for both tensile and DMA test. The bone-shaped sample is pushed and pulled with a settled frequency (1~3~10~0.3 Hz) under increasing temperature (room temperature~180 ℃, 10℃/min) until it breaks.

3.5.4 Differential scanning calorimetry (DSC) analysis

A Differential scanning calorimetry (DSC) is a technique that measures the differences in heat flow rate between a sample and an inert reference as a function of time and temperature, in order to investigate how polymers response to a change of the temperature. The DSC consists of a computer and a measurement chamber. In the chamber, the sample slot (the one that contains the material) and the reference slot (which is empty) go through a heating-cooling process. Meanwhile the computer regulates the heating rate and makes a real-time measurement and record the heat flow by the sensors connected to the slots.

At increasing temperature, the heat flows into the polymer sample as a result of endothermic (heating) processes. The heat capacity of the sample system can be found by dividing the heat flow by heating rate (the temperature increased of every unit of heating time). Once the temperature hit the melting point (T_m) of the sample, its polymer chains can move around freely. If the sample in its molten stage cools down, it will reach the glass transition temperature (T_g). At that point the sample will change from an elastic material to a more brittle one because of the mobility of its polymer chains. The T_g of polymers can be observed in the change of heat flow over the range of

between the before and after hitting the T_g point. At some temperature above T_g some of the polymer chains have enough energy to arrange a steady form and make some crystallized sections (T_c). The polymer at the crystallization point goes through an exothermic process and the polymer sample will need less heat than the empty reference slot, which result in a decrease in the recorded heat flow curve and create a crystallization peak.

Not all the type of polymers can go through the three transitions (T_m, T_g and T_c). Only purely amorphous polymers can undergo a glass transition. While the crystallization and melting peak can be observed in the polymers that form crystals.

In this study, the curves acquired from DSC were used to study the heating behavior of CNWs-PHBV composite. The analysis was performed using DSC 404 F1 Pegasus® under dry nitrogen flow (80 ml/min). The weight of the sample fragment used in this test is pre-measured. The scanning was carried out in cycles of heating-cooling and for every sample it repeats three times. The temperature goes from 30 ℃ to 180 ℃ at a scanning rate of 10 ℃/min in the first time heating. Once it reaches to 180 ℃ the sample start to be cooled naturally. In the second cycle the scanning rate is 30 ℃ and in the third cycle it is 40 ℃. That is to say, every sample will be heated three times and cooled three times.

4) RESULTS AND DISCUSSION

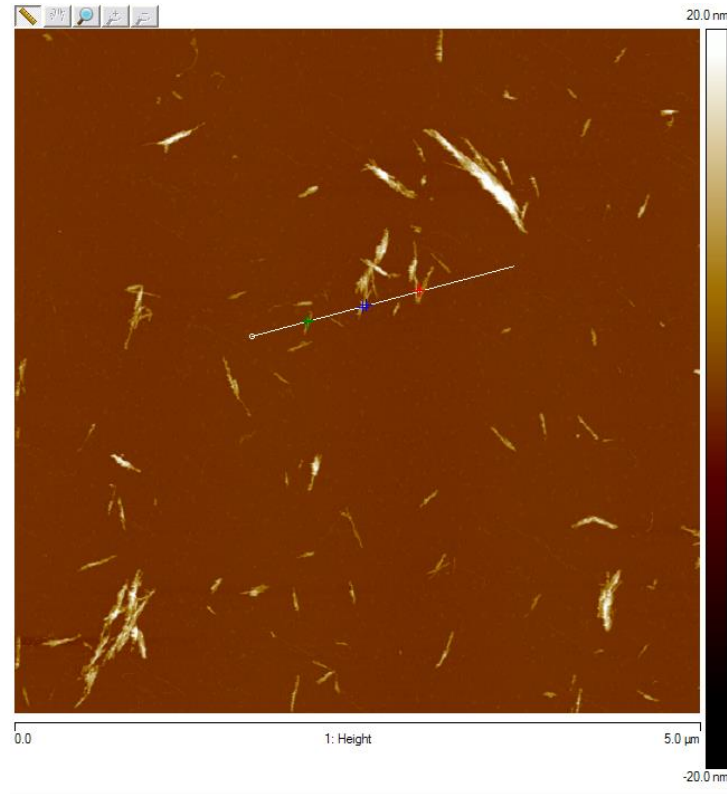### 4.1 Observing the CNWs using an atomic-force microscope (AFM)

Figure7. AFM images of dispersed CNWs and the table of measurement

Pair	Horizontal Distance	Vertical Distance	Surface Distance	Angle	Rmax	Rz	Rz Count	Rms	Ra (Frequency Cutoff)	Frequency Cutoff	Radius	Radius Sigma
1	0.030 (µm)	-10.394 (nm)	0.033 (µm)	-18.8...	10.388 (nm)	0.000 (nm)	0.000	0.089 (nm)	5.450 (nm)	2.491 (µm)	0.013 (µm)	0.005 (µm)
2	0.020 (µm)	-5.588 (nm)	0.022 (µm)	-15.4...	5.584 (nm)	0.000 (nm)	0.000	0.097 (nm)	3.389 (nm)	2.491 (µm)	0.027 (µm)	0.161 (nm)
3	0.020 (µm)	9.053 (nm)	0.022 (µm)	24.06...	9.057 (nm)	0.000 (nm)	0.000	0.097 (nm)	4.122 (nm)	2.491 (µm)	0.335 (µm)	0.002 (nm)

Table3. Measurement of the radius of three CNWs individuals

The morphology and dispersion of CNWs in deionized water is observed by AFM. (Figure7.) The individuals of CNWs that have been chosen for a measurement can be clearly distinguished of the image, although it can still observe whiskers agglomeration which causes problem of determine of the true length of them. The ratios of chosen CNWs individuals that have been detected and represented in the tables3 are from the minim 13nm to the maxim 335 nm, which meets the standard from the previous reviews of CNWs.

4.2 Result of tensile test

- The Stress-Strain curves

Figure9 shows the stress-strain curves of the first group of 4 different type of sample. It can be observed that the CNW-PHBV-1 has the most stiffness meanwhile the CNW-PHBV-3 and CNW-PHBV-5 ones are more ductile during the process. As to the CNW-PHBV-0 one, it is stiff at first but doesn't elongate much as others, which fit the real situation. That is to say, the CNWs have created hydrogen bond among the matrix polymers, which indicates that there is a good interfacial adhesion. Therefore, this interfacial adhesion improved the toughness of the matrix material in this case.
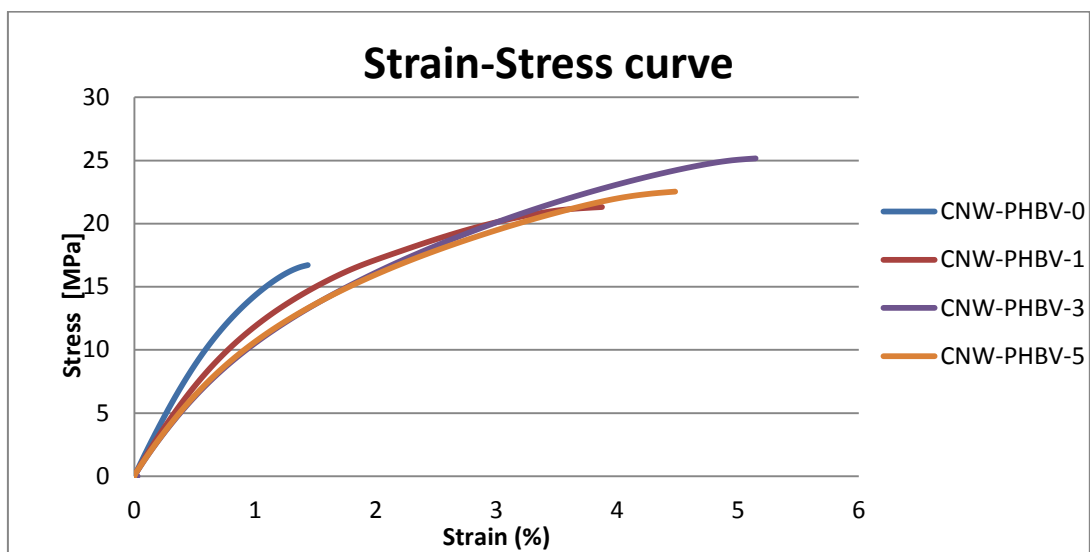

Figure 8. Stress-Strain curve of four different types of samples

- The yielding stress and Elastic modul at the yielding point

The yielding stress of every sample is calculated by knowing the static force of the yielding point and the dimension of the sample. The elastic modul is calculated by the yielding stress and the strain of the yield point which is given by the stress/strain curve. The figure9 compares the average number of every type of sample of these two data. Surprisingly, the CNW-PHBV-3 has a better stress meanwhile the CNW-PHBV-1 show the highest value of Elastic modulo. Moreover, the pure matrix sample obtains a larger elastic modulus than CNW-PHBV-3 and CNW-PHBV-5, which means that the obtained result of tensile test doesn't meet the expecting result.
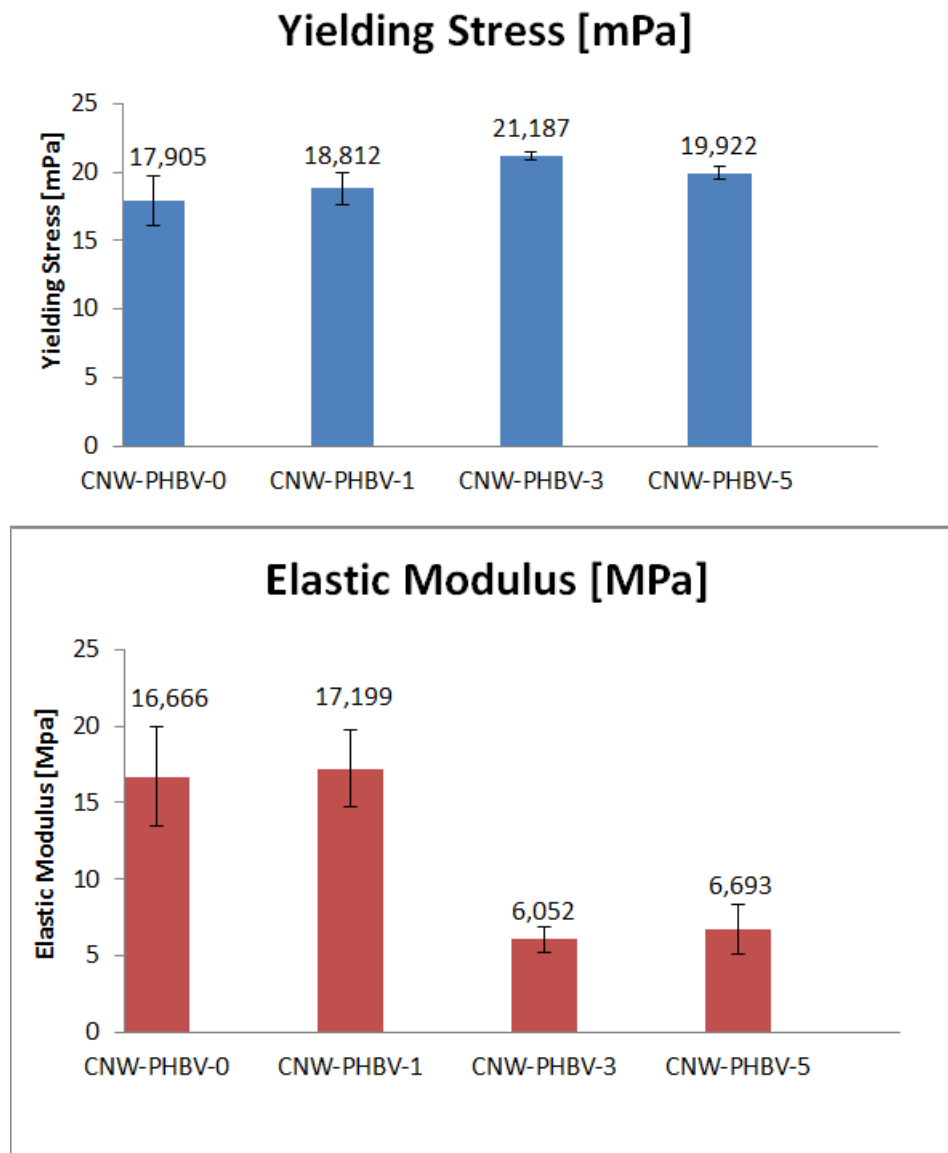

Figure 9. Diagram of yield stress and elastic modulus of every type of sample

4.3 Result of DMA analysis

Observing the curves of Tanδ and Storage modulus of the four different samples that shows in figure 10, it is very noticeable the more CNWs the sample contains, the less storage modul it has. And the curve of the Tanδ shows that for every type of samples the phase angle differences of osilation are basically the same. However, as early expected the result should be totally opposite.
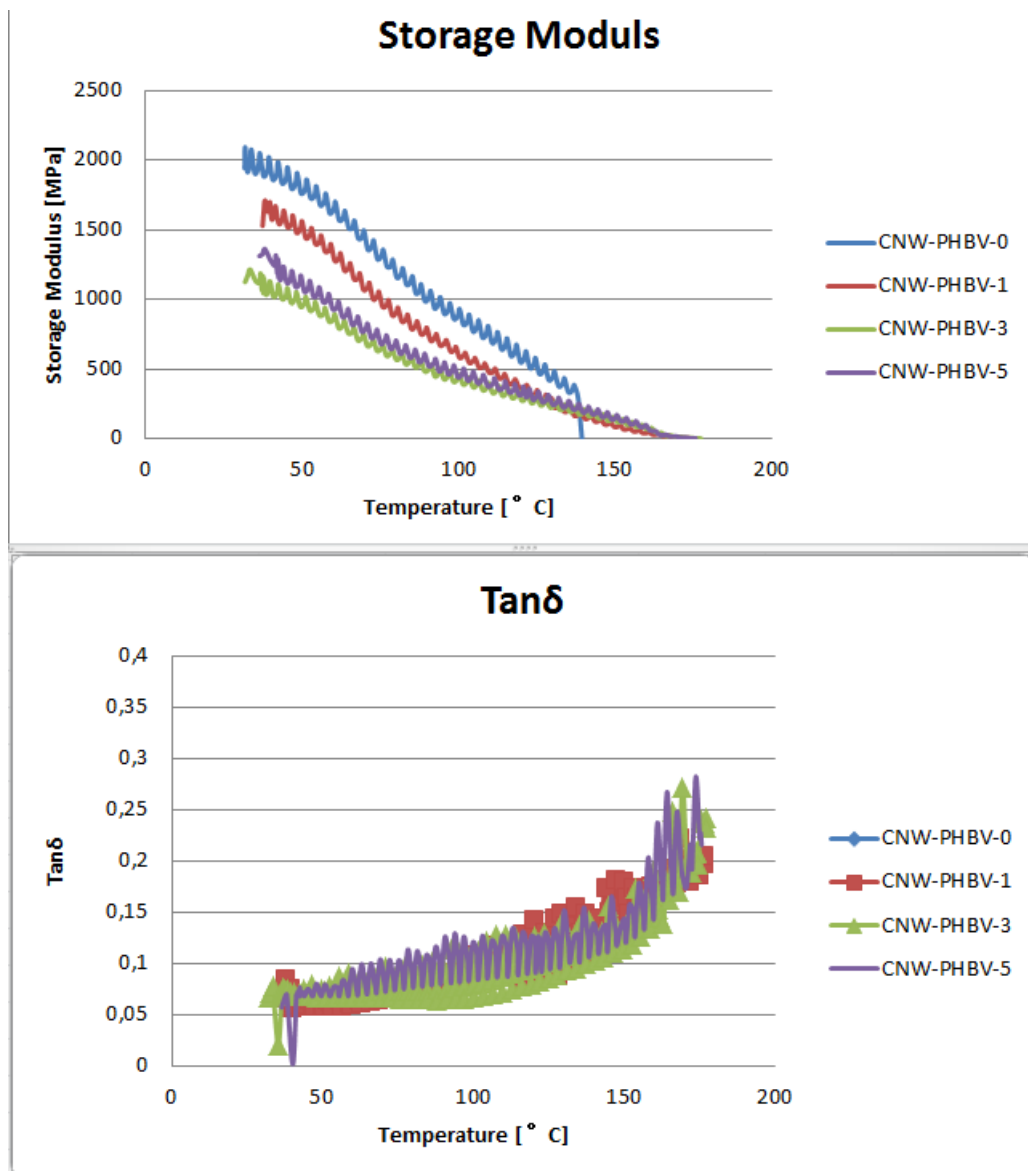

Figure 10. the curves of Tanδ and Storage modulus of the four different samples

4.4 Result of DSC analysis

In figure11, the curve of the glass transition, cold crystallization, and melting of PHBV can be clearly observed. The glass transition temperature (T_g) of PHBV was not noticeably affected by the addition of various contents of CNWs. In the other hand, the temperature of crystallization and melting has been slightly increased as the increasing of the percentage of CNWs.
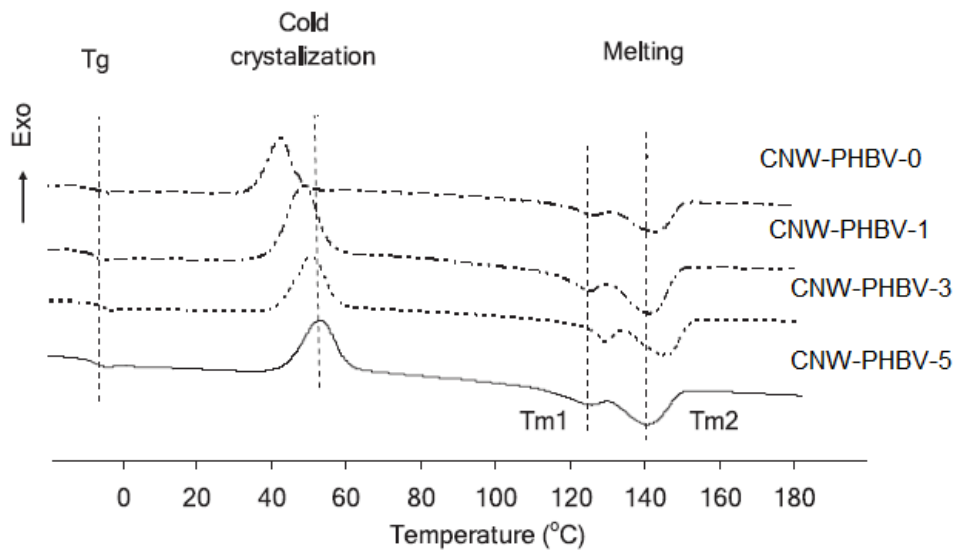

Figure 11. DSC curves of the first cycle of 4 different samples

5) CONCLUSION

This study has evaluated the investigation of the biocomposite formed by PHBV and CNWs. Following are the conclusion made from this study:

- The objective of obtention of CNWs has been archived. The acid hydrolysis method with sulfuric acid has been approved usefully.

- The whole obtaining procedure of PHBV/CNWs biocomposite sample has been a plain sailing applying the double solvent casting method.

- Morphological study using AFM revealed good dimensions and dispersion of CNWs.

- The DSC test, at the same time, has given a reasonable result. The glass transition, cold crystallization, and melting of the composite has been slightly mortified by the existence of CNWs.

- In the tensile test, the CNWs have created hydrogen bond among the matrix polymers and reinforced the elongation of the matrix material. Meanwhile, the sample CNW-PHBV-3 has a better stress and the sample CNW-PHBV-1 shows the highest value of Elastic modulo. It means that when the percentage of CNWs is 1%- 3%, the composite are tougher and more ductile.

- The DMA test shows that the more CNWs the sample contains, the less storage modul the composite will has. At the same time, the change of quantities of CNWs will not affecte the viscosity of matrix material.

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