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Thermogravimetric analysis of Polyhydroxyalkanoates (PHA)/nano-Calcium Phosphate (nCaP)/chitosan biocomposite for heat-related manufacturing process

Najah Mat Isa[b)], Aisyah Razak[c)], Aiman Haiqal[d)] and Sharifah Adzila[a)]

Fakulti Kejuruteraan Mekanikal dan Pembuatan, Universiti Tun Hussein Onn Malaysia (UTHM), Parit Raja, 86400, Batu Pahat, Johor, Malaysia

[a)] *Corresponding author: adzila@uthm.edu.my*
[b)] *najahanismatisa@gmail.com*
[c)] *sitiaisyahrazak94@gmail.com*
[d)] *gd220027@student.uthm.edu.my*

Abstract. Nowadays, the adaptability of biodegradable Polyhydroxyalkanoates (PHA) polymer has been thoroughly assessed to its full potential including shape, mechanical characteristics, thermal properties and biocompatibility. Additionally, PHA has been produced as biomaterials with potential applications in bone regeneration. Similar to the mineral apatite found in human bones, nano calcium phosphates (nCaP) are minerals that are necessary for tissue development and cell adhesion, which have an impact on newly formed apatite. This study aims to investigate the influence of additives on thermal stability of biocomposite via thermogravimetric analysis (TGA) machine. PHA pellets were mixed with nCaP as filler ranging from 3-15wt% and 10wt% of chitosan binder via conventional melt compounding. Thermal analysis showed that the addition of bio-filler improves thermal stability of PHA with highest onset temperature recorded at 208.1°C. Morphological analysis showed that the addition of nCaP and chitosan alters the flow characteristics of the composite resulting in surface roughness. Subsequently, in order to prevent the biocomposite from decomposing, all heat-related activities particularly those involving machining, it is recommended to perform at temperatures that do not exceed the onset temperature.

INTRODUCTION

Natural biopolymer indicated by the prefix bio are biodegradable plastics because of their multidirectional qualities and receiving a great deal of attention in the medical and scientific disciplines nowadays [1-3]. During the past two decades, there has been a surge of interest in biocompatible and biodegradable natural biopolymers that are being used in biomedical application [4,5]. One of the natural biopolymers that extracted from biomass is Polyhydroxyalkanoates (PHA) [6]. PHA are flexible polyesters generated by a wide range of bacteria as intracellular granules in response to metabolic stress [7]. PHA has piqued the interest of researchers since it can be made from a range of renewable resources and is fully biodegradable and highly biocompatible thermoplastic material [8]. Microorganisms may collect several forms of PHA, including homopolymer, copolymer, and polymer mixes [9]. PHA copolymer properties are greatly influenced by the kind, content, and distribution of comonomer units that form the polymer chains, as well as the molecular weight distribution [10,11]. All of the PHAs that are currently available have been discovered to be both in vitro and in vivo degradable. [12-14].

However, PHA have several weaknesses such as low mechanical properties and poor biological properties like less appetite growth on PHA. Bioceramic was combined with PHA to enhance its bone-related properties, which resulted in gains in compressive elastic modulus, maximum stress, and osteoblast responses, such as cell growth and

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alkaline phosphate activity, both in vitro and in vivo [15,16]. Recently, researchers have focused their attention calcium phosphate (CaP) that have a mineral composition that is quite similar to regular bone because of their biocompatibility, and effective bone replacements [17]. CaP are non-toxic biomaterials that can be functionally integrated with natural bone without the need for fibrous tissue encapsulation. They also do not trigger the normal bone mineralization process or cause inflammatory or foreign body reactions. In addition, a CaP biomaterial offers a physical matrix that is ideal for the deposition of new bone, and it has the potential to exhibit growth-guiding qualities, leading bone to extend its development into places that it would not otherwise inhabit [18,19].

In order to process PHA with CaP, thermal analysis is important factor to achieve suitable temperature for processing. Thermal analysis is a branch of materials science where the properties of materials are studied as they change with temperature [20]. Thus, in terms of research publications, thermal analysis is a key tool in the discovery of new knowledge and is comparable in importance to processing method. Thermogravimetric is one of the thermal analysis branches. It involves recording mass as a function of temperature [21]. These experiments are carried out using modified balances that include a furnace which allows the control of a temperature programmed with the simultaneous measurement of the mass of the sample. The first step in processing methods, especially for polymer and composite materials, is to determine a material's thermal stability and the percentage of volatile components by understanding the weight change that takes place as a sample is heated at a constant rate [22].

This research will focus on thermogravimetric analysis of various percentage of PHA/nCaP/chitosan biocomposite in order to achieve suitable temperature for processing this biocomposite. This bioceramic that is being utilized in this study is derived from a natural source (eggshell waste), rather than manufactured bioceramic, which is an advance over sustainability [23,24]. In order to obtained high quality of completed plastic parts, it is necessary to find the befitting process parameters such as melting temperature, mould temperature and decomposition temperature [25]. Hence, this study will focus on discovering the suitable manufacturing temperature for any heat-related machining processing of PHA/nCaP/chitosan biocomposite for example extrusion, injection molding, 3D printing and many more.

METHODOLOGY

Materials

PHA of the medical grade was provided in pellet form by Ecomann Biotechnology, Inc. in the United States under the name *Ecomann® Bioresin EM10080*. The melt mass-flow rate (MFR) is 1 g/10 min (170°C/2.16 kg), and the true density or specific gravity is 1.27 g/cm^3, both in accordance with ASTM D570 standards.

The osteoconductive filler of nano-Calcium Phosphate (nCaP) powder was synthesized via wet chemical precipitation at previous work [23,24]. It contains 1.63 Ca/P ratio with composition of 80% HAP + 20% β-TCP and purity is 96% along with less than 10% of impurities such as iron, cobalt, cadmium, copper, nickel, zinc, chlorine ion and sulfate ion. Its typical particle size ranges from 150 to 300 nm.

Chitosan powder, also known as Poly (D-glucosamine) Deacetylated Chitin ($C_8H_{13}NO_5$)n), was supplied in a 500g quantity by *Ecomann Biotechnology, Inc.* (United States), and it will acts as a binder.. It has 30–40 µm of average particle size with 99.9% purity and density of 0.25 g/ml.

Manufacturing of PHA/nCaP/Chitosan biocomposites

In order to remove any moisture that might still be present and minimise any chemical breakdown of a compound as a result of reaction with water (hydrolysis effects), PHA pellets, nCaP powder, and chitosan were separately dried beforehand at 60°C for at least 6 hours in a drying oven. The materials were then blended in a brabender plastography machine for approximately 20 minutes at a temperature of 150°C and rotational speed of 30 rpm. These parameters were made for all PHA biocomposite compositions with various nCaP contents that are shown in Table 1.

A Brabender plastography machine as in Fig.1 with integrated control panels that displays actual values of speed and temperature (3 zones), die heads, mixer blades geometries, and extruder screws with real torque measurement up to 200 Nm was used to melt-compound the various ratios of PHA, nCaP, and chitosan mixtures, each weighing 50 g. All of the blended mixtures were then crushed into pellets using a crusher machine before being examined.

TABLE 1. Compositions of the samples prepared in accordance to the weight content (wt%) of PHA, nCaP and chitosan.

Sample	PHA [wt%]	nCaP [wt%]	Chitosan [wt%]
100PHA	100	–	–
87PHA	87	3	10
83PHA	83	7	10
79PHA	79	11	10
75PHA	75	15	10

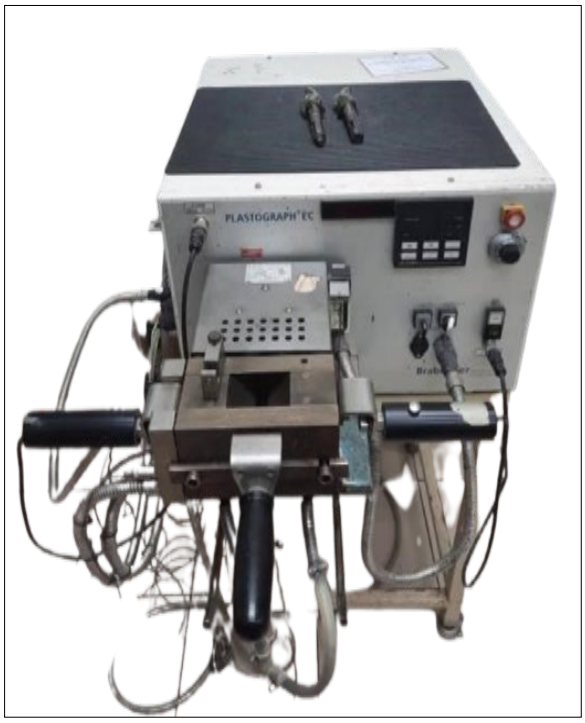


FIGURE 1. Brabender plastography machine for blending PHA/nCAP/chitosan biocomposite.

Microscopic characterization of PHA/nCaP/chitosan biocomposites

A scanning electron microscope (SEM) brand *HITACHI U151O* was used to characterize the surface morphology of blending sample after crushing. Sample were subjected to metallization process with gold by sputtering in a sputter coater. Besides, Energy Dispersive X-Ray Analyzer (EDX) also being used to detect all element in PHA/nCaP/chitosan biocomposite.

Thermal analysis of PHA/nCaP/chitosan biocomposites

Thermogravimetric analysis (TGA) was carried in a TGA/Simultaneous Differential Scanning Calorimetry thermobalance brand *LINSEIS (STA PT 1600)*. Sample with weight range 15–25 mg was placed in alumina crucibles and subjected to a heating program from 25 to 700°C at a heating rate of 20°C/min in nitrogen gas with flow rate of 66 mL/min.

RESULT & DISCUSSION

Thermal Analysis

Thermal decomposition of PHA/nCaP/chitosan biocomposites were evaluated by TGA. Figure 2 display TGA curves, while Table 2 gathers the main thermal stability parameters obtained for the neat PHA part and the PHA/nCaP/chitosan biocomposites with different nanoparticle contents. According to Fig. 3, all samples have a similar degradation profile with two degradation phases and reasonably flat curves from ambient temperature to roughly 150°C, with only about 16 percent mass loss. This is frequently referred to as moisture evaporation during the hydrolysis breakdown of susceptible bonds in polymer chains. In an article wrote by Lyu (2009), hydrolysis is one of the four major degradation mechanisms for polymers used in biomedical devices which had been extensively studied especially for biodegradable polymers including polyesters [26]. Next, the onset temperature at 10% mass loss ($T1_{10\%}$) also known as point where material start to decompose showed a considerably decrease from the neat PHA part to PHA filled with nCaP loadings from 208.1°C to 169.9°C. However, a significant decrease of onset temperature for higher loadings was observed from 3wt% of nCaP to 15wt% of nCaP. The highly hydrophilic nature of nano CaP and its susceptibility to moisture might cause the PHA polymer to be particularly vulnerable to the chain scission of PHA molecules thus offer less thermal stability at high temperatures. The reduction of onset degradation temperature also affected by volatile emission from PHA matrix for most samples.

All samples experienced two degradation phases and it is found that when the first degradation (T_1) happened over the temperature range from 300°C to 324°C, due to disruption of amorphous and crystalline region of PHA polymer. The thermostability of PHA/nCaP/chitosan were observed by increasing the amount of nCaP content at second degradation temperature (T_2) where it attributes to final decomposition of nCaP at the temperature range of 300°C to 370°C even with small increments. This trends also reported in similar work by Martinez (2020) where the onset degradation temperature decreased when nHA contents were added to P(3HB-co-3HHx). The shifted readings were attributed to nCaP agglomerates forming in the polymer matrix [27]. Besides, Ferri (2017) and Najah (2022) mentioned that when utilising larger quantities of nCaP in the PHA matrix, the agglomeration issues was impossible to be avoided [28,29]. When large volumes of nanosized filler aggregates are generated, the structure changes from nanocomposite to microcomposite reducing the shielding effect of the nano particles.

All biocomposite samples loaded with nCaP shows better thermostability starting from 3wt% of nCaP up to 15wt% compare to neat PHA polymer. During the homogenizing process, strong hydrogen connections and Van der Walls forces developed between inorganic particles and the polymeric chain resulting increasing in thermal stability of the composite. On the other hand, the maximum thermal degradation (T_{max}) was observed to be decrease with increasing in nCaP content as it indicates point where material decomposed optimally (approximately at 413°C ± 0.2). Finally, percentage of residual mass was observed at 700°C where it gradually increases with increase in nCaP content which could be because the residue is mainly related to nCaP content and the high thermal stability of the mineral nanoparticles. As tabulated data shown, composite containing 15wt% of nCaP show a 52% of residual mass which corresponds to the nCaP filler content in comparison to percentage of residual mass of neat polymer which is half to 3wt% nCaP content which is 22%.

TABLE 2. Main thermal stability and degradation parameters of PHA/nCaP/chitosan biocomposites in terms of onset temperature of degradation ($T_{10\%}$), first degradation temperature (T_1), second degradation temperature (T_2), maximum thermal degradation (T_{max}), and residual mass at 700°C.

Sample	$T_{10\%}$ [°C]	T_1 [°C]	T_2 [°C]	T_{max} [°C]	Residual mass [%]
100PHA	208.1	324.0	366.0	408.6	22
87PHA	177.0	314.4	377.1	415.1	40
83PHA	175.3	311.8	375.4	414.5	43
79PHA	171.9	310.0	374.9	413.2	47
75PHA	169.9	307.4	372.3	412.5	52

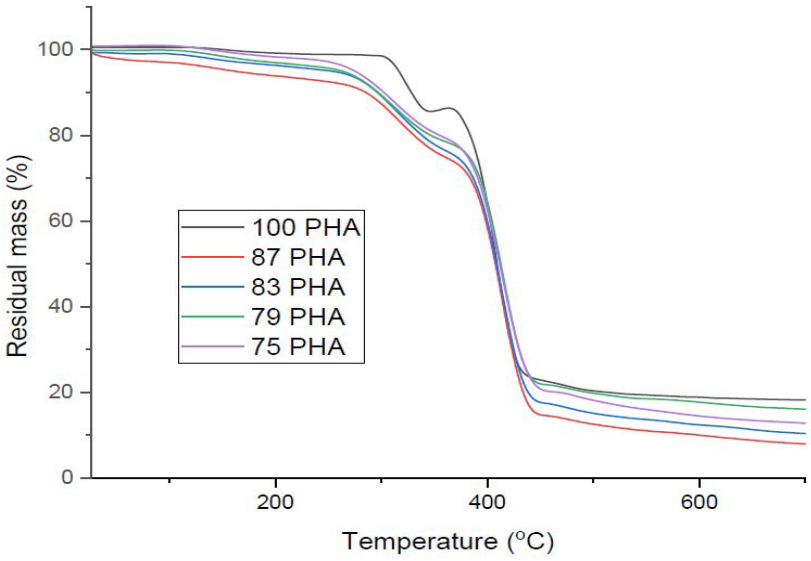
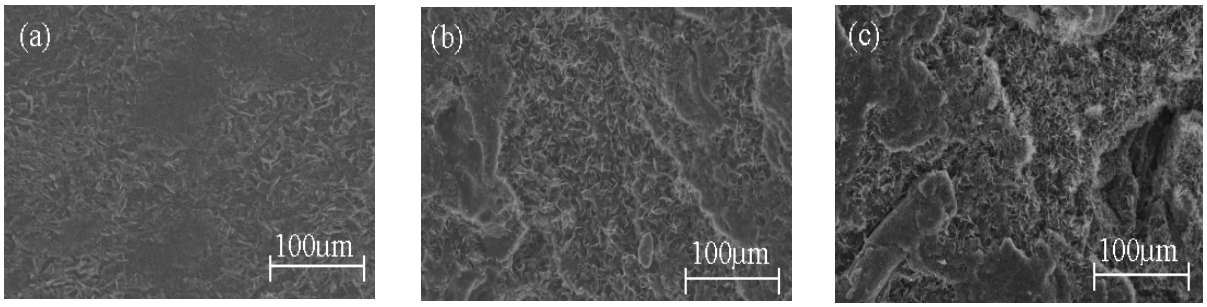


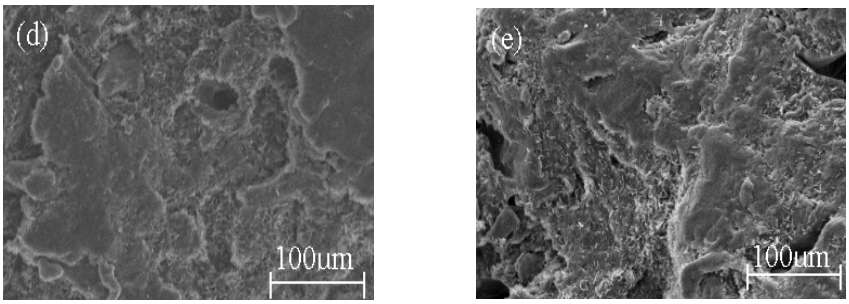
Figure 2. Thermogravimetric analysis (TGA) curves for all samples.

Morphological Analysis

The presence of nCaP in the polymer matrix has also been shown to have an effect towards the morphology of the biocomposite. The morphological analyses of neat PHA and PHA/nCaP/chitosan biocomposite were shown in Fig. 3. Figure 3(a) shows typical surface of neat PHA with soft surface, without any specific morphological characteristic. As the nCaP content increase, the surface of the sample become rougher. This trend is shown in Fig. 3(b), (c), (d) and (e) where the surface of the sample become rougher when nCaP content increase due to the presence of porous filler. Figure 3(b) shows starting of porous microstructure with additional of 3 wt% of nCaP, followed by Fig. 3 (c) that shows more porosity than Fig. 3(b), (d) and (e) shows more porous structure and uneven surface as the nCaP content increasing up to 15 wt%. The existence of a porous structure on the biocomposite offers various advantage in a texture for cell adhesion which this PHA/nCaP/chitosan biocomposite able to achieve. The particle dispersion is quite good and nCaP particles seem to be embedded in PHA matrix as proven in TGA curves that all samples experienced the same degradation profile with two degradation phases.



(a) (b) (c)



(d) (e)

Figure 3. SEM micrographs of (a) neat PHA, (b) 87PHA, (c) 83PHA, (d) 79PHA and (e) 75PHA

CONCLUSION

In this work, biocomposite of PHA based matrix and nano Calcium Phosphate as its osteoconductive filler were obtained by conventional blending process. According to thermal research from TGA analysis, all samples, from neat PHA to PHA/nCaP/chitosan, went through two phases of degradation with a comparable degradation profile and fairly flat curves from room temperature to about 150°C. The thermostability of the biocomposite increased with the addition of more nCaP, with sample 75PHA that has 15wt% exhibiting the highest thermostability, demonstrating that the nCaP filler was distributed uniformly throughout the PHA matrix. Additionally, because nanoparticles have a high thermal stability, the percentage of residuals matches the amount of its content in the PHA matrix where, biocomposite containing 15wt% of nCaP show a 52% of residual mass which is the highest compare to all PHA/nCaP/chitosan samples. Conclusively, this work suggested that any heat-related manufacturing process must be carried out below that temperature range since the PHA/nCaP/chitosan biocomposite exhibits a restricted onset temperature range from 170°C to 200°C. In addition, it also to prevents the biomaterial-based composite from going through any physical processes that could harm the materials, like oxidation and decomposition.

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