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# The Development of Temporary Bone Scaffolds from High Density Polyethylene (HDPE) and Calcium Carbonate ($CaCO_3$)

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**Abstract**
In this modern era, Calcium Carbonate ($CaCO_3$) is widely used as one of the main materials in bone tissue engineering. The use of porous material scaffolds made from bioceramic and polymer components to support bone cell and tissue growth is a longstanding area of interest in biomedical field. The aim of this research is to extract $CaCO_3$ from cockle shells and analyze the mechanical and physical properties of powder and samples built by mixing $CaCO_3$ with synthetic polymer High Density Polyethylene (HDPE) to produce stronger and more flexible composite for bone replacement application. Unused cockle shells were collected and cleansed before crushed to make 100 µm-sized $CaCO_3$ powder. The process of mixing HDPE and $CaCO_3$ is by using brabender mixing machine in temperature of 170°C based on differences in their weight percentages. Samples were produced using injection molding method. The samples were tested for mechanical testing. Then, the powder and samples were analyzed using SEM, EDX and FTiR analysis to observe the microstructure and content of $CaCO_3$ as well as the sample structure to determine which ratio sample is the most suitable to be used in the mentioned field. The flexural modulus of the composite from flexural test for sample 1 to sample 3 was between 2.45 GPa to 2.77 GPa while Young's modulus achieved from tensile test samples was between 0.43 GPa to 0.59 GPa. The impact strength achieved for samples 1 to 3 was between 0.69 J/mm$^2$ to 0.74 J/mm$^2$. The results show the weight percentage of $CaCO_3$ affected the mechanical and physical strength of the samples greatly.

***Keywords:*** *Cockle shell; $CaCO_3$; HDPE; Mechanical test; Bone implant; Biomaterial; Bio-polymer*

## 1. Introduction

This engineering field is taking advantages of the use of porous 3D scaffolds to provide an appropriate situation for tissue and organ regenerations including upgrading current materials involved in the field for future use. Scaffolds act as a template for tissue formation and are seeded with cells including growth factors. These cell-seeded scaffolds are either cultured in vitro to synthesize tissues which can then be implanted into an injured site, or are implanted directly into the injured site, using the body's own systems, where regeneration of tissues or organs is induced [1].

Many studies have used approaches such as carbonation or solution to produce inorganic calcium carbonate raw material over the past few years [2][3]. However, scientists and researchers have found new ways of employing nature-based materials such as mineral products extracted from calcium carbonate. The new trend is highly preferable in terms of environmental preservation and due to its nature, cockle shells can provide raw material at reasonable low cost besides having good purity of mineral components naturally [4].

Calcium Carbonate ($CaCO_3$) is found naturally in our environment such as seashells, rocks and eggshells. In a related research, $CaCO_3$ was extracted from cockle shells and known for its potential in multiple tissue engineering applications. Cockle shells are commonly found in Malaysia as waste products of food and can also be easily found alongside beaches. The increasing demands of natural materials based on $CaCO_3$ can easily be fulfilled by using cockle shells as the main source due to their low cost and availability [5]. Cockle shells consist of about 96% of $CaCO_3$ whilst other components include organic substances and oxides like Silicon Dioxide ($SiO_2$), Magnesium Oxide (MgO) and Sulfur Dioxide ($SO_3$) [5].

Researches in making biodegradable materials are clinically and experimentally studied to design scaffolds with particular characteristics. The combination of biodegradable polymers and bioactive inorganic materials are being widely used. In addition, the rate of degradation and the by-products of biodegradable materials are also critical in the role of bone regeneration. The aim of this study is to produce and assess mechanical properties of $CaCO_3$ nanoparticles and HDPE as a potential bone matrix for tissue engineering and strengthening material.

## 2. Methodology

### *2.1 Cockle shells $CaCO_3$ powder preparation*

The preparation process started by collecting wasted cockle shells from a seafood restaurant. Before the crushing and grinding of cockle shells can be done, the collected shells must first be cleaned up and left to dry in the open air to assure that it did not adhere to the sharp edge of the granulating machine as it could be difficult to wash after being utilized. The pieces of the crushed cockle shells then needed to experience similar procedure several times to obtain the smallest size.

The powder of the cockle shells was sifted once with the extent of 200µm, experiencing the procedure known as ball mills. The estimated time for powder delivered by milling process was less than 100 minutes. After completion, the $CaCO_3$ powder was sieved with the sponge of the strainer of 100µm to get a uniform size powder.

### *2.2 Sample preparation*

Mixing process was required to blend $CaCO_3$ uniformly which was synthesized from cockle shells, altogether with high density polyethylene (HDPE) as natural polymer. The final product was stronger composite with more tolerance in flexibility compared to metal material. The mixture from two different materials, which are $CaCO_3$ and HDPE pallets, were mixed together using brabender mixing machine at the temperature of 170°C to make the samples. Nine samples for three different ratios were prepared using the following combinations; ratio 1: $CaCO_3$ 10%, HDPE 90%, ratio 2: $CaCO_3$ 20%, HDPE 80%, and ratio 3: $CaCO_3$ 30%, HDPE 70%.

The weight for each material was calculated using brabender machine weight calculation:

$$m = V \times y_c \times k$$

Where $m$ is sample weight, $V$ is mixer chamber volume (55 $cm^3$), $y_c$ is composite density and $k$ is constant (0.60).

Brabender mixing machine was heated to 170 °C while $CaCO_3$ powder and HDPE pallets were weighted according to calculated weight for certain ratios. As the HDPE pallets were melted, the powder was put inside the mixture little by little and left to fully mix for 10 minutes before repeating the process for different ratio.

### *2.3 Crushing process*

The samples were crushed using the crushing machine to produce smaller size to be used for injection molding. Figure 1 shows the raw material before and after crushing in the crushing machine.

(a) Before crushing (b) After crushing

Figure 1: Cockle shell

### *2.4 Injection molding*

All samples for mechanical testing were generated through the process of injection molding using the Nissei NP7 Real Mini machine. Each mechanical testing has different sample shape depends on the standard that used for each test. Table 1 shows the ratio and how many samples should be fabricated for each mechanical testing.

Table 1: Ratio of samples

| Sample | $CaCO_3$ (%) | HDPE (%) | No. of sample for Flexural test | No. of sample for Impact test | No. of sample for Tensile Test |
|---|---|---|---|---|---|
| S1 | 10 | 90 | 3 | 3 | 3 |
| S2 | 20 | 80 | 3 | 3 | 3 |
| S3 | 30 | 70 | 3 | 3 | 3 |

### *2.5 Mechanical testing*

In this research, flexural, tensile and impact tests were the chosen testing method to obtain the mechanical properties of the composite. These tests were referred to ASTM D790 (flexural test), ISO 179 (Impact test and ASTM D638 (Tensile test). The results obtained later should be able to determine whether the composite material is firm

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and suit to apply for load received as temporary bone implant.

Flexural test or three point bending test was used to determine the bending properties of the material by using Universal Testing Machine (UTM) with 10kN load cells. The specimen underwent injection molding procedures. The specimen dimension was 80mm in length, 10mm in width and 4mm in thickness.

The Charpy impact test was performed to measure the energy capacity absorbed by the material when subjected to a sudden load. The impact test was important to determine the toughness of the material by measuring the amount of energy absorbed before fracture or fail. The reading of the experiment will be taken in unit Joule. The specimen dimension was 80mm in length, 10mm in width and 4mm thickness and 2mm notch at the middle. This research used Charpy impact test equipment.

Tensile tests were carried out to measure the force required to break a plastic sample specimen and to determine to what extend the specimen stretches and elongate to the specific breaking point. Using Universal testing machine (5kN load cells) with standard ASTM D638 for tensile test, the test will provide data for tensile strength, stress, tensile strain and tensile modulus. The sample shape used was Narrow-waisted Dumbbell.

*2.6 Material Characterization*

Examination of prepared samples and $CaCO_3$ powder was done by Scanning Electron Microscopy (JEOL JSM 6400 SEM ATTACHED WITH EDX, Germany). Tensile and impact test samples were coated in platinum coating prior to studies on electron conductivity, microstructural characterization and sample structure at breaking point for further study. Few milligrams of cockle shell $CaCO_3$ powders were affixed to a stub with carbon paint placed on the sample holder. For solid samples, they were stuck to a metal bar using carbon tape before tested. The sample holder was mounted on a rotatable disc inside the machine and all samples were prepared for both SEM and EDX analyses. The surface morphology of the sample was observed on SEM operated under low vacuum at an accelerating voltage of 15kV.

The same sample was analyzed using EDX to investigate the elemental constituents after the spectrum was determined.

The FTIR analysis method used infrared light to scan test samples and observe chemical properties. The chemical functionality of the samples were analyzed using the spectroscopic method utilizing a Fourier Transform Infrared (FTIR). Spectrophotometer (Perkin Elmer) was used over the range of 400 $cm^{-1}$to 4000 $cm^{-1}$ with $CaCO_3$ powder, HDPE pallets, sample 1, sample 2 and sample 3, respectively.

## 3. Results and Discussion

### *3.1 Flexural test*

Data shows such in Figure 2 depicted the highest maximum force applied to the sample which is 35.83N for S3 with the ratio of 70% HDPE and 30% $CaCO_3$. It was then followed by 35.27N for S2 with the ratio of 80% HDPE and 20% $CaCO_3$, and 32.88N for S1 with ratio of 90% HDPE and 10% $CaCO_3$. The flexural stress and strain is shown to be the highest for S3 followed by S2 and S1. Using the flexural modulus formula, the calculation shows that S3 has the highest reading compared to S1 and S2 respectively. As the weight percentage of $CaCO_3$ increases, the flexural stress also increases. This shows that S3 can withstand more stress that acts upon it than S1 with less $CaCO_3$.

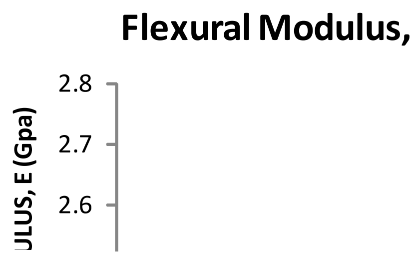


Figure 2: Bar chart for flexural modulus, E versus weight percentage of $CaCO_3$

Flexural modulus was calculated using the formula to indicate a material's stiffness when it flexed. HDPE is originally a polymer that has high elasticity. However, due to the increasing weight percentage of $CaCO_3$ in each of the sample, it can be concluded that the higher the amount of $CaCO_3$ mixed with HDPE, the higher the flexural modulus of the material. HDPE has relatively high elasticity and will not break easily when stress is acted upon it. The more weight percentage of $CaCO_3$ is added with HDPE, the longer the time taken for the material to achieve its maximum force [6].

### *3.2 Impact test*

The test was done using three different ratios with three specimens each. The calculated impact energy and impact strength are tabulated in the table. The impact energy between the three samples gave a stable reading with differences about 0.01J and the

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calculated impact strength has moderate differences of around 0.01 J/mm$^2$. S1 with ratio of 10% $CaCO_3$ have the highest value for impact strength of 0.72 J/mm$^2$. S3 with the ratio of 30% $CaCO_3$ and S2 with the ratio of 20% $CaCO_3$ have the same value for impact strength of 0.71 J/mm$^2$. Figure 3 shows the bar chart for impact test.

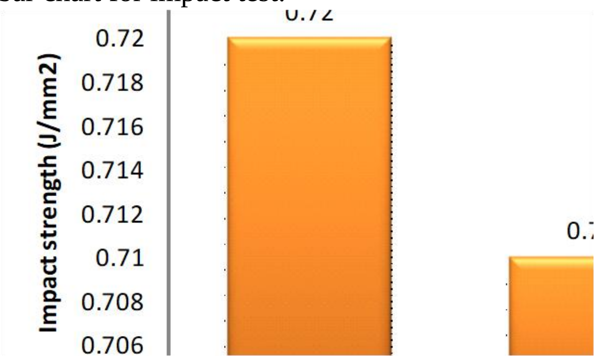



Figure 3: Bar chart for Impact strength versus weight percentage of $CaCO_3$

As analyzed, the increasing weight percentage of $CaCO_3$ affected the impact strength of the materials. In consequence, the impact strength of the composites completely decreased. The decreasing value over the samples indicated the brittle/weak material.

### *3.3 Tensile test*

Results obtained for tensile test as shown in Figure 4, where the values of Young's Modulus are changing according to the weight percentage of $CaCO_3$. S1 with 10% $CaCO_3$ ratio has the lowest maximum force as it can bear with 259.09N and the lowest Young's Modulus value of 0.43 GPa. The second highest maximum force is S2 with 20% of $CaCO_3$ ratio for 265.98N maximum force and 0.47 GPa for Young's Modulus value. The highest maximum force value was made by S3 with 30% of $CaCO_3$ ratio for 291.99N.

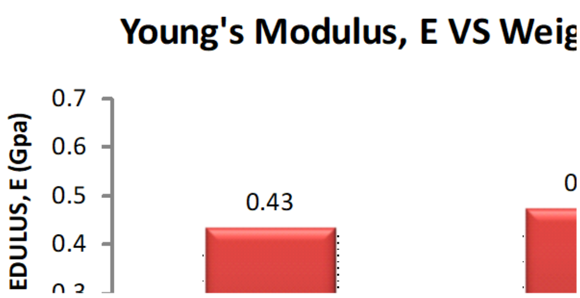



Figure 4: Bar chart of young's modulus versus weight percentage of $CaCO_3$

Young's Modulus is also known as the elastic properties of material to return back to its original shape after force is applied on it. The weight percentage of $CaCO_3$ in the sample increases the Young's Modulus of the materials based on Figure 4. It appears that S3 with the highest value can withstand the most maximum force applied on it compared to S1 and S2.

### *3.4 SEM and EDX Analysis*

SEM analysis of synthesized cockle shells $CaCO_3$ powder with different magnifications to have clearer vision of the particles of a) 150x magnification, b) 1000x magnification and c) 3000x magnification in Figure 5 was compared to previous study by Myeroff et al. in Figure 6 where Figure 6(b) and (c) reveal the prepared cockle shell, $CaCO_3$ powder has rod-like or its cluster structures are shown using arrow in the images of Figure 5 (a) and (b). These rods/needle-like structures are typical aragonite.

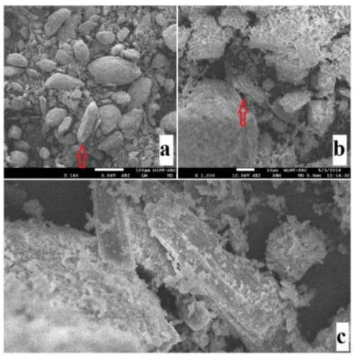



Figure 5: SEM analysis of synthesized $CaCO_3$

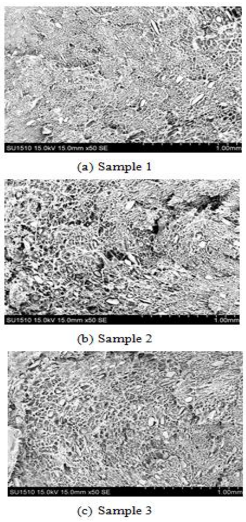



Figure 6: SEM analysis with 50x magnification for impact test

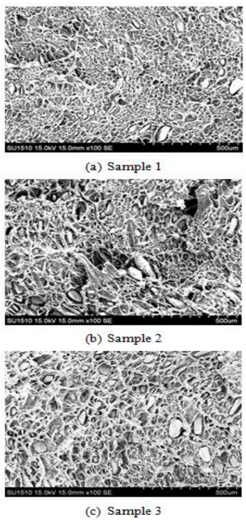
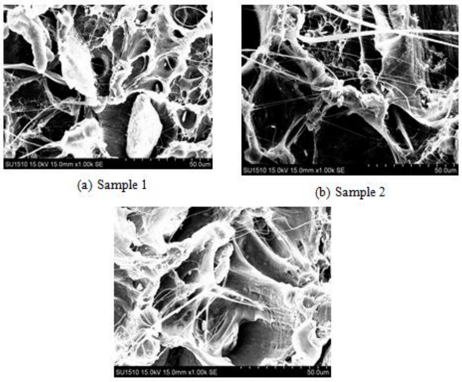


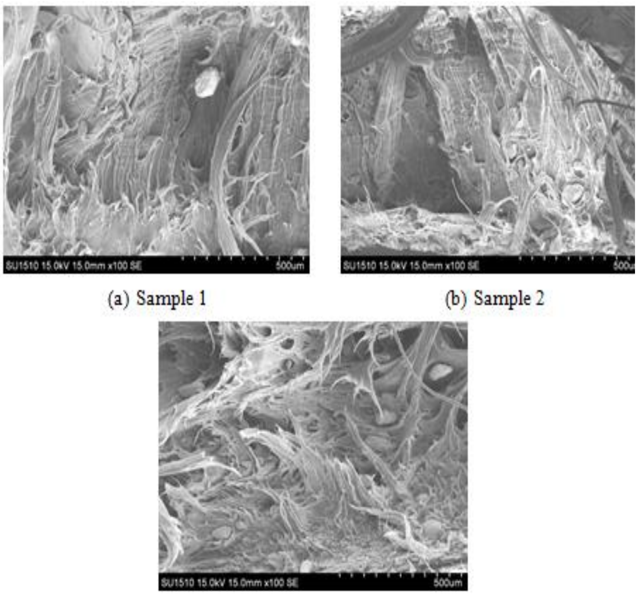

Figure 7: SEM analysis with 100x magnification for impact test



(a) Sample 1 (b) Sample 2 (c) Sample 3

Figure 8: SEM analysis with 1000x magnification for impact test

Figure 6, Figure 7 and Figure 8 show the structure of sample 1 containing 10% of $CaCO_3$ ratio where less $CaCO_3$ particles were present in the structures compared to sample 2 containing 20% of $CaCO_3$ and sample 3 containing 30% of $CaCO_3$ particles. Sample 3 had the most micron-sized $CaCO_3$ particles in the structures followed by sample 2 and sample 1. The results proved that the differences in weight percentage of $CaCO_3$ were visible through SEM analysis and the samples were mixed evenly as the particles were found everywhere.



(a) Sample 1 (b) Sample 2 (c) Sample 3

Figure 9: Tensile test samples under 100x magnification

Tensile test samples were scanned under 100x magnification to determine the structure after it has been pulled until failure occurred. Sample 1 in Figure 9 (a) showed less hair as well as less $CaCO_3$ particles on the sample's breaking point because it had the lowest weight percentage of $CaCO_3$. Sample 3 in Figure 10 (c) has the highest hair visible under the SEM analysis and lots of $CaCO_3$ particles seemed to appear at the sample's breaking point.

The elemental constituents of the synthesized cockle shell, $CaCO_3$ powder, was investigated at each spectrum found in cockle shell $CaCO_3$ powder. Figure 10 (a) shows the EDX result for $CaCO_3$ and the results were compared with Bharatham [7] as in Figure 10 (b). From these figures, it is proved that this element used as the bioactive materials in the research is pure $CaCO_3$ that extracted from cockle shell. All the essential ions that should be contained inside the $CaCO_3$ were all detected which are Carbon (C), Calcium (Ca) and Oxygen (O). It also can be seen from Figure 11 (b) that the peak for Ca is higher than the other ions. This shows that $CaCO_3$ extracted from cockle shell is able to produce element with rich in Calcium where good for bone and teeth development.

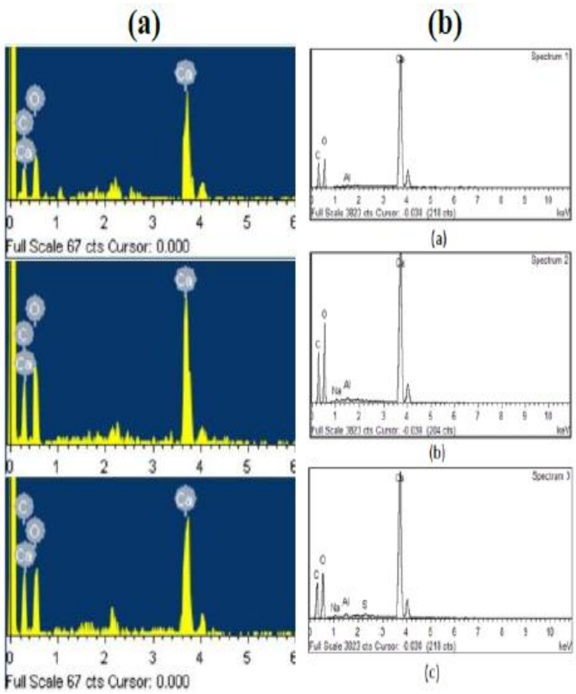



Figure 10: (a) EDX result of CaCO3 powders and (b) EDX result by Bharatham et. al.[7]

Analyzed samples were investigated at each spectrum graph, and elements such as C, O and Ca were found in each scaffold sample tested.

*3.4 FTiR Analysis*

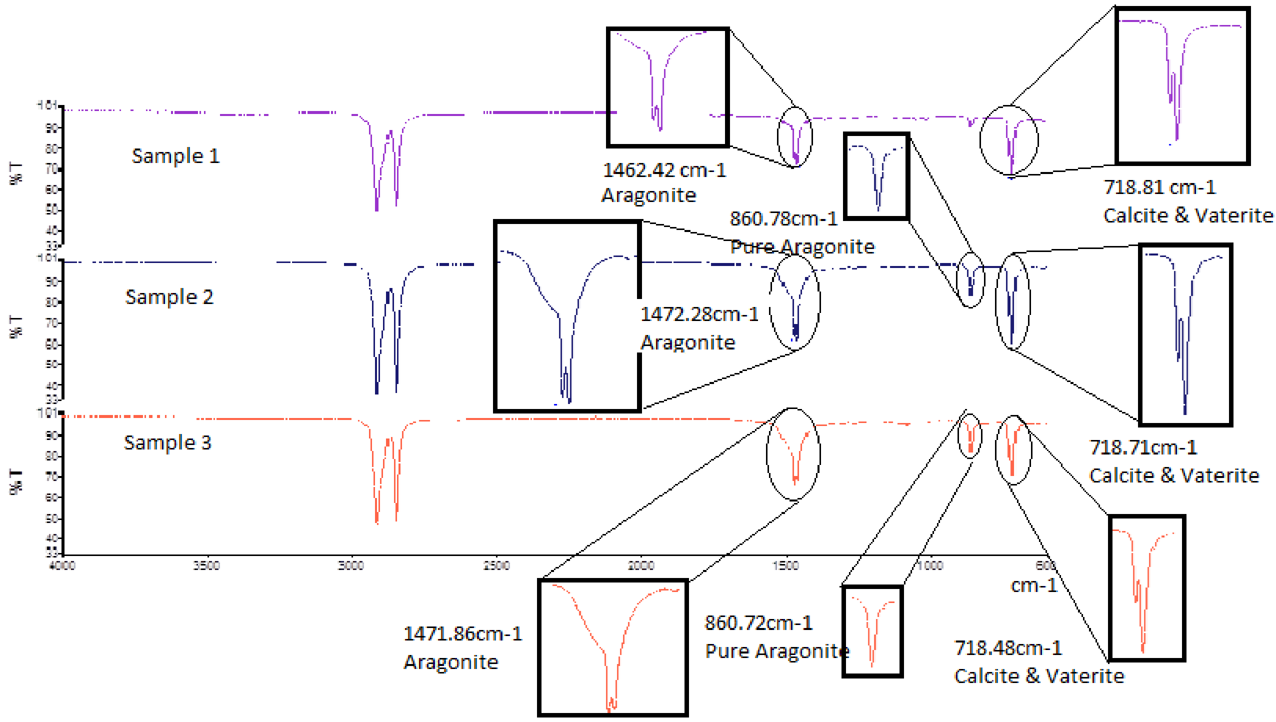



Figure 11: Sample contains polymorphs of $CaCO_3$; aragonite, calcite and vaterite

Analysis using Perkin Elmer Fourier Transform Infrared machine to confirm the material contained in the samples is really mixtures of $CaCO_3$ and HDPE. Figure 11 indicates that the samples containing polymorphs of $CaCO_3$; aragonite, calcite and vaterite are based on the graph of $CaCO_3$[8,9].

The phase of cockle shells $CaCO_3$ powder was confirmed by FTiR Analysis where the spectrum showed the peak ranging from 1460 $cm^{-1}$ to 1473 $cm^{-1}$ can be attributed to aragonite characteristics and peak range between 860.72 $cm^{-1}$ to 860.78 $cm^{-1}$ confirming that the powder was pure aragonite. Peak near the range from 718.43 $cm^{-1}$ to 718.81 $cm^{-1}$ shows that there were calcite and vaterite in the powder respectively.

## 4. Conclusion

The unused cockle shells were successfully extracted to $CaCO_3$ powder and HDPE pallets were mixed with different weight percentage or ratio. 100 micron-sized $CaCO_3$ powder was produced through the crushing and grinding process of cockle shells. Three different ratios of samples; 90% HDPE and 10% $CaCO_3$, 80% HDPE and 20% $CaCO_3$ and 70% HDPE and 30% $CaCO_3$ were mixed evenly using brabender machine. Mechanical testing that has been done proved that the increasing weight percentage affected the physical and mechanical strength of the scaffold samples greatly. This research occupying and implementing the mechanical tests to predict the strength of the material and how efficient $CaCO_3$ is as the bioceramic material in attracting the Calcium and Phosphorus minerals to encourage the development of new bone formation. Besides the suitable elements contained in the samples for biomedical use, the mechanical properties also meet the requirements to match the host bone properties and have proper load transfer especially when force is acted upon it based on previous studies.

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