

Characterisation of *Polymesoda bengalensis* Shell Powder

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Abstract

*Seafood shell is abundant and has no eminent use and thus, commonly regarded as waste. Reusing and converting it into a useful material can decrease the amount of waste. Therefore, a study of the crystalline structure should be performed before identifying the potential use of the material. The aim of this study is to identify the element and polymorph of *Polymesoda bengalensis* shell. The characterisations involved the usage of X-ray powder diffraction (XRD), scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDX). The XRD study revealed that the shell powder mostly consisted of aragonite. The analysis from SEM also revealed that the aragonite was in the form of rod-like crystal. The morphology of sectional, inner and outer surfaces of the shell was found that the aragonite was arranged in the form of a cross-lamellar structure of various sizes. The elemental content of the shell showed that CaCO_3 in this shell contained large amounts of calcium and carbon.*

Keywords: *Polymesoda bengalensis, Clam Shell, Polymorph; Aragonite.*

1. Introduction

Calcium carbonate (CaCO_3) can be obtained either by mining processes from limestone or from biological resources such as seashells and eggshells. Its powder is mostly used in industrial applications such as paper, inks, plastics and medicines [1]. In composite production, CaCO_3 is one of the widely used fillers infused in the plastics matrix composite [2].

Basically, CaCO_3 has three types of polymorph: calcite, aragonite and vaterite [3]. Commercial CaCO_3 from limestone is in the form of calcite [4]. Calcite can be found in Strombidae shell such as *Crassostrea gigas* [5] and *Strombus gigas* [6], and at the outer layer of several bivalves [7, 8], such as Pterioidea and Mytiloidea [9, 10]. Natural aragonite is of biogenic origin and it is denser than calcite. Due to its higher density and biocompatibility, aragonite is suitable for producing scaffold to repair fractured bone [11]. It can also be produced in the lab but in a small quantity [12]. Vaterite is a metastable CaCO_3 that has been detected in the upper layer and at the surface of freshwater cultured pearls [13]. Currently, calcium carbonate polymorph can be synthesised in the lab under a controlled environment in terms of, for example, temperature [14] and chemical solution [15].

This research investigates the characteristics of *Polymesoda bengalensis* shell and its powder. The findings from this research is beneficial for industrial application such as in wastewater treatment and biomaterial. The finding is also provide a green resource due to the conversion of waste to a

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useful product. In addition, the use of CaCO₃ derived from local seafood shell waste may reduce the amount of waste in the landfill and the dependence on CaCO₃ resources from limestone quarries.

In Malaysia, edible brackish water bivalves are on the top list of the major seafood production (54%), followed by shrimp (17.3%) and marine fish (6.3%) [16]. Among the edible bivalves are *Polymesoda bengalensis* from the Corbiculidae family. The sample of *Polymesoda bengalensis* used for this study was collected from Kota Samarahan, Sarawak. [17]. It was distributed in the mangrove and muddy areas in the maximum divisions of Sarawak, the biggest state in Malaysia. The shell is dark green, hard and thick, and it is sub-trigonal in shape with eroded umbo. *Polymesoda spp.* provides a cheap protein source [17] and can grow up to 10 cm [18]. By far, there is no official documentation on the characterisation of this clam shell and its usage. In Malaysia, most of the researches on molluscs has only focused on cockles *Anadara granosa* [4, 19], *Perna viridis* [20] and razor clam [21, 22]. Most of the literature reported on the utilisation its body tissue as biomonitors for heavy metal at estuaries and in coastal water [23]. Other countries such as Japan, South Korea and United Kingdom convert the disposed oyster shell to be fertilizer, food additive and natural mineral supply [24, 25].

Table 1 shows a comparison of the polymorphs of other mollusc shells studied by other researchers with *Polymesoda bengalensis*. Islam *et al.*[4] found that there were other trace elements such as Aluminium, Copper and Tellurium present in *Anadara granosa* shell powders after the powders were sieved with aperture size of 90 µm. Compared to *Anadara granosa*, *Polymesoda bengalensis* does not contain any trace elements in the powder when it passed through a 63 µm sieve. *Strombus gigas* Linnaeus shell from a family of Strombidae largely contained aragonite. On the other hand, calcite was only found at the outmost layer of the *Strombus gigas* shell.

Table 1. Polymorph of different species of bivalve shells.

Polymorph	Family	Species
Entirely Aragonite	Unionidae ^a	<i>Amblema costata</i> , <i>Elliptio dilitatus</i> , <i>Fusconia subrotunda</i> , <i>Fusconia undata</i> , <i>Legumia recta latissima</i> , <i>Megaloniaias gigantean</i> , <i>Pleurobema cordatum</i> , <i>Plelhobasus cyphus</i> , <i>Quadrula pustulosa</i> , <i>Quadrula quadrula</i> . [6]
	Lampsilinae ^a	<i>Actionaias carinate</i> , <i>Lampsilis anadontoides</i> , <i>Lampsilis ventricosa</i> , <i>Leptodea fragilis</i> , <i>Obovaria subrotunda</i> , <i>Obliquaria reflexa</i> , <i>Proptera alata</i> , <i>Ptychobranthus fasciolaris</i> , <i>Trucilla donaciformis</i> . [6]
	Anodontinae ^a	<i>Lasmigona complanata</i> , <i>Lasmigona costata</i> , <i>Strophitus rugosus</i> . [6]
	Arcidae	<i>Anadara granosa</i> . [4]
Calcite and Aragonite	Strombidae	<i>Strombus gigas</i> Linnaeus [6]
Entirely calcite	Ostreidae	<i>Ostrea sp</i> [6]

^aThe samples were taken from the Muskingum River in Ohio.

^bThe samples were taken from Kota Samarahan, Sarawak, Malaysia

This research is to determine the polymorph and crystalline structure of the calcium carbonate from the clamshell of *Polymesoda bengalensis*. Knowing the polymorph is very important because different polymorph has different potential application. Aragonite CaCO₃ from seafood shell is applicable for removing heavy metal from polluted wastewater. It is able to adsorb lead and cadmium better than calcite CaCO₃ [26, 27] Aragonite CaCO₃ is also biocompatible compared to other elements such as CaO and MgO [28].

2 Materials and methods

2.1 Sample collection

The muddy clam shells were scrubbed to remove grits on the shell. Then, the shells were soaked in the boiled water for 15 minutes to allow the shell and flesh to be separated easily. After that, the shells were dried in the oven at a temperature of 85°C for 6 hours. One of the shells was cut as shown in Figure 1 by using a Struers cutting machine. A grinding process was performed on the cut surface area by applying grit paper of grade 1000 before the shells were washed and dried again in the oven for 8 hours. Finally, the surfaces of outer, inner and sectional parts of the specimen were gold-sputtered and then mounted under the SEM for morphology observation.



Figure 1. Sample of *Polymesoda bengalensis* shell

2.2 Preparation of the shell powder

The small particle was produced by crushing the dried shell using mortar and pestle. To transform the particles into a powder form, the particles were ground using a blender. The powders were then sieved through aperture size of 63 μm . Only powder passed through 63 μm sieve size was packed and sealed in a polyethylene plastic bag for morphology and polymorph analysis.

2.3 Analysis

(1) Polymorph, elemental and morphological test

The type of crystal of the shell powder was identified using PANalytical X'Pert PRO powder diffractometer using Copper anode in wavelength of 0.154 nm and taking 2θ in the range of 0° to 80° and step size of 0.01° . The data was analysed by X'Pert HighScore software. For morphological study, clam shell and its powder were first coated with gold using gold sputter machine before it was observed using SEM (model Hitachi S3400N). The elemental study was performed at three different areas of the shell namely inner, cross-section and outer parts using energy dispersive X-ray analyser (EDX-Horiba EMAX model) at 10-15 kV.

(2) Powder size and specific surface area measurement

The powder mean size was determined using particle size analyzer (model Malvern Mastersizer 2000). 5 g clam shell powder was mixed in the distilled water and stirred to obtain the homogeneity and to avoid the particle settling. Laser diffractor was used to measure the mean size of the powder. The specific surface area of the powder was measured using Brunauer-Emmett-Teller (BET) apparatus by applying the liquid nitrogen, degassed at 100 °C for 24 hours and at 10^{-5} mmHg pressure.

3. Results and Discussion

3.1 Identification of type of polymorph by XRD analysis

Figure 2 demonstrates the XRD patterns of *Polymesoda bengalensis* shell powder. It shows that all peaks matched with CaCO_3 [29] from 0° to 80° of 2θ . The dominant peak of 2θ correlated with (hkl) indices of (111), (102), (201) and (122) were found at 26.192° , 27.46° , 33.090° and 45.823° , respectively. From these indices, it can be said that the polymorph of CaCO_3 is aragonite according to ICSD collection code no. 157994. Bragg's equation is used to determine the crystal structure and polymorph of the crystal.

$$d_{hkl} = \frac{\lambda}{2 \sin \theta}$$

Where d is a lattice interplanar spacing of the crystal, θ is an x-ray incidence angle (Bragg angle) and λ is a wavelength of the characteristic x-ray.

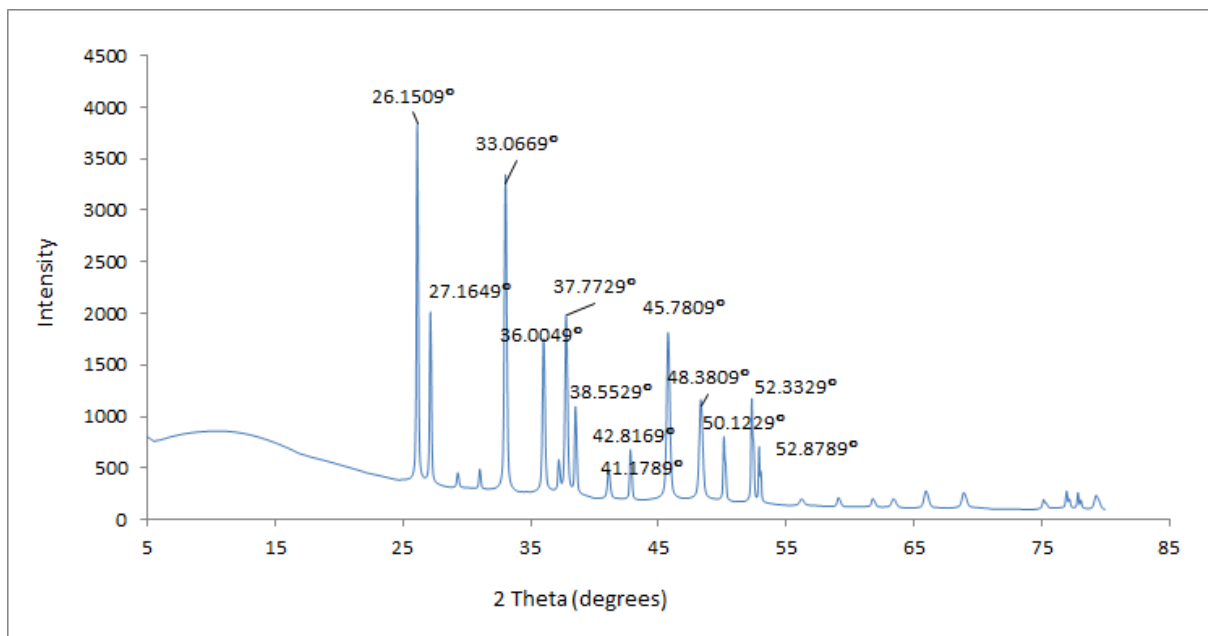


Figure 2. Diffractogram of *Polymesoda bengalensis* shell powder.

Aragonite CaCO_3 has high potential for biomaterial. It can be used for bone repair due to its density is higher than commercial CaCO_3 [12]. Nanocrystalline aragonite CaCO_3 impregnated with hydraulic acid allows young hyaline cartilage regeneration results in animal bone. This shows that the scaffold made from aragonite CaCO_3 could be useful for treating both chondral and osteochondral defect in human bone [30].

Aragonite CaCO_3 can be an agent to remove heavy metals in wastewater such as cadmium, cobalt, copper, lead and zinc. Ground aragonite CaCO_3 powder has high capacity to adsorb heavy metals, exceed the capacity level gained by chitin and chitosan [31]. The submicron carbonate structure in the shell powder provide the organic matrix for the heavy metal adsorption. Compared to calcite CaCO_3 , aragonite CaCO_3 is more effective to adsorb lead in wastewater [32].

3.2 Surface morphology by SEM

Figure 3 illustrates that most of the particles in aragonite clam shells were in the form of rod-like. The powder was also arranged in similar direction. Interestingly, it was found to be attracted to each other due to high surface energy [33, 34]. High surface energy is normally occurred between untreated CaCO_3 particles.

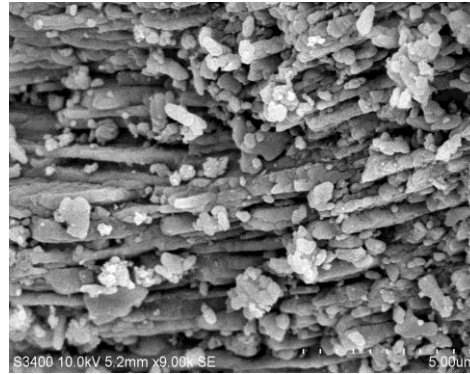


Figure 3. SEM image of the surface morphology of *Polymesoda bengalensis* clam shell particle

Figure 4(a) shows that the outer surface of the shell is rough with the presence of concentric rings. The colour is green but the umbo at the ventral is found to be eroded. On top of that, the inner surface of the shell is shiny in white colour except at the dorsal part where the muscles attached is yellow. The sectional part of the shell in Figure 4(b) reveals that the aragonite layers were arranged in the form of cross-lamellar structure. Similar arrangement was also found in a mollusc shell of *S. Marginatus* [35]. It is fundamental to note that crossed-lamellar structure with various levels was also found in lamellar bone, which can be used to reduce the anisotropy, thus that material may perform satisfactorily under various loading conditions [36]. Figure 4(c) highlights the discovery of a porous structure at the ventral part of an outer surface of the shell. Different structure was detected at dorsal part of outmost surface of the shell as shown in Figure 4(d). The structure was finer than the ventral part. The aragonite structure was clearly observed at a shiny white colour surface of the inner part. At the yellow-coloured area, the coarser particle with longer rod-like aragonite was detected but not in the white area as shown in Figure 4(f).

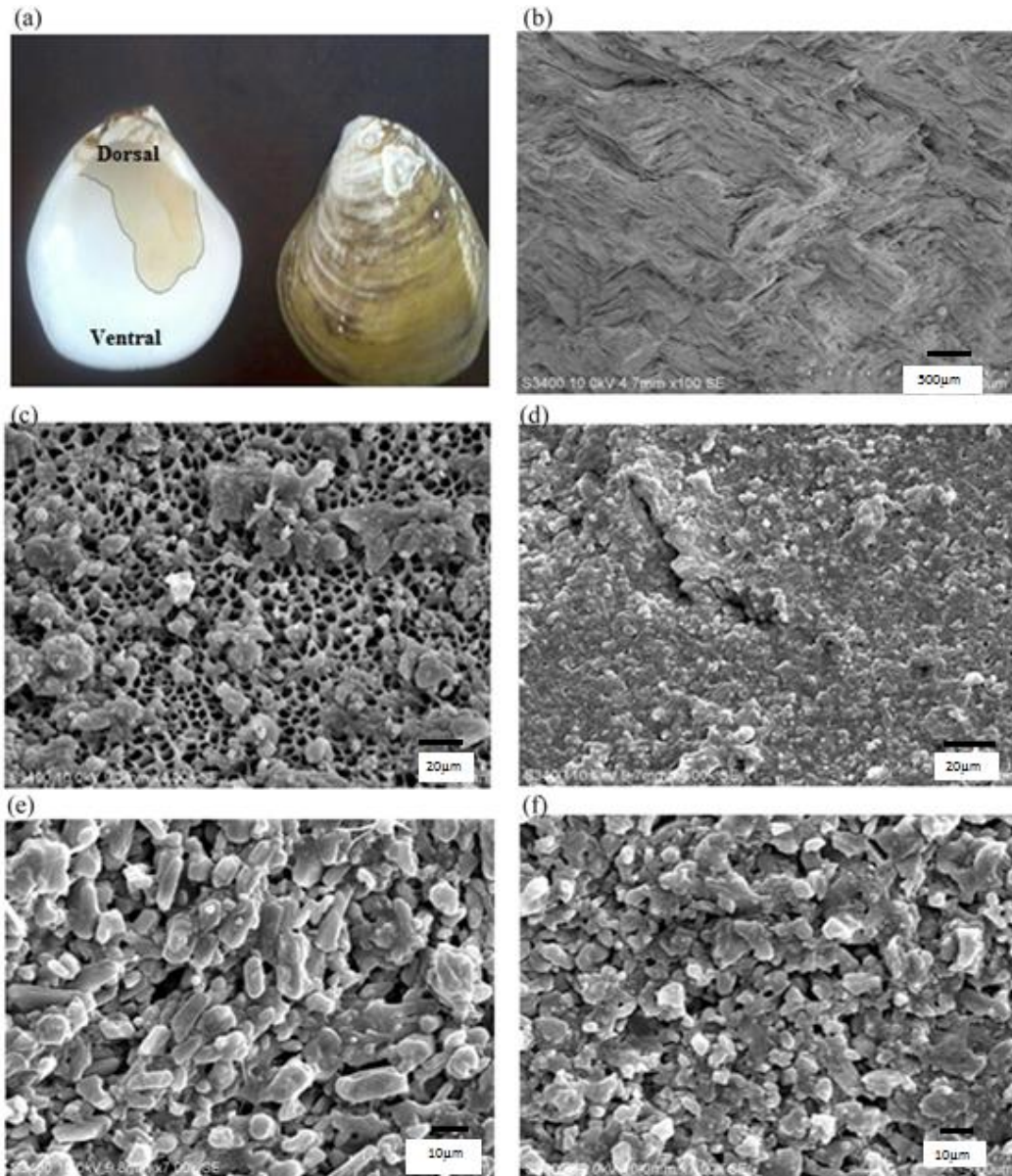


Figure 4. Image of (a) inner and outmost surfaces, and SEM images of the (b) cross-sectional area, (c) ventral part of the outmost surface, (d) dorsal part of the outer surface, (e) yellow area of the dorsal part at the inner surface, and (f) white area of the dorsal part at the inner surface of the *Polymesoda bengalensis* shell

3.3 Elemental analysis by EDX spectrum

Table 2 shows the EDX results of elemental content of the powder derived from *Polymesoda bengalensis* shells. The findings revealed that the powder, which was less than 63 µm, contained pure calcium carbonate without any trace elements. The calcium carbonate powder was rich in oxygen and calcium. The carbon content in this shell is 14.18% compared to only 11.91% in commercial calcium carbonate [4]. Figure 5 shows the spectrums at spot 1 to 3 of the shell.

Table 2. Elemental content of particle derived from *Polymesoda bengalensis* clam shell

Spot	C (%)	O (%)	Ca (%)	Total (%)
Spot 1	11.23	33.69	55.08	100
Spot 2	15.94	50.63	33.43	100
Spot 3	15.38	45.37	39.25	100
Mean	14.18	43.23	42.59	
Std. Deviation	2.58	8.67	11.20	
Max	15.94	50.63	55.08	
Min	11.23	33.69	33.42	

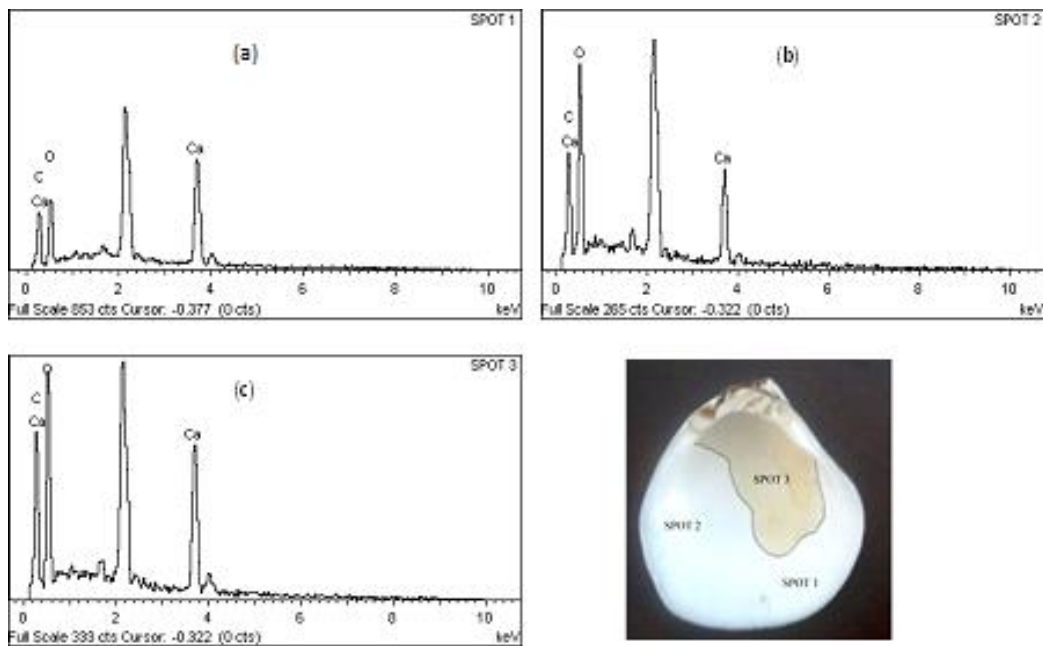


Figure 5. EDX spectrum at spot 1, 2 and 3 at the inner surface of the clam shell

3.4 Mean diameter size and specific surface area

Table 3 shows the mean diameter size and the surface area of the clam shell powder. It shows that the finer the size of the powder, the more specific area of the powder. Larger specific surface area produced higher surface energy. This further explains why the finer particles tend to attract among themselves and produce particle cluster. The excess energy at the surface of the finer particles compared to bulk is higher than coarse particles. The active bond between the particles create the formation of particles cluster. Coarser particles have less specific surface area because the excess surface energy is lower than bulk particles. Therefore, coarser particles normally less tendency to coagulate or agglomerate.

Agglomeration is uninvited in particulate filler composites. The particle cluster is a point of where failure starts in composite material due to higher stress concentration. To avoid this in nano-particle reinforced polymer matrix composites, the CaCO_3 particles are normally treated with stearic acid [30]. The reduction of surface energy minimises particle-particle interaction, thus encourages better homogeneity of filler in the polymer matrix.

For pharmaceutical tablet, particles agglomeration is very crucial. The compactness of the tablet is higher if the powder is finer. The tablet strength is controlled by the surface energy or particles bonding. Therefore, contradicted with particulate composite system, the agglomeration is invited in pharmaceutical tablet.

Table 3. Particle sizes and specific particle surface areas of the investigated particle

Particle size (μm)	Specific surface area (m^2/g)
29.84	7.143
35.06	2.217
46.30	0.589

4. Conclusions

Aragonite, which is a valuable mineral polymorph, was detected in the clam shell of *Polymesoda bengalensis* species by XRD analysis. The result appeared to be well substantiated by applying SEM, where rod-like aragonite was observed, and its elements were found to be rich in oxygen and calcium without any detection of trace elements. The specific surface area of the powder was found to be increased as the particle size decreased.

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