Crystal Structure and Morphology Dependence of the Phase of Mollusc Shell: A Case Study of XRD, SEM and ESR

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Abstract. The aim of this study is to investigate the physical properties in the Paphia undulate and Amusium pleuronectes shells collected from the coastal area of Chonburi province, Thailand. The crystal structure of the shells was studied by x-ray diffraction (XRD). The XRD patterns spectra reveal that the Paphia undulate shells and Amusium pleuronectes shells are made of a pure aragonite phase, and a mixture phase of aragonite and calcite, respectively, the identification and quantitative analysis were performed by using the Rietveld method. In this study, we also used the scanning electron microscope (SEM) to study the Morphology of the Paphia undulate shells and Amusium pleuronectes shells. The results on SEM micrographs agree well with those of XRD. In addition, the ions in the shell samples were also studied by Electron Spin Resonance Spectroscopy (ESR). The ESR spectra show that the samples of both shells were consisting of Mn²⁺ ions. The pattern of the ESR spectrum is thus strongly influenced by the environment of the paramagnetic ions, and then it can be used to identification of crystal structure of mollusc shell

1. Introduction

Mollusc shells are fascinating organic-material bio-composites with high mechanical performances. The marine mollusk exoskeletons are expected to contain carbonaceous matter as they form themselves towards the carbonate exoskeletons by absorbing much of atmosphere emissions [1]. The shells are found to have two common mineral forms of CaCO₃; calcite and aragonite. The crystal structures of calcite and aragonite and their inter-conversion have been studied [2]. Aragonite shells and aragonite mineral deposits are known to undergo a slow metamorphosis into calcite. The major and minor parts of organic materials are intercrystalline and intracrystalline, respectively [2-4].

Sea shells are found as two common mineral forms of CaCO₃, calcite and aragonite. Sea shells are composed of 97-99% CaCO₃ (calcite, aragonite, or vaterite) with lesser amounts of MgCO₃, (Al,Fe)₂O₃, SiO₂, Ca₃P₂O₇, CaSO₄, protein, and mucopolysaccharides. In addition to these major and minor constituents, trace amounts of Sn, Mo, Mn, Cd, Ti, B, Pb, Au, Ag, Ni, Co, Bi, Cu, Sr, and Rb. As have also been found in varying amount [1].

The purpose of this study was to obtain information of the shell structures of marine mussel shells. The information on microstructure is one of the most significant relative data for clarification of evolutionary trends of shell structure, and assessment the potentiality of using the Amusium pleuronectes shell and Paphia undulate shell as alternative biomaterials. It is of great importance to understand the formation and stability conditions for all crystal modifications of calcium carbonate. A better understanding of this phenomenon will aid in the development of new approaches, especially
for the formation of complex metamorphic for uses as composite materials, bone replacement, device fabrication in microelectronics and optoelectronics.

2. Materials and Method

The *Paphia undulate* and *Amusium pleuronectes* samples were obtained from the coastal area of Chonburi province, the eastern part of Thailand. First of all the soft parts inside the marine mussel samples were removed after opening the bivalve of the shell. The bivalve shell was brushed and washed with distilled water. Afterward the bivalve samples were cleaned by soaking in dilute HCl solution for several minutes to remove all organic materials, followed by washing thoroughly with distilled water. The cleaned sample was ground into fine powder to desired sizes by sieves and labels. The powder samples are denoted as M-CL and M-SC where the labeling numbers indicate *Paphia undulate* (Carpet Clam) and *Amusium pleuronectes* (Radoated Scallop), respectively.

The crystal structure was characterized by a powder X-ray diffractometer. Powder diffraction data were recorded at room temperature using a powder diffractometer, Bruker AXS D8 Advance [CuKα (Ni filtered) with scintillation detector; 20 range from 20-80° and step size of 0.02°]. The diffraction data were analyzed by the Reitveld method using the computer program FULLPROF SUITE-2000. Therefore, this method was used to estimate the mineral percentage in the sample. Another that, all shell samples were cut and polished for Scanning Electron Microscopy. The morphology were characterized by Field-emission scanning electron microscopy (FE-SEM, Hitachi S-4700, Japan)

The Metal ions in the exoskeletons were studied by ESR spectrometer. All ESR spectra of the powder samples were measured on Bruker E500 CW ESR spectrometer operating at X-band microwave frequency of 9.8 GHz using 0.5 mT field modulation amplitude with a time constant of 0.03 s.

3. Results and Discussion

![Figure 1. Powder XRD patterns of *Paphia undulate* shell (M-CL) and *Amusium pleuronectes* shell (M-SC) samples](image)
The crystal structure of $M$-$CL$ shell samples was made of aragonite phase, a common form of CaCO$_3$ mineral. For $M$-$SC$ shell has a mixture phase of aragonite and calcite as shown in Figure 1. The mineral phases of the bivalve samples all species were identified from the diffractograms by reference to the Joint Committee on Powder Diffraction Standard (JCPDS) numbers 00-005-0453, and 01-086-2339 corresponding to aragonite phase and calcite phase respectively.

The powder X-ray diffraction pattern of M-CL in Figure 2 shows that the crystal structure of $M$-$CL$ shell sample was made of a pure aragonite phase, a common form of calcium carbonate (CaCO$_3$) mineral. The X-ray diffraction patterns of $M$-$SC$ shell sample in Figure 3 exhibits peak characteristics of calcite and aragonite. For all refinements, lattice parameters and phase fractions of M-SC sample are in good agreement. The percentage of calcite and aragonite is 94% and 6%, respectively.

Figure 4 shows the SEM micrographs of the $M$-$CL$ shell. It is seen that the $M$-$CL$ shells composed of aragonite plates with the porous scattered all over the shell sample, a rod-like shape of CaCO$_3$ is obtained in these SEM images, which is the typical shape of aragonite. For $M$-$SC$ shell samples, the columnar structures of mixture phase of aragonite and calcite can be observed in Figure 5. It is clear that amount of aragonite and calcite material adheres to the SEM micrographs of the shell.

![Figure 2](image1.png)

**Figure 2.** Difference X-ray diffraction profiles for the Rietveld refinement of *Paphia undulate* shell (M-CL) sample. Experimental (○mark), calculated (solid line) and difference (bottom) powder diffraction profile. $R_{wp} = 8.17$, $\chi^2 = 1.37$.

![Figure 3](image2.png)

**Figure 3.** Difference X-ray diffraction profiles for the Rietveld refinement of *Amusium pleuronectes* (M-SC) shell sample. Experimental (○mark), calculated (solid line) and difference (bottom) powder diffraction profile. $R_{wp} = 7.31$, $\chi^2 = 1.31$. 
Figure 4. The figure and microstructure of *Paphia undulate* (M-CL).

Figure 5. The figure and microstructure of *Amusium pleuronectes* (M-SC).

The ESR spectra of the M-CL shell samples are shown in Figure 6. The six hyperfine lines are obtained due to a trace of the Mn$^{2+}$ ions present in the carbonate of aragonite structure. The ground state $^6S_{5/2}$ in the absence of an external magnetic field splits due to spin-spin interaction, into three Kramer’s doublets with separations of 4D and 2D. In the presence of an applied magnetic field along the symmetry axis, these Kramer’s doublets split further and the energy between successive levels, correct to first order, is $g\beta B + 4D$, $g\beta B + 2D$, $g\beta B$, $g\beta B - 2D$, $g\beta B - 4D$, with the result that they give rise to five equally spaced resonance. Since the nuclear spin of $^{55}$Mn is 5/2 each of these five lines split further to a sextet and hence one can observe 30 line pattern per molecule per unit cell.

The broad absorption lines in M-CL shell could be due to low symmetry environment. The ESR spectra of M-SC shell shown in the Figure 7. The ESR spectra of M-SC shell, the Mn$^{2+}$ ions present in the carbonate deviate from the higher symmetry environment. Each spectrum consists mainly of six strong Mn$^{2+}$ lines are more intense. The spectrum is obviously attributed to the $m_S = -1/2 \leftrightarrow +1/2$ sextet fine structure transition of Mn$^{2+}$ ions (electron spin $S = 5/2$ and nuclear spin $I = 5/2$) according to the selection rule $\Delta m_S = \pm 1$, $\Delta m_I = 0$. The weaker pairs of peaks between the main peaks are so called the forbidden transitions in which both electron and nuclear spin state change, each Mn$^{2+}$ peak splits into a doublet, which results from the formation of calcite phase. This in equivalence gives rise to a doublet splitting of each fine-structure transition. The ESR spectra results are in good agreement with those of XRD and SEM as previously described.
Figure 6. X-band ESR spectra of *Paphia undulate* (M-CL) shell samples in the magnetic field range 300-400 mT.

Figure 7. X-band ESR spectra of *Amusium pleuronectes* (M-SC) shell samples in the magnetic field range 300-400 mT.

4. Conclusion

The *M-CL* shell and *M-SC* shell samples obtained from the coastal area of Chonburi province, the eastern part of Thailand consists mainly of CaCO$_3$. The aragonite phase is not the only type of calcium carbonate crystal to make up the shell structure in mussel. The calcified part of the shell consists of two types of calcium carbonate crystals, either pure aragonite and mixture phase between aragonite and calcite. Moreover, the ESR spectroscopy can be profitably used to examine information concerning structural organization. Then biomineralized can provide unusual metal ion environments, related to heavy metals control in coastal areas involve monitoring a series of metal in water, which to study crystal structure on transition metal ions. The information from ESR spectroscopy microstructure is one of the most significant relative data for clarification of evolutionary trends of shell structure and assessment the potentiality of using the mussel shell as biomonitors of heavy metal pollution.

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5. References

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