



RESEARCH ARTICLE

Hidden engineering in molecular silence: Examination of biomineralization structure in the shell of *Magallana gigas* (Thunberg, 1793) species using X-ray diffraction (XRD)

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ABSTRACT

This study was conducted using X-ray diffraction (XRD) analysis to determine the mineralogical composition of the shell structure of *Magallana gigas* (Pacific oyster). The analyses revealed that the shell is predominantly composed of calcium carbonate (CaCO_3), with the structure predominantly found in the crystalline calcite phase. XRD patterns were thoroughly evaluated in the 20° – 80° 2θ range, and high-intensity diffraction peaks specific to the calcite phase were detected, particularly in the 40° – 49° and 60° – 78° regions. Signals related to the aragonite phase were limited and of low intensity. The biomineralization process plays a central role in organisms' adaptation to environmental factors and structural protection. Marine mollusks like *M. gigas* provide physical protection and gain resilience to chemical variability in their habitats through biomineralization mechanisms that govern shell formation. The dominance of calcite in the shells is demonstrated comprehensively by our XRD data, as the preferential formation of the calcite phase in this species' shell structure is favored for its advantages in long-term environmental stability and biological energy efficiency. Additionally, the obtained data make significant contributions to understanding the biochemical and environmental interactions involved in shell formation in marine organisms. In this regard, the study makes significant contributions for future research on the formation, function, and ecological importance of biogenic minerals.

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Introduction

The evolution of biomineralization in mollusks represents a key development in the history of life over approximately 500 million years, marking the beginning of the production of hard structures such as shells, skeletons, or exoskeletons by organisms. This biological mechanism is known as biomineralization, and it has not only supported the structural integrity of organisms but also enabled them to effectively protect their soft tissues from external factors. In this way, organisms have been able to adapt to environmental changes and challenges, laying the foundation for the emergence of evolutionary diversity and adaptation strategies (Marin et al., 2007; Murdock, 2020; Louis et al., 2022). The importance of biomineralization lies not only in making organisms more resistant to environmental stress factors but also in paving the way for increased diversity at the ecosystem level (Gilbert, 2022). Living organisms can develop different strategies in the process of mineral production. These strategies range from passive external biological mineralization (also known as organomineralization) processes, which are influenced by environmental conditions, to biogenic mineralization that are directed by the organism internal biochemical mechanisms and occur in a more controlled manner. These processes play a significant role in the formation, organization, and biological functionality of the mineral structure, directly affecting the ability of organisms to gain environmental adaptation and structural resilience (Weiner & Dove, 2003; Dupraz et al., 2009; Louis et al., 2022; Chen et al., 2019; Qin et al., 2024). The growth process of hard biological structures such as shells and skeletons is largely determined by environmental factors and the organism physiological condition. The development of these structures can slow down or even come to a complete halt due to fluctuations in environmental variables such as temperature, food availability, and pH levels, or due to a slowdown in the organism metabolic activities. This demonstrates how closely the calcification mechanism is related to the vital balance of living organisms (Iglikowska et al., 2018; Figuerola et al., 2021; Ehrlich et al., 2021; Campodoni et al., 2021; Louis et al., 2022). The hard tissues produced by many organisms not only provide structural durability but also serve various biological functions depending on the species and location. For example, shells provide physical protection against external factors, while bones support the body structure and enable movement in vertebrate animals. On the other hand, otoliths found in certain fish species play a role in balance and hearing by being located in the inner ear. This diversity

demonstrates how versatile biomineralized tissues are in terms of their adaptation in the evolutionary process (Louis et al., 2022). Marine bivalves actively regulate to synthesize a wide variety of mineral types, such as silica, bioapatite, iron oxides, and hydroxides. However, the most commonly produced mineral component in the shell mineralization is CaCO_3 deposition system. This component can appear in three different crystal forms (polymorphs). While calcite and aragonite are the most commonly encountered forms, vaterite can be produced more rarely. These polymorph selections may vary depending on the organism genetic traits and environmental conditions (Skinner & Jahren, 2003; Louis et al., 2022). Shells make up a significant portion of the total weight of bivalve organisms, typically ranging 56-61%. Approximately 94% of the structural composition of a shell is made up of CaCO_3 . This indicates that shells are largely mineralized and durable structures. Marine organisms use shells, which are hard external structures, to provide protection and structural support. These shells are typically formed by the aggregation of CaCO_3 crystals, which are covered by a thin organic membrane. This structure enhances the protection of organisms against external factors and increases their chances of survival (Hamester et al., 2012; Ituen, 2015; Mititelu et al., 2022; Kızılkaya et al., 2024).

Material and Methods

Sample Collection and Preparation

In this study, samples were collected in 2015 from Bandırma, Turkey, to examine the mineralogical composition of the shell structure of *Magallana gigas* (Pacific oyster). The samples were taken from the local marine environment and prepared for analysis under laboratory conditions. The collected *M. gigas* shells were initially washed with clean water to remove marine water and external contaminants. This process aimed to clean the shells of dirt, algae, and other organic residues. Care was taken during the cleaning process to ensure that the mineral structure of the shells was not damaged and their natural surfaces were preserved. After cleaning, the shells were ground to obtain a homogeneous texture. The grinding process was carefully carried out in the laboratory to ensure the samples were reduced to an appropriate powder size. This step is necessary to obtain fine powder samples for XRD analysis. The ground samples were homogenized to ensure they could be analyzed in the XRD device. This ensured that the data obtained from the mineralogical analysis would be accurate and reliable.

X-Ray Diffraction (XRD) Analysis

In this study, XRD analysis technique was used to determine the mineralogical composition of the shell structure of *M. gigas*. The shell samples were analyzed using the PANalytical Empyrean XRD device located at the Central Laboratory of Çanakkale Onsekiz Mart University. This device is equipped with an advanced diffraction system to ensure high precision and accuracy for mineralogical analyses. XRD is a widely used technique for determining the internal structure and mineral composition of samples. The PANalytical Empyrean device used in this study has the capacity to detect different mineral phases in samples with various diffraction angles and high-resolution features. Located at the Central Laboratory of Çanakkale Onsekiz Mart University, this device provides an ideal environment for examining diffraction patterns in the 20°–80° 2θ range. The analyses were conducted on the powdered forms of the samples. Before being analyzed in the XRD device, the powdered samples were properly released and homogenized.

Results and Discussion

XRD examination of bivalve shell structure reveals which calcium carbonate form (calcite, aragonite) these organisms prefer. In this way, the biomineralization process, adaptations to environmental conditions and changes in shell structure can

be understood. In addition, XRD data provide a scientific basis for the evaluation of shells for biotechnological and environmental applications. The shells of marine organisms such as *M. gigas* are composed of CaCO₃ polymorphs crystals formed as a result of biogenic mineralization. Calcite and aragonite are the two most commonly found polymorphs in these processes. According to XRD analysis results, the higher concentration of calcite in this oyster species reflects the organism biological response to environmental conditions (such as temperature, ion concentration, pH, etc.). This provides important data for understanding the role of environmental factors on shell mineralogy. XRD analysis allows for detailed information about the crystal structure of the material. This method is widely used, especially in determining the crystal phases of biogenic CaCO₃ deposition minerals. Organisms are known to form these phases through a process called biomineralization. In this process, the organism directs calcium and carbonate ions to produce specific crystal structures through particular proteins and organic compounds. The formation of crystal phases is known to depend on environmental factors (such as temperature, pH, magnesium/calcium ratio) and the genetic structure of the organism. In particular, aragonite forms more easily at higher magnesium concentrations and may be preferred when rapid shell formation is required.

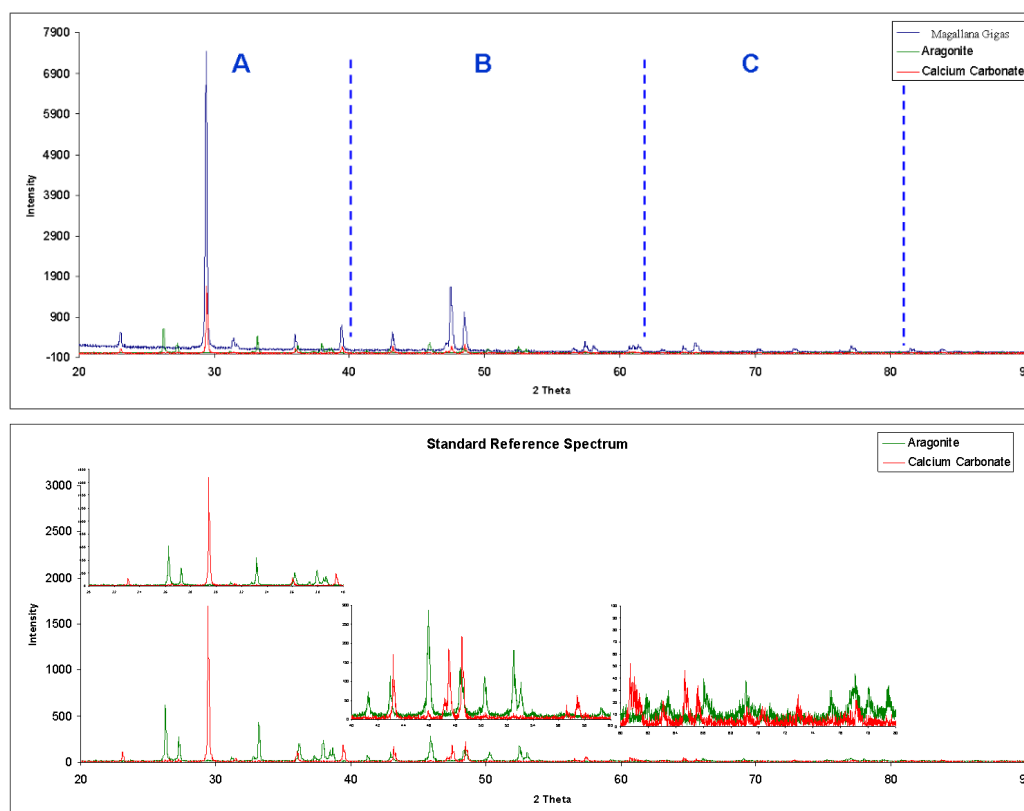


Figure 1. XRD spectra (20°–90° 2θ) of *Magallana gigas*, CaCO₃ and aragonite

Such analyses are crucial for understanding the different crystal phases of biogenic CaCO_3 . Aragonite, with its properties such as high resolution and biocompatibility, has potential for use in environmental applications, biomaterial production, and particularly in the biomedical field. The use of shells obtained from species like *M. gigas* as a natural source of aragonite is therefore noteworthy. Additionally, these types of analyses contribute to the industrial utilization of shell waste and the development of appropriate methods for recycling processes.

In this study, the mineralogical composition of the shell structure of *M. gigas*, a bivalve mollusk species, was investigated using XRD method. The results obtained from the analysis and the reference materials are shown in Figure 1. In Figure 1, the XRD spectrum has been divided into three regions (20-40° 2 θ , Figure 2; 40-60° 2 θ , Figure 3; and 60-80° 2 θ , Figure 4) to allow for a detailed interpretation. The *M. gigas* sample (blue line) was compared with two different standard reference materials: aragonite (green line) and CaCO_3 deposition (red line, calcite form). Through this comparison, the crystal forms that make up the shell structure were attempted to be determined. In the analysis of crystal structures, not only the position of the peaks but also their shape (sharpness, width), and intensity provide important technical data. Sharp, symmetrical, and high-intensity peaks indicate that the crystals within the sample are highly ordered, that is, crystallized. Peak width can allow for the calculation of nanocrystal sizes, which can provide information about the structural quality of biomineralization products. Additionally, the content of the amorphous phase can be evaluated by the increase in the background level and the formation of broad peaks. When the XRD pattern of the shell is examined, it can be seen that the pattern of the *M. gigas* sample (blue line) largely aligns with the CaCO_3 polymorphs pattern (red line). A particularly dense peak is observed in the 2 θ range of approximately 26°–30°. The peaks in this region directly overlap with the crystal planes characteristic of CaCO_3 . Similarly, other significant peaks in the 30°–50° range are also found to match with the aragonite and CaCO_3 phases. Notwithstanding, while there are some limited similarities between the specific peak points of aragonite in the calcite form (green line) and the *M. gigas* shell, the intensity of these overlaps is low. In particular, the peak around 2 $\theta \approx 29.4^\circ$, characteristic of calcite, overlaps with CaCO_3 , making it difficult to distinguish. In contrast, considering the overall pattern, it can be inferred that the aragonite content is quite low. The sharp and high-intensity peaks observed in the obtained XRD pattern indicate that the crystal structure of the shell is well-ordered

and highly crystalline. This suggests that the *M. gigas* shell has undergone a biogenically regulated crystallization process and possesses a homogeneous phase content. It can be concluded that the shell is predominantly composed of the CaCO_3 phase, with a smaller proportion of amorphous or other crystalline phases. Based on the XRD analysis, it has been determined that the mineralogical composition of the *M. gigas* shell is largely in the form of the CaCO_3 phase.

In Figure 2, the XRD analysis of the shell sample of the *M. gigas* species was examined by enlarging the 2 θ values between 20° and 40°. This detailed spectral analysis enables a more precise and thorough evaluation of the crystal phases present in the shell structure. This range is particularly critical for distinguishing the main crystal forms of CaCO_3 polymorphs, namely the aragonite and calcite phases. These phases are commonly found together in biogenic-origin materials, and phase determination by XRD is a fundamental step in mineralogical characterization. In XRD analysis, the 20°–40° 2 θ range is one of the critical regions for the mineralogical characterization of biomineralization products. This range contains characteristic diffraction signals that allow for the differentiation of biogenic CaCO_3 phases, such as aragonite, calcite, and sometimes vaterite. During the biomineralization process, organisms synthesize one or more of these phases in accordance with environmental and biological control factors. The type of synthesized mineral can vary depending on many parameters, such as the growth environment, ion concentration, temperature, pH level, and biological templating. The most striking diffraction peak in the analyzed region is located at approximately 29.4° 2 θ and shows a very high intensity. This peak represents a common reflection plane for both the sample and calcite phases, and by itself, it is not sufficient for phase differentiation. Notwithstanding, the other characteristic signals observed along with this main peak, particularly those that align with the diffraction patterns specific to CaCO_3 phases, indicate that CaCO_3 is the dominant structure in the *M. gigas* shell. Notably, diffraction peaks around approximately 23°, 26.2°, and 29.5° 2 θ are characteristic reflections of CaCO_3 crystal phases. These signals are clearly detected in both the reference aragonite and calcite patterns as well as in the *M. gigas* sample. The high degree of overlap of these peaks with the sample further confirms that the shell is predominantly composed of CaCO_3 mineral. Additionally, the high intensity, sharpness, and symmetry of the peaks observed in the diffraction pattern reveal that the shell material has a highly crystalline structure. The narrow width of the peaks and

the smooth termination of their bases suggest that the crystal sizes are regularly and homogeneously distributed, with very limited amorphous content. These features indicate that the shell mineralization in *M. gigas* shells occurs in a highly controlled manner, optimizing the crystalline structure to enhance the mechanical strength of the shell. As a result, the detailed XRD analysis performed in the 20°–40° 2 θ range demonstrates that the mineralogical composition of the *M. gigas* shell is predominantly CaCO₃ deposition -based. These detailed analyses in this range are important not only for phase identification but also for understanding the formation conditions of biogenic calcium carbonates and the mechanisms of biocrystallization. This mineralogical structure provides valuable insights into both environmental adaptation processes and potential biomaterial applications.

Figure 3 presents the XRD analysis of the *M. gigas* shell sample in the 40°–60° 2 θ range, which provides significant information for determining the crystal phases present in the sample. In this range, characteristic diffraction peaks related to CaCO₃ polymorphs, such as aragonite and calcite, can be examined in more detail. This specific range is particularly

critical for identifying the different crystal structures (polymorphs) of CaCO₃. In the examined region, particularly in the 40°–49° 2 θ range, two prominent peaks are observed in the *M. gigas* sample. These peaks represent characteristic diffraction signals of CaCO₃ deposition phases and strongly align with the reference CaCO₃ patterns. This indicates that the calcite phase is predominantly present in the sample, and a significant portion of the shell structure is composed of this crystal form. Comparisons with reference CaCO₃ patterns clearly show a strong correlation between these two peaks and the calcite phase. Additionally, within the 42°–44° and 50°–58° 2 θ ranges, some minor peaks characteristic of aragonite is also detected. Although these peaks are of low intensity, they confirm that aragonite is present in trace amounts in the sample. While some peaks related to aragonite are present in the same region, they are much lower in intensity and weaker when compared to the *M. gigas* sample. This suggests that the aragonite content is quite limited. Interestingly, the contribution of this phase remains secondary in the overall mineralogical structure of the sample, leading to the conclusion that the dominant phase is still calcite.

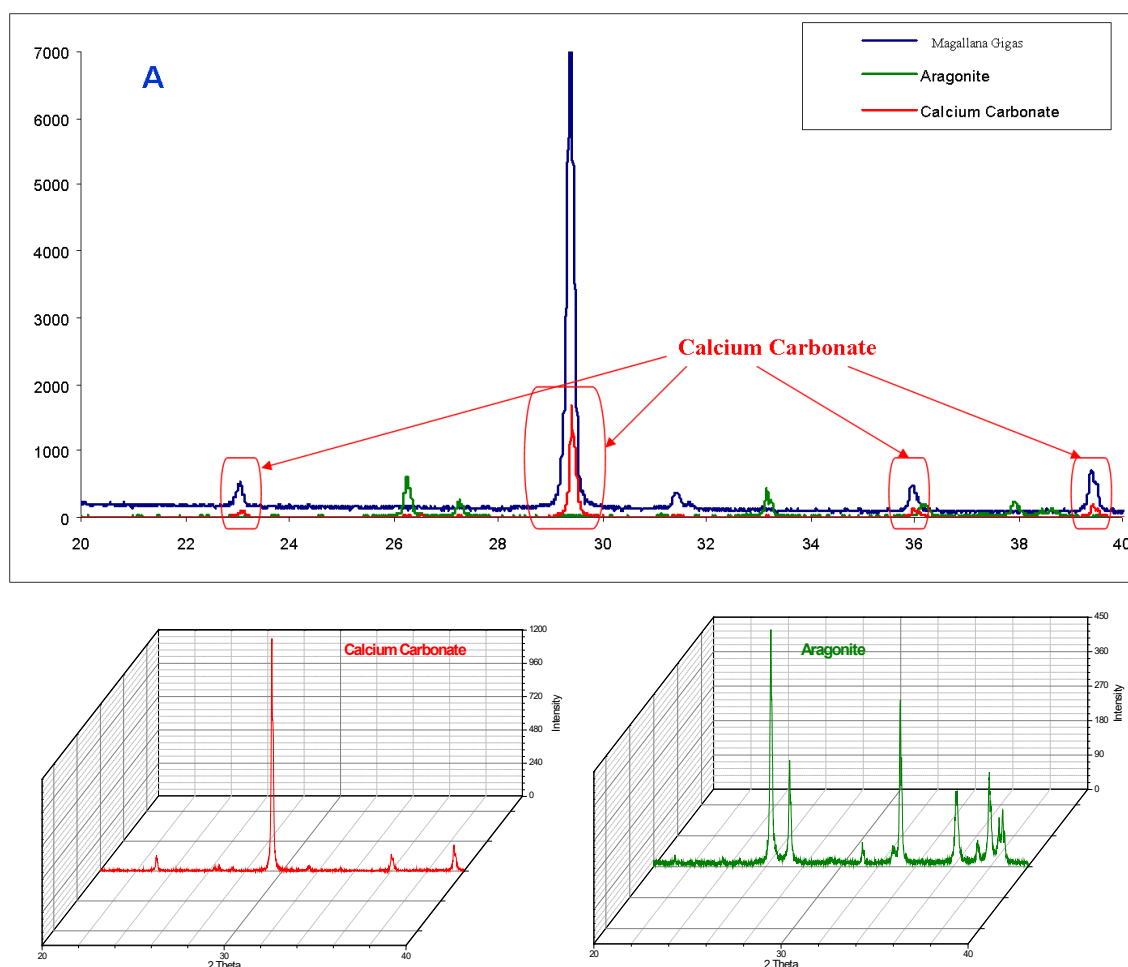


Figure 2. Comparative XRD spectra in the 40°–60° 2 θ range

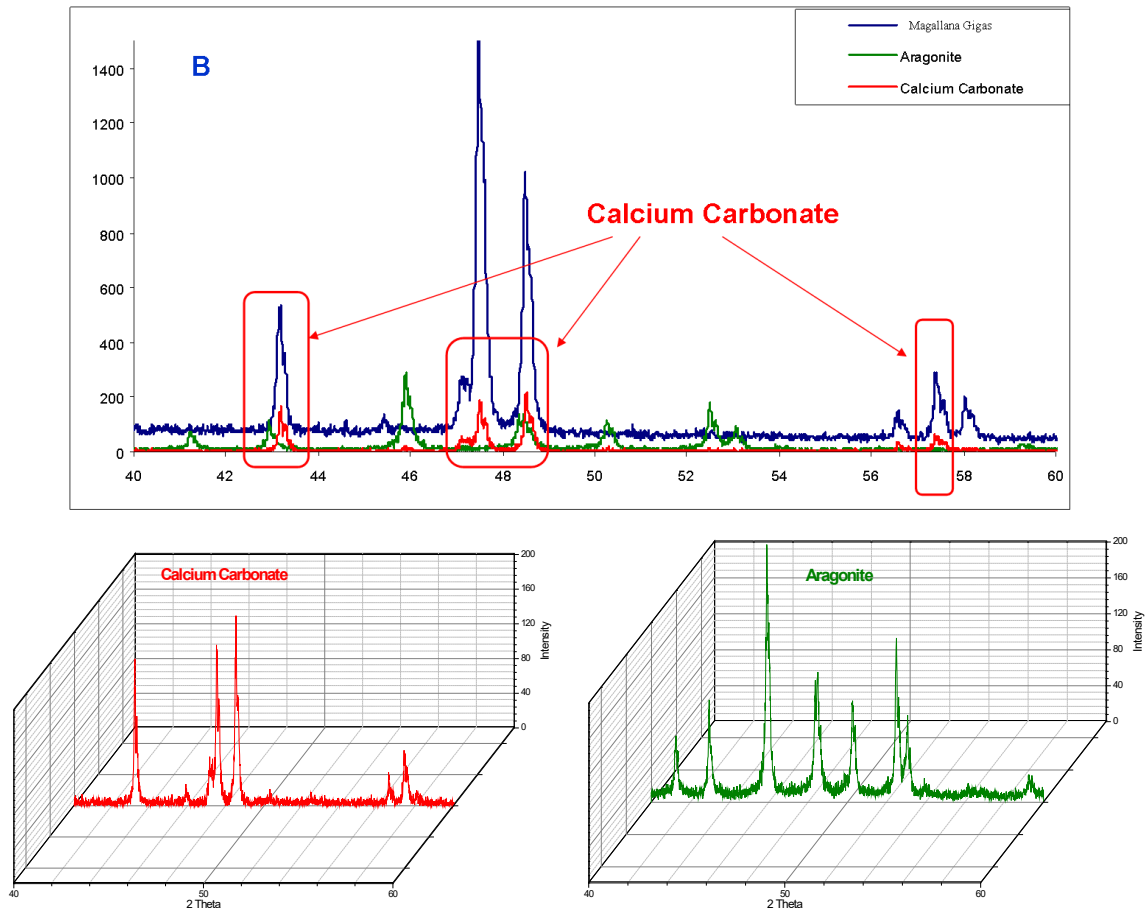


Figure 3. Comparative XRD spectra in the 40° – 60° 2θ range

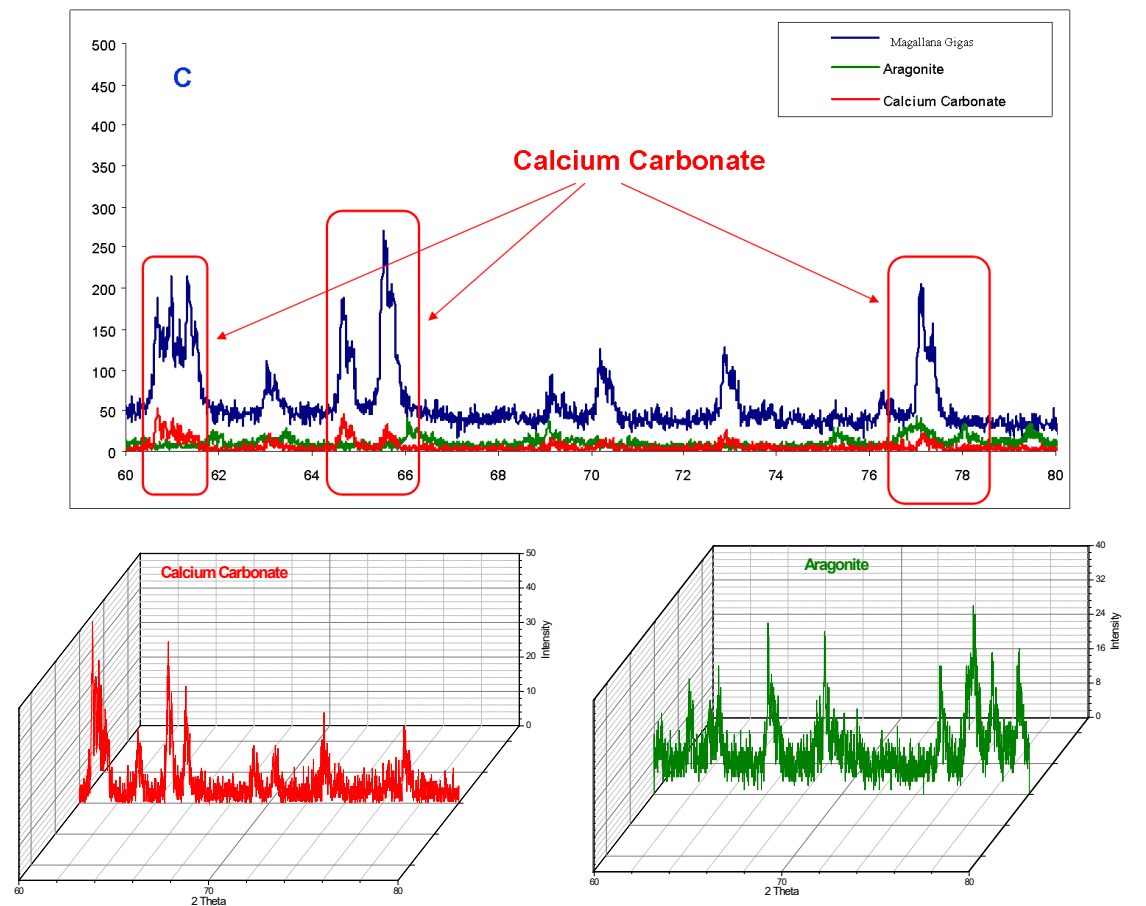


Figure 4. Comparative XRD spectra in the 60° – 80° 2θ range

In Figure 4, XRD analysis of the *M. gigas* shell sample, the 60°–80° 2 θ range was zoomed in on, providing significant insights into the mineralogical structure of the shell. The diffraction peaks observed in this range particularly confirm the presence of crystal phases of CaCO₃. The intense and distinct peaks found in the regions marked with red boxes on the graph reflect characteristic signals of CaCO₃, specifically the calcite form. These peaks are concentrated at approximately 61–62°, 65–66°, and 77.5–78° 2 θ values. These peak points indicate that the crystals in the *M. gigas* shell are highly ordered and highly crystallized. Moreover, the signals in these regions are stronger and more distinct compared to the aragonite phase (green), indicating that the calcite phase is dominant in this range. Additionally, there is a high degree of overlap between the general CaCO₃ (red line) pattern and the sample. Consequently, this analysis shows that the mineral composition of the *M. gigas* shell consists of a high percentage of CaCO₃, which is predominantly in the crystalline calcite form. This structure contributes to the shell durability and provides insights into the direction of calcification.

The preference for CaCO₃ phases in the shells of marine organisms like *M. gigas* can be explained by biological, environmental, and physical reasons. It is well-known that these organisms use their shells as protective structures against environmental influences. The crystal phases of CaCO₃, especially aragonite and calcite, are particularly suitable for providing this protection. The use of CaCO₃ in this form can also provide energy efficiency for the organism. Biological systems require less energy to form these structures, which can be advantageous for survival (Reddy, 2013; Muhammad Mailafiya et al., 2019). Additionally, CaCO₃ crystals isolate the organism internal structure from the chemical effects of the external environment, regulate ion exchange, and can act as a buffer against environmental changes such as salinity. While aragonite has a denser and harder structure, calcite is more stable and durable (McCauley, 1981; Hossain & Ahmed; 2023). These crystal phases enhance the mechanical strength of the shell, providing resistance against cracking and breaking. As a result, this detailed XRD analysis shows that the shell structure of *M. gigas* is largely composed of CaCO₃. This suggests that the crystalline formation of these biogenic structures is directly shaped by environmental conditions and the organism's biomineralization mechanisms. The selection of CaCO₃ phases in the shell structure of *M. gigas* offers numerous advantages, such as maintaining structural integrity, resisting environmental conditions, and making biological processes

energy-efficient. It is proposed that this represents an evolutionary adaptation resulting from an evolutionarily optimized biomineralization strategy.

Conclusion

This study provides important insights into how biogenic materials are optimized through natural processes by characterizing the mineralogical structure of *M. gigas* shells. The results obtained serve as a valuable resource for biomaterial research and the understanding of marine organisms' adaptation mechanisms. In this study, the mineralogical composition of the shell of *M. gigas* was examined in detail using XRD methodology. Biomineralization is a complex process in which organisms integrate inorganic and organic components to produce optimized structures. In the case of *M. gigas*, shell mineralogy reflects both the organism's survival strategy and its environmental interactions. Research in this field offers significant implications for materials science and ecology.

The mineralogical composition of the *M. gigas* shell was analyzed using XRD, and the data obtained revealed that the shell is largely composed of CaCO₃, with the calcite phase being dominant. Detailed examinations of the XRD spectra in the 20°–80° 2 θ range showed high-intensity and sharp characteristic peaks specific to calcite (e.g., 29.4°, 39.4°, 43.2°, and 47.5°), indicating that the shell has a highly crystalline structure. Additionally, peaks corresponding to the aragonite phase (e.g., 26.2° and 45.9°) were detected at low intensity, suggesting that this mineral is present in limited quantities within the shell structure. The narrow and symmetrical nature of the peaks supports the idea that the crystal sizes are homogeneously distributed and that the amorphous phase is minimal. The high intensity and sharpness of the peaks observed in the analyzed regions indicate that the crystalline structure of the *M. gigas* shell is highly crystallized. This regularity in crystal structure is directly related to the material mechanical strength properties. It can be inferred that this provides a significant clue in understanding how the shell structures of marine organisms' function as natural defense mechanisms. The findings show that the shell is predominantly composed of crystalline forms of CaCO₃, particularly aligning with the characteristic peaks of the calcite phase. The low intensity of signals from the aragonite phase suggests that this mineral is present in a secondary capacity in the shell structure. The sharp, symmetrical, and high-density peaks observed in the XRD analysis further confirm the shell's highly crystalline and

homogeneous structure. These findings suggest that the biomineralization process of *M. gigas* occurs through a controlled and optimized strategy. Thus, it can be concluded that the CaCO₃ polymorphs of the *M. gigas* shell is a well-regulated and optimized mechanism.

The fact that the mineralogical composition of the shell is predominantly made up of calcite is considered to provide an evolutionary advantage in terms of mechanical durability, environmental adaptation, and energy efficiency for *M. gigas*. The stable structure of calcite enhances the shell's resistance to external physical and chemical influences, while also allowing the biological production process to require less energy. As a result, this study has detailed the mineralogical composition of the *M. gigas* shell using XRD data and highlighted the importance of this structure in terms of biological resilience and environmental adaptation. The findings contribute to the understanding of biomineralization mechanisms and the field of biomaterials research. In future studies, a more detailed examination of the physical properties of this mineralogical structure (such as hardness, fracture resistance, etc.) and its relationship with environmental factors could provide further insights into the evolutionary and functional significance of these biomineralized shells.

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Compliance With Ethical Standards

Authors' Contributions

HY: Conceptualization, Investigation, Writing- original draft
DŞB: Investigation, Data curation, Formal analysis, Writing – review & editing

BK: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

Generative AI (e.g., ChatGPT 4.0, DeepSeek) was used for grammatical review of the introduction and discussion sections. The authors validated all outputs and assume full responsibility for the content.

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