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Biogenic structure of seashells: Mineralogical and surface properties of *Flexopecten glaber* (Linnaeus, 1758) collected from Bandırma Bay, the Sea of Marmara

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ABSTRACT

The aim of this study was to determine the mineralogical structure and surface properties of sea shells belonging to the species *Flexopecten glaber* (Linnaeus, 1758) and to obtain information about their adaptation to environmental conditions. For this purpose, PZC (point of zero charge) analysis was used to evaluate the surface charge behavior, XRD (X-ray diffraction) to identify the crystalline phases, SEM-EDS (Scanning Electron Microscopy-Energy Dispersive Spectroscopy) to investigate the surface morphology and elemental composition, and FT-IR (Fourier Transform Infrared Spectroscopy) to characterize the functional groups and organic-inorganic components of the shell. As a result of the PZC analysis, the zero point charge of the shell surface was determined to be at pH 8.33, indicating that the surface carries a positive charge below pH 8.33 and a negative charge above this value. XRD analyses revealed that the crystal structure of the shells was predominantly showed as calcium carbonate ($CaCO_3$) form. EDS results showed that the shell composition contains 54.9% oxygen, 31.1% carbon, 7.5% calcium, and 6.1% nitrogen. Additionally, trace amounts of sodium (0.2%), magnesium (0.1%), and sulfur (0.1%) were detected. These data indicate that the shell structure is a complex biomaterial composed of both inorganic and organic components. The results provide important data for understanding the sensitivity of *F. glaber* shells to environmental factors and the responses of marine organisms to environmental changes. This study offers significant scientific contributions for understanding environmental adaptation mechanisms and the sustainable management of marine resources.

INTRODUCTION

Bivalves are a widely distributed group of shelled invertebrates found in both marine and freshwater environments. Species such as oysters, mussels, scallops, and clams are ecologically important, contributing to ecosystem functions like water filtration and nutrient cycling. As filter feeders, bivalves enhance water quality by removing suspended particles from the environment and also serve as a vital food source for various aquatic organisms. Although calcium carbonate is the primary structural component of bivalve shells, trace amounts of various inorganic elements are also present, varying across species. These elements can affect the physical characteristics of the shells and play a significant role in the physiological processes and life cycle of bivalves. Consequently, the study of shell composition offers valuable insights into both the biology of marine organisms and the dynamics of their ecosystems (Chakraborty et al., 2020; Mititelu et al., 2022). The shells of bivalve organisms account for a substantial proportion of their total body weight, typically ranging from 56% to 61%. Composed of approximately 94% calcium carbonate ($CaCO_3$), these shells represent highly mineralized and mechanically robust structures, reflecting their essential protective and structural functions. Marine organisms utilize their shells for both protection and structural support. These shells are formed through the aggregation of calcium carbonate ($CaCO_3$)

crystals, which are embedded within a thin organic matrix. This composite structure enhances resistance to external stressors and contributes to the overall survival and adaptability of the organisms in their natural habitats (Hamester et al., 2012; Ituen, 2015; Chakraborty et al., 2020; Mititelu et al., 2022; Kizilkaya et al., 2024a,b). Marine organisms employ a range of strategies for mineral formation, from passive processes influenced by environmental factors during organomineralization to active biomineralization mechanisms that are biochemically regulated. These processes critically influence the crystal structure, morphology, and functional properties of the minerals, enabling organisms to adapt to their environment and achieve enhanced structural durability (Louis et al., 2022; Chen et al., 2019; Qin et al., 2024). This study investigates the shell structure of the marine scallop species *Flexopecten glaber* from mineralogical and chemical perspectives, revealing how biomaterials are formed through natural processes. The results highlight the adaptation mechanisms of marine organisms to environmental conditions and emphasize the potential of these biomaterials to serve as a natural model for biomaterial research.

MATERIAL AND METHODS

Sample Collection and Preparation

In this study, *F. glaber* shells were collected from Bandırma, Türkiye. The samples were obtained from their natural marine environment and prepared for laboratory analyses. The shells were carefully washed with clean water to remove dirt, algae, and other organic residues, taking care not to damage the mineral structure during this process. After cleaning, the shells were ground into powder of appropriate sizes under laboratory conditions.

Determination of the Point of Zero Charge (PZC) in Shells

The point of zero charge (PZC) was determined by examining the behavior of homogenized and ground *F. glaber* shells under different pH conditions (Mahmood et al., 2011; Kızılkaya et al., 2024a; Kızılkaya et al., 2024b). For this purpose, solutions each with a volume of 100 mL and one gram sample containing 0.01 M KNO_3 were prepared. The initial pH values (pH_i) of the solutions were adjusted to range between 4 and 10 using 0.1 M HCl and 0.1 M NaOH. A specific amount of the modified shell sample was added to each solution with a different pH. After sample addition, the solutions were stirred for 48 hours at a constant temperature using a magnetic stirrer at 30°C and 100 rpm. At the end of the stirring period, the final pH values (pH_F) of each solution were measured and recorded. For each solution, the difference between the initial and final pH ($\Delta pH = pH_i - pH_F$) was calculated and plotted against the initial pH values (pH_i). The point where the resulting curve intersects the x-axis was accepted as the point of zero charge (PZC) of the sample. This method revealed the pH-dependent change of surface charge and allowed the determination of the PZC value, an important parameter for the shell structure.

Chemical Structure Analysis in Shells

Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) analyses were performed to determine the surface morphology and chemical composition of the samples. These analyses were conducted using a JEOL JSM-7100F SEM device located at the Central Research Laboratory of Çanakkale Onsekiz Mart University. The instrument offers a magnification range from ×40 to ×300,000 and an adjustable accelerating voltage between 0.2 and 30 kV. To increase the surface conductivity of the samples and improve image quality, a conductive coating of gold-palladium alloy (80% Au-20% Pd) was applied prior to analysis using a Quorum coating device. During the coating process, a metal film approximately 2-3 nm thick was deposited under a vacuum of 8×10^{-1} mbar/Pa with a current of 10 mA. Fourier Transform Infrared Spectroscopy (FT-IR) analysis using ATR technique was performed to determine the chemical bond structures and functional groups of the shell samples. The analyses were carried out using a Perkin-Elmer Spectrum One FT-IR spectrometer. Spectral measurements were taken within the wavelength range of 650–4000 cm^{-1}. The *F. glaber* shells used in the analysis were previously ground and homogenized, and no additional pre-treatment was applied before measurement. X-Ray Diffraction (XRD) analyses (20-90 theta) were conducted to determine the crystal structures and mineralogical compositions of the shell samples. These analyses were performed using a PANalytical Empyrean XRD device located at the Central Research Laboratory of Çanakkale Onsekiz Mart University.

RESULTS AND DISCUSSION

The point of zero charge (PZC) is a fundamental parameter widely used in the fields of physical chemistry and surface chemistry, and it is particularly important for understanding adsorption processes. PZC refers to the pH value at which the total electrical charge on a solid surface is zero. In other words, at this point, the positive and negative charges on the surface completely balance each other, rendering the surface electrically neutral (Kosmulski, 2002; Zuyi and Taiwei, 2003; Al-Maliky et al., 2021; Kosmulski, 2023). The electrical charges of solid surfaces vary depending on the pH of the solution. If the solution's pH is lower than the PZC value, the concentration of hydrogen ions (H^+) on the surface will be high, causing the surface to acquire a positive charge (Kosmulski, 2002; Kosmulski, 2018; Bayram et al., 2023; Ghaedi et al., 2024; Monroyet al., 2025). This charging behavior directly affects the mechanism by which ions bind to the surface, and therefore, the PZC plays a decisive role in adsorption capacities and selectivities (Faizal et al., 2022; Bayram et al., 2023; de Moura et al., 2023). Therefore, the point of zero charge (PZC) of the surface plays a critical role in thoroughly evaluating surface-adsorbate interactions in adsorption studies (Fiol and Villaescusa, 2008; Mahmood et al., 2011). The PZC value can vary depending on the chemical nature of the material used as the adsorbent, the surface functional groups, and the ionic composition of the solution. For example, the PZC values of natural calcium carbonate-based materials are generally around neutral pH, whereas for some oxide- or carbon-based materials, this value may shift to more acidic or basic pH ranges. Therefore, a specific PZC value should be determined for each material (Khan et al., 2021; Kosmulski, 2021; Fallah et al., 2023; Alkhaldi et al., 2024). The determination of the PZC is generally carried out using potentiometric titration, the salt solution method (commonly using 0.01 M KNO_3), or zeta potential measurements (Tan et al., 2008; Cristiano et al., 2011; Bakatula et al., 2018). One of the most common methods involves adjusting the pH of an electrolyte solution with a constant ionic strength to various values and adding adsorbent samples to these solutions (Mahmood et al., 2011; Kızılkaya et al., 2024a,b). The point where ΔpH equals zero, meaning the initial and final pH values are the same, is considered the point of zero charge (PZC) of the system (Kızılkaya et al., 2024a,b).

In this study, the point of zero charge (PZC) of *F. glaber* shell particles was determined based on their surface characteristics. This analysis was conducted to understand the surface properties of *F. glaber* shell particles. The data presented in the graph illustrate how the electrical charge on the shell surface responds to changes in pH. The PZC value of *F. glaber* shells was found to be 8.33 (Figure 1). PZC refers to the pH value at which the total electrical charge on a surface is neutral, and this value can vary depending on the protonation and deprotonation of chemical groups present on the surface. When the solution pH is below the PZC, the environment contains more protons, causing the surface to attract protons and gain a positive charge. This facilitates the binding of negatively charged ions (anions) to the surface. Conversely, when the solution pH is above the PZC, the surface loses protons and becomes negatively charged, increasing the adsorption of positively charged species (cations). In this context, the shells have been shown to be effective adsorbents with the potential to retain negatively charged anions under pH conditions above 8.0. In Figure 1, the ΔpH results corresponding to different initial pH (pHi) values are presented graphically, and the PZC value determined by linear regression analysis is also indicated on the graph.

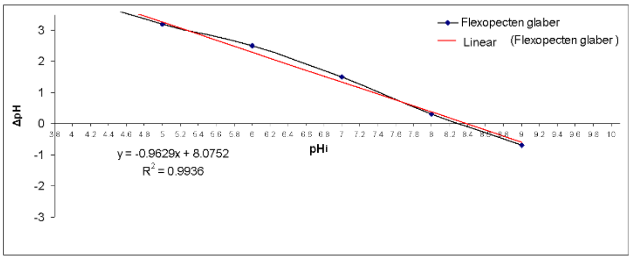


Figure 1. Graphical representation of the zero-charge point of *F. glaber* shell particles

The shells of the species *F. glaber* were analyzed using X-ray Diffraction (XRD), and the obtained data were compared with the crystal forms of calcium carbonate, specifically aragonite ($CaCO_3$). In Figure 2, the XRD spectrum of a mixture consisting of calcite (C) and aragonite (A), provided by the RRUFF project, is presented as a reference for comparison (Lafuente et al., 2015). Reference spectra facilitate the evaluation of the shells' XRD analysis results and help identify the existing structural phases. This method, used to analyze the atomic and molecular structures of shells, is based on the principle that crystals diffract X-rays in a specific order due to their unique atomic arrangements. The resulting diffraction patterns are characteristic to each crystal, acting like a fingerprint that helps distinguish one from another. Calcium carbonate, the main component of bivalve shells, is shaped through a process called biomineralization. The formation of the crystal structure is influenced by various factors such as the species' biological traits, developmental processes, and environmental conditions. In Figure 2, the most intense peak in the XRD spectra corresponds to the calcite peak. Each peak in the XRD spectrum corresponds to the diffraction of X-rays by a specific atomic plane within the mineral's crystal structure. The intensity of these peaks reflects the amount of X-rays diffracted, while the peak angles represent the planes where the diffraction occurs. Since calcite and aragonite have different crystal structures, they exhibit distinct diffraction patterns. Thus, the chemical composition and crystal structure of a mineral can be distinguished through X-ray diffraction. Examination of the XRD spectra shown in Figure 2 reveals that the shell structure of the *F. glaber* species largely consists of the aragonite form of calcium carbonate. Similarly, in Figure 2, blue color represents the analyzed samples, red represents calcium carbonate, and green indicates the aragonite phase.

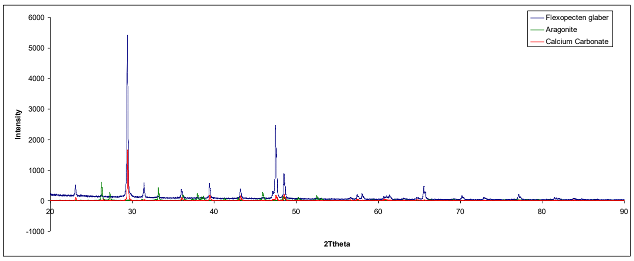
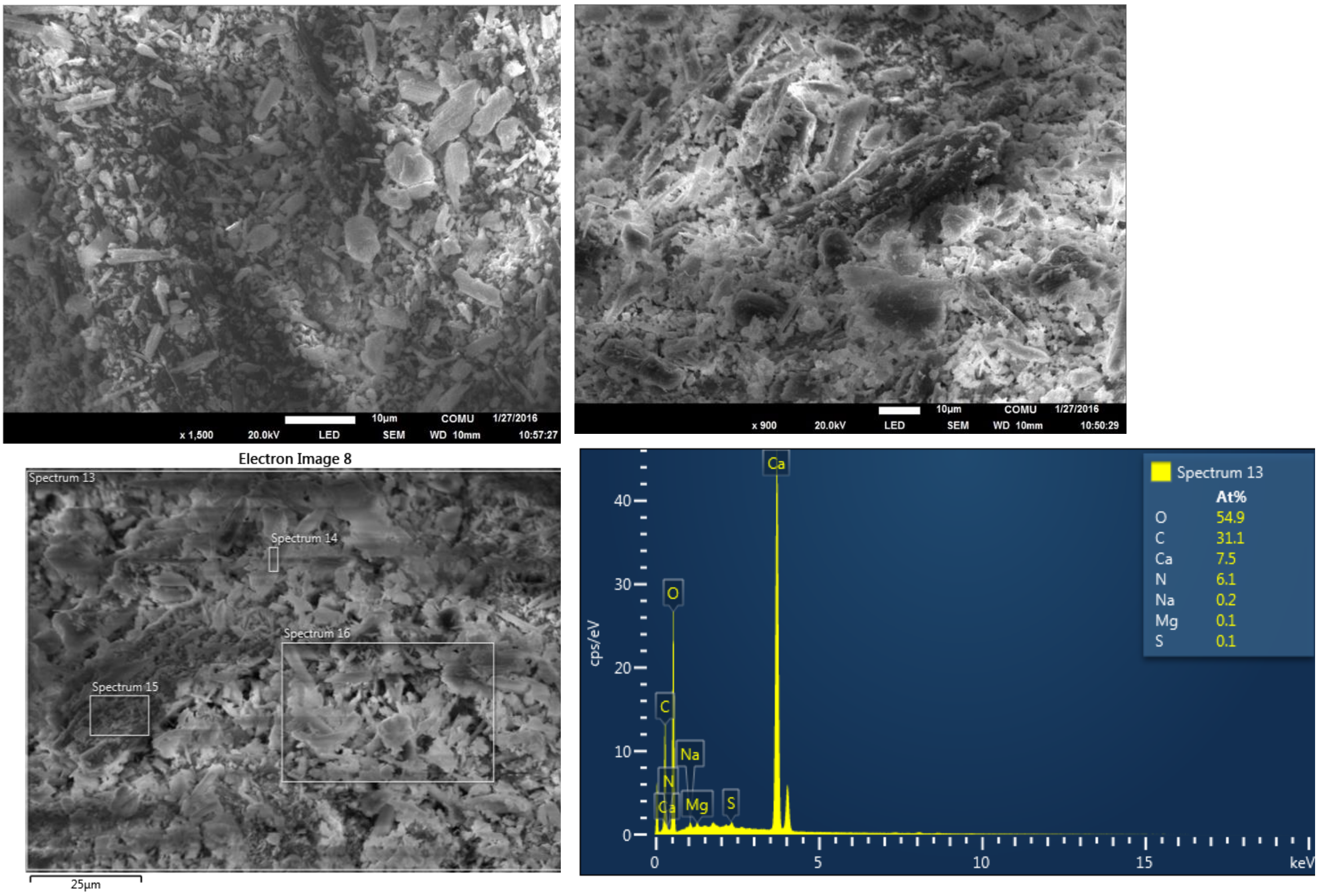


Figure 2. XRD spectra of *F. glaber* shell particles and reference calcium carbonate-aragonite (Lafuente et al., 2015)

Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDS) is an integrated analytical technique that allows detailed examination of a sample surface structure as well as determination of its elemental composition. This method utilizes a focused electron beam directed onto the sample surface to obtain high-resolution surface images, while simultaneously enabling the identification of existing elements. Due to its capability to provide precise surface analyses, SEM-EDS is widely employed across diverse research and industrial fields, including materials engineering, geology, biology, chemistry, and environmental sciences. Its widespread preference stems from offering both morphological and chemical information, allowing a comprehensive evaluation of the sample. Figure 3 presents the SEM and EDS images of the shells. Energy Dispersive Spectroscopy (EDS) is an analytical technique used to determine the elemental composition of a material.



Spectrum 13

	At%
O	54.9
C	31.1
Ca	7.5
N	6.1
Na	0.2
Mg	0.1
S	0.1

Figure 3. SEM and EDS images of *F. glaber* shell particles

The resulting spectrum from this analysis reveals the presence of elements such as carbon (C), oxygen (O), calcium (Ca), magnesium (Mg), sodium (Na), and nitrogen (N). The high intensities of calcium (Ca), carbon (C), and oxygen (O) elements, which are the main components of the $CaCO_3$ structure, indicate that the mussel shells predominantly consist of calcium carbonate. The carbon element is present both in the crystal structure of calcium carbonate and in the organic components of the shell (such as proteins). Oxygen, as an indispensable part of these inorganic structures, occupies a significant place in the spectrum, while calcium is the fundamental inorganic building block of the mussel shell's calcium carbonate. These results reveal that the mussel shells possess a complex structure rich in both mineral and organic components. In this study, the elemental composition of *F. glaber* shell particles was analyzed by Energy Dispersive Spectroscopy (EDS). The analysis results provide important information about the chemical structure of the shell and reveal the effects of environmental factors on the biomineralization process. The elements with the highest proportions in the shell chemical composition were determined as carbon (31.1%) and calcium (7.5%), confirming that the shell primarily consists of calcium carbonate ($CaCO_3$). Calcium carbonate is the most commonly used biomineral in the shell formation of marine organisms and is generally found in aragonite or calcite crystal forms. Nitrogen, detected at 6.1%, indicates a significant presence of organic components in the shell structure. This organic matrix is considered to be conchiolin (a protein organic matrix), a structure mainly composed of proteins. The organic matrix facilitates the orderly precipitation of calcium carbonate crystals, thereby increasing the mechanical strength of the shell. Sodium (0.2%) and magnesium (0.1%) are basic components of seawater and are incorporated minimally into the shell structure. Sulfur (0.1%) may originate from sulfur-containing amino acids in the organic matrix or sulfate ions in seawater. It should be noted that EDS analysis provides surface characterization. Due to the heterogeneous nature of the sample, different compositions may be obtained in different regions. Additionally, difficulties in detecting light elements (especially hydrogen) limit the complete characterization of organic components. These findings contribute to understanding biomineralization processes and offer valuable data for pollution monitoring studies in marine environments.

Fourier Transform Infrared Spectroscopy (FT-IR) is a spectroscopy technique used to examine the chemical bonds and structural characteristics of molecules. In this method, infrared radiation is directed at the sample, and the amount of light absorbed by the molecules is measured as a function of wavelength. These measurements provide detailed information about the chemical composition and functional groups of the sample. FT-IR is widely used in various fields such as biological research, drug development, food analysis, and materials science due to the molecular-level insights it offers. In this study, FT-IR analysis was conducted to identify the main components of the shells. Figure 4 shows the FT-IR spectra of *F. glaber* shell particles. The primary component of the shells, calcium carbonate, was detected through its characteristic absorption bands. The strong band at 1411 cm^{-1} (asymmetric stretching vibration of the carbonate ion), along with bands at 875 cm^{-1} and 712 cm^{-1} (carbonate bending vibrations), indicate the presence of calcium carbonate. These findings demonstrate that the mineral portion of the shell is predominantly composed of calcium carbonate. The bands at 1652 cm^{-1}, 1540 cm^{-1} and 1238 cm^{-1} confirm the presence of a protein-rich organic matrix within the shell structure. These proteins represent the organic fraction of the shell. The broad band at 3440 cm^{-1} (O-H stretching) reflects the hydrated nature of the shell. Similar results were found in the studies of Bayraklı et al. (2024). Furthermore, traces of environmentally derived sulfate and lead accumulation were detected. These findings provide insight not only into the structural features of the shells but also into the chemical conditions of their living environment.

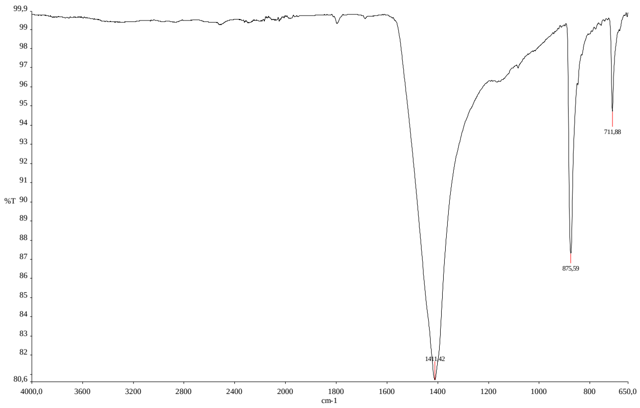


Figure 4. FT-IR spectrum of *F. glaber* shell particles

Calcium carbonate is a widely occurring mineral in nature and plays an important role for living organisms. Its crystal phases, especially aragonite and calcite, possess notable properties essential for protection and structural support of organisms. The crystalline structures of calcium carbonate, such as aragonite and calcite, contribute significantly to the formation of external skeletons and internal frameworks of organisms due to their hardness and durability. Marine shells, corals, and certain algae utilize calcium carbonate to shield themselves from external factors and provide structural integrity. These structures enhance the survival chances of organisms and help maintain balance within ecosystems. Although aragonite and calcite share the same chemical composition, they differ in their crystal structures. Aragonite has an orthorhombic crystal system, whereas calcite is trigonal. This difference influences the physical and chemical properties of both minerals. For example, aragonite is generally less stable and can gradually transform into calcite over time. However, some organisms prefer aragonite because it is thought to precipitate faster and form more complex structures. The use of aragonite and calcite forms of calcium carbonate provides energy efficiency for the organism. Biological systems expend less energy to form these structures, which is a significant advantage, especially for organisms with limited resources. Energy savings allow organisms to allocate more energy to growth, reproduction, and other vital activities, thereby increasing their chances of survival and reproductive success in competitive environments. The processes by which biological systems produce calcium carbonate are also noteworthy. Organisms control the precipitation of calcium carbonate by utilizing calcium and carbonate ions from seawater or other sources. This process is often guided by proteins and other organic molecules, enabling organisms to create calcium carbonate structures of desired shapes and sizes. In conclusion, the crystal phases of calcium carbonate such as aragonite and calcite are crucial for protection, structural support, and energy efficiency in living organisms. These minerals enhance the survival and reproductive success of many organisms, ranging from marine shells to corals. A better understanding of the role of calcium carbonate in biological systems is important for both basic and applied sciences. In particular, comprehending biomineralization processes can inspire the design and development of new materials. Furthermore, the significance of calcium carbonate should not be overlooked in efforts to conserve marine ecosystems and achieve sustainable development goals (Reddy, 2013; Muhammad Mailafiya et al., 2019; Hossain and Ahmed; 2023).

CONCLUSION

This study examines the mineralogical and surface properties of *F. glaber* shells, shedding light on the natural optimization of biogenic materials. The shells are mainly composed of calcium carbonate ($CaCO_3$), primarily in the forms of aragonite and calcite, along with trace minerals that influence their mechanical strength and resistance to environmental factors. Factors such as temperature, salinity, and pH affect the shell's mineral composition, and *F. glaber* can modify its shell structure to adapt to changing conditions. Surface characterization identified a point of zero charge (PZC) at pH 8.33, indicating that the shell's electrostatic properties vary with environmental pH. X-ray diffraction (XRD) confirmed the dominance of aragonite crystals, while scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) revealed a multilayered surface structure and the presence of a protein-rich organic matrix. Fourier Transform Infrared Spectroscopy (FT-IR) further confirmed the composite nature of the shells, consisting of both inorganic minerals and organic compounds. Overall, the study provides valuable data on the adaptation mechanisms of marine organisms and offers a scientific basis for biomaterial research and marine conservation.

Compliance with Ethical Standards

Authors' Contributions

HY: Conceptualization, Investigation, Methodology, Writing-review & editing, Supervision, Funding acquisition, Project administration

BK: Conceptualization, Writing-original draft, Data curation, Formal Analysis, Visualization, 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

The authors declare that formal consent is not required for this type of study.

Data Availability

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

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