Identification of CaCO₃ polymorphs of shellfish by FTIR spectroscopy and evaluation of metals adsorption by powdered exoskeleton shell

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Shellfish are found in the coastal region throughout the tropical, sub-tropic and temperate zone. A variety of shellfish species are known in nature. Being filter-feeding organisms, organic and inorganic substances easily gain entry into them. Very often, heavy metals such as Cd, Pb, Cu, and even Hg can accumulate in their bodies. The presence of these heavy metals in shellfish is an indicator of environmental pollution, and a threat to humanity as these fish are widely consumed in coastal regions. Here, biochemical analysis of exoskeleton shell mainly composed of polysaccharides, proteins and calcium carbonate (CaCO₃) - have been studied. Calcium carbonate (CaCO₃) which is one of the major constituent of the exoskeleton part of the shellfish exists in three polymorphs viz., calcite, aragonite and vaterite. The identification of CaCO₃ polymorphs in shellfish is extremely important as it gives information on the structural arrangement of the calcium carbonate lattice in these organisms. Here, we present a convenient method for the identification of CaCO₃ polymorphs in shellfish collected from Goa, west coast of India, with Fourier-Transform Infrared Spectroscopy (FTIR) and further confirmed by powder X-ray analysis. Additionally, the potential of the powdered shell biomass for metal removal from an aqueous medium has also been investigated.

[Keywords: Bio-mineralization, Calcium carbonate, Infrared spectroscopy, Polymorph, Shellfish]

Introduction

Shellfish are commonly found in the coastal regions, and their various species are known in nature. They do not only form an essential cuisine for coastal people but are also a source of nutrients. Being filter-feeding organisms, organic and inorganic substances can easily enter their body leading to the accumulation of toxic materials, such as organic pollutants and heavy metals, including Cd, Pb, Hg, etc. The bioaccumulation of these compounds is an immense threat to human beings as a vast population in the coastal region feed on shellfish. The exoskeleton of shellfish is chiefly composed of polysaccharides, such as chitin, chitosan, proteins and calcium carbonate (CaCO₃). Intriguingly, several marine organisms, including shellfish, produce CaCO₃ using a sophisticated bio-mineralization process¹,², and it was claimed that biologically produced CaCO₃ have superior mechanical properties compared to the naturally occurring CaCO₃ (refs. ³,⁴). The biomineralization of CaCO₃ by marine organisms is vital in the carbon cycle for the fixation of carbon dioxide from the atmosphere. Further, the mechanical properties of CaCO₃ lattice depend on several factors, e.g. size and arrangement of crystal units, polymorph composition (calcite/aragonite ratio), degree of calcium substitution in CaCO₃ by Mg and Sr, and the quality and quantity of organic matrix⁵,⁶. Although mineralogy in shells is mainly biologically controlled, the chemistry and environmental factors also play an essential role. Indeed, CaCO₃ crystal size and structure are partially determined by environmental factors, including pH, salinity and temperature⁷,⁸. For instance, a higher water temperature leads to enhanced aragonite/calcite proportion, while low salinity reduces its proportion⁹,¹⁰. On the other hand, a decrease in pH in the environment can alter the composition and mechanical properties of shellfish¹¹.

CaCO₃ exists in three significant polymorphs, viz. aragonite, calcite and vaterite, of which aragonite and calcite are more stable and commonly found in shells. Calcite has trigonal symmetry, whereas aragonite has orthorhombic symmetry, a compact structure composed of triangular carbonate ion groups (CO₃²⁻), with carbon at the centre of the triangle and three oxygen atoms at each corner. Aragonite can be transformed into more stable calcite under physical and chemical conditions. In nature, aragonite is readily converted into calcite at a temperature above 380 °C (ref. ¹²). Previously, Checa et al. reported that by
Numerous methodologies are utilised for identifying CaCO₃ polymorphs, such as FTIR, X-ray diffraction and surface analysis techniques, namely XPS and TOF-SIMS. Although the FTIR technique is often used for the identification of the carbonate polymorph of CaCO₃, its utility in the identification of the carbonate polymorph present in shellfish is scarce. The swift and cost-effective identification of the CaCO₃ structure is necessary to understand the nature of carbonate allotropy of shells as it could correlate to the metal accumulation in the shellfish exoskeleton. For instance, aragonite had higher adsorption affinity towards Cd²⁺, whereas calcite had higher adsorption capacity toward Pb²⁺. Thus, structural information of the shell exoskeleton could give preferential metals that could accumulate into the shell biomass.

An extensive study was carried out on the biochemical analysis of shellfish to understand their nutritional value. Moreover, the application of chitosan extracted from shellfish exoskeleton has been studied in various domains, such as catalysis and materials and biomedical applications. However, a limited study is done on the application of shell biomass for removing metals and organic contaminants. In 2014, Wu et al. reported the removal of Cu by the exoskeleton of waste oyster shells. In 2019, aragonite crystals synthesized from egg shells were employed for the efficient removal of heavy metals, such as cadmium and lead. Heavy metals removal by waste shell biomass is an attractive and viable method as shell wastes are cheap and readily available. Furthermore, finding a sustainable use of these shell wastes could help in waste management through routine harvesting of the biomass.

In the present study, a fast and cheap identification method for CaCO₃ polymorphs, present in shellfish collected from Goa, has been described. Moreover, the exoskeleton biomass of shells has been investigated for metal adsorption from an aqueous solution.

Materials and Methods
The shells used in this study were either collected from the Goa coast or obtained from the local market. Shellfish studied in this study include Oyster (Villortia cyprinoids), Clam (Meretrix casta and Perna erosa), molluscs (Perna viridis), gastropods (Bolinus brandaris) and an unidentified gastropod sp. In the case of clams, 2-3 organisms were sampled, whereas for other organisms, only one sample of each organism was analysed. Shells were thoroughly washed with water, and the exoskeleton was separated from the soft body matrix. The exoskeleton was treated with dilute HNO₃ (5%), washed several times with distilled water and finally, dried in a hot air oven. The dried exoskeleton was grounded to powder by a mechanical rotor. The powder was directly used for FTIR and metal adsorption studies.

FTIR spectroscopy
FTIR spectra were recorded in the 400 – 4000 cm⁻¹ spectral region at room temperature using about 1 mg of sample mixed with KBr (1:20) and grounded into powder in a mortar. Spectra were recorded at 4 cm⁻¹ resolution with 20 scans on a Shimadzu Infinity-I infrared spectrophotometer equipped with Diffuse Reflected Spectrophotometer assembly, whereas pH and the Total Dissolved Solids (TDS) were measured using the OKTON-510 bench top instrument.

Determination of metal adsorption by shell biomass
1 g of the salt was dissolved in 1000 ml of distilled water. Then 50 ml of each of the metal solutions were added to 1 g of the grounded calcite/aragonite sample in a 100 ml conical flask. The flasks were covered and kept aside for 2 days. After 2 days, the solution was filtered. The pH and TDS were recorded for the standard metal solution as well as the solution obtained after filtration through a column packed with shell biomass.

Results and Discussion
FTIR spectroscopy was used to identify CaCO₃ polymorphs present in the shellfish collected from the Goa coast. FTIR is a powerful tool for the identification of allotropes of crystalline carbonate viz. calcite, aragonite and vaterite. Usually, calcite structure showed IR absorption frequencies for the carbonate ion at around 1087 (v1), 881 (v2), 1432 (v3) and 713 (v4) cm⁻¹. In the case of aragonite, the absorption at 713 (v4) split into two peaks at 710 and 714 cm⁻¹, which is characteristic of the aragonite structure, whereas for vaterite absorption peak for v4 appeared at 741 cm⁻¹ (Table 1).

In this work, the dried and powdered exoskeleton of shellfish was used to study the carbonate stretching frequency in the mid-IR region. Figure 1(a, b) showed
the FTIR spectra of shellfish in the carbonate stretching frequency region. The significant absorption frequencies (cm\(^{-1}\)) of shells under investigation are summarized in Table 2. The FTIR spectrum of powdered oyster (*Villortia cyprinoids*) contained two broad bands at 1083 and 877 cm\(^{-1}\) assignable to v(1) and v(2), respectively. The strong absorption band at around 1427 cm\(^{-1}\) and a weak band at 713 cm\(^{-1}\) are due to the normal mode of vibrations, v3 and v4, respectively. The spectrum showed the characteristic calcite band for v2 at 877 cm\(^{-1}\), and a single band for v1 at 713 cm\(^{-1}\), confirming the calcite nature of carbonate polymorph for *Villortia cyprinoids*.

In the case of clam, *Meretrix casta* and *Perna erosa*, the FTIR spectrum displayed characteristic aragonite absorption bands at 862 cm\(^{-1}\) and 864 cm\(^{-1}\), respectively and a weak peak corresponding to v1 vibration appeared at around 712 cm\(^{-1}\), which split into two bands (711 and 700 cm\(^{-1}\)). Similarly, the FTIR spectra of *Perna viridis*, *Bolinus brandaris* and the unidentified gastropod showed a similar pattern of the

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**Table 1 — Fourier transform stretching frequency (wave numbers in cm\(^{-1}\)) of carbonate ion for calcite, aragonite and vaterite\(^{[a]}\)**

<table>
<thead>
<tr>
<th>S No.</th>
<th>Calcite</th>
<th>Aragonite</th>
<th>Vaterite</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(v_1)</td>
<td>(v_2)</td>
<td>(v_3)</td>
<td>(v_4)</td>
</tr>
<tr>
<td>1</td>
<td>874</td>
<td>1430</td>
<td>710</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1087</td>
<td>881</td>
<td>1432</td>
<td>712</td>
</tr>
<tr>
<td>3</td>
<td>870</td>
<td>1430</td>
<td>1430</td>
<td>715</td>
</tr>
<tr>
<td>4</td>
<td>1079</td>
<td>876</td>
<td>713</td>
<td>712</td>
</tr>
</tbody>
</table>

\(^{[a]}\)Source: Anderson & Breccevic (1991) references therein\(^{17}\)

![Fig. 1 — a) FT-IR spectra of: Clam (*Meretrix casta*) (up), Mollusc (*Perna viridis*) (down); and b) FT-IR spectra of: oyster (*Villortia cyprinoids*) (up), Gastropod (*Stramonita haemastoma*) (down)\(\)](image-url)
FTIR bands confirming the carbonate present in them is of aragonite polymorph (Table 2).

The polymorph nature inferred from FTIR spectra was corroborated by powder XRD analysis. Powder XRD of CaCO₃ crystals exhibited prominent peaks of 2θ at 29.4° for calcite, whereas aragonite exhibited two strong peaks of 2θ at 26.2° and 27.2°, respectively. In the powdered X-ray pattern of the oyster sample, *Villortia cyprinoids* exhibited a strong peak for 2θ at 28.6° (Fig. 2), on the other hand, clam sample, *Meretrix casta* showed two peaks for 2θ at 26.7° and 28.2° (Fig. 3) confirming that CaCO₃ polymorph present in *Villortia cyprinoids* is calcite and that of *Meretrix casta* is aragonite. The powder XRD graphics of *Villortia cyprinoids* and *Meretrix casta* are shown in Figures 2 & 3, respectively. The XRD pattern of *Villortia cyprinoids* and *Meretrix casta* are in agreement with the calcite and aragonite structures.

The metal absorption property of the exoskeleton powder was investigated by measuring the pH and TDS of the standard solution (control) and solution after treatment with shell powder. In a typical experiment, the pH of salt solutions, namely, FeCl₃(anhy), Fe(NO₃)₃.9H₂O, Cu(NO₃)₂.3H₂O, CdCl₂.H₂O and Pb(NO₃)₂ were measured before and after the treatment with the shell powder, viz. calcite and aragonite carbonates. As evident from Table 3, the pH of FeCl₃ (anhy.) standard solution (pH ≈ 2.65) increased to 7.97 and 7.94, post-treatment with the shellfish powder oyster (calcite) and clam (aragonite), respectively, indicating a significant absorption of iron by the shell powder. The increase in the pH was most significant for FeCl₃ (anhy.) and the least for Pb(NO₃)₂ (Table 3). The metal absorption property of the shell powder was also studied by measuring the TDS of the standard solution and the metal solution after treatment with the shell powder (calcite and aragonite). TDS of the metal solution decreased.

### Table 2 — The infrared peaks for various shells collected from Goa in KBr (500-4000 cm⁻¹ ranges)

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of edible shell</th>
<th>IR Band (cm⁻¹)</th>
<th>Asymm. and bending frequency of CO₃²⁻ (cm⁻¹)</th>
<th>Allotrope type of carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oyster <em>(Villortia cyprinoids)</em></td>
<td>2513, 1795, 1685, 1512, 1427, 1159, 1083, 1010, 877, 1427, 1083, 1010, 877, 713</td>
<td>1483, 1082, 862, 713, 700</td>
<td>Calcite</td>
</tr>
<tr>
<td>2</td>
<td>Clam <em>(Meretrix casta)</em></td>
<td>2546, 2520, 2497, 1786, 1483, 1082, 862, 713, 700</td>
<td>1483, 1082, 862, 713, 700</td>
<td>Aragonite</td>
</tr>
<tr>
<td>3</td>
<td>Clam <em>(Perna erosa)</em></td>
<td>2546(m), 2520(m), 2497(m), 1786(s), 1435(s), 1082(m), 864(s), 844(w), 713(s), 700(m)</td>
<td>1483, 1082, 862, 713, 700</td>
<td>Aragonite</td>
</tr>
<tr>
<td>4</td>
<td>Mollusc <em>(Perna viridis)</em></td>
<td>2546(m), 2520(m), 2497(m), 1786(s), 1479(s), 1082(m), 862(s), 844(w), 713(s), 700(m)</td>
<td>1479(s), 1082(m), 862(s), 844(w), 713(s), 700(m)</td>
<td>Aragonite</td>
</tr>
<tr>
<td>5</td>
<td>Gastropod*</td>
<td>2546, 2520, 2497, 1786, 1483, 1082, 862, 844(vw), 711, 700</td>
<td>1481, 1082, 862, 844(vw), 711, 700</td>
<td>Aragonite</td>
</tr>
<tr>
<td>6</td>
<td>Gastropod <em>(Bolinus brandaris)</em></td>
<td>2542, 2520, 2498, 1786, 1082, 862, 713(m) and 700 (m)</td>
<td>1483(s), 1082(m), 862(m), 713(m) and 700 (m)</td>
<td>Aragonite</td>
</tr>
</tbody>
</table>

*Species not identified; Abbreviations: s - strong; m - medium; w - weak; vw - very weak
significantly from the corresponding standard solution after treatment with the shell powder (Table 4). The results showed that the exoskeleton of shellfish, which is usually discarded, is good metal absorber. Hence, the exoskeleton of shell has the potential as an alternative method to remove metals/heavy metals from water. The powdered shell may be explored for the treatment of toxic metals from industries, especially, electroplating industries, for the removal of metals from its outlet before entering the environment.

**Conclusion**

This communication presents a fast identification of CaCO₃ polymorphs present in shellfish by FTIR. Carbonate polymorphs present in clams, molluscs and gastropods are primarily aragonite in nature, whereas in oyster, it was found to be calcite. FTIR gave fast and cost-effective structural information for the identification of carbonate polymorphs present in shellfish. This identification was confirmed by XRD analysis of the dried and powdered exoskeleton shell. Further, the shell biomass was studied for metal adsorption using TDS and was found to be effective for the removal of metals thus; the biomass can be used for the treatment of water contaminated by metals/heavy metals. Therefore, this study enables an understanding of the structural arrangement of CaCO₃ in shellfish and the possible use of these waste materials for metal/heavy metal absorption from polluted water. It can be further explored as a remedial measure to treat the contaminated water released from electroplating industries before entering the environment.

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**Conflict of Interest**

The authors have no conflict of interest to declare.

**Ethical Statement**

This work does not involve animal experiments which need ethical approval.

**Author Contributions**

KSS designed the experiments, analysed the data and wrote the manuscript. SGS performed the experiments and helped with data analysis.

**References**