

# **STUDY OF BIOMINERALIZATION IN MOLLUSCAN SHELL – COMPARATIVE ANALYSIS BETWEEN CLASS BIVALVA AND GASTROPODA**

Submitted in partial fulfilment of the requirements for the award of  
Bachelor of Technology degree in Biotechnology

By

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**APRIL – 2024**



# **SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY**

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**BONAFIDE CERTIFICATE**

This is to certify that this project report is the bonafide work of **SURIYARAJ S A S (40230046)**, who carried out his project work entitled **"STUDY OF BIOMINERALIZATION IN MOLLUSCAN SHELL – COMPARATIVE ANALYSIS BETWEEN CLASS BIVALVA AND GASTROPODA"** under our supervision from September 2023 to April 2024.

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**Submitted for Viva voce Examination held on** 06/04/2024

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## **DECLARATION**

I, Suriyaraj S A S (40230046), hereby declare that the project work entitled “**STUDY OF BIOMINERALIZATION IN MOLLUSCAN SHELL – COMPARATIVE ANALYSIS BETWEEN CLASS BIVALVA AND GASTROPODA**” done by me under the guidance of **Dr. D. Inbakandan** (external guide) and **Dr. S. Usha Nandhini** (internal guide) at Centre for Ocean Research, Sathyabama Research Park, Sathyabama Institute of Science and Technology is submitted in the partial fulfillment for the requirements of Bachelor of Technology degree in Biotechnology.

**Date:**

**Place:** Chennai

**SIGNATURE OF THE CANDIDATE**

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## **ABSTRACT**

Mollusk inhabit coastal regions across tropical sub-tropical and temperate zones, showcasing diverse species. As filter feeders, they readily absorb organic and inorganic substances. During its different stages of life, it has undergone distinct development of shells and its associated structures. Strength of shells depend upon the composition of the shells. Compared to the early life stages shells were become harder in later stages. In this study we analyzed the composition of shells at three different stages of gastropod *Chicoreus virgenus* and bivalve *Perna viridis*. Spectroscopic analysis of  $\text{CaCO}_3$  reveals two different polymorphs: calcite and aragonite. Identifying these polymorphs is crucial for understanding the structural arrangement of the calcium carbonate lattice within the shells. Analyzed shells with three different spectroscopic technique such as FTIR, XRD and Raman spectroscopy showed significant variation in the composition of calcite and aragonite. Result indicates that green mussel with absence of calcite while gastropods showing presence of both calcite and aragonite.

### **Keywords:**

Biom mineralization, Calcium Carbonate, Crystal Structures, Mollusc Shells, Comparative Analysis.

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## LIST OF ABBREVIATIONS:

|      |                                            |
|------|--------------------------------------------|
| FTIR | Fourier Transmission Infrared Spectroscopy |
| SMP  | Shell Matrix Protein                       |
| vSub | Ventral Subiculum                          |
| XRD  | X- ray Diffraction                         |
| BLA  | Basolateral amygdala                       |
| HFS  | High Frequency                             |
| LFS  | Low Frequency                              |

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## **CHAPTER – 1**

### **INTRODUCTION**

Biomineralization is the formation of Calcium carbonate structures by organisms to protect themselves or perform a life function. These structures are majorly seen in marine organism such as sea sponges, corals, mollusc etc as such. Due to the marine environment being slightly basic the formation of these structures is favored greatly.

Mollusc are one major organism that produces carbonate structures within their body cavity. These shells are full of microstructures that are inter connected to form a bigger shell and increasing layers added to the present shell layers during growth period. The presence of different minerals within the shell aggloramate determine the color and other factors. Some major organisms under this family are bivalve and gastropods. The most common microstructure being Aragonite and Calcite.

Bivalvia or bivalve is a class of mollusc found in marine and fresh waterbodies, where they are attached to an anchor for support or buried under sand. Their bodies are compressed between two shells with a bilateral symmetry and a hinge at its dorsal point. The shell opens for ingestion of food and other activities. This compression helps it against strong tides and predators as such. Some major examples include Oysters, Mussels, clams, and scallops.

Certain types of bivalves that are seen to have an attachment on a support has seen to have a flat side compared to its counterparts who have high mobility, these factors of sedentary and others affect the shape and composition of Bi valve structures (Regan et al., 2021). Identification of species based on color of the shell is inconsistent as study shows same species had a different coloration pattern within themselves. (Reunov et al., 2021).

Gastropoda forms their shell in a spiral pattern creating a torsion majorly in the right-hand direction. These organisms are generally called sea snail and feed on phytoplankton and zooplanktons. These organisms being motile moves at a very slow

pace. Some gastropod buries themselves in the sand to hide from predators and harsh tides. The ability of a mollusk to form a shell to protect itself from predators and harsh environmental factors. This process of creating a calcium outer shell is called Biomineralization. This happens as a multi-process step.

The process starts with protein and other organic compounds formed into a net-like matrix called SMP (Shell matrix protein) around the area of requirement. Apart from shell formation these SMPs also help the mollusk in the development of immunity and environmental adaptation (Arivalagan et al., 2017). The region created by SMPs is called privileged space (Gilbert et al., 2022) where calcium and  $(\text{CO}_3)^{2++}$  ions are filled to create multiple layers of bio composites to form a shell (Yarra, Blaxter, et al., 2021).

Seawater has a lower concentration of  $(\text{CO}_3)^{2++}$ , thus the organism raises the amounts of  $(\text{CO}_3)^{2++}$  inside the shells to bring the calcium ratios to an even level (Gilbert et al., 2022). This is achieved with the help of extrapallial fluid in marine mollusks. It contains a mixture of protein polysaccharides and altered carbon chemistry within itself (Langer et al., 2018).

The calcium and carbonate ions present in the extrapallial fluid create calcium carbonate in various steps. Calcite and aragonite are thermally stable forms of calcium, but there are other states as well, including amorphous calcium carbonate, which is meta-stable and serves as a precursor to calcite and aragonite (Huang et al., 2018). The presence of water is not significant but plays a role in the hydration of the protein in the non-classical pathway and the nucleation process of the shell formation. (Lu et al., 2021). Based on the types of organic matrix created the composition of the Mg, Sr, and Ca varies. This determines whether the matrix is soluble or insoluble which tells us the ratio of metals present in it (Schnecko et al., 2010). The presence of these metals also helps us in determining the calcification rate or rate of crystal formation where Sr is a major player (Carré et al., 2006). This aragonite shell formed through the process of biomineralization was studied and the mechanical strength was far superior compared to synthetic aragonite.

The dorsal mantle epithelium produces these SMP macromolecules. The gene mantle secretosome determines and regulates the deposition rate and the pattern of

the shell (Kocot et al., 2016). The process of deposition starts with periostracum a non-mineralized layer followed by the prismatic layer and the nacreous layer where aragonite crystals are formed (Suzuki et al., 2017).

Due to weather conditions or availability of nutrients the deposition pattern of the shell changes. Presence of Mg ions within the shell matrix produces a different shell composition and pattern. Nutrient intake such as carotenoid and other minerals helps in determining the vibrancy and shell pattern and color while it is formed.

This study helps in studying these local colonies and creates a bookmark of data acting as a reference for future.

## CHAPTER – 2

### LITERATURE SURVEY

#### **2.1 BIOMINERALIZATION MECHANISM**

Bivalves have evolved a range of complex shell forming mechanisms that are reflected by their incredible diversity in shell mineralogy and microstructures. A suite of proteins exported to the shell matrix space plays a significant role in controlling these features, in addition to underpinning some of the physical properties of the shell itself. There is a consensus that a minimum basic protein tool kit is required for shell construction, to date, this remains undefined. In this study, the shell matrix proteins of four highly divergent bivalves were analyzed in an identical fashion using proteomics pipeline. This enabled us to identify the critical elements of a "Basic tool kit" for calcification processes, which were conserved across the taxa irrespective of the shell morphology and arrangement of the crystal surfaces. (Arivalagan et al., 2017)

Calcium carbonate biomineralizing organisms have played major roles in the history of life and the global carbon cycle during the past 541 Ma. Both marine diversification and mass extinctions reflect physiological responses to environmental changes through time. An integrated understanding of carbonate biomineralization is necessary to illuminate this evolutionary record and to understand how modern organisms will respond to 21st century global change. Biomineralization evolved independently but convergently across phyla, suggesting a unity of mechanism that transcends biological differences. The model provides a framework for understanding the environmental sensitivity of marine calcifiers, past mass extinctions, and resilience in 21st century acidifying oceans. (Gilbert et al., 2022)

Mollusc shells are a result of the deposition of crystalline and amorphous calcite catalyzed by enzymes and shell matrix proteins. Despite this, a minimal biomineralization toolbox comprising proteins and protein domains critical for shell

production across species has been identified. Using a matched pair design to reduce experimental noise from inter-individual variation, combined with damage-repair experiments and a database of biomineralization SMPs derived from published works, proteins were identified that are likely to be involved in shell calcification. These data expand the number of candidates biomineralization proteins in bivalve molluscs for future functional studies and define a minimal functional protein domain set required to produce solid microstructures from soluble calcium carbonate. (Yarra, Blaxter, et al., 2021)

Inferring features of a biomineralization mechanism from partitioning is complicated, because not all partitioning coefficients show vital effects in every calcium carbonate producing organism. The partitioning coefficient is not the only aspect of partitioning. Polymorph specificity of Me partitioning was explicable in terms of inorganic precipitation in the case of Sr and Mg, but not Li and B. Seawater carbon chemistry did not have the effect on B partitioning that was expected based on data on inorganic precipitates and foraminifera. (Langer et al., 2018)

The relationships with crystal growth rate  $G$  were investigated thanks to well defined periodic growth structures providing a precise shell chronology. Our results show that for both species, environmental parameters only have minor influence, whereas crystal growth rate strongly influences trace elements concentrations, especially for Sr. The relationship between  $G$  and Sr/Ca exhibits variability among the shells as well as inside the shells. For a same growth rate value, Sr/Ca values are higher in more curved shell sections, and the growth rate influence is stronger as well. (Carré et al., 2006)

## **2.2 *ENVIRONMENTAL FACTORS LEADING TO VARIATION***

The transformation of the original signal by the wavelet results in coefficients, which are another expression of the signal. In the discrete wavelet transform, two wavelets are used: the mother wavelet and the scaling function. In conclusion, the

signal is decomposed in two series of coefficients for each scale of observation. (Verrecchia, n.d.)

There is a relation between extrapallial fluid pH and the opening/closing behavior of the shell valves of *Crassostrea rhizophorae* when exposed to air. During a 2 hr period of emersion EPF (Extra Pallial Fluid) pH fell from a mean of 7.58 to 7.15; reflecting an onset of anaerobiosis and a buildup of acidic metabolites. Although pH continued to fall during emersion, air-gaping behavior facilitated the maintenance of a relatively high pH. Full valve adduction did not appear to be correlated with a specific EPF pH value. (Littlewood & Young, 1994)

The inorganic composition of the extrapallial fluids of *Mercenaria mercenaria*, *Mytilus edulis* and *Crassostrea virginica* was significantly different from sea water. Calcium was the principal ion bound in the extrapallial fluids. This binding was accomplished by a non- dialyzable component that appeared to be a glycoprotein (Carré et al., 2006).

Numerous recent and fossil molluscs have microstructural development increments inside their shells, which are thought to be reflections of alternating phases of shell deposition and disintegration that took place during aerobic and anaerobic respiration, respectively. The calcium carbonate from the shell neutralizes the acidic byproducts of anaerobic metabolism, leaving a somewhat insoluble organic residue at the mantle-shell contact. This organic material is reincorporated inside the shell with the restoration of oxygenated conditions and the restart of aerobic respiration. We are led to the almost paradoxical conclusion that, due to shell-destructive processes, a relatively complete and detailed record of both short- and long-term growth is frequently preserved within the molluscan exoskeleton. This is because metabolic changes are frequently synchronized with lunar or solar cycles (or both). (Lutz & Rhoads, 1977)

### **2.3 GEOGRAPHIC VARIABILITY**

We find shell calcification decreased towards high latitude, with mussels producing thinner shells with a higher organic content in polar than temperate regions. In polar, subpolar, and Baltic low-salinity environments, mussels produced thin shells with a thicker external organic layer, and an increased proportion of calcite and organic matrix, providing potentially higher resistance against dissolution in more corrosive waters. Conversely, in temperate, higher salinity regimes, thicker, more calcified shells with a higher aragonite proportion were deposited, which suggests enhanced protection under increased predation pressure. Interacting effects of salinity and food availability on mussel shell composition predict the deposition of a thicker periostracum and organic-enriched prismatic layer under forecasted future environmental conditions, suggesting a capacity for increased protection of high-latitude populations from ocean acidification. (Telesca et al., 2019)(Telesca et al., 2019)

Molluscs use controlled calcium carbonate crystal deposition inside a protein framework as part of their biomineralization process to create intricate bio composite structures. Aquaculture and animal resilience in the face of future climate change depend heavily on effective biomineralization. A lack of fundamental understanding of the genes and processes involved prevents us from fully comprehending biomineralization, despite the fact that many enzymes and structural proteins from the shell and mantle tissue have been found. During development, maintenance, and repair in adult bivalves, the mantle tissue secretes the shells; the healing process is particularly accessible to experimental dissection at the transcriptome level in individual animals. (Yarra, Ramesh, et al., 2021)

Bivalves, from raw oysters to steamed clams, are popular choices among seafood lovers and once limited to the coastal areas. Over the years, oysters, mussels, scallops, and clams have been the focus of research for improving the production, managing resources, and investigating basic biological and ecological questions. One aspect that is also taking momentum is the use of bivalves as a model system for

human health. In this review, we highlight some of the aspects of the biology of bivalves that have direct implications in human health including the shell formation, stem cells and cell differentiation, the ability to fight opportunistic and specific pathogens in the absence of adaptive immunity, as source of alternative drugs, mucosal immunity and, microbiome turnover, toxicology, and cancer research. (Fernández Robledo et al., 2019)

Bivalve *Arctica islandica* shells are employed as biogeochemical proxies, or biogenic elements that are closely connected to environmental factors at the time of shell formation, to reconstruct paleo-environmental conditions (for example, temperature). Numerous studies have demonstrated that shell development and microstructure can have an impact on proxies like element and isotope ratios. To verify these biogeochemical proxies for a wider variety of environmental circumstances, it is crucial to assess the influence of changing environmental factors such as high pCO<sub>2</sub> and subsequent changes in carbonate chemistry on shell characteristics. Growth tests with *Arctica islandica* from the Western Baltic Sea held at various pCO<sub>2</sub> levels (from 380 to 1120 atm) demonstrate no effect of increased pCO<sub>2</sub>, showing that *A. islandica* shows no signs of being affected by elevated pCO<sub>2</sub>. (Stemmer et al., 2013)

Within Breiðafjörður Bay, a large and shallow bay in W-Iceland, genetic differentiation was demonstrated between whelks from sites separated by just 20 km. Here, we extended our previous studies on the common whelk in Breiðafjörður Bay by quantifying phenotypic variation in shell morphology and color throughout the Bay. Whelk in Breiðafjörður Bay displayed fine-scale patterns of spatial variation in shape, thickness, and color diversity. Whelk shells in the innermost part of Breiðafjörður Bay were thick with an elongate shell, round aperture, and low color diversity, whereas in the outer part of the bay the shells were thinner, rounder, with a more elongate aperture and richer color diversity. (Magnúsdóttir et al., 2018)

Using Geometric Morphometric Analysis, the right and left valve interiors of the three species of bivalves from family Veneridae - Lyrate asian hard clam, Quahog or

little neck, and Manila clam from Tubod, Lanao del Norte, Philippines were explored for phenotypic variations. Multivariate Analysis of Variance/Canonical Variance Analysis scatter plot was used to graphically present the clustering of each species. Relative Warp Analysis and Kruskal-Wallis Test, was used to obtain consensus morphology of the three species and their variations along the negative and positive axes, and to test if there are significant differences on the anatomical landmark of the three species, respectively.

Results in ANOVA yielded significant differences in the three species, CVA showed a distinct clustering of species population, and Kruskal-Wallis supported this clustering by obtaining significant results.(Stephen et al., 2014)

## ***2.4 STRESS INDUCED ALTERATIONS IN SHELL MORPHOLOGY***

The nucleus accumbens receives emotional information from the basolateral amygdala (BLA) and contextual information from the ventral subiculum (vSub) of the hippocampus, respectively (NAc). We investigated how acute and persistent restraint stress changes the plasticity of the vSub and BLA afferent pathways using in vivo extracellular recordings from NAc neurons. Applying high frequency (HFS) and low frequency (LFS) stimulation to the vSub allowed researchers to examine how vSub and BLA inputs affected NAc responses. Additionally, the role of dopamine in the NAc in mediating the effects of stress on plasticity was investigated by iontophoretic administration of the D2-antagonist sulpiride. The NAc's BLA- and vSub-evoked responses were affected differently by acute and recurrent constraint. When restrained repeatedly but not when restrained severely. (Gill & Grace, 2013)

Reef-dwelling large benthic foraminifera are particularly sensitive to changes in light exposure, but their cryptic behavior allows them to colonize different habitats across a light gradient. Reduced light below their optimum level for growth and calcification caused a significant re-duction in antioxidant capacity, and a significant decline in the density of newly added chambers. The capacity to regulate symbiont density and to host a diversity of symbiotic diatoms within Amphistegina could be a

reason for their ability to acclimate rapidly to different light levels. Prolonged exposure to reduced light may result in a decrease in shell density and, consequently, a decline in carbonate production in reef environments. (Prazeres et al., 2016)

Depression is closely associated with alterations in dendritic spine morphology and spine density. Several chronic stress models, including chronic restraint stress, chronic unpredictable mild stress, and chronic social defeat stress, have been used to recapitulate depression-like behaviors in rodents and study the underlying mechanisms. Generally, chronic stress causes dendritic atrophy and spine loss in the neurons of the hippocampus and prefrontal cortex. These alterations induced by chronic stress are often accompanied by depression-like behaviors. (Qiao et al., 2016)

In 5 post-larval size classes using the alkalinity anomaly method. results show that calcification rate decreased with lower pH in all 5 size classes measured. Increased calcification rate with size allowed the larger sizes to overcome dissolution pressure and deposit new shell material under corrosive conditions. Size dependency of pH effects on calcification is likely due to organogenesis and developmental shifts in shell mineralogy occurring through the post-larval stage. (Waldbusser et al., 2010)

The author investigated that carbon, oxygen and trace element records from different shell layers in the mussels *Mytilus galloprovincialis* combined with detailed investigations of the shell ultrastructure. Most prominently, the shells recorded the shock of transplantation, both in their shell ultrastructure, textural and geochemical record. Shell calcite, precipitated subsequently under acidified seawater responded to the pH gradient by an in part disturbed ultrastructure. These field experiments showed that care is needed when interpreting potential ocean acidification signals because various parameters affect shell chemistry and ultrastructure. (Hahn et al., 2012)

## **2.5 CHARACTERIZATION**

### **2.5.1 STEREO MICROSCOPY**

Analytical light microscopy encompasses any type of microscopical work that provides information about a sample beyond observation in a brightfield view. The applications of microscopy in the forensic sciences are many and diverse because of the microscope's ability to detect, resolve, and image even the smallest items of evidence. Many types of sophisticated analyses can be conducted on the smallest of samples, often without alteration or destruction. As a result, microscopes continue to be indispensable in all forensic disciplines involving the natural sciences (Houck, 2013).

### **2.5.2 FTIR**

In FTIR spectrometer, there are two beams Michelson interferometer is present. A beam is emitted by a source and splits it two by beam splitter. The reflected part of the beam travels to the fixed mirror, is reflected there and heat the beam splitter again. Because the two split beams are spatially coherent, they interfere on recombination. The beam, modulated by the movement of the mirror, leaves the interferometer and finally focused on the detector. The signal is then detected by detector, the interferogram, is thus radiation of intensity of the combined beams as a function of the movable mirror. One of the most essential steps for obtaining an FTIR spectrum are First; to produce an interferogram with and without a sample in the beam, second; to transforms these interferograms into spectra of the source with and without sample absorption (Perkins, 1986)

### **2.5.3 X-RAY DIFFRACTION ANALYSIS**

X-ray diffraction is used most often to identify crystalline phases by comparison to a known database, measure atomic spacings (with the use of appropriate standards), measure structural properties of the crystalline phases present (e.g., preferred

orientation and particle size), and to probe atomic arrangements in amorphous materials. The identification of crystalline materials is often aided by chemical analysis in order to identify the elements present. The mass fractions of the phases present in a sample can be determined by comparison to known standards. Since XRD is relatively quick and is usually a nondestructive technique, it is often one of the first methods employed in the characterization of nanoparticles. The electron diffraction pattern is similar to an X-ray diffraction pattern (discussed below) in that diffraction occurs when the Bragg condition is satisfied. The Bragg equation ( $n \lambda = 2 d \sin \theta$ ) relates the wavelength ( $\lambda$ ) of the electrons, the angle ( $\theta$ ) of the electron beam with respect to a set of diffracting planes, and the spacing ( $d$ ) between those planes, which is determined by the crystal structure of the material examined. One challenge when working with electron diffraction is that electrons are often multiply scattered by the sample, which can complicate the interpretation of electron diffraction patterns (Burleson Garcia et al., 2009).

#### **2.5.4 RAMAN SPECTROSCOPY**

It is based on Raman Effect, i.e., frequency of a small fraction of scattered radiation is different from frequency of monochromatic incident radiation. Raman spectra arise due to inelastic collision between incident monochromatic radiation and molecules of sample. Much of this scattered radiation has a frequency which is equal to frequency of incident radiation and constitutes Rayleigh scattering. Only a small fraction of scattered radiation has a frequency different from frequency of incident radiation and constitutes Raman scattering. When the frequency of incident radiation is higher than frequency of scattered radiation, Stokes lines appear in Raman spectrum. When the frequency of incident radiation is lower than frequency of scattered radiation, anti-Stokes lines appear in Raman spectrum. Scattered radiation is usually measured at right angle to incident radiation. (Bumrah & Sharma, 2016) (Chalmers et al., 2012) (Thompson et al., 2014)

## **CHAPTER – 3**

### **AIM AND OBJECTIVES**

- Collection, identification, and study of morphological properties of selected shells of three different sizes.
- Preparation and qualitative assessment of gastropod and bivalve shells through spectroscopic techniques
- Intra-specific and interspecific comparative analysis between composition of selected two molluscan shells.

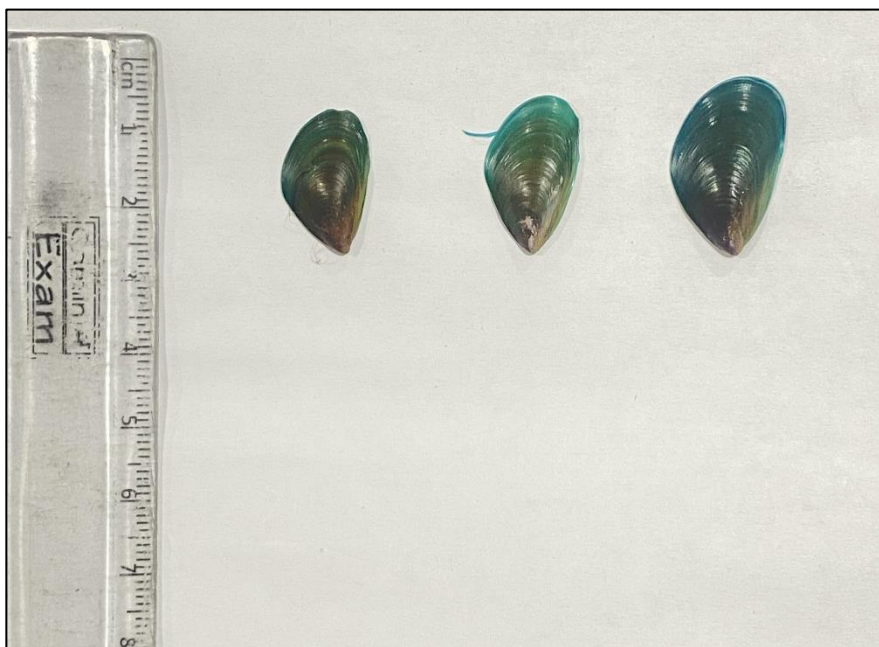
## CHAPTER – 4

### MATERIALS AND METHODS

All chemicals used for the study were analytical grade purchased from Sigma-Aldrich himedia, Merck, India.

#### 4.1 COLLECTION OF SHELLS

Bivalve shells are collected from the shore of Kalpakkam and gastropod shells are collected from the coast of Rameshwaram. Shells of *Perna viridis* and *Chicoreus virgineus* are collected based on increasing size of shells. Shells with external damages were avoided for analysis. Recently washed away shell which are preferred for the study. Collected shells were stored in plastic Ziplock bag cover.



**Fig 4.1.1: *Perna viridis* Size wise  
PV1 (left), PV2 (middle), PV3 (right)**



**Fig 4.1.2: *Chicoreus virgineus* collected size wise  
CV1 (left), CV2 (middle), CV3 (right)**

## **4.2 PROCESSED OF SHELLS**

These collected shells were washed with distilled water to remove larger debris such as algae and biofoulers. Organic and inorganic debris which are adhered on the shells were removed with fine brush followed by washing the shells with 3% v/v sodium hypochlorite (NaClO). Cleaned shells are dried for 24 hours under 60° C. *Perna Viridis* were segregated as PV1 (1.954cm – length, 1.04cm – width), PV2 (2.07cm – length, 1.186cm – width) and PV3 and *Chicoreus virgineus* were segregated as CV1 (4.54cm – length, 2.65cm – width) CV2 (4.84cm – length, 3.438cm – width) and CV3 (6.70cm – length, 4.31cm width) based on their size. The dried shells were drilled with A Hand drill (BOSH GSB 450 Impact) with a 2mm tungsten drill bit was used collect fine powder shell samples and the broken fragments for further analysis. Larger fragments were used for imaging and morphological observation while the fine powder was used for characterization studies.

### **4.3 CHARACTERIZATION OF SHELL POWDER**

#### **4.3.1 STEREO MICROSCOPY**

Stereo Microscope (Leica Stereo Zoom M205C, Germany) was used to capture surface and fractured images of bivalve and gastropod shells. These images were captured under different magnification utilizing a moving contraption with a microscopy lens and a camera to observe surface morphology.

#### **4.3.1 ATR - FTIR**

The standard procedure for FTIR spectra acquisition involves some preliminary steps to remove any factor that could influence the spectra interpretation. A first measure is carried out without the sample holder. Data acquisition was performed by The Agilent Microlab Software. A total number of 6 scans for each separate sample were taken and the intensity for the wavenumber between  $500\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$  is recorded. Afterwards, the numerical processing and display of the data was carried out. Particular reference spectra of the two forms of calcium carbonate (calcite ( $\text{CaCO}_3$ ), aragonite ( $\text{CaCO}_3$ )) from the database of the RRUFF<sup>TM</sup> Project of the University of Arizona.

#### **4.3.2 XRD**

The structure of the prepared samples was recorded by X-ray powder diffraction using an X-ray diffractometer X' Pert Pro – PAnalytic) with Cu K radiation ( $\lambda = 0.1546\text{ nm}$ ) operating at the condition of 40 kV and 40 mA. The specimen was pulverized into a fine powder and used for the analysis. The diffraction angle was continuously scanned from  $10^\circ$  to  $90^\circ$  in  $2\theta$  at a scanning rate of  $2^\circ/\text{min}$ . A range of  $10\text{--}90^\circ$  is shown in the figures because no relevant peaks occurred in the excluded region. Particular reference spectra of the two forms of calcium carbonate (calcite

(CaCO<sub>3</sub>), aragonite (CaCO<sub>3</sub>)) from the database of the RRUFF<sup>™</sup> Project of the University of Arizona.

#### **4.3.3 RAMAN SPECTRSCOPY**

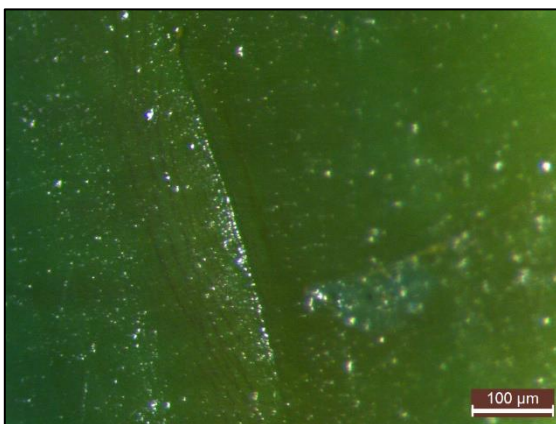
Bivalve spectral acquisitions were recorded using a Micro-Laser Raman (Seiki, Japan). Unless stated otherwise, shells were investigated using an argon-ion 488-nm excitation (9.0 mW, 10%) for a 20-s accumulation time. The laser intensity was between 10% and 50% on shell, adjusted to avoid damage to the specimen. Selected shells. Particular reference spectra of the two forms of calcium carbonate (calcite (CaCO<sub>3</sub>), aragonite (CaCO<sub>3</sub>)) from the database of the RRUFF<sup>™</sup> Project of the University of Arizona

## CHAPTER – 5

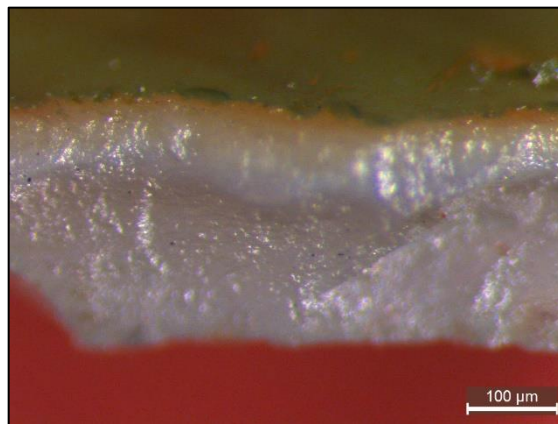
### RESULTS AND DISCUSSION

#### 5.1 CHARACTERIZATION

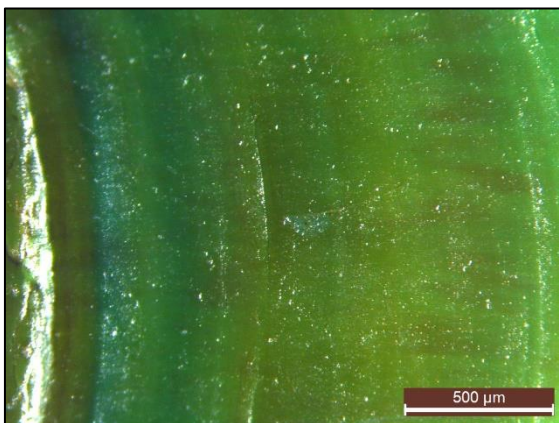
##### 5.1.1 Stereo Microscopy



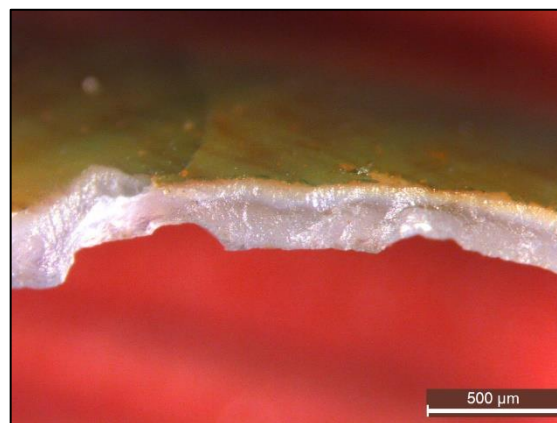
***Fig 5.1.1: Surface image of PV1 100µm***



***Fig 5.1.2: Fracture image of PV1 100µm***



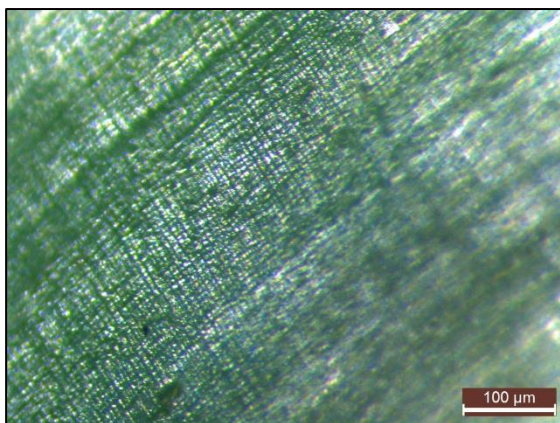
***Fig 5.1.3: Surface image of PV1 500µm***



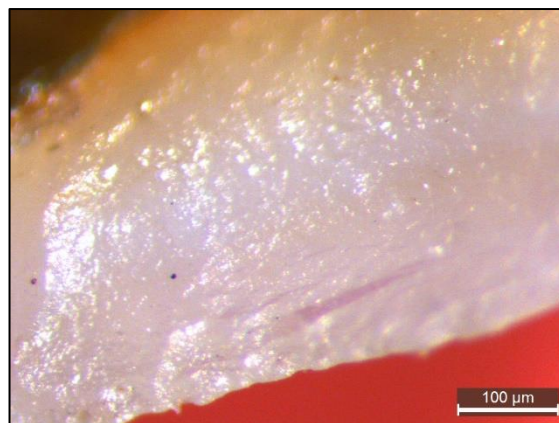
***Fig 5.1.4: Fracture image of PV1 500µm***

The shell surface was seen with irregular protrusion figure 5.1.1. PV1 is characterized with low density as the fracture has a translucent texture allowing the

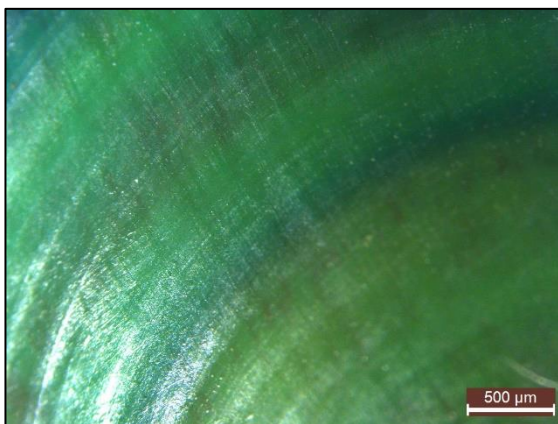
light to pass through. Indugival growth lines within the fracture is hard to identify in figure 5.1.4 but can be distinguishable in figure 5.1.2 moving towards the right of the image. The shell surface is seen with the same growth pattern with streaks of color variation in the figure 5.1.3



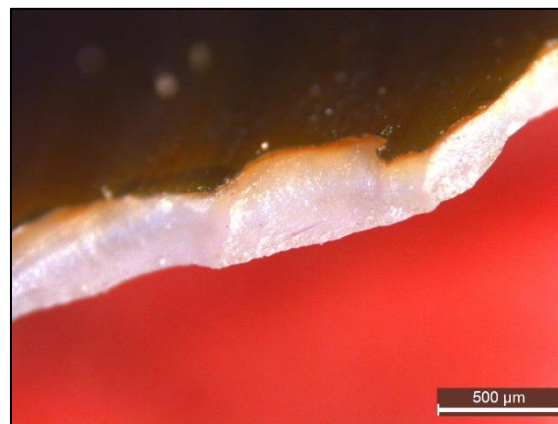
***Fig 5.1.5: Surface image of PV2 100µm***



***Fig 5.1.6: Fracture image of PV2 100µm***



***Fig 5.1.7: Surface image of PV2 500µm***

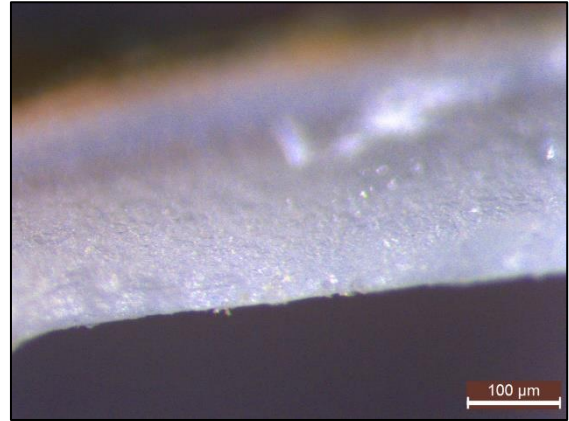


***Fig 5.1.8: Fracture image of PV2 500µm***

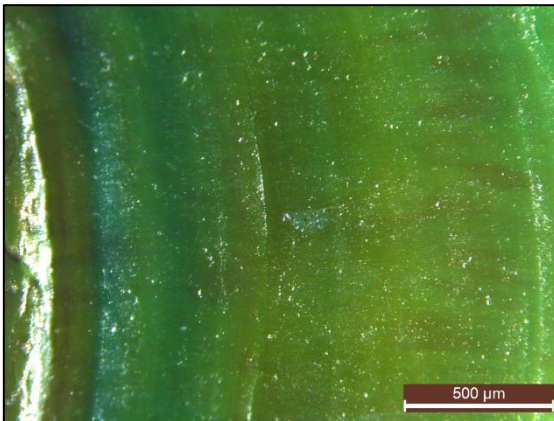
The surface image of PV2 figure 5.1.5 and figure 5.1.7 have a flat surface and even growth rings with significant green shading. Figure 5.1.6 and figure 5.1.8 have a denser carbonate structure with visible growth rings stretching in the horizontal direction.



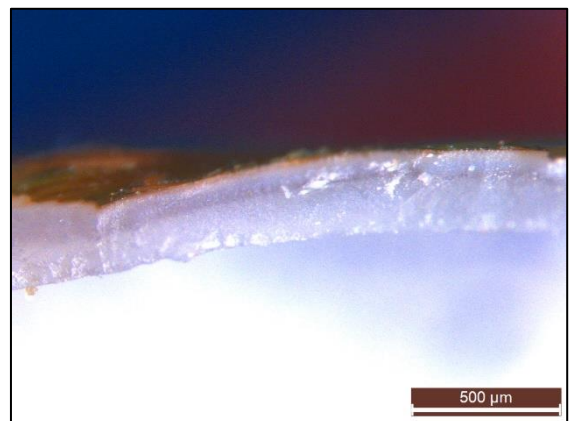
***Fig 5.1.9: Surface image of PV3 100μm  
100μm***



***Fig 5.1.10: Fracture image of PV3***



***Fig 5.1.11: Surface image of PV3 500μm  
500μm***

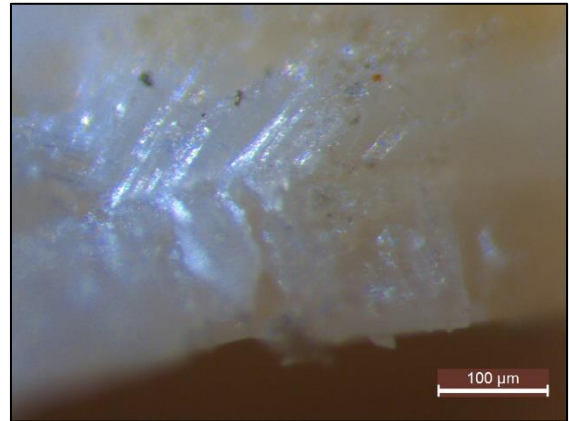


***Fig 5.1.12: Fracture image of PV3***

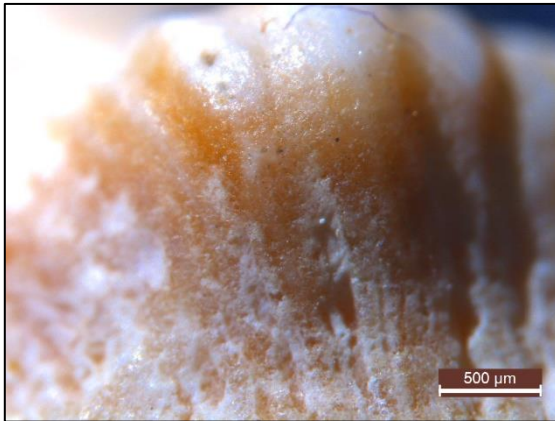
Figure 5.1.9 and Figure 5.1.11 shows similar surface protrusions of that of PV1. Visible growth rings in the horizontal direction are observed. Figure 5.1.10 and figure 5.1.12 shows fracture without growth rings and a distinct breakage.



***Fig 5.1.13: Surface image of CV1 100μm***



***Fig 5.1.14: Fracture image of CV1 100μm***



***Fig 5.1.15: Surface image of CV1 500μm***

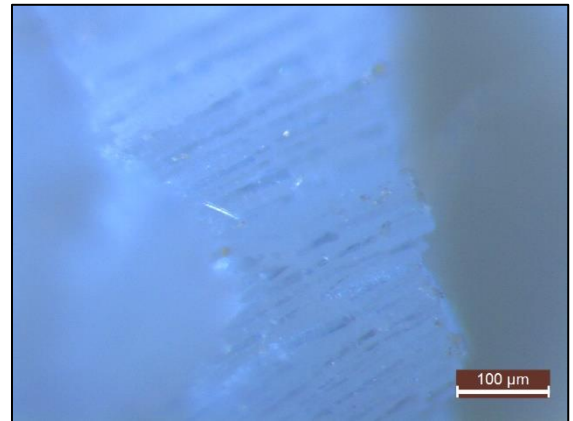


***Fig 5.1.16: Fracture image of CV1***

The surface images of the sample CV1(fig. 5.1.13, fig 5.1.15) is characterized by a strong orange hue with irregular surface morphology. Growth pattern is seen within the image of the fracture (Fig.5.1.14, Fig.5.1.16) having a mild translucent nature. The direction cannot be determined by figure as the shell have a tortional growth.



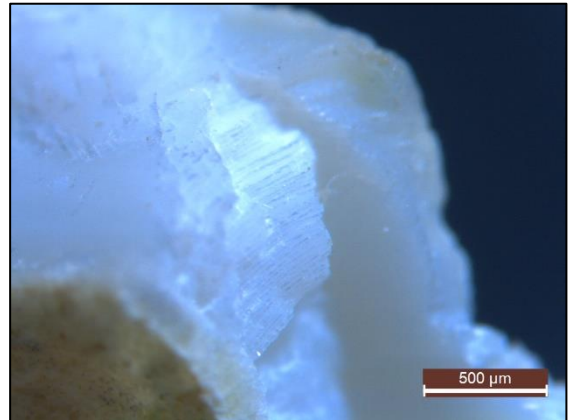
***Fig 5.1.17: Surface image of CV2 100μm***



***Fig 5.1.18: Fracture image of CV2***

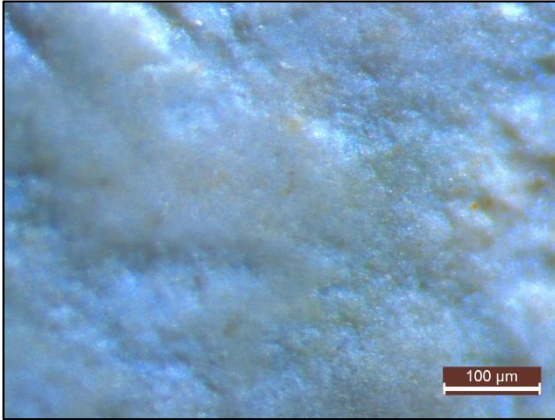


***Fig 5.1.19: Surface image of PV2 500μm***

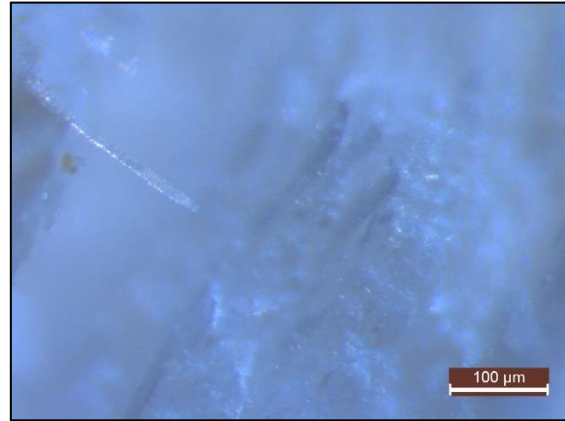


***Fig 5.1.20: Fracture image of PV2***

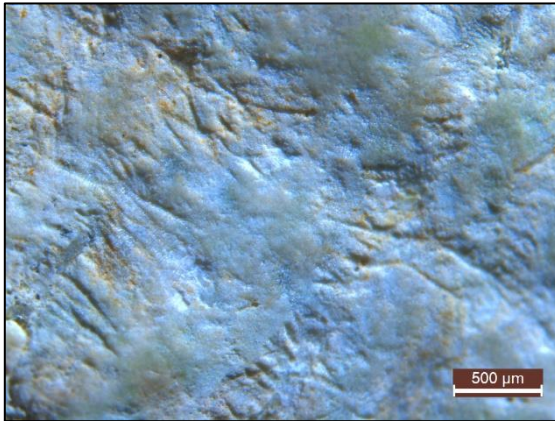
Similar to CV1, CV2 have a milder orange hue and many white specks in the surface image (Fig.5.1.17, Fig.5.1.19). The fracture image (Fig.5.1.18, Fig.5.1.20) shows clear growth ring pattern but not growth direction. Compared to Fig.5.1.14 the orange hue is not seen within the fracture of Fig.5.1.18.



***Fig 5.1.21: Surface image of CV3 100μm***



***Fig 5.1.22: Fracture image of CV3 100μm***



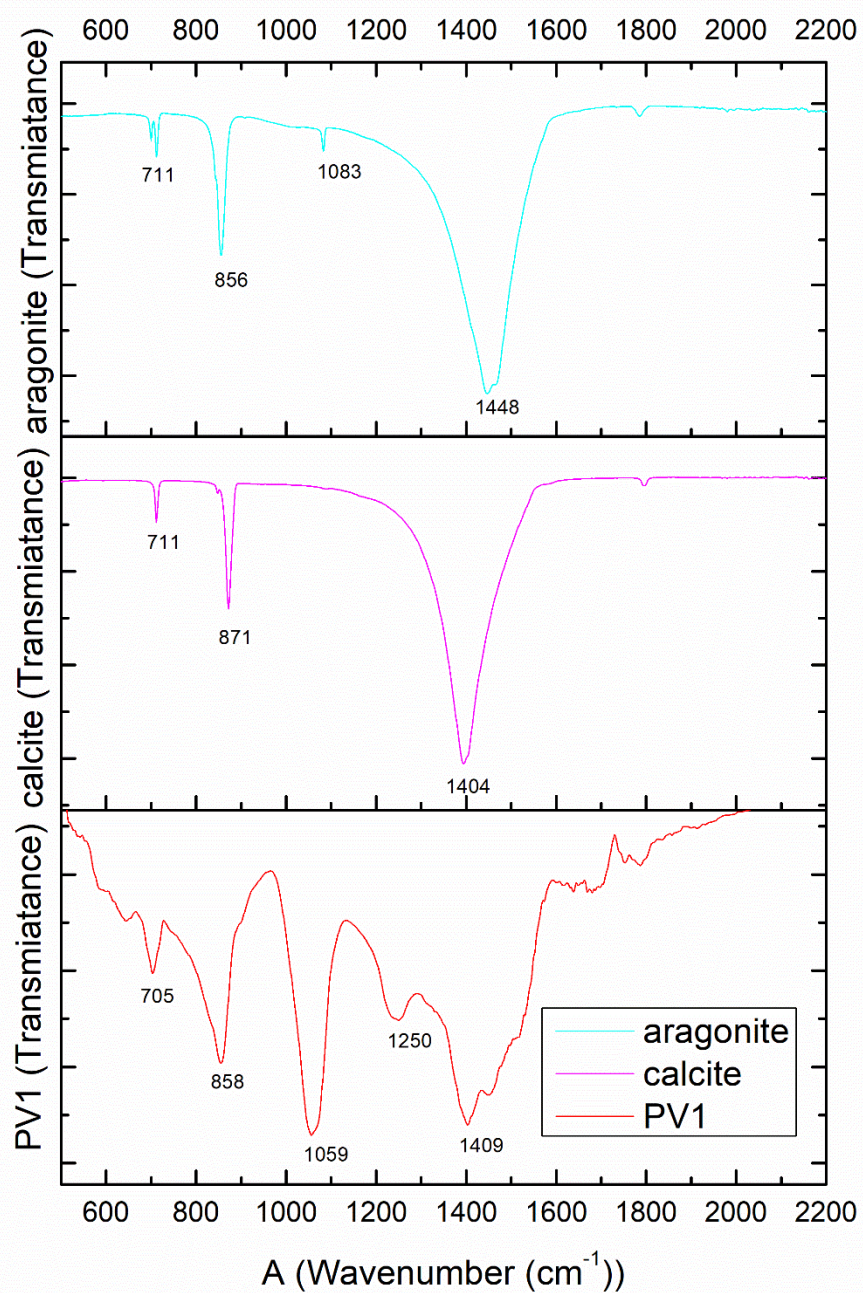
***Fig 5.1.23: Surface image of CV3 500μm***



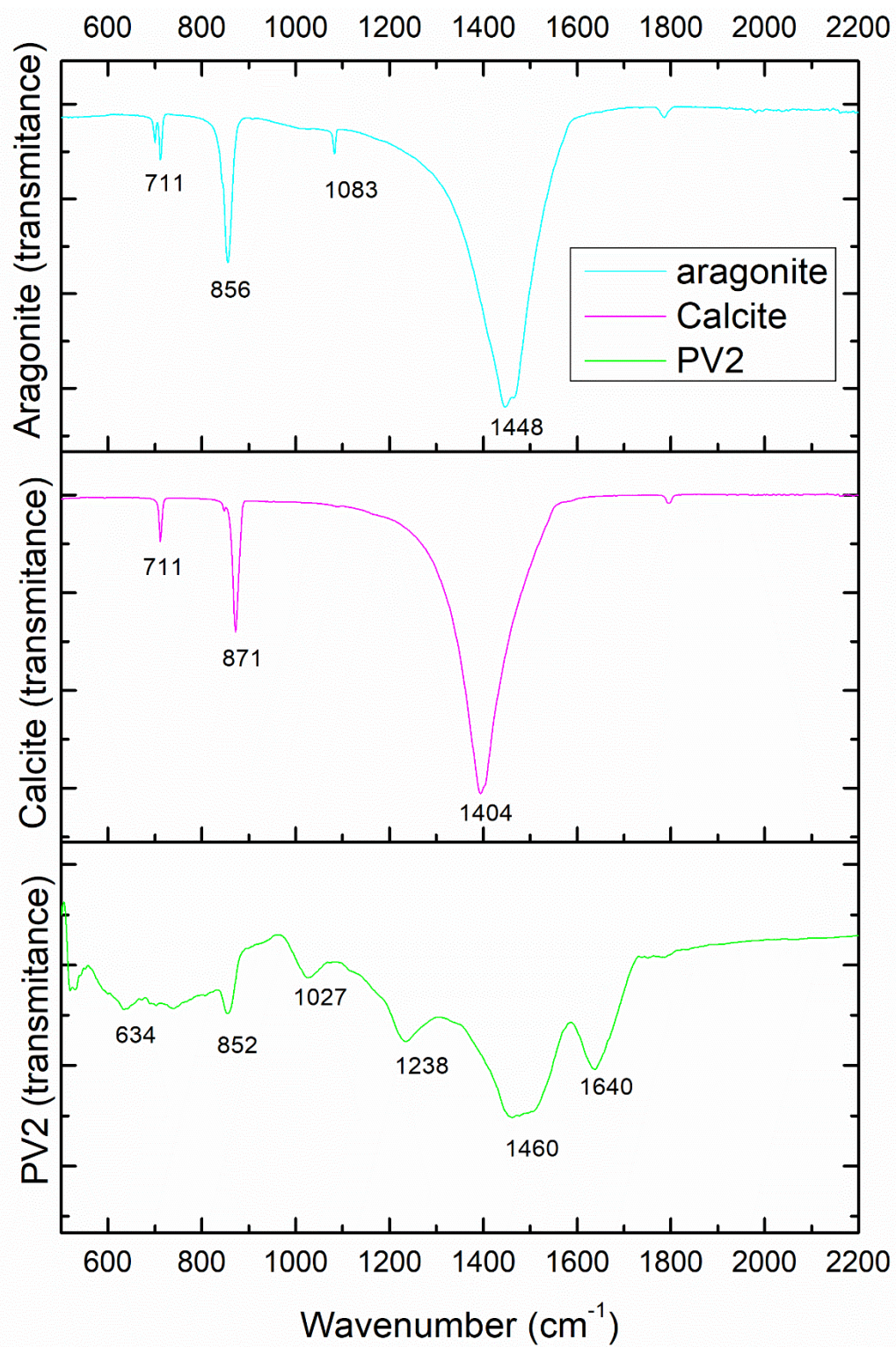
***Fig 5.1.24: Fracture image of CV3 500μm***

Surface images (fig.5.1.21, fig.5.1.23) CV3 have more white specks with irregular streaks of orange hue. The surface is filled with deeper crevices and crests. Fracture image of CV3 (Figure 5.1.22, 5.1.24) is well distinguishable and significant similar to CV2.

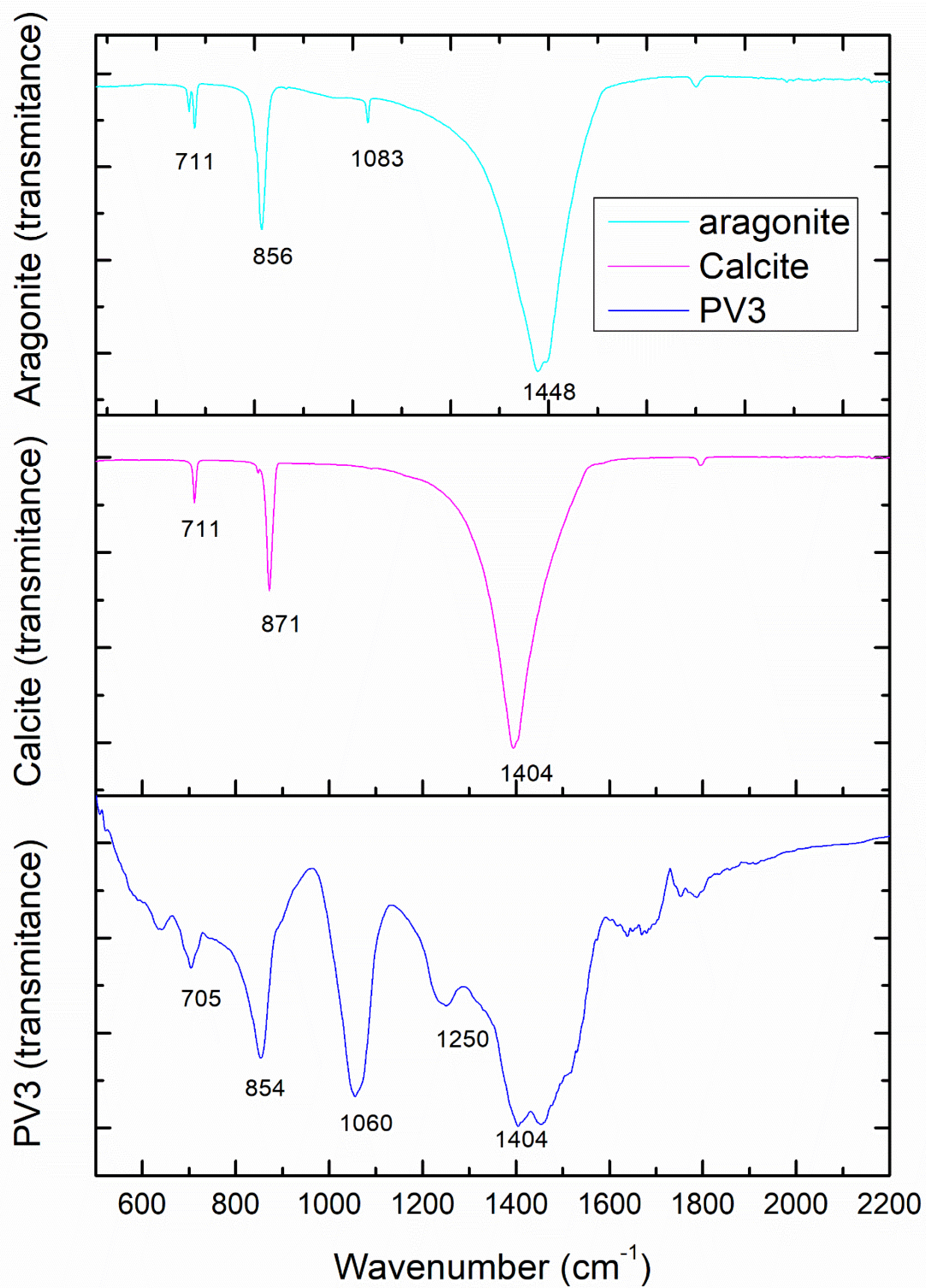
### 5.1.2 ATR-FTIR



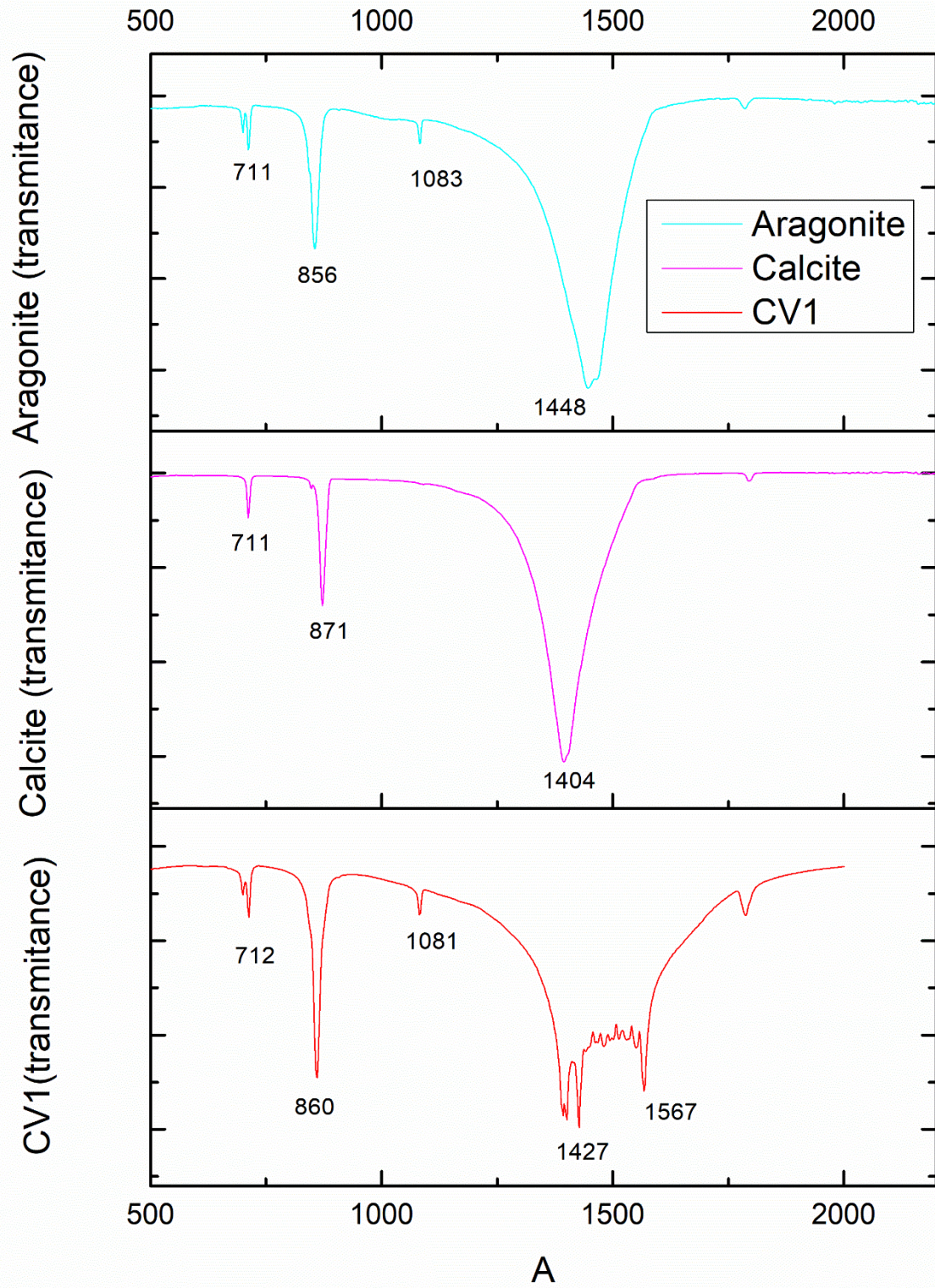
**Fig 5.2.1: FTIR pattern of specimen PV1**



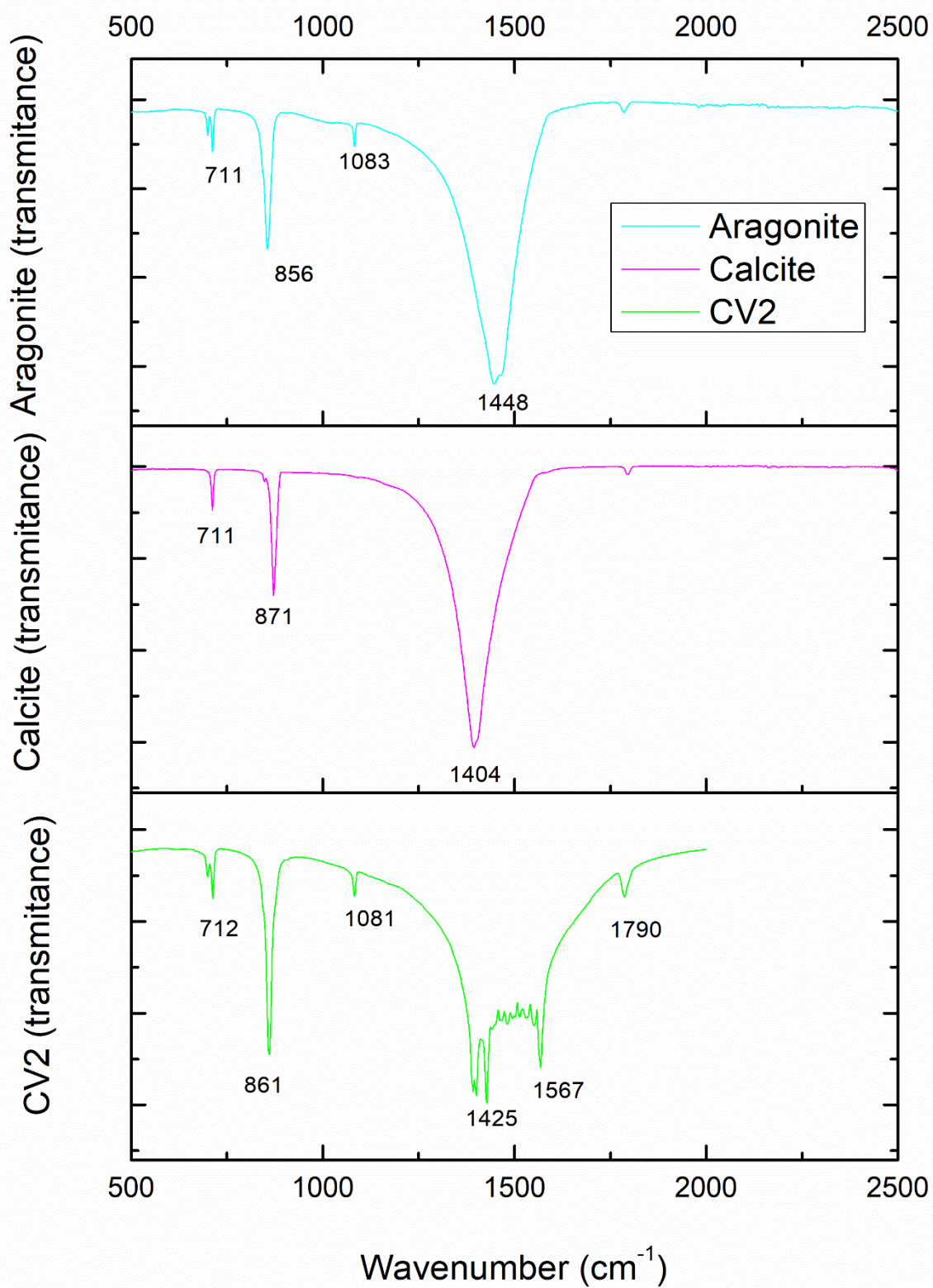
**Fig 5.2.2: FTIR pattern of specimen PV2**



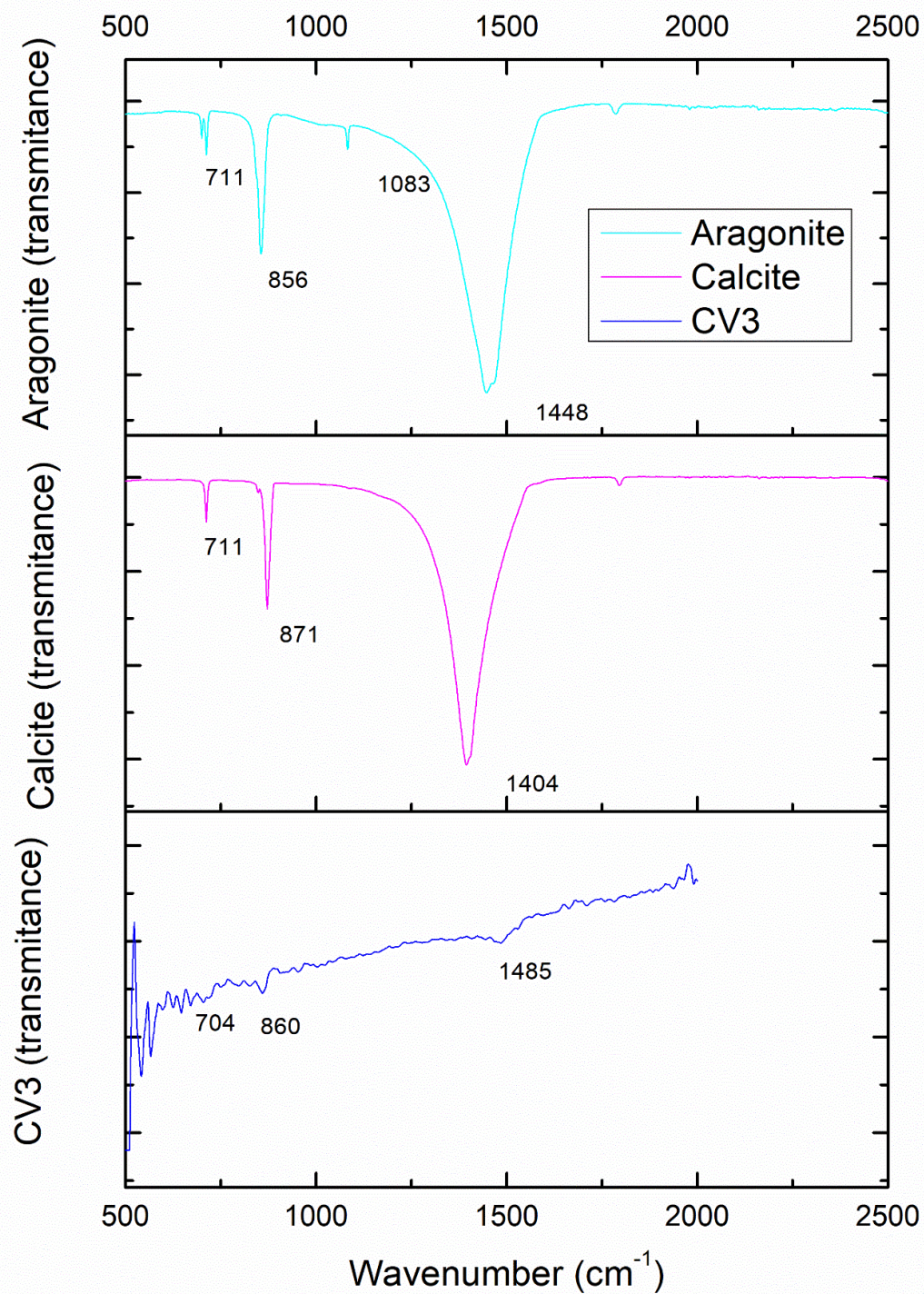
**Fig 5.2.3: FTIR pattern of specimen PV3**



**Fig 5.2.4: FTIR pattern of specimen CV1**



**Fig 5.2.5: FTIR pattern of specimen CV2**



**Fig 5.2.6: FTIR pattern of specimen CV3**

The FTIR data shows the presence of Aragonite in the PV1 (figure: 5.2.1) and PV3 (figure: 5.2.3) from the distinct peaks around the range of  $705\text{ cm}^{-1}$ ,  $858\text{ cm}^{-1}$ ,  $1060\text{ cm}^{-1}$  and  $1404\text{ cm}^{-1}$ . These pertain to Double degenerate planar bending, aragonite out of carbonate bending, aragonite symmetric carbonate stretching and asymmetric carbonate stretching. FTIR of PV2(figure: 5.2.2) shows peaks of both calcite and aragonite. This trend is seen similarly in samples of CV1(figure: 5.2.4) and CV2(figure: 5.2.5) showing peaks of both calcite and aragonite. CV3(figure: 5.2.6) shows major similarity towards calcite indicating the presence of its structure.

Somkiat Seesanong reported six normal vibrational modes are a nondegenerate symmetric stretch ( $\nu_1$ ;  $A'_1$ : Raman active), nondegenerate asymmetric (out of plane) bend ( $\nu_2$ ;  $A''_1$ : IR active), doubly degenerate asymmetric stretch ( $\nu_3$ ;  $E'$ : Raman and IR active), and doubly degenerate symmetric (in-plane) bend ( $\nu_4$ ;  $E'$ : Raman and IR active). The FTIR spectra of the CSP, MSP, and OSP samples were analyzed according to this theory. Two strong intense bands at  $696\text{ cm}^{-1}$  and  $863\text{ cm}^{-1}$  are assigned to the  $\nu_4$  and  $\nu_2$  modes, respectively. A weak band at  $1030\text{ cm}^{-1}$  is contributed to  $\nu_1$  mode. A band at  $1413\text{ cm}^{-1}$ , looking like a mountain, is related to  $\nu_3$  mode. A weak band observed at  $1782\text{ cm}^{-1}$  may be respected as the combination bands of  $\nu_4 + \nu_1$  modes. A weak band at  $2520\text{ cm}^{-1}$  and a broad band around  $2875\text{ cm}^{-1}$  may be regarded as a combination of or/and overtone of  $\nu_4$ ,  $\nu_3$ , and  $\nu_1$  modes (Seesanong et al., 2021). J. Balmain reported that the peaks of the carbonate ions in Bivalve (Oyster) shell were due to the internal vibration modes of the  $\text{CO}_3^{2-}$  ions,  $713$ ,  $700$ ;  $864$ ,  $844$ ;  $1090$  and  $1490\text{ cm}^{-1}$ . Apart from these fingerprint bands of the minerals they reported various other C=C and C=O bonds (Balmain et al., 1999). Singh stated these standard peaks and also reported dual peaks at  $710\text{ cm}^{-1}$  and  $713\text{ cm}^{-1}$  which was a minor characteristic of aragonite structure seen in shellfish study. These particular peaks are seen in the FTIR graphs of CV1 and CV2. Huang Zengqiong reported the stretching vibration of S=O group having a number  $1026\text{ cm}^{-1}$  in the species *Solen grandis* ((Zengqiong & Gangsheng, 2011). This band is seen in the FTIR plot of PV2 showing presence of S=O bonds (Yasuo Kikuchi & Nobuo Tamiya, 1983)

FTIR analyses reported by Madhuvilakku were made for uncalcined bivalve shell powder and bivalve shell of *Margaritifera falcata* were calcined at 850 °C for 2.5 h. In the uncalcined shell powder, the major absorption bands occurred at 1443, 869, and 712  $\text{cm}^{-1}$ , attributed to the asymmetric stretch, out-of-plane band, and in-plane bend vibration modes, respectively, for  $\text{CO}_3^{2-}$  molecules. After the calcination, bivalve shells lost carbonate ions and absorption bands of  $\text{CO}_3^{2-}$  shift to higher energy (i.e., 1471, 1090, 874  $\text{cm}^{-1}$ ). This has been attributed to the decrease of the reduced mass of the functional group attached to the  $\text{CO}_3^{2-}$  ions. After the calcinations process, a new peak appears at 3642  $\text{cm}^{-1}$  indicating the presence of  $\text{Ca}(\text{OH})_2$ , formed from exposure of CaO with atmospheric air (Madhuvilakku et al., 2013)

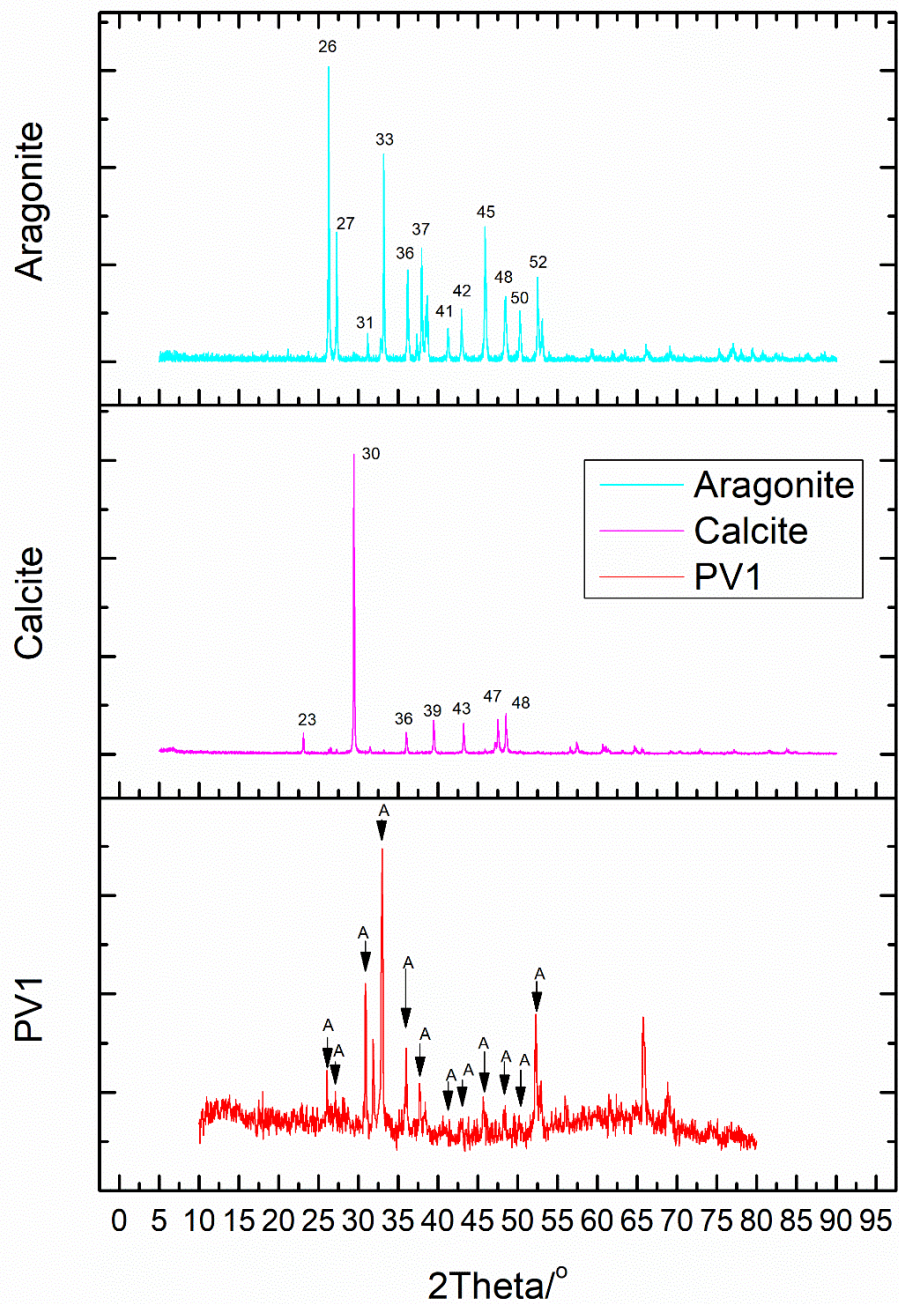
The spectral peak at 1086  $\text{cm}^{-1}$ , was due to the C–O stretching, which perhaps denoted the chemical glycosylation in proteins of the snail shell matrix. The existence of spectral peaks for the  $\text{COO}^-$  functional group, C=C bond, and C–O stretching is perhaps due to the presence of  $\text{CO}_3^{2-}$  ions in snail shells as well as glycosylated proteins forming in the snail shell matrix. The characteristic carbonate  $\nu_4$  bands of aragonite were at 701.9 and 713.5  $\text{cm}^{-1}$  and the characteristic carbonate  $\nu_2$  band of aragonite at 865.8  $\text{cm}^{-1}$  revealed the availability of aragonite form of calcium carbonate in the shells of *B. bengalensis*, *P. globose* and *B. costula* in the work carried out by Parveen Saida (Parveen et al., 2020)

The FTIR spectrum showed organic bands of lower intensity; the band at 1786.52  $\text{cm}^{-1}$  was attributed to the carboxylate (carbonyl) groups of the acidic proteins in the organic matrix. Four bands characteristic of aragonite, corresponding to the  $\text{CO}_3^{2-}$  ions, were identified:  $\nu_3$  at 1467.32  $\text{cm}^{-1}$ ,  $\nu_1$  at 1081.43  $\text{cm}^{-1}$ ,  $\nu_2$  at 863.39  $\text{cm}^{-1}$ , and  $\nu_4$  at 702.31–713.73  $\text{cm}^{-1}$ . The  $\nu_4$  band corresponded to the planar bending mode of the carbonate vibration and the  $\nu_1$  band to the symmetric stretching mode. Moreover, the characteristic carbonate  $\nu_4$  bands of the aragonite were at 713.73 and 702.31  $\text{cm}^{-1}$  and the characteristic carbonate  $\nu_2$  band of the aragonite was at 863.39  $\text{cm}^{-1}$ , indicating the availability of the aragonite form of  $\text{CaCO}_3$  that is seen in the shell powders of snails. This was reported by Hülya ŞEREFLİŞAN based on the work on the bivalve

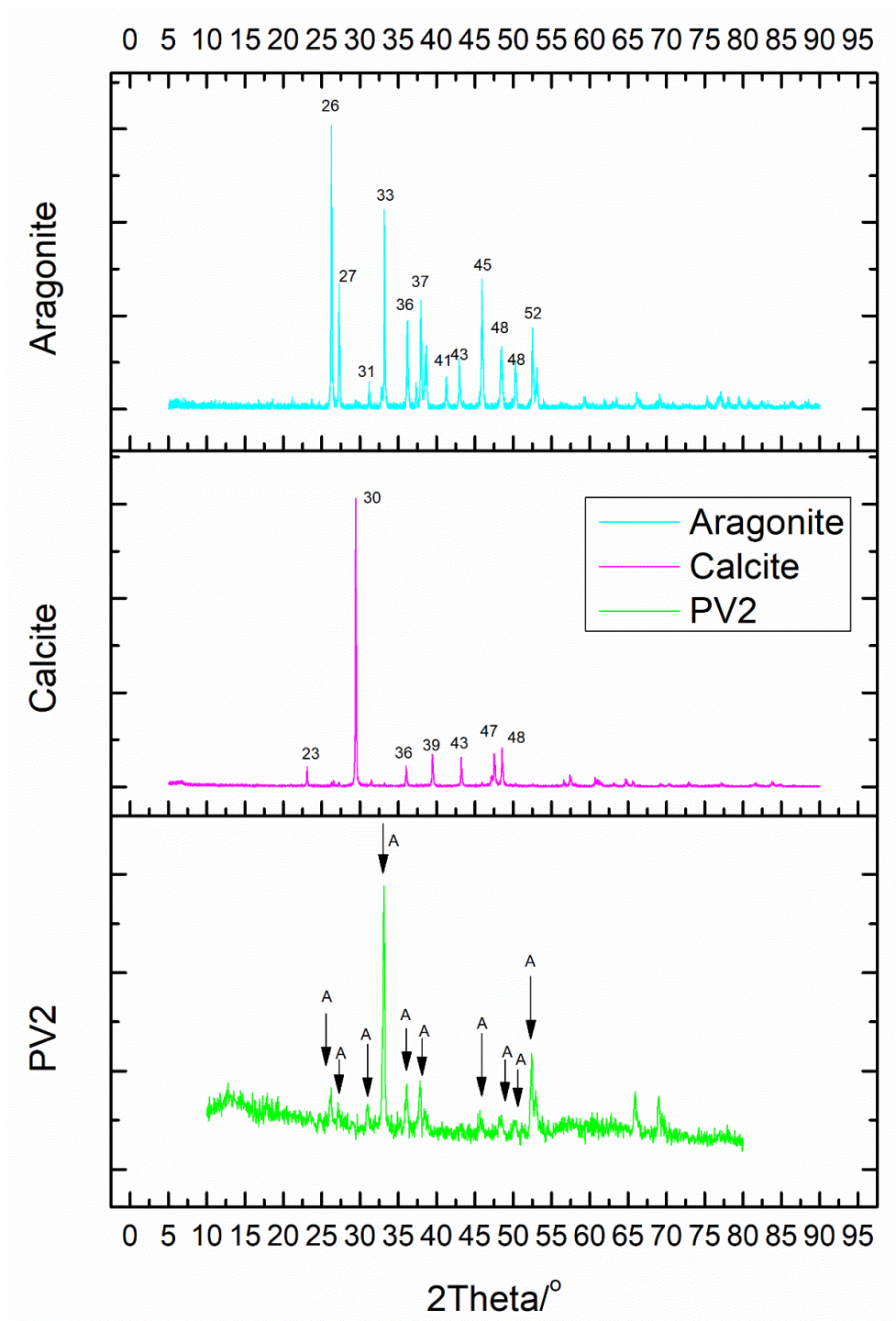
Unionidae.(ŞEREFLİŞAN, 2023)

FTIR analysis of the specimens P.Virdis PV1, PV2 and PV3 shows similar peaks to that of standard calcite and aragonite. Sample PV2 shows significant peaks around 1500 to 2000 indicating the presence of C=O, C=N, C=C. Samples PV1, PV3 have similar peaks of  $706\text{ cm}^{-1}$  to  $707\text{cm}^{-1}$ ,  $854\text{cm}^{-1}$  to  $856\text{cm}^{-1}$ ,  $1403\text{cm}^{-1}$  to  $1405\text{cm}^{-1}$  showing the presence of aragonite.

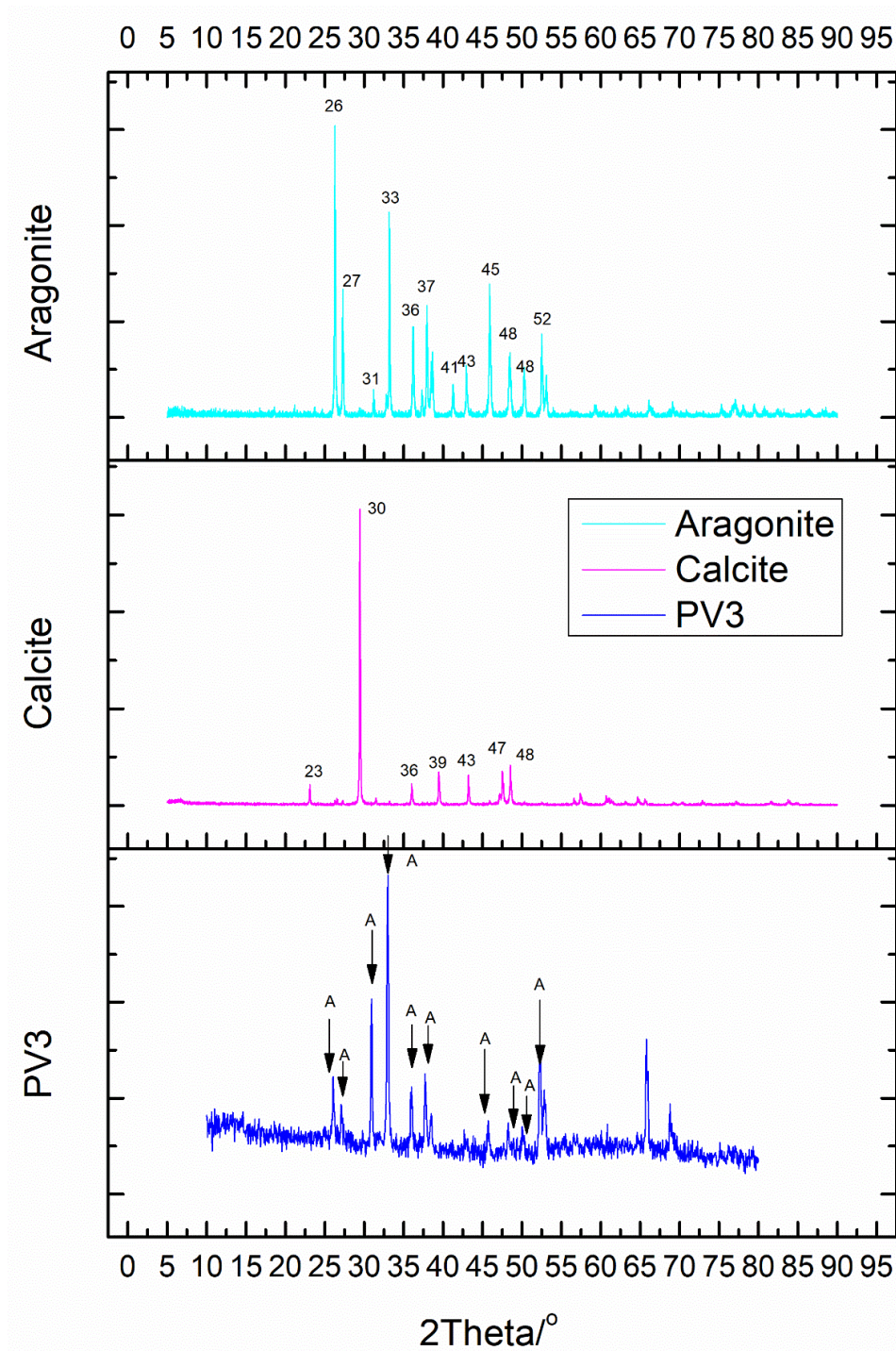
### 5.1.3 XRD



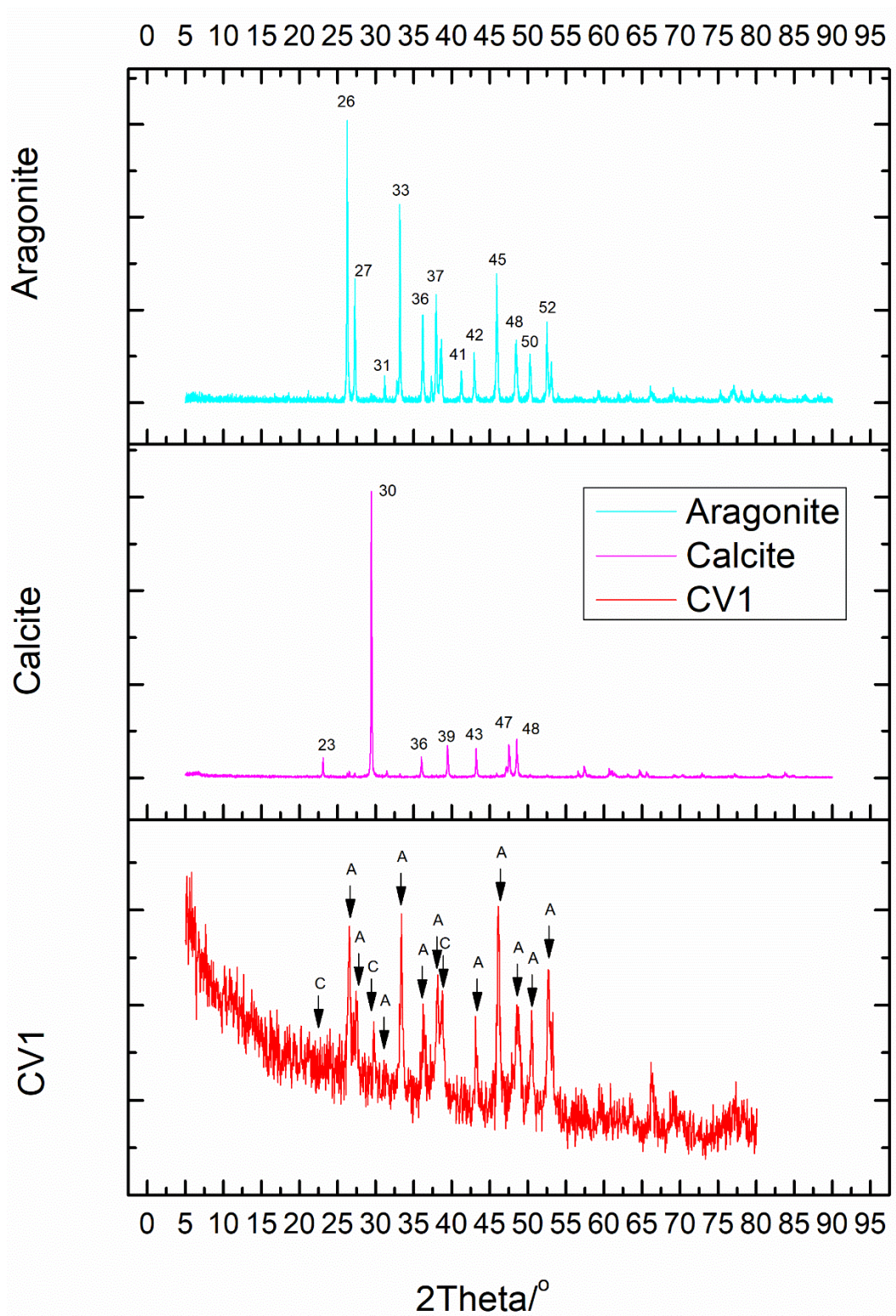
**Fig 5.3.1: XRD pattern of specimen PV1**



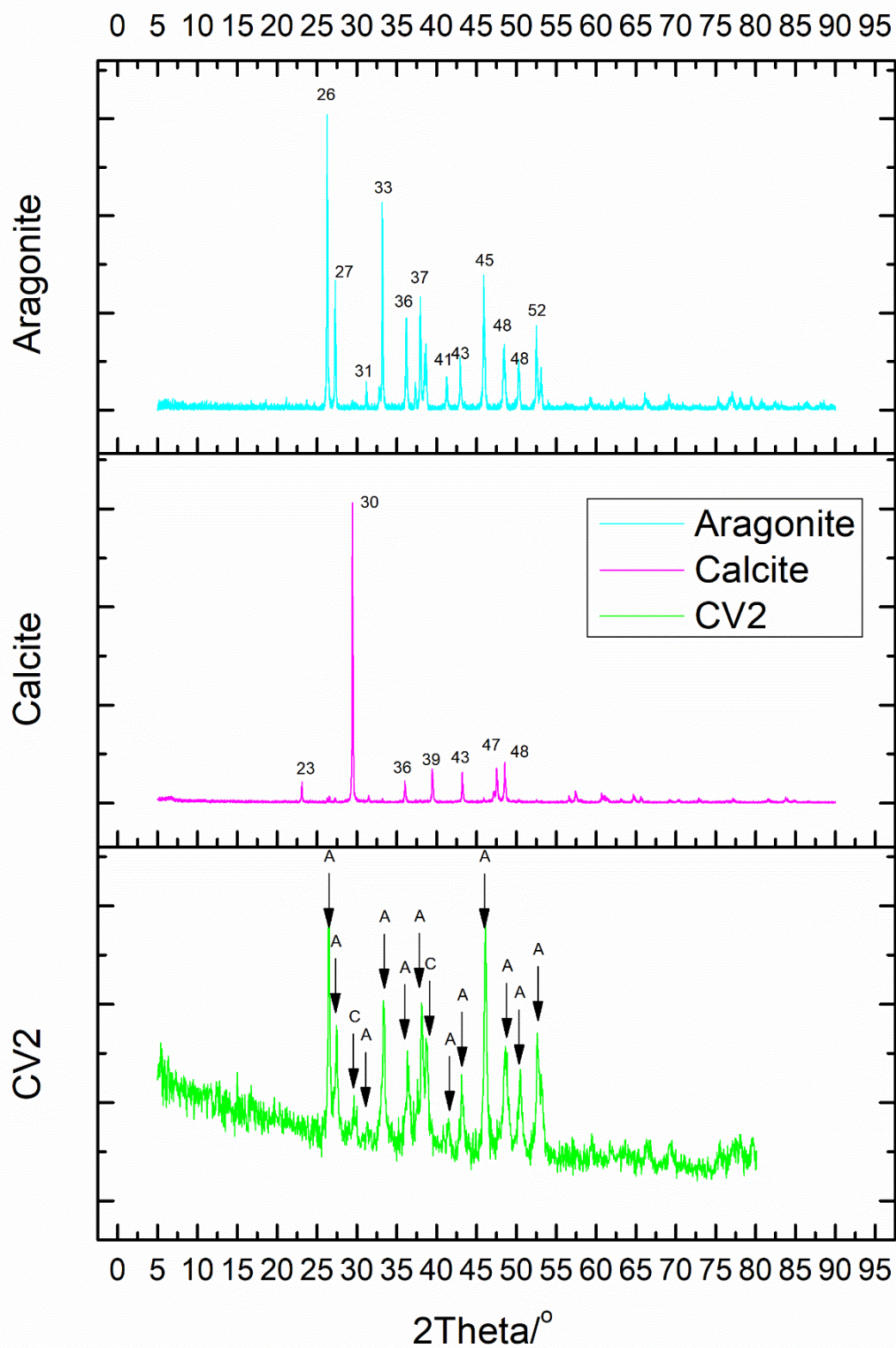
**Fig 5.3.2: XRD pattern of specimen PV2**



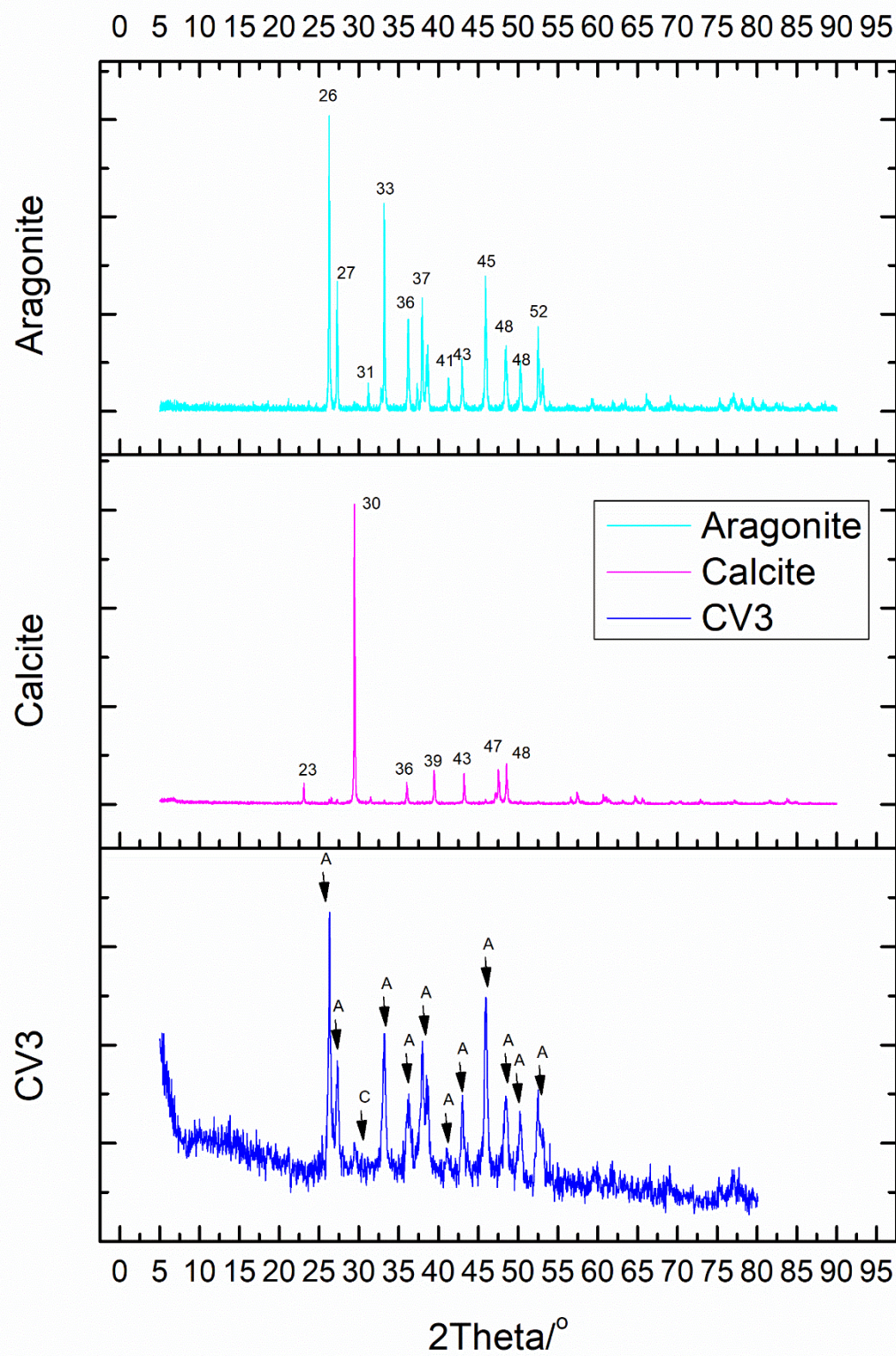
**Fig 5.3.3: XRD pattern of specimen PV3**



**Fig 5.3.4: XRD pattern of specimen CV1**



**Fig 5.3.5: XRD pattern of specimen CV2**



**Fig 5.3.6: XRD pattern of specimen CV3**

The XRD spectroscopy data of PV1, PV2 and PV3 were observed showing that these shells have calcium carbonate present in aragonite crystal structure when compared to the standards. Similarly, CV1, CV2 and CV3 shows peaks of aragonite and major peaks of calcite with lower intensity. The slighter curve in the graphs of CV1, CV2 and CV3 shows the presence of an amorphous components.

The XRD spectroscopy data of PV1, PV2 and PV3 were observed showing that these shells have calcium carbonate present in aragonite crystal structure when compared to the standards. Similarly, CV1, CV2 and CV3 shows peaks of aragonite and major peaks of calcite with lower intensity. The slighter curve in the graphs of CV1, CV2 and CV3 shows the presence of an amorphous components.

In general, all the peaks had  $26^{\circ}$ ,  $27^{\circ}$ ,  $31^{\circ}$ ,  $33^{\circ}$ ,  $36^{\circ}$ ,  $37^{\circ}$ ,  $41^{\circ}$ ,  $42^{\circ}$ ,  $45^{\circ}$ ,  $48^{\circ}$ ,  $50^{\circ}$  and  $52^{\circ}$  as standard peaks of aragonite. On comparison PV1 (figure 5.3.1) had low intensity peaks at  $41^{\circ}$ ,  $42^{\circ}$ ,  $48^{\circ}$  and  $50^{\circ}$  due to improper or inadequate mixing of the sample. This can also be seen at the intensity of the peaks of  $26^{\circ}$ ,  $27^{\circ}$ ,  $41^{\circ}$ ,  $43^{\circ}$  in the sample PV2 (figure 5.3.2) and  $31^{\circ}$  in the sample PV3 of figure (figure 5.3.3).

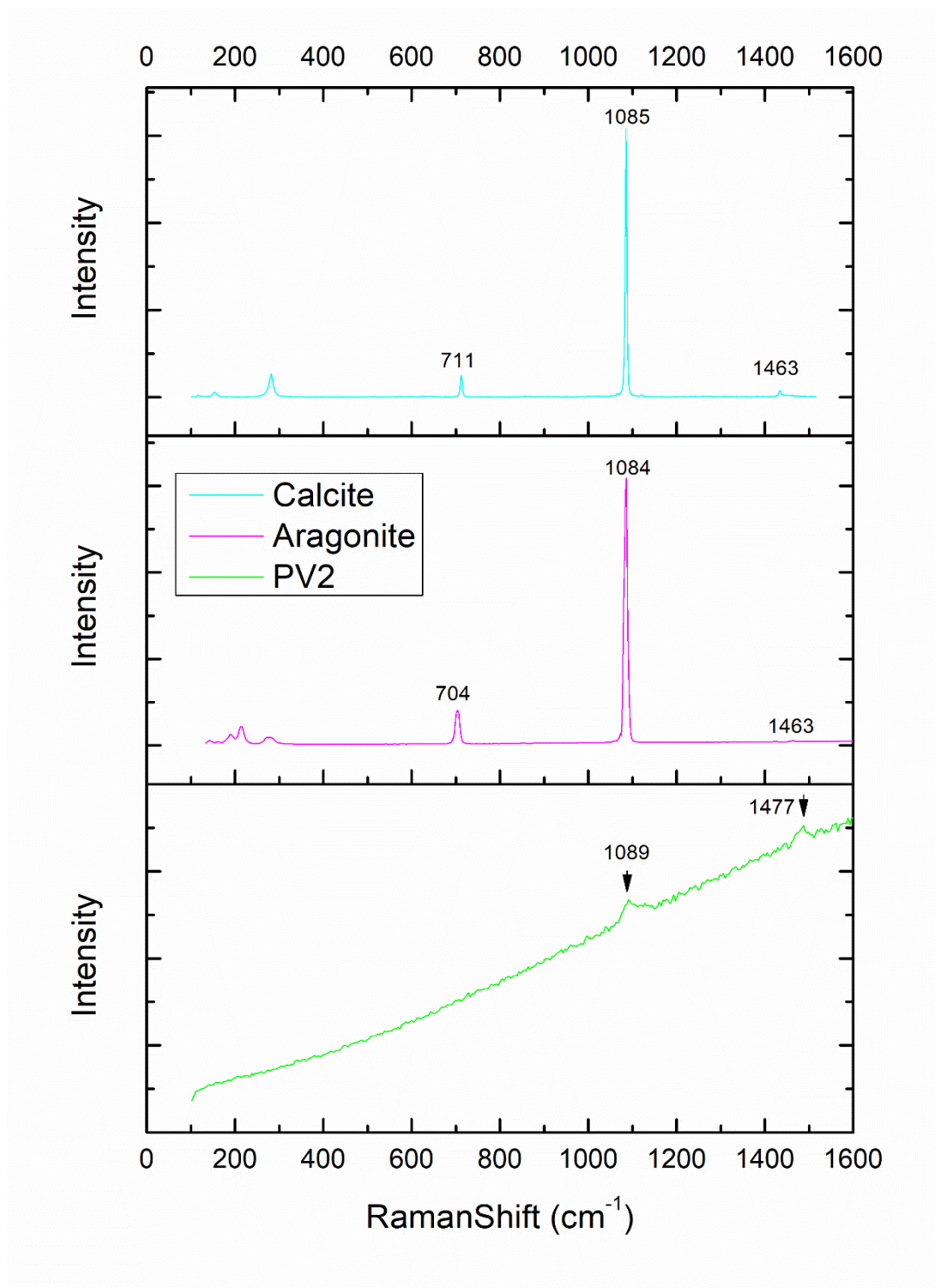
This trend is seen in the calcite peaks which is dominated by the presence of aragonite. Further analysis shows low intensity peaks at  $23^{\circ}$ ,  $30^{\circ}$ ,  $39^{\circ}$  in the sample CV1 (figure 5.3.4) which pertains to calcite. Peaks of  $30^{\circ}$  is seen to have lower intensity through CV2 and CV3 (figure 5.3.5, figure 5.3.6) which can be hard to spot in the graphs.

The X-ray diffraction data reported by Saida Parveen were acquired in steps of  $0.02^{\circ}$  at scattering angles ( $2\theta$ ) ranging from  $20^{\circ}$  to  $90^{\circ}$ . The XRD phase analysis of aragonite, portrayed bears high intensity peaks at  $2\theta = 26.21^{\circ}$ ,  $27.22^{\circ}$ ,  $33.15^{\circ}$ ,  $36.17^{\circ}$ ,  $37.89^{\circ}$ ,  $38.42^{\circ}$ , and  $45.86^{\circ}$  with monochromatic Cu-K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ). The XRD pattern reflected the intense peaks at (111) and (012) planes, providing the evidence of the

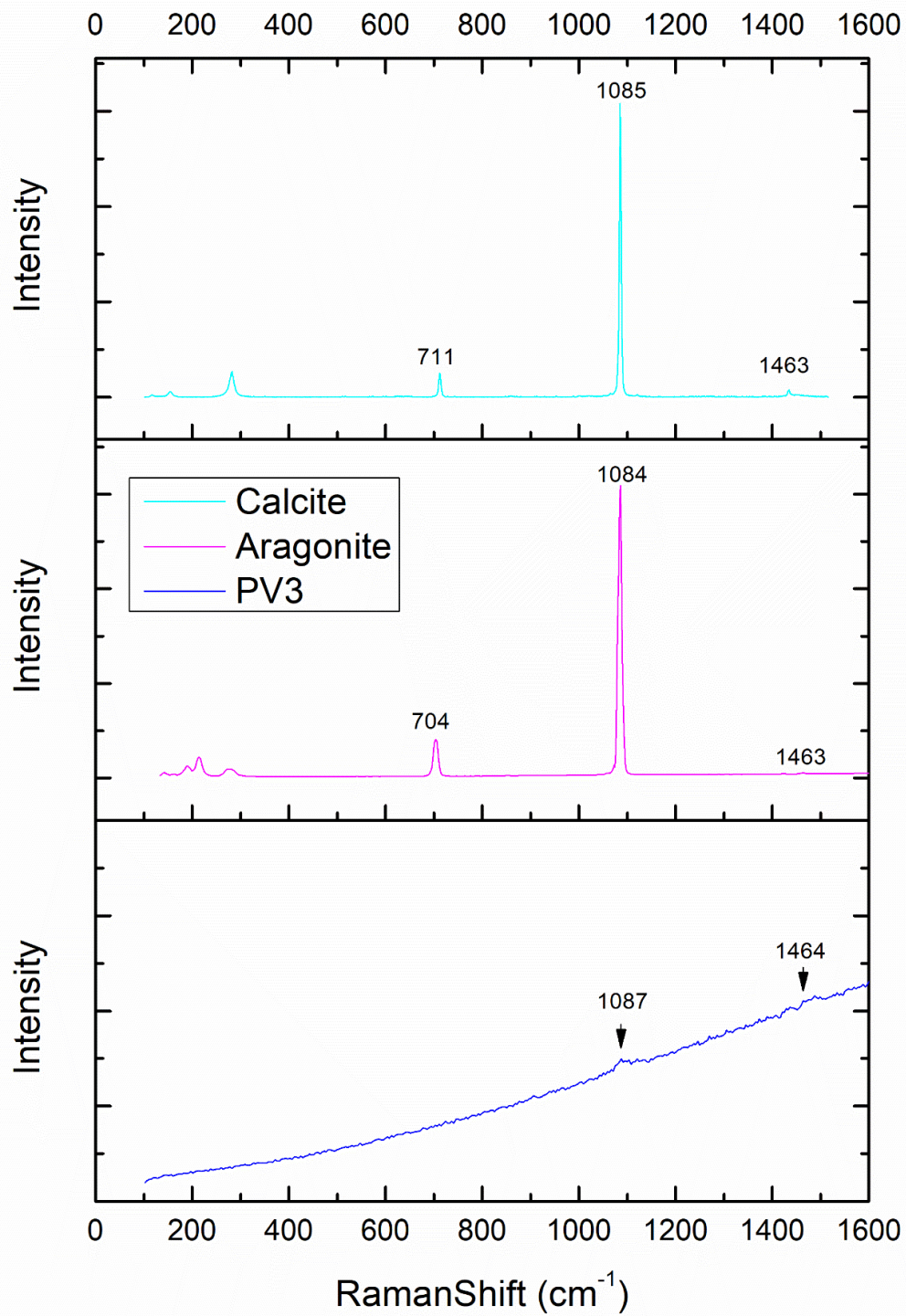
existence of orthorhombic aragonite phase.(Parveen et al., 2020)

The diffraction intensity is the calcite (104) = 29.81 and the next to strongest are the calcites at {113}, {110}, {2 0 2}, {116} and {1010}, respectively. The XRD analysis revealed that the crystalline form of calcium carbonate in the sclerites was calcitic polycrystals. The diffraction angle, 29.81 in the calcite (104) indicates Mg-calcite because the diffraction angle for pure calcite is 29.31 as reported by M. Azizur Rahman. (Rahman & Oomori, 2008)

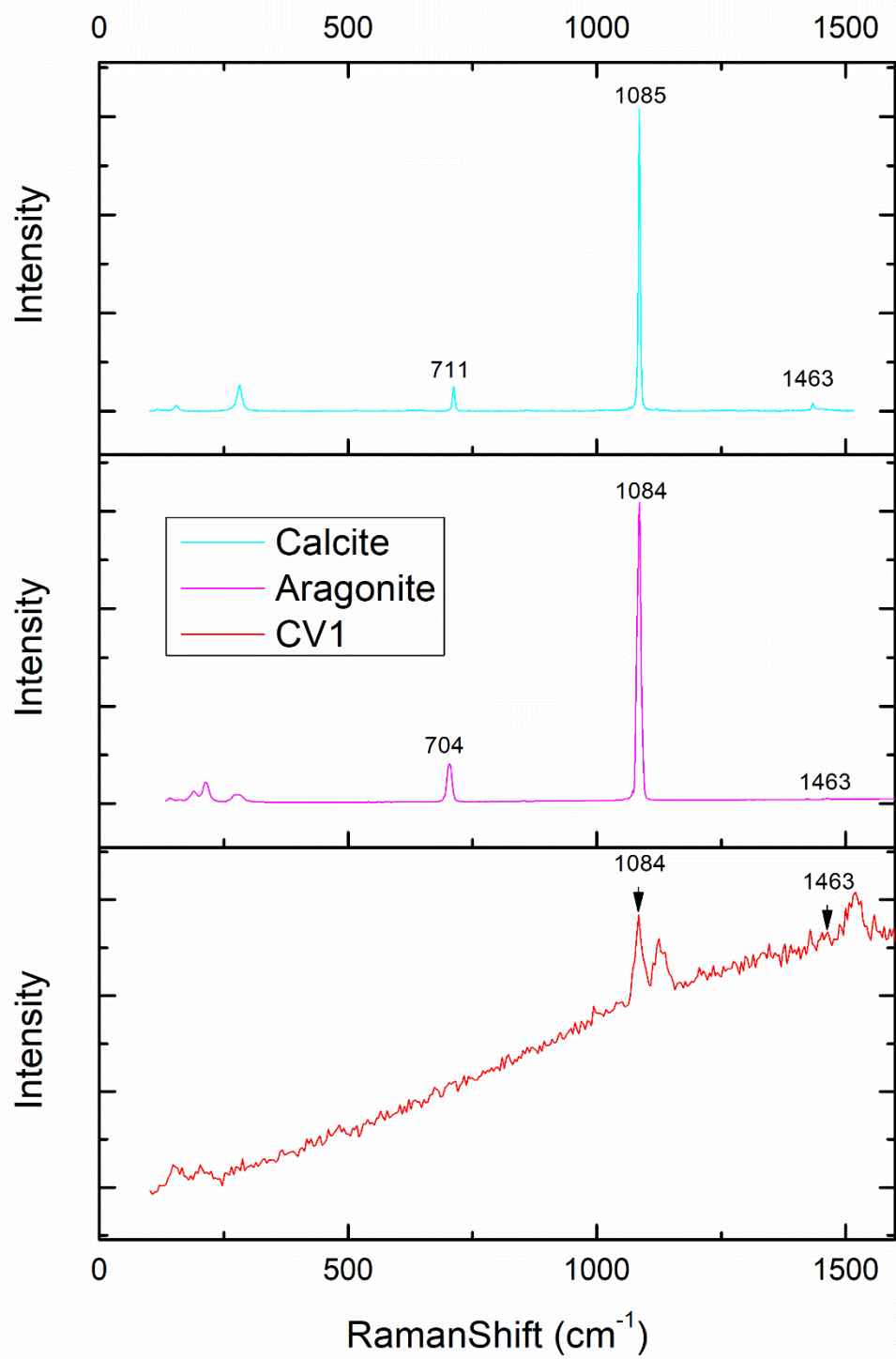
#### 5.1.4 RAMAN SPECETROSCOPY



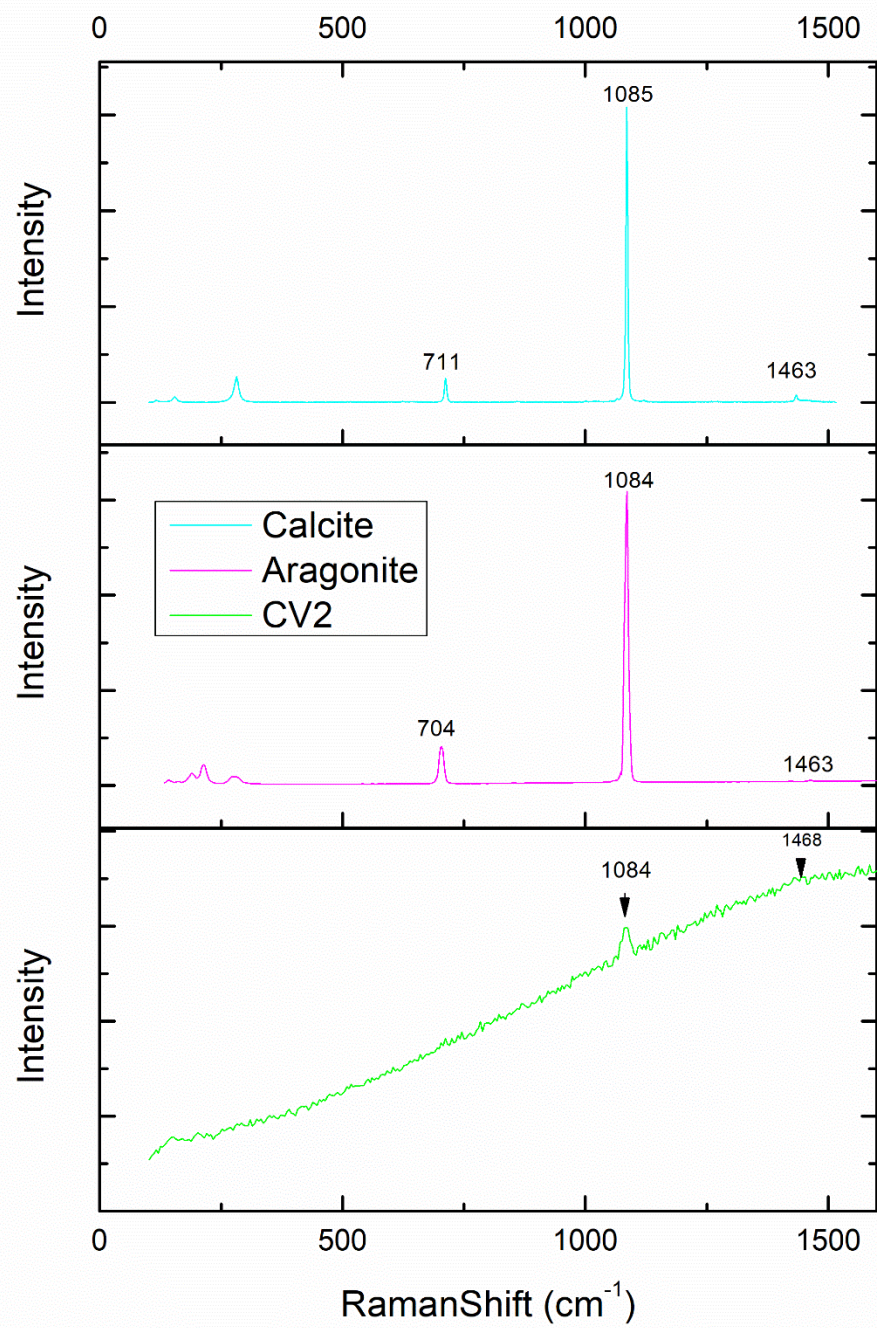
**Fig 5.4.1: Raman Spectra of specimen PV2**



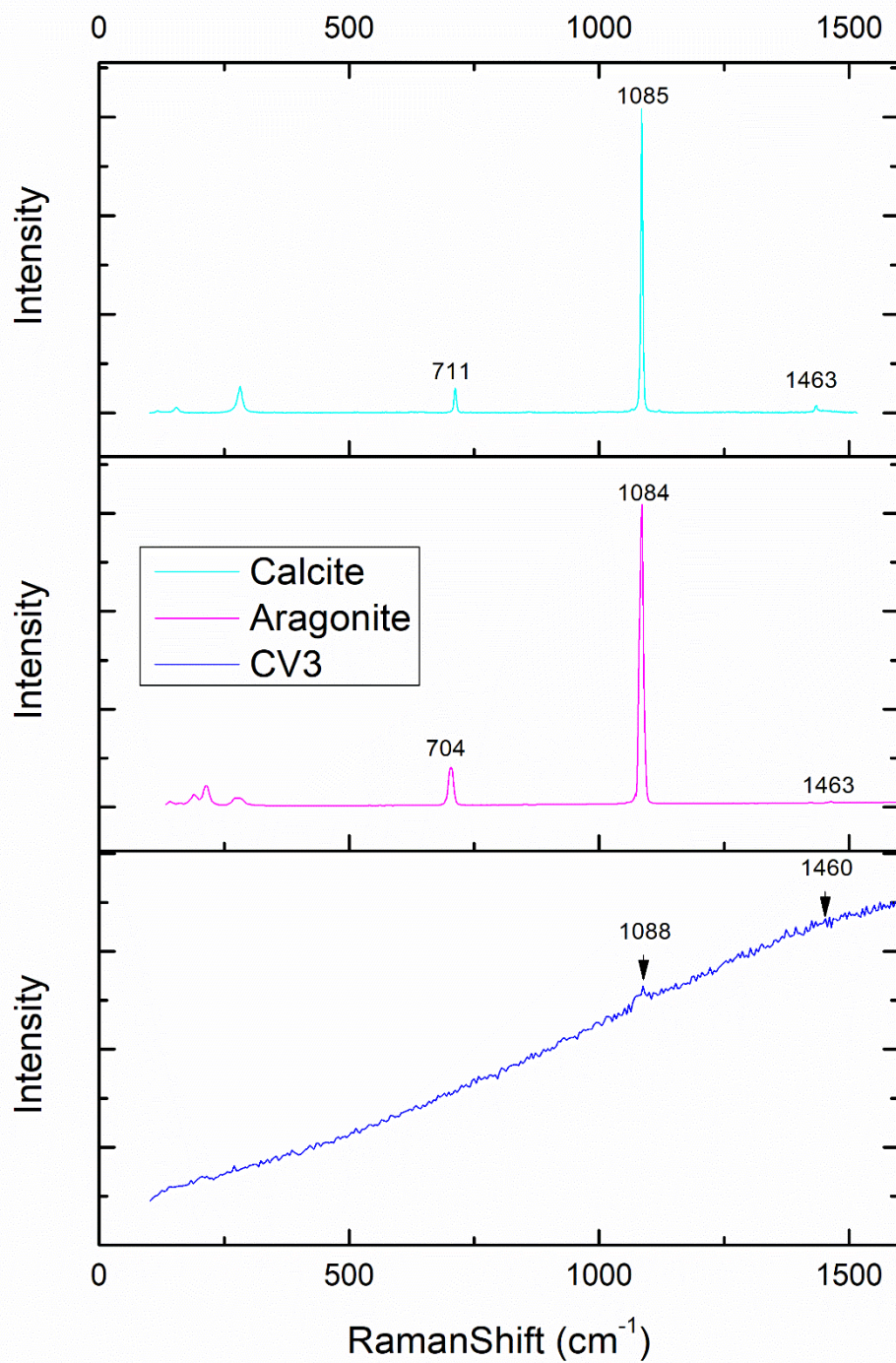
**Fig 5.4.2: Raman Spectra of specimen PV3**



**Fig 5.4.3: Raman Spectra of specimen CV1**



**Fig 5.4.4: Raman Spectra of specimen CV2**



**Fig 5.4.5: Raman Spectra of specimen CV3**

The raman spectra plot of PV1 could not be plotted as it couldn't give any distinct lines. The plot of PV2 and PV3 (fig 5.4.1 and fig 5.4.2) shows significant peaks at 1087 to 1089  $\text{cm}^{-1}$  and 1464. These pertain to the character of aragonite when compared to the standard obtained from the RRUFF database. Thompson reported that spectra from four to six individuals of each of the seven bivalve species were averaged to examine general differences between species and all of the spectra showed the sharp characteristic bands from aragonite at 705 and 1085  $\text{cm}^{-1}$ .(Thompson et al., 2014)

As mentioned, the distinct bands at 1085 were visible than the bands at 704 due to loss of resolution as a result of grinding the sample to a fine powder. These bands at 1085  $\text{cm}^{-1}$  represent for both calcite and aragonite. These peaks were of lower precision due to fine powdering of samples. Related to Raman analysis, in all spectra a very intense band at 1085  $\text{cm}^{-1}$  ( $\nu_1$ ) was observed, which can have the contribution of both calcite and aragonite. For lime-stone, scallop and oyster shells, bands at 155 and 280  $\text{cm}^{-1}$  (translational lattice vibrations), 711  $\text{cm}^{-1}$  ( $\nu_4$ ), 1085  $\text{cm}^{-1}$  ( $\nu_1$ ), 1435  $\text{cm}^{-1}$  ( $\nu_3$ ) and 1747  $\text{cm}^{-1}$  ( $\nu_1+\nu_4$ ) were observed which confirm the presence of calcite, reported by (Ferraz et al., 2019)

In the present study conducted by the author, they obtained six tyrosinase genes from *P. maxima*, *P. margaritifera* and *P. f. martensii* that did not encode the six conserved histidine residues within the copper-binding sites. In *P. f. martensii*, PmTyr-4 and PmTyr-6 were nacreous-layer-specific SMPs. PmTyr-4 played a crucial role in the formation of mother of pearl by inhibiting calcium carbonate precipitation and modifying crystal morphology. This type of tyrosinase mutants of *Pinctada* spp. may participate in the formation of mother-of-pearl through similar functions. Their results improved our understanding of the function of nacreous-layer-specific tyrosinase in pearl oysters.(Xiong et al., 2022)

## CHAPTER – 6

### SUMMARY AND CONCLUSION

Bivalve and gastropod shells spectroscopic investigations can have several purposes, among which (a) the understanding of biomineralization processes in the formation of shells, that vary according to species of molluscs, (b), the assessment of the vulnerability of these organisms to stress factors (i.e., ocean acidification) and (c) the investigation of the chemical composition of these biomaterials.

Results showed a fair differentiation between the shell structures of the two molluscan species. A good adherence to the standards of the carbonate forms of aragonite and was identified for mussel, while the prevailing calcite and aragonite composition in *C. virgineus* samples was detected. The study demonstrated the advantages of FTIR, XRD and Raman spectroscopy technique for bivalve and gastropod shells investigation aiming at different purposes from environmental studies to biomaterial characterization. The age wise analysis shows that both of the species carrying the same  $\text{CaCO}_3$  polymorphs from juvenile to adult. But the composition between the species shows significant differences from the analysis. This will be helpful for future research on ocean acidification and its impact on two different species with distinct composition patterns.

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## ANNEXURE



|     |                    | 5 <sup>th</sup> International Conference on<br>RECENT TRENDS IN MICROBIOLOGY (RTM-2024)<br>February 26 <sup>th</sup> & 27 <sup>th</sup> , 2024                                                                                                                           |                     |
|-----|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| 72. | ABS_64 RTM2024_064 | Antibacterial potential of Indian cooperative spider ( <i>Stegodyphus sarastnorum</i> ) web against pathogenic bacteria<br><b>Karuppusamy Sasikala<sup>1</sup>, Nagarajan. N<sup>1*</sup>, Santhanam Selvaraj<sup>2</sup> and Krishnamoorthy. S<sup>2</sup></b>          |                     |
| 73. | ABS_65 RTM2024_065 | Heavy metal containing Wastewater treatment using Microbial Fuel Cells<br><b>P. Aswaja<sup>1</sup>, A. Arun<sup>1*</sup></b>                                                                                                                                             |                     |
| 74. | ABS_66 RTM2024_066 | EXPANDING UPON CURRENT APPLICATIONS AND ENVISIONING FUTURE PERSPECTIVES OF LACTIC ACID BACTERIA AS PROBIOTICS<br><b>S. Yuvarani<sup>1</sup>, VS. Roshini<sup>1</sup> and S. Padmavathy<sup>1*</sup></b>                                                                  |                     |
| 75. | ABS_67 RTM2024_067 | Study of Biomineralization in bi valve and gastropod due to geographic, stress and other factors<br><b>Suriyaraj Senthilrajan<sup>1</sup>, S. Usha Nandini<sup>1</sup>, D. Inbakandan<sup>2</sup>, Naren Kumar<sup>2</sup></b>                                           |                     |
| 76. | ABS_68 RTM2024_068 | Evaluating the Antibiofilm Capabilities of <i>Commiphora Caudata</i> Stem & Leaf Essential Oil Against Diabetic Wound Biofilm Micro-Organisms<br><b>Karthick Kumar M<sup>1</sup>, Manikandan S<sup>1</sup>, Palpperumal S<sup>1</sup> and Renuga Devi R<sup>1*</sup></b> |                     |
| 77. | ABS_69 RTM2024_069 | Copper Nanoparticles synthesized from seaweed ( <i>Caulerpa scapelliformis</i> ) and antifungal activity and efficiency of fungal pathogens<br><b>M.Suganthi<sup>1</sup> and S.R. Sivakumar<sup>1*</sup></b>                                                             |                     |
| 78. | ABS_70 RTM2024_070 | Phytol as novel drug candidate against carbapenem resistant <i>Klebsiella pneumoniae</i> for wound Infections<br><b>Shibasini Murugan<sup>1</sup> and Kavitha Thangavel<sup>1*</sup></b>                                                                                 |                     |
|     |                    | HOME                                                                                                                                                                                                                                                                     | << PREVIOUS NEXT >> |



## GRADUATE APTITUDE TEST IN ENGINEERING 2024

अभियांत्रिकी स्नातक अभिक्षमता परीक्षा २०२४

ORGANISING INSTITUTE: INDIAN INSTITUTE OF SCIENCE, BENGALURU

### SCORE CARD

Name of the Candidate

**SURIYARAJ S A S**

Name of the Parent/Guardian

**SUCITHRA**

Registration No.

**BT24S57402310**

Test Paper

**Biotechnology (BT)**

Date of Examination

**February 10, 2024**

GATE Score

**351**

Marks out of 100

**39.0**

All India Rank (AIR)  
in the test paper

**2815**

**Qualifying Marks**

General

**38.9**

EWS/OBC-NCL

**35.0**

SC/ST/PwD

**25.9**

Number of candidates  
appeared for the test paper

**17078**



*Suriyaraj S A S*

*Prof. Chandra Sekhar Seelamantula*

Prof. Chandra Sekhar Seelamantula  
Organising Chairperson, GATE 2024  
On behalf of NCB-GATE  
Ministry of Education (MoE)



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A candidate is considered **qualified** if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with this Score Card.

This Score Card is valid  
up to 31<sup>st</sup> March 2027.

