

**PHYSICAL CHARACTERIZATION AND
MICROSCOPIC STUDIES OF *PORITES*
SPECIES FROM SABAH**

FADHLIA ZAFARINA BINTI ZAKARIA

**SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH
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**THESIS SUBMITTED IN FULFILMENT FOR THE
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DECLARATION

I hereby declare that this submission is my own work and that to the best of my knowledge and belief, it contains neither materials published/written by another person nor material which to a substantial extent has been accepted for the award of any other degree of a university or other institutions of higher learning except where due acknowledgement is made in the text of the paper.

27 November 2008

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FADHLIA ZAFARINA BINTI ZAKARIA



ABSTRACT

PHYSICAL CHARACTERIZATION AND MICROSCOPIC STUDIES OF *PORITES* SPECIES FROM SABAH

Physical characterization of *Porites* species from Sabah have been studied by using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Fourier Transform Raman Spectroscopy (FT-Raman) and Scanning Electron Microscopy (SEM). The purpose of this research is to study physical characteristic of 3 powder particle sizes; ≤ 38 , ≤ 53 , and $\leq 106 \mu\text{m}$ of *Porites* species. *Porites* skeletons used in these studies were *Porites australiensis*, *Porites cylindrica*, *Porites lutea*, *Porites lichen*, *Porites digitata*, *Porites nigrescens*, *Porites rus*, *Porites annae* and *Porites* sp. It was found that crystallinity is increased from *Porites lutea* (lowest crystallinity) to *Porites cylindrica* (highest crystallinity). Low value of FWHM indicates that the crystals are defect free and periodically arranged while high value of FWHM indicates that the crystals are randomly arranged or have low degrees of periodicity. Crystallite sizes were found to be in the range of $1007.781\text{--}1706.040 \text{ \AA}$ and *Porites* species are belonged to two groups (smaller and larger crystallite sizes respectively). It is found that all *Porites* species exhibit orthorhombic crystal structures. From the values of M1, *Porites* species are categorized into two groups; the first group shows high values of M1 with biogenic aragonite cell constant while the second group shows high values of M1 with synthetic aragonite cell constants. It can be concluded that species with high value of M1 has peak data profiles which are compatible with the fitted cell constants. FTIR spectra show the variations in the v_1 , v_2 , v_3 and v_4 bands strength as a function of particle size. Each band decreases in strength with decreasing particle sizes for all species. The size of particulate matter will affect the strength of absorptions because electromagnetic radiation will interact with particulate matter differently for different particle sizes as the ratio of surface to volume scattering changes. FT-Raman spectra show the effect of particle size on the wavenumber and intensity of carbonate symmetric stretching mode, v_1 bands. It is found that the intensity of v_1 band decreases with decreasing particle size. These changes in the wavenumber of Raman bands with particle size are inherent properties of individual minerals. From morphological study, corallites distribution of *Porites* species can be divided into even and uneven. SEM at low magnification (100X) shows fine structures of intact coral skeletons. SEM at high magnification (2000X) however shows broken and fragmented rod-shaped aragonite crystals of some *Porites* species due to the effects of grinding process. In conclusion, there are significant differences of physical characteristic between these three particle sizes of *Porites* powder samples.

ABSTRAK

Pencirian fizikal karang spesis *Porites* dari Sabah telah dijalankan menggunakan Pembelauan Sinar-X (XRD), Spektroskopi Inframerah Penjelmaan Fourier (FTIR), Spektroskopi Raman Penjelmaan Fourier (FT-Raman) dan Mikroskopi Elektron Pengimbas (SEM). Tujuan kajian ini adalah untuk mengetahui cirian fizikal bagi 3 saiz butiran serbuk iaitu ≤ 38 , ≤ 53 , and $\leq 106 \mu\text{m}$ bagi spesis *Porites*. Rangka *Porites* yang digunakan dalam kajian ini ialah *Porites australiensis*, *Porites cylindrica*, *Porites lutea*, *Porites lichen*, *Porites digitata*, *Porites nigrescens*, *Porites rus*, *Porites annae* dan *Porites sp.* *Porites lutea* didapati mempunyai kristaliniti paling rendah dan *Porites cylindrica* mempunyai kristaliniti paling tinggi. Nilai FWHM rendah menunjukkan hablur yang sempurna dan tersusun secara berkala manakala nilai FWHM tinggi menunjukkan hablur tersusun secara rawak dan mempunyai darjah kalaan yang rendah. Saiz kristalit adalah dalam julat $1007.781 - 1706.040 \text{ \AA}$ dan spesis *Porites* tergolong kepada dua kumpulan (saiz kristalit kecil dan besar). Semua spesis *Porites* menunjukkan struktur hablur ortorombik. Daripada nilai M_1 , *Porites* terbahagi kepada dua kumpulan (nilai M_1 yang tinggi dengan sel malar aragonit biogenik dan aragonit sintetik. Dapat disimpulkan bahawa spesis yang mempunyai nilai M_1 yang tinggi mempunyai profil data puncak yang bersesuaian dengan sel malar. Spektra FTIR menunjukkan perbezaan keamatan jalur v_1 , v_2 , v_3 dan v_4 dengan saiz butiran yang berbeza. Setiap jalur menunjukkan pengurangan keamatan dengan penurunan saiz butiran bagi setiap spesis. Saiz bahan berbutir mempengaruhi keamatan penyerapan kerana sinaran elektromagnetik akan bertindakbalas dengan bahan berbutir secara berbeza untuk saiz butiran yang berbeza apabila nisbah permukaan kepada isipadu penyerapan berubah. Spektra FT-Raman menunjukkan kesan saiz butiran kepada nombor gelombang dan keamatan mod regangan simetri karbonat, jalur v_1 . Dapat dibuktikan bahawa keamatan jalur v_1 berkurang dengan pengurangan saiz butiran. Daripada kajian morfologi, taburan koraliit bagi spesis *Porites* dapat dibahagikan kepada sekata dan tak sekata. SEM pada pembesaran rendah (100X) menunjukkan struktur rangka karang yang sempurna. Walau bagaimanapun, SEM pada pembesaran tinggi (2000X) menunjukkan hablur aragonit berbentuk rod yang pecah dan berserpihan bagi beberapa spesis *Porites* akibat proses menumbuk. Dengan ini, jelas menunjukkan bahawa terdapat perbezaan signifikan dari segi cirian fizikal antara tiga saiz butiran serbuk yang berbeza bagi spesis *Porites*.



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LIST OF SYMBOLS

| | |
|--------------------|---------------------|
| m | Metre |
| mm | Millimetre |
| θ | Angle (theta) |
| n | Reflection order |
| λ | Wavelength (lambda) |
| d | Interplanar spacing |
| \AA | Angstrom |
| Π | Phi |
| km | Kilometre |
| km^2 | Kilometre square |
| cm | Centimetre |
| kV | Kilovolt |
| $^{\circ}\text{C}$ | Degree Celcius |
| g | Gram |
| μm | Micrometre |
| V | Volt |
| A | Ampere |
| MA | Milliampere |
| F | Frequency |
| Z | Atomic number |



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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Skeletal structures of organisms recently became the object of broad interdisciplinary studies as their functionally optimized properties arouse inspiration for rapidly growing interest in nanotechnology and biomaterial research domains (Jelinski, 1999). Various skeletal biominerals are the target of studies, including calcium carbonate compounds that occur most widely in nature. For example, mollusk nacre and skeletons of scleractinian corals (especially reef-builders) are known for their mechanical strength, which is partly attributed to their composite structure; the crystallographically ordered biomineral nano-components and micro-components are closely associated with the organic molecules and form complex hierarchical structures (Gre' goire, 1967; Mutvei, 1969, 1979; Currey & Kohn, 1976; Cuif & Dauphin, 2005a; Rousseau *et al.* 2005).

Natural coral (*Porites*) consists of a mineral substance, principally calcium carbonate in the structural form of aragonite with impurities such as Sr, Mg and F ions, and an organic matrix (Guillemin *et al.* 1987; Richard *et al.* 1998). Hydroxylapatite of natural origin used in biomedical applications are derived from human or bovine bone or from coral, as found in marine invertebrate life (Damien & Revell, 2004). Sea corals of *Porites* species possess anatomical structure, physical, and chemical characteristics that simulate human bone (Ripamonti, 1992; Preidler *et al.* 1996; Jamshidi *et al.* 1988; Holmes *et al.* 1986; Sartoris *et al.* 1986). Natural coral, submitted to rigorous protocols of preparation and purification, can be used as a replacement biomaterial for bone grafts in orthopaedic surgery. It can replace bony tissue without inappropriate response from the human body (biocompatibility); it develops a chemical bond with the bone surface (bioactivity) and is able to form bony tissue when it is in contact with bone (osteoconductivity) (Guillemin *et al.* 1987).



Natural biogenic carbonates species of sea origin attract special interest in biomaterials, science and technology. Due to their porous structure comparable to that of bone they are suggested to be suitable natural materials for dental and bone restorations (Westbroek & Marin, 1998). The pores allow invasion of the blood and bone marrow cells, followed by bone formation (Nicolaides *et al.* 2000). There are two main types of porous coralline hydroxyapatite differing in microstructure which are used as bone substitutes (Table 1.1) and the species of marine invertebrates exploited in medical applications are identified in Table 1.2.

1.2 Research Materials

Porites skeletons used in these studies were obtained from the Institute for Borneo Marine Research, Universiti Malaysia Sabah. The list of coral skeletons are as follows:

1. *Porites australiensis*
2. *Porites cylindrica*
3. *Porites lutea*
4. *Porites lichen*
5. *Porites digitata*
6. *Porites nigrescens*
7. *Porites rus*
8. *Porites annae*
9. *Porites* sp. (species that has not been identified and named as yet)

1.3 The Purpose of this Research

This study was designed to elucidate the physical properties of natural coral (*Porites*) as a potential source of coral biomaterial. The coral biomaterial (coralline hydroxyapatite) is manufactured by the hydrothermal conversion of the calcium carbonate skeleton of natural coral to hydroxyapatite (Damien & Revell, 2004). Study of different particle sizes was conducted to see this effect on the physical properties of the corals. This is because microcrystalline materials would display physical properties that may differ from those of their corresponding bulk materials (Alivisatos, 1998).

1.4 Objectives

The main objective of this research is to characterize *Porites* species of corals by using x-ray diffraction, scanning electron microscopy, Fourier transform infrared spectroscopy and Fourier transform Raman spectroscopy.

1.5 The Benefits of this Research

Research works on *Porites* are important to enable their physical and chemical properties being verified. If *Porites* needs to be used as source for bone substitution that is hydroxylapatite in the medical field, all aspects of this material need to be studied. Scientists have always focused on the production of coral biomaterial, its architectural properties and compatibility of the biomaterial as bone substitute, neglecting research at the basic level of that material. At present, there are limited data available on natural *Porites* species (before it is converted into a coral biomaterial). This thesis reports for the first time a physical characterization study of nine different natural *Porites* coral skeletons especially from Sabah. The understanding of natural coral in its original form is very useful to elucidate several important properties of the corals which contribute to their usage and the reasons why it is being chosen as a potential source of producing coral biomaterials in medical application.

Table 1.1: Microstructure of corals used in biomedical applications

| Origin of CHA Genus | Microscopical similarity | Total porosity | Closed pore size (μ) | Interconnecting pores (μ) |
|---------------------|---|----------------|----------------------------|---------------------------------|
| Porites | Cortical bone, pore size corresponds to average diameter of an osteon in human bone | <60% | 200-230 | 190 |
| Goniopora | Cancelloous bone | >70% | 500-600 | 220-260 |

Source: Ripamonti (1992), Preidler *et al.* (1996), Jamshidi *et al.* (1988), Holmes *et al.* (1986), Sartoris *et al.* (1986)

Table 1.2: Classification of corals in biomedical use

| | |
|---------|--------------------------------------|
| Kingdom | Animalia |
| Phylum | Coelenterata |
| Order | Scleractenia |
| Family | Poratidae |
| Genera | Porites species Goniopora species |

Source: Damien & Revell (2004)

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter discusses about *Porites* species corals, introduction to crystallography, previous works and findings on *Porites* coral and other species of corals, biogenic aragonites, biomaterials derived from coral and data analysis regarding instruments that would be used in this research.

2.2 *Porites*

Porites is a genus of Scleractinian coral. Colonies of this highly variable genus could occur rounded, plate-like, column-like, finger-like, branching or encrusting. There are many common names given to the corals of the genus *Porites* including Christmas Tree Worm Rock Coral, Boulder Coral and Encrusting Coral. These corals are found throughout tropical and subtropical areas of the Atlantic, Indian and Pacific Oceans (Veron, 2000).

2.3 Aragonite

Aragonite is a carbonate mineral. Aragonite and the mineral calcite are the two common, naturally occurring polymorphs of calcium carbonate, CaCO_3 . Aragonite forms naturally in almost all mollusk shells and the calcareous endoskeleton of warm- and cold-water corals (Scleractinia). Crystal structure of aragonite is shown in Figure 2.1 (Oganov *et al.* 2005).



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