UNIVERSITI TEKNIKAL MALAYSIA MELAKA

PREPARATION OF CALCIUM PHOSPHATE POWDER FROM FISH BONE WASTE

This report is submitted in accordance with requirement of the University Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Engineering Materials) (Hons.)

By

MUHAMMAD ISWAN SHAH BIN ISMAIL

B051610191

950415-04-5331

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TIDAK TERHAD

Disahkan oleh:

Alamat Tetap:

542-D Jalan Tengkera,
75200, Melaka

Cop Rasmi:

Dr. Toibah Binti Abd. Rahim
Pensyarah Kanan / Senior Lecturer
Fakulti Kejuruteraan Pembuatan
Universiti Teknikal Malaysia Melaka
Hang Tuah Jaya 76100 Durian tunggal Melaka

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I hereby, declared that this report entitled "Preparation of Calcium Phosphate Powder from Fish Bone Waste" is the result of my own research, under the guidance of Dr. Toibah bt Abd Rahim as project supervisor, except for as cited in references.

Signature : ....

Author's Name : MUHAMMAD ISWAN SHAH BIN ISMAIL

Date : ....15/9/2020...

APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for Degree of Manufacturing Engineering (Engineering Materials) (Hons). The member of the supervisory committee is as follow:

(DR. TOIBAH BT ABD RAHIM)

ABSTRAK

Calcium Phosphate (CaP) telah digunakan sebagai biomaterial dalam kebanyakkan aplikasi perubatan sejak beberapa dekad. Tulang ikan bole digunakan sebagai sumber bio-CaP yang murah dan pada masa yang sama ia juga dapat membendung dan mengurangkan kesan buruk terhadap alam sekitar. Di dalam kajian ini, bio calcium phosphate $Ca_3(PO_4)_2$ telah diperolehi daripada sisa tulang ikan yang mana tulang ikan tersebut diperolehi daripada kawasan tempatan. Proses sintesis CaP dilakukan melalui proses pengkalsinasi diantara 600°C dan 1000°C. Kajian difraksi sinar-X menunjukkan bahawa fasa dominan adalah Hydroxyapatite (HA) pada semua sampel. Oleh kerana peningkatan suhu, intensiti HA yang diperoleh juga meningkat sehingga 800°C. Tetapi selepas pemanasan diatas suhu 800°C, terdapat penurunan intensiti dimana dapat dilihat daripada analisa yang diperolehi, puncak HA telah menurun dan semakin melebar. Ini kerana berkemungkinan HA telah bertukar kepada TCP. Kajian mikroskopi telah mendedahkan iaitu serbuk HA dihasilkan oleh percantuman zarah kecil membentuk zarah yang lebih besar. Kajian ini juga membuktikan bahawa CaP telah Berjaya dihasilkan dengan pengunakan tulang ikan patin dengan fasa HA sebagai fasa utama di dalam kesemua sampel.

ABSTRACT

Calcium Phosphate (CaP) has been developed as biomaterial in many biomedical applications since decades ago. Fish bones can be used as a cheap source of biological CaP, which simultaneously offers added value for by-products and decreases unnecessary environmental impact. In this study, biological calcium phosphate was synthesized from fish bone waste from *Pangasius* fish which were collected from local area. Simple calcination process between 600°C and 1000°C was used to produce the CaP powder. X-ray diffraction study reveals that the dominant phase was hydroxyapatite (HA) in all calcined powders and were identical to standard HA pattern. The increasing of calcination temperature had led to the formation of minor phase of β-Tricalcium phosphate (β-TCP). Moreover, as the temperature was increased, the intensity of HA obtained was higher but there was a sudden drop after heating above 800°C. The peak becomes less narrower and broader as HA might transform into another phase; β-tri-calcium phosphate (TCP) phase. Scanning electron microscopy (SEM) reveals the HA powders have been produced by agglomeration of small particles into larger particles. This study reveals that the CaP can be produce by utilizing the waste from *Pangasius* fish with HA as the main phase of the calcined powders.

DEDICATION

My beloved father, Ismail bin Selamat

My appreciated mother, Mastura bt Udi

My respected supervisor, Dr. Toibah bt Abd Rahim

My adored siblings, sisters and brothers who always support me through thick and thin

Marni bt Ismail

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For giving me support, money, cooperation, encouragement and understandings

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LIST OF ABBREVIATIONS, SYMBOLS AND NOMENCLATURES

CaP	- Calcium Phosphate
HA	- Hydroxyapatite
TCP	- Tricalcium Phosphate
β-TCP	- Beta Tricalcium Phosphate
α-TCP	-Alpha Tricalcium Phosphate
XRD	- X-Ray Diffraction
SEM	- Scanning Electron Microscopy
PSA	- Particle Size Analysis
UHMPWE	- Ultra high molecular weight poly ethylene

CHAPTER 1

INTRODUCTION

The first chapter of this report includes a few of important elements for research purposes which are background of the project, problem statement, objectives of the study, scope and project outline. The background project context approximately illuminates the overall research subject overview and provides information. The surface of the research will be influenced by the basic knowledge regarding calcium phosphate. Next, problem statement will focus on current problems that triggered this research. Whereas the objectives of the study will explicate the aim of the project and describing what the study is trying to be accomplish. Next, the scope of the study will briefly describe about the limitation of the research. And lastly project outline which include details of each chapter. Lastly, this research focuses only using *Pangasius* fish waste since this fish can only be found in Malaysia.

1.1 Background of Study

Calcium phosphate is a class of materials which has the component and ability to be used as an implant material for bone. Hydroxyapatite (HA) is one of the components under calcium phosphate. Hydroxyapatite and related calcium phosphate ceramic material has been widely used as an implant material due to the similarity of its component and properties to bone. The implant material needs to be either bioactive, bioinert or biodegradable. In this case, HA has a bioactive property. There are two ways to derived HA its either synthetically or from natural waste. According to Ozawa & Suzuki (2004), fish waste has inorganic bone which contains hydroxyapatite. The weight of fish bone and animal waste contribute to roughly about 60 to 70 percent of Hydroxyapatite as the main component (Piccirillo et al., 2013).

Hydroxyapatite is classified as one of the practical prospects for bioactive bone implants (Prakasam et al., 2015). In addition, development of HA for biomedical use can be categories as a perfect option as it doesn't require a high cost of process and has an unlimited supply of raw materials which can be obtain from animal waste (Sunil & Jagannatham, 2016). These animal waste refers to fish bone, bovine bone, eggshells and corals. Development of HA from animal waste can help on reducing daily waste and eventually helps to improve the environment.

HA is bioactive material but it is non-biodegradable. Therefore, the application of beta tricalcium phosphate (β-TCP) takes place. β-TCP is a biodegradable material which degrades out of the human body after 86 weeks. Based on Wiltfang et al. (2002), small amount of the ceramic residue is present after a 86 weeks which these small residue ceramic is presented inside of the patient bone. Many researches nowadays have focused on the use of high strength bone implants of HA in the form of dense ceramic. To maximize the strength of HA, the grain need to be in a smaller size which it need to be densified quickly after being obtained (Prakasam et al., 2015).

1.2 Problem Statement

In Malaysia, fish is consumed daily and there is a lot of waste produced from fish. According to Rustad (2003), about 50 to 60 percent from 91 million tons of fish caught globally is used for consumption while the rest is discard as waste. Therefore, a huge amount of by product is discard which leads to environmental pollution and biohazards threats to human beings. In order to help to reduce the overexploitation as well as contributing to the environment, these study aims to produce calcium phosphate from fish bone waste as these by-products contains high value which could benefit in terms of medical. Moreover, producing calcium phosphate synthetically is hard and cost consuming. By using this waste, it can be an easy route to obtain calcium phosphate as the fish bone waste is abundant and the process to produce calcium phosphate is simpler which is using simple heat treatment, calcination and sintering method. Comparing synthetic calcium phosphate and the one from natural resources, synthetic calcium phosphate has a non-uniform nanostructure and nonstoichiometric composition with less content of hydroxyl (Leventouri, 2006). Therefore, in this study, a natural calcium phosphate will be produced using fish bone waste by simple heat treatment process at temperature of 600°C, 800°C and 1000°C.

1.3 Objectives

Objectives of the study were correlated with the problem statement. The objectives are:

i. To prepare calcium phosphate powder from fishbone waste using simple calcination process at different calcination temperatures.
ii. To study the effect of calcination temperatures on the properties of as-produced calcium phosphate derived from fish bone waste using XRD, PSA and SEM.

1.4 Scopes

Calcium phosphate can be derived from many animals by product and waste. This project focused on using only the *Pangasius* fish as the raw material. Calcium phosphate obtained from the fish waste can be used as a biomaterial for implantation. Dry and clean fish bone waste is calcined based on the temperature given by TGA analysis on the fish bone. The as synthesized CaP are characterized on their microstructure, crystallinity, phase transformation and particle size. The characterization on the calcined HA powder at different temperature will be conducted using SEM, XRD and PSA.

1.5 Significance of Study

This study significantly promotes the production of calcium phosphate from biowaste to help in reducing environmental hazards. This study will also benefit in medical field as ceramic biomaterial such as hydroxyapatite has a lot of benefits. One of the benefits is it can be used as a coating material to trigger the connection between human bones and implant material since it is a bioactive material and has a similar calcium to phosphate ratio as inorganic part of bones and teeth. In addition, this research will serve as a reference in the future to the researches about producing calcium phosphate from natural waste.

1.6 Chapter Overview

The organization of this report is as follow; Chapter 1 will explain what the whole research is about with a research background followed by problem statement, objective, scope of research and the significance of this study. Chapter 2 comprise of literature review from previous research regarding the crystal structure, crystallinity, types of calcium phosphate and others. Chapter 3 covers preparation and characterization method of the research which includes flowchart, raw material, sample preparation, characterization method and analysis. Chapter 4 presents the comprehensive discussion on the results and data obtained throughout the entire study. Conclusion and recommendation for this experiment is presented in Chapter 5

CHAPTER 2

LITERATURE REVIEW

In this chapter, topics that will be discussed generally about types of biomaterials and its application, source of natural calcium phosphate, types of calcium phosphate derived from biowaste and types of calcium phosphate focuses on the crystal structure. Next, this chapter also will elaborate regarding synthesis method to obtain calcium phosphate which tells about the size, phases and crystallinity of the obtained calcium phosphate. Lastly, types of human bones will be discussed together.

2.1 Introduction to Biomaterials

Tissue engineering has been an alternative solution to repair and regenerate human damaged bone. Scaffolds can act as a tissue-forming host and be a tissue-regenerating house for them to grow. There are several requirements for scaffolds to enable tissue formation for example high porosity, large pores for minerals to pass through and specific surface properties which allows the clamping of cell tissues. CaP- based material helps in triggering the reaction between implant material and tissue (Davies, 1996). These statement is also supported by Anselme (2000) which states that CaP based product support osteoblast adhesion and proliferation. The drawbacks of these material is it cannot be used in high load bearing application due to brittleness and low fatigue resistance. Porosity of the bioceramics which is greater than 100μm is needed to ensure cell colonization (Hench, 1991). This is to guarantee the nutrients can pass thought the implants so that it doesn't prevent body from getting enough nutrients. Calcium phosphate is a type biomaterial under ceramic. There are 3 major categories of biomaterials which are metallic, polymer, and ceramic. The combination of two or more classes will create a composite. These categories of biomaterials will be further explained in this chapter.

2.2 Classification of Biomaterials

Crystal structure, bonding and macrostructure can differentiate the materials into different classes. If the types of bonding are observed carefully, these materials can be separated into three big categories which is metals, polymers and ceramics. Firstly, metallic bonded materials are called metals. Metallic bond or known as ionic bond is a bond between metal and non-metal atoms. Since the valence electron are abundant in metallic bonds, metals have the electrical and thermal conductivity properties. Next, polymer are materials with a long carbon chain and covalently bonded together via secondary bonding. These materials don't prohibit conductivity characteristic because their valence electrons are bonded using covalent bond.

Covalent bond happens between two non-metals atoms which they share the valance electron together. Figure 2.1 shows the difference between ionic bond and covalent bond. Lastly are ceramic materials. Materials that mainly ionic and/or covalently bonded are referred to as ceramics (Surgery, 2009). Considering that ionic and covalent bonds doesn't provide free electrons, in general ceramics are thermally and electrically non-conducting materials. The combination of either any two of the classes will provide a new class of material called composite.

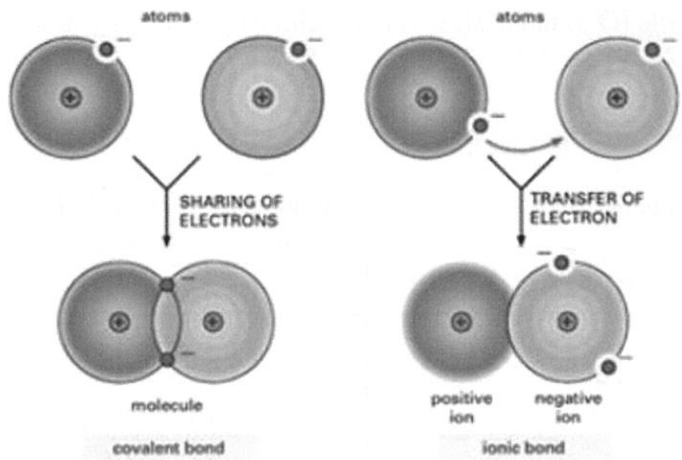

Figure 2.1: Covalent Bonds and Ionic Bonds (Libretexts, 2019)

2.2.1 Metallic Biomaterials

Metallic biomaterial is often used to create biomedical devices due to high strength, high toughness and good biocompatibility. Biocompatibility is one of the desired properties in biomaterials. The meaning of biocompatibility is the material doesn't cause any harm or reaction towards the surrounding tissue and living system. Example of components of a biological system are bone and intracellular tissues. Another important properties that required in a metallic biomaterial is a good mechanical properties (Hussein et al., 2015). When there is a mismatch of modulus of elasticity between human bone and implant materials, stress shielding takes place. Modulus elasticity of the bone varies from 4 to 30 GPa (Peppas, 2000). In addition, the material should possess a low modulus and a high strength to ensure the service life of the device is prolonged thereby eliminating the need for reconstruction of implant.

Metallic biomaterial trustworthiness in mechanical efficiency has resulted in mainly for the manufacture of hard tissue replacement medical devices, such as artificial hip joints, bone sheets and dental implants (Niinomi, 2008). Next, metallic biomaterials is used in load bearing application due to its high strength, high fracture toughness, and high modulus of elasticity, E. However, high modulus elasticity contributes to stress shielding because of mismatch between bone and implants. Table 2.1 summarize the chemical composition of alloys such as Titanium (Ti) alloys, stainless steels and Cobalt (CO) alloys which commonly used as biomaterial.

Table 2.1: Titanium alloys for biomedical applications (Niinomi, 2002)

No	Alloy	Microstructure
1	Pure Ti	(ASTM F67-89)
2	Ti-6Al-4V ELI (ASTM F136-84, F620-87)	α+β type
3	Ti-6Al-4V (ASTM F1108-88)	α+β type
4	Ti-6Al-7Nb (ASTM F1295-92, ISO5832-11)	α+β type (Swiss)
5	Ti-5Al-2.5Fe (ISO5832-10	α+β type (Germany)
6	Ti-5Al-3Mo-4Zr	α+β type (Japan)
7	Ti-15Sn-4Nb-2Ta-0.2Pd	α+β type (Japan)
8	Ti-15Zr-4Nb-2Ta-0.2Pd	α+β type (Japan)
9	Ti-13Nb-13Zr (ASTM F1713-96)	near β type (U.S.A.), Low modulus
10	Ti-12Mo-6Zr-2Fe (ASTM F1813-97	β type (U.S.A.), Low modulus
11	Ti-15Mo	β type (U.S.A.), Low modulus
12	Ti-16Nb-10Hf	β type (U.S.A.), Low modulus
13	Ti-15Mo-5Zr-3Al	β type (Japan), Low modulus
14	Ti-15Mo-2.8Nb-0.2Si-0.26O	β type (U.S.A.), Low modulus
15	Ti-35Nb-7Zr-5Ta	β type (U.S.A.), Low modulus
16	Ti-29Nb-13Ta-4.6Zr	type (Japan), Low modulus
17	Ti-40Ta, Ti-50Ta	β type (U.S.A), High corrosion resistance

Titanium and titanium alloys are used due to its high biocompatibility and it is more preferable compared to another metallic biomaterial. It has good mechanical strength, good corrosion resistance due to strong oxidation layer, low Young's modulus, lighter compared to stainless steels and cobalt alloys and it has a non-magnetic behavior. However, there are some drawback of using titanium as biomaterial which is it has a relatively low resistance to plastic shearing, and low surface oxide protection (Alam & Haseeb, 2002).

2.2.2 Polymer Biomaterials

Polymers are by far the most commonly used material in biomedical application. Polymers are organic materials forming long chains consisting of many repetitive units. Polymers are known to have a unique property such as it is flexible, high resistance to biochemical attack, good biocompatibility, it is light and available in several other compositions with appropriate physical and mechanical characteristics. It also can be easily produced well into the desired form products.

Polymer has a characteristic in which it is biodegradable. Polymer degrade from the body after a it has completed is job. For example, polylactic acid (PLA) when it degrades, its by product is lactic acid which it can be eliminated from the body through sweat. In addition, Mechanical characteristics of polymers rely on some factors including macromolecular chains and molecular weight composition (Patel & Gohil, 2012). Several mechanical properties of selected polymer biomaterials are listed in Table 2.2.

Some example of current application that uses polymer as biomaterial are contact lenses, breast implantation, artificial heart, heart valves, drug capsule and tablet coatings, cardiac assisting devices, artificial blood vessel and skin, supply of medications and treating inflammatory sites or tumors and lastly transportation bag for blood plasma.