



UNIVERSITI TEKNIKAL MALAYSIA MELAKA

**PREPARATION AND CHARACTERIZATION OF CHITOSAN
POWDER FROM SHRIMP SHELLS**

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

by

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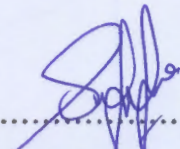
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ABSTRAK

Pada masa kini, pengeluaran Chitin dan Chitosan telah dipertingkatkan dan dibangunkan secara besar-besaran dalam skala komersial. Melebihi jutaan tan metrik Chitosan dihasilkan setiap tahun daripada cengkerang ketam dan udang. Chitosan diiktiraf sebagai bahan yang mempunyai pelbagai manfaat dan faedah seperti bebas toksik, sistem pelupusan yang menyeluruh, keserasian biologi yang baik dan rintangan yang mantap terhadap aktiviti mikrob-mikrob negatif. Dengan meletakkan kepentingan ciri-ciri biobahan yang terdapat di dalam komposisi Chitin dan Chitosan, satu eksperimen telah dijalankan dengan mengekstrak Chitin daripada cengkerang udang dan menukarkan Chitin ke Chitosan melalui deasetilasi. Chitin tersebut terhasil daripada cengkerang dan operkulum udang spesis *Penaeus Monodon* atau Udang Harimau (oleh demineralisasi dan deproteinisasi) dan Chitosan terhasil melalui proses deasetilasi daripada Chitin. Keputusan kajian ini akan membuka jalan dan menyediakan maklumat asas bagi penggunaan Chitosan dalam pembangunan aplikasi bioperubatan. Kepingan Chitosan seterusnya dihancurkan menjadi serbuk menggunakan peralatan makmal “*Planetary Ball Milling*” dengan kelajuan putaran yang berbeza. Beberapa kaedah pencirian bahan akan dijalankan untuk mengkaji morfologi, struktur dan komposisi secara fizikal setelah sintesis dan pengekstrakan Chitosan dilakukan seperti; *Scanning Electron Microscope* (SEM), *Energy Dispersive X-ray* (EDX), *X-ray Diffraction* (XRD), *Particle Size Analyzer* (PSA) dan *Fourier Transform Infrared Spectrometry* (FT-IR).

ABSTRACT

Nowadays the production of Chitin and Chitosan are developed in a commercial scale. More than billion tons of Chitosan are manufactured each year from the shells of crabs and shrimps. Chitosan are recognized as beneficial materials that are non-toxicity, good biodegradability, universal biocompatibility and having a good resistance in the term of antimicrobial activity. Keeping the importance of chitin and Chitosan in mind, an attempt has been made on the extraction of Chitin from the shell and conversion of Chitin into Chitosan through deacetylation. The Chitin was prepared from shell and operculum of *Penaeus Monodon* or Tiger Shrimp (by demineralization and deproteinization) and Chitosan by the deacetylation of Chitin. The results of the present study pave the way and provide the baseline information for the utilization of Chitosan in the development of biomedical application. The resulted Chitosan flakes will be milled into powder using Planetary Ball Milling equipment with different rotational speed. Several material characterization method will be done to examine the morphology, structure and composition in the term of physically after the synthesis and extraction of the Chitosan have been done such as; Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD), Particle Size Analyzer (PSA) dan Fourier Transform Infrared Spectrometry (FT-IR).

DEDICATION

This report is lovingly dedicated to my respective parents; Mr. Mat Yusoff Bin Mat Ali and Mrs. Sakyah Binti Rasidi, my beloved younger sisters and brothers; Nurul Hidayah Binti Mat Yusoff, Muhammad Izzat Bin Mat Yusoff, Nurul Atiqah Binti Mat Yusoff, Muhammad Imran Bin Mat Yusoff, Muhammad Izzuddin Bin Mat Yusoff and Nurul Adlina Binti Mat Yusoff, who have been my constant source of inspiration. They have given me the drive and discipline to tackle any task with enthusiasm and determination. Without their love and support this project would not have been made possible.

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LIST OF ABBREVIATIONS AND NOMENCLATURE

UK	-	United Kingdom
USA	-	United States of America
XRD	-	X-Ray Diffraction
SEM	-	Scanning Electron Microscope
FT-IR	-	Fourier Transform Infrared Spectrometry
NaOH	-	Sodium Hydroxide
HCL	-	Hydrochloric acid
Al ₂ O ₃	-	Alumina
HCP	-	Hexagonal Closed Packard
BCC	-	Body Centered Cubic
STA	-	Heat Treating and Aging
WBC	-	Water Binding Capacity
FBC	-	Fat Binding Capacity
	-	Alpha
	-	Beta
MM	-	Mechanical Milling
°C	-	Degree Celsius
MPa	-	Megapascal
µm	-	Micronmeter
gm	-	Gram
nm	-	Nanometer
N	-	Newton
HV	-	Hardness Vickers
Wt.%	-	Weight Percent
kgf	-	Kilogramforce
g/cm ³	-	Gram per cube centimeter
N/mm ²	-	Newton per square milimeter

LIST OF SYMBOLS

	-	Alpha
	-	Beta
MM	-	Mechanical Milling
°C	-	Degree Celsius
MPa	-	Megapascal
µm	-	Micronmeter
gm	-	Gram
nm	-	Nanometer
N	-	Newton
HV	-	Hardness Vickers
Wt. %	-	Weight Percent
kgf	-	Kilogramforce
g/cm ³	-	Gram per cube centimeter
N/mm ²	-	Newton per square milimeter

CHAPTER 1

INTRODUCTION

1.1 Research Background.

Chitosan is collective name for a group of partially and fully deacetylated chitins. Chitosan was first discovered in 1811 by Henri Braconnot, director of the botanical garden in Nancy, France. Braconnot observed that a certain substance (chitin) found in mushrooms did not dissolve in sulfuric acid. Over the last 200 years, the exploration of Chitosan has taken on many different forms (Ruiz-Herrera, 1978). Several other researchers continue to build on the original finding of Braconnot, discovering new uses for chitin as they find different forms of it in nature.

Chitosan and chitin are polysaccharide polymers containing more than 5,000 glucosamine and acetylglucosamine units, respectively, and their molecular weights are over one million Daltons. Chitin, the polysaccharide polymer from which chitosan is derived, is a cellulose-like polymer consisting mainly of unbranched chains of N-acetyl-D-glucosamine (Austin *et al.*, 1981). Deacetylated chitin, or Chitosan, is comprised of chains of D-glucosamine.

Chitosan, the partially deacetylated polymer of N-acetyl-D-glucosamine, is water-soluble (Tolaimate *et al.*, 2000). Rheology, flocculation and film formation testing have been performed with Chitosan, demonstrating its usefulness in medical and analytical applications. Biodegradable and biocompatible properties of Chitosan films have been studied with good outcomes. N-carboxymethylchitosan solubility and structure have been reported, along with its ability to chelate metal ions and to enhance binding of dyes (Knaul *et al.*, 1999).

Chitosan have several advantages in their properties such as insoluble in water or alkali solution and soluble in inorganic acid like diluted hydrochloric acid and nitric acid or most organic acid (Knorr, 1984). In diluted acid solution, backbone chain of Chitosan will hydrolyze slowly. Consequently, the special characteristics make Chitosan essential in several applications. Unfortunately, Chitosan cannot withstand a high temperature as it will decompose easily at that temperature range (Prerna P. Dawade *et al.*, 2010).

In biomedical applications, Chitosan has a set of unique characteristic which makes it an excellent candidate to be used as scaffold for tissue regeneration purposes. In addition to being biodegradable and non-immunogenic, Chitosan supports the attachment and the subsequent proliferation and growth of different kind of cells, such as chondrocytes and mesenchymal cells, which is attributed to the cationic nature of Chitosan. Chitosan also can exhibits a number of favorable biological activities, which include stimulation of cellular growth and maintenance of the chondrogenic phenotype (M. Kucharska *et al.*, 2010).

Chitin and Chitosan possess very interesting biological properties, therefore, they have been used in many applications, mainly in the medical and pharmaceutical fields such as; non-toxicity, biodegradability, biocompatibility, citocompatibility, antimicrobial activity, anticholestrolemic activity, antioxidant activity, anti-inflammatory action, analgesic action, haemostatic action, mucoadhesion, angiogenesis stimulation, macrophage activation, granulation and scar formation, absorption enhancer and mucoadhesion (Moorjani *et al.*, 1978).

The biological properties of these compounds depend strongly on their solubility in water and other commonly used solvents. In its crystalline form, Chitosan is normally insoluble in aqueous solutions above pH 7; however, in dilute acids, the protonated free amino groups facilitate the solubility of the molecule (Niekraszewicz *et al.*, 2006). The pKa of primary amino groups depends closely on DA, so the solubility of Chitosan is also dependent on DA. Being a highly insoluble and chemically rather unreactive material, chitin has a much lower applicability than Chitosan.

The preparation of Chitosan powder involving four steps such as raw material preparation, demineralization, deproteinization and deacetylation. The resulted Chitosan flakes will be milled with planetary ball mill with different rotational speed such as 100rpm, 150rpm, 200rpm and 250rpm for 1 hour. Several material characterizations will be done to observe the Chitosan in the term of physically characteristics such as; Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD), Particle Size Analyzer (PSA) and Fourier Transform Infrared Spectrometry (FT-IR).

1.2 Problem Statement.

Chitosan powder from shrimp shells are believed to be very useful in biomedical usage as the properties of the Chitosan are very helpful in surgical application (Prerna P. Dhawade *et al.*, 2012). Chitosan powder can be prepared by extracting crustaceans shell such as crab and shrimp shell using several methods. However, in Malaysia, crabs are sold with a higher price in the market (retrieved on 26 May 2013 from <http://www.dof.gov.my/en/faq;jsessionid=3B4C4BC89B808B19C9B733C6F117CEBD>). As an alternative, shells from shrimp's type *Penaeus Monodon* or "Tiger Shrimp" is used to be extracted into Chitosan sample. Based on Dilyana Zvezdova (2010), the Chitosan preparation are not included with decolouration steps. The Chitosan formed are in yellowish colour. Consequently throughout the project, the steps involved during preparation of Chitosan flakes are modified to get Chitosan with a higher degree of deacetylation, whiter colour of powder and lower cost of production with an effective time consumed. The shrimp shells are immersed in 2N HCl for 12 hours during demineralization and 2N NaOH is added with distilled water during deproteinization to reduce the concentration of caustic soda used. Several characterization techniques such as Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD), Particle Size Analyzer (PSA) and Fourier Transform Infrared Spectrometry (FT-IR) are done to make sure the existence of Chitosan inside the extracted material powder.

1.3 Objective.

The objectives of this research are:-

- i. To prepare Chitosan powder from shrimp shells of *Penaeus Monodon* or “Tiger Shrimp”.
- ii. To study the effect of Chitosan powder milled with different rotational speed of Planetary Ball Milling; 100 rpm, 150 rpm, 200 rpm and 250 rpm.
- iii. To characterize the properties of Chitosan powder using Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD), Particle Size Analyzer (PSA) and Fourier Transform Infrared Spectrometry (FT-IR).

1.4 Scope.

The scope of this project lies on the preparation of Chitosan powder itself in term of its experimental procedures, characterisation techniques as well as its properties. The preparation of Chitosan powder involving four steps; raw material preparation, demineralization, deproteinization and deacetylation (Dilyana Zvezdova, 2010). Since, this project involved the tiny particles of the Chitosan powder extracted from shrimp shell, the microstructural analysis is to be carried out by using Scanning Electron Microscope (SEM) and XRD (X-ray Diffraction) that are capable of analyzing the samples at such scale. The observation to ensure the existence of the Chitosan composition from the powder extracted is then analyzed using XRD machine. Only five method of material characterization will be done for the synthesis and extraction of the Chitosan; Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD), Particle Size Analyzer (PSA) and Fourier Transform Infrared Spectrometry (FT-IR).

1.5 Outline of Project.

This final year project is divided into five chapters comprising of introduction, literature review, methodology, results and discussion as well as conclusion and future work respectively. The first introductory chapter elaborates briefly the research background, problem statement, objectives, scope of study and the outline of project.

Chapter two, literature review chapter presents the published literatures that are relevant to particular topic of this research, demonstrating the knowledge of any previous work and awareness of related theories, debates and controversies. Also, this chapter provides background to the new research, linking the new research to what has preceded it.

On the other hand, chapter three discusses the review of the methodology carried out in order to produce the desired product or outcome of the project. The most appropriate method was chosen, allowing the sample to be further analysed by suitable material characterization methods.

Chapter four provides the details of the results acquired throughout the experiment as well as the discussion on the results. The discussion consists of the justification and problems that have been undergone. The data have been given in the forms of tables and figures.

The conclusions and recommendation about this study are discussed in Chapter five, concluding all other chapters and recommending the possible betterment to get the more satisfactory outcome in the future work.

CHAPTER 2

LITERATURE REVIEW

This section provides the literature review that is related to the project development. It reviews about the basically materials used and its properties in various field especially in medical application. In addition, the existence recent techniques of producing Chitosan also have been discussed in this chapter.

2.1 Biomaterials

Biomaterials is a term used to indicate materials that constitute parts of medical implants, extracorporeal devices, and disposables that have been utilized in medicine, surgery, dentistry, and veterinary medicine as well as in every aspect of patient health care. The National Institutes of Health Consensus Development Conference defined a biomaterial as “any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body” (Boretos and Eden, 1984).

Biomaterials are materials (synthetic and natural, solid and sometimes liquid) that are used in medical devices or in contact with biological systems. Biomaterials can be produced either in nature or synthesized in the laboratory using a variety of chemical approaches utilizing metals and alloys, polymers, and ceramics. Because the structures of these materials differ, they have different properties and, therefore, different uses in the body.