CHAPTER 1

INTRODUCTION

During the past decade, the shrimp industries in Thailand have expanded rapidly, yielding high export earnings each year. In the year 1994 alone, the export earnings of shrimp products from Thailand reached US$ 4000 million. High-value shrimp products include frozen and canned shrimp while value-added products include breaded products, sushi-shrimp and shrimp cocktail. To manufacture these products, the shrimp heads and shells are removed. This biowaste is sold to feed mills at a low price (approx. US$ 0.01/ kg). The weight ratio of this waste to the raw materials is 30-40% which means that, if the capacity of production in one plant is 20 tonnes/day, around 8 tonnes of shrimp biowaste is produced. At present, there are about 100 factories in Thailand producing frozen and canned shrimp. The biowaste production per day is estimated to be at least 800 tonnes [1]. This has led to scientists becoming interested in converting this biowaste into value-added products such as chitin and chitosan for use in a wide range of specialist applications.

1.1 Sources and Structures of Chitin and Chitosan

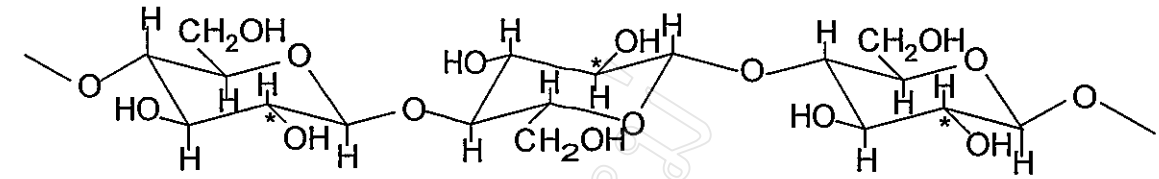
Chitin $(C_8H_{13}NO_5)_n$ has the elemental composition: C 47.29%, H 6.45%, N 6.89% and O 39.37% [2]. Its systematic name is poly(1,4-2-acetamido-2-deoxy-β-D-glucose) [3,4] and it is the one of the most abundant polysaccharides found in nature. It is often considered to be a cellulose

derivative although it does not occur in organisms producing cellulose [5]. Cellulose, poly(1,4-β-D-glucose) [3], consists of β-(1-4)-D-glucopyranose units. In contrast, chitin has the same backbone but the 2-hydroxy group is replaced by an acetamide group [5].

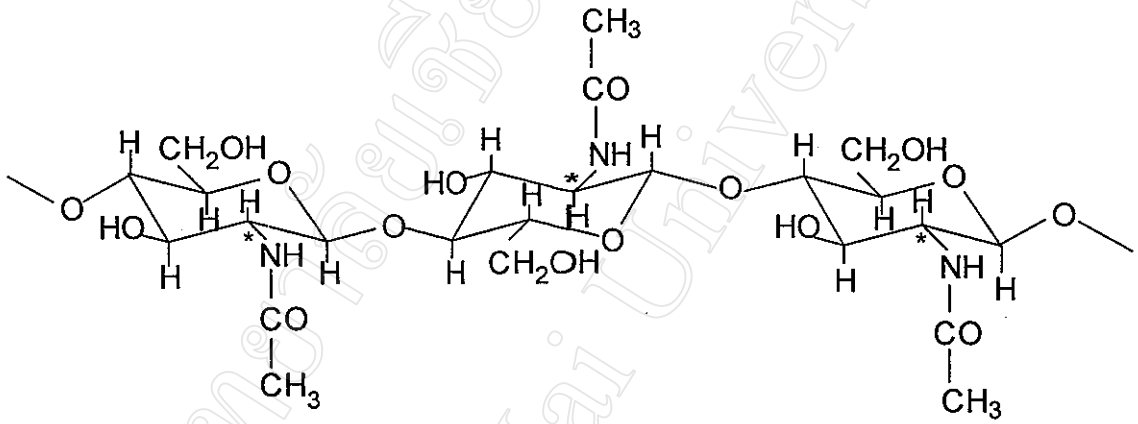
Chitosan, poly(1,4-2-amino-2-deoxy-β-D-glucose) [3], is the N-deacetylated derivative of chitin, although this N-deacetylation is almost never complete. Generally, according to convention, the name ***chitosan*** is used when the degree of deacetylation exceeds 50%. The chemical structures of cellulose, chitin and chitosan are compared in Figure 1.1. While cellulose is a genuine homopolymer, chitin and chitosan are heteropolymers in which not every repeating unit is the same.

Chitin can be found in many species of lower animals and plants where it is used as a cell reinforcement. The polymer exists widely in the cell walls of fungi, moulds and yeast, and in cuticular and exoskeletons of invertebrates such as crabs, shrimps and insects. Crustacean shells are the largest source of commercial chitin as large quantities of shell waste is available from seafood factories throughout the world processing crabs, shrimps and krill.

Crustacean shells are typically composed of three major components: chitin, minerals and proteins. During the commercial extraction process, the shell waste is first ground to small pieces. The minerals and proteins are then removed by successive treatment of the crushed shells with aqueous acidic and alkali solutions. Typically, the dry mass of shellfish waste contains about 14-35% by weight of chitin.



(a)



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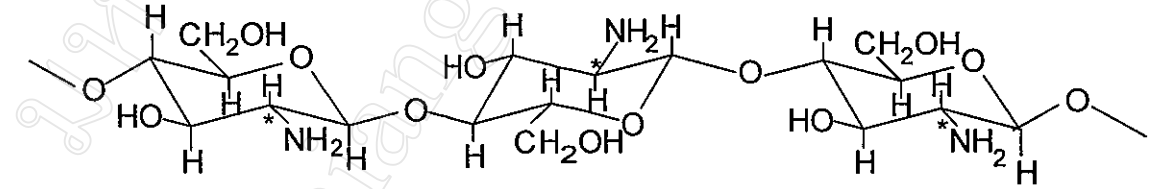

(c)

Figure 1.1: Chemical structures of:

(a) cellulose, poly(1,4-β-D-glucose)

(b) chitin, poly(1,4-2-acetamido-2-deoxy-β-D-glucose)

(c) chitosan, poly(1,4-2-amino-2-deoxy-β-D-glucose)

Note : * shows the positions of the C-2 carbon atoms

In contrast to chitin, chitosan rarely exists naturally. It is usually obtained by deacetylating chitin with concentrated alkali solution. As shown in Figure 1.1, the main difference between chitin and chitosan is in the nature of the amino group on the C-2 position of the glucose residue, being a primary amine in chitosan and part of an acetamide group in chitin. In reality, chitinous materials always possess a mixture of primary and acetylated amine groups, while it is also possible to convert chitosan back to chitin by acetylating the primary amine group [3].

1.2 Chitin Polymorphism

Chitin occurs in three polymorphic forms, each of which differ in the arrangement of the molecular chain within the crystal cell. α-Chitin is the tightly compacted, most crystalline polymorphic form where the chains are arranged in an anti-parallel fashion (Figure 1.2); β-chitin is the form where the chains are parallel (Figure 1.3) and γ-chitin is the form where two chains are "up" to every one "down".

By far the most abundant polymorphic form in nature is α-chitin which is found in arthropod cuticles and in certain fungi. β-Chitin exists as a crystalline hydrate which accounts for its lower stability since water can penetrate between the chains in the crystal lattice. γ-Chitin has been found in the cocoons of the beetles *Ptinus tectus* and *Rhynchaenus fagi.* The three forms of chitin have been also found in different parts of the same organism, namely in the squid *Loligo* whose beak contains α-chitin, whose pen contains β-chitin and whose stomach linings contain γ-chitin, indicating that the three

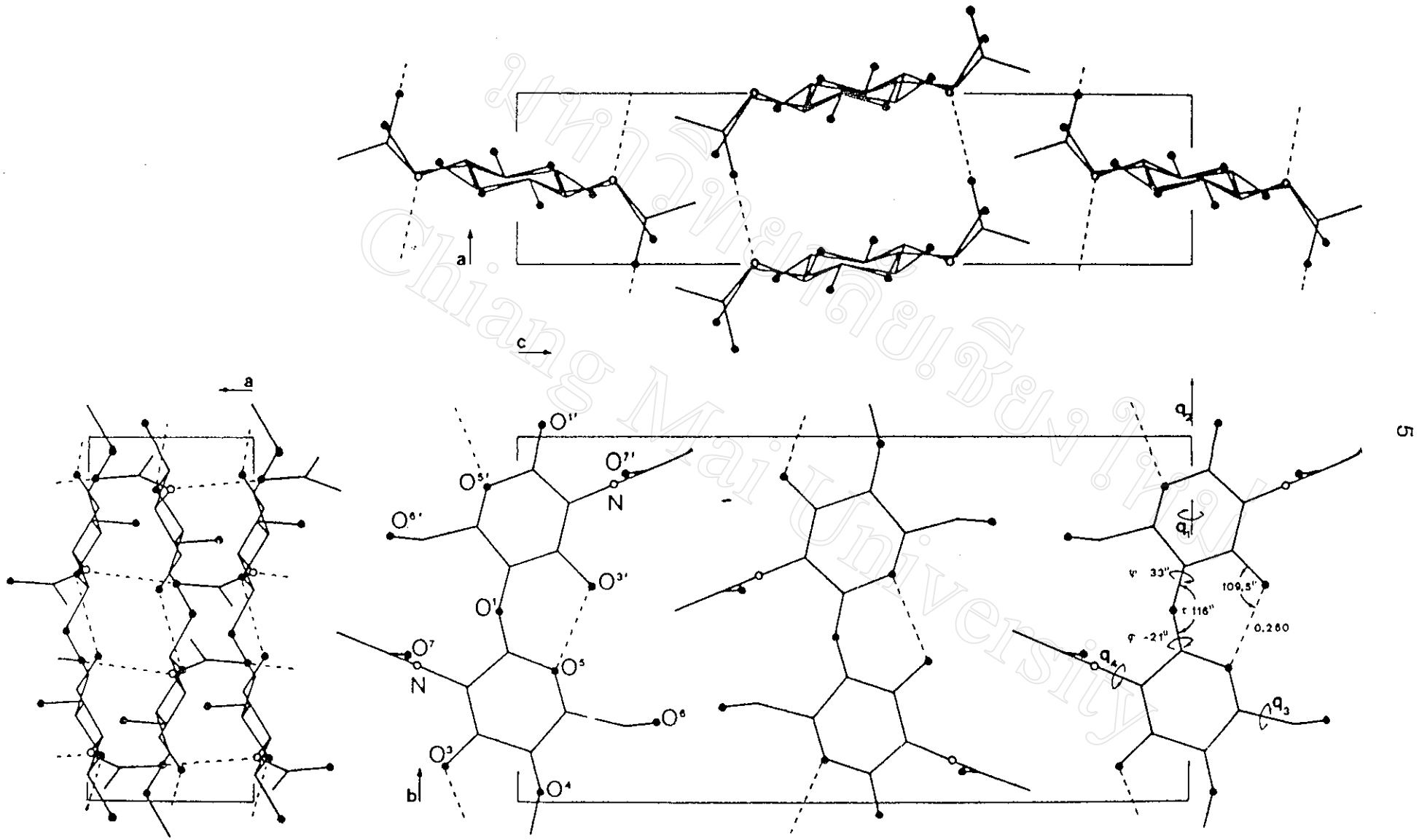

Figure 1.2: Projections of the proposed model for α-chitin [7].

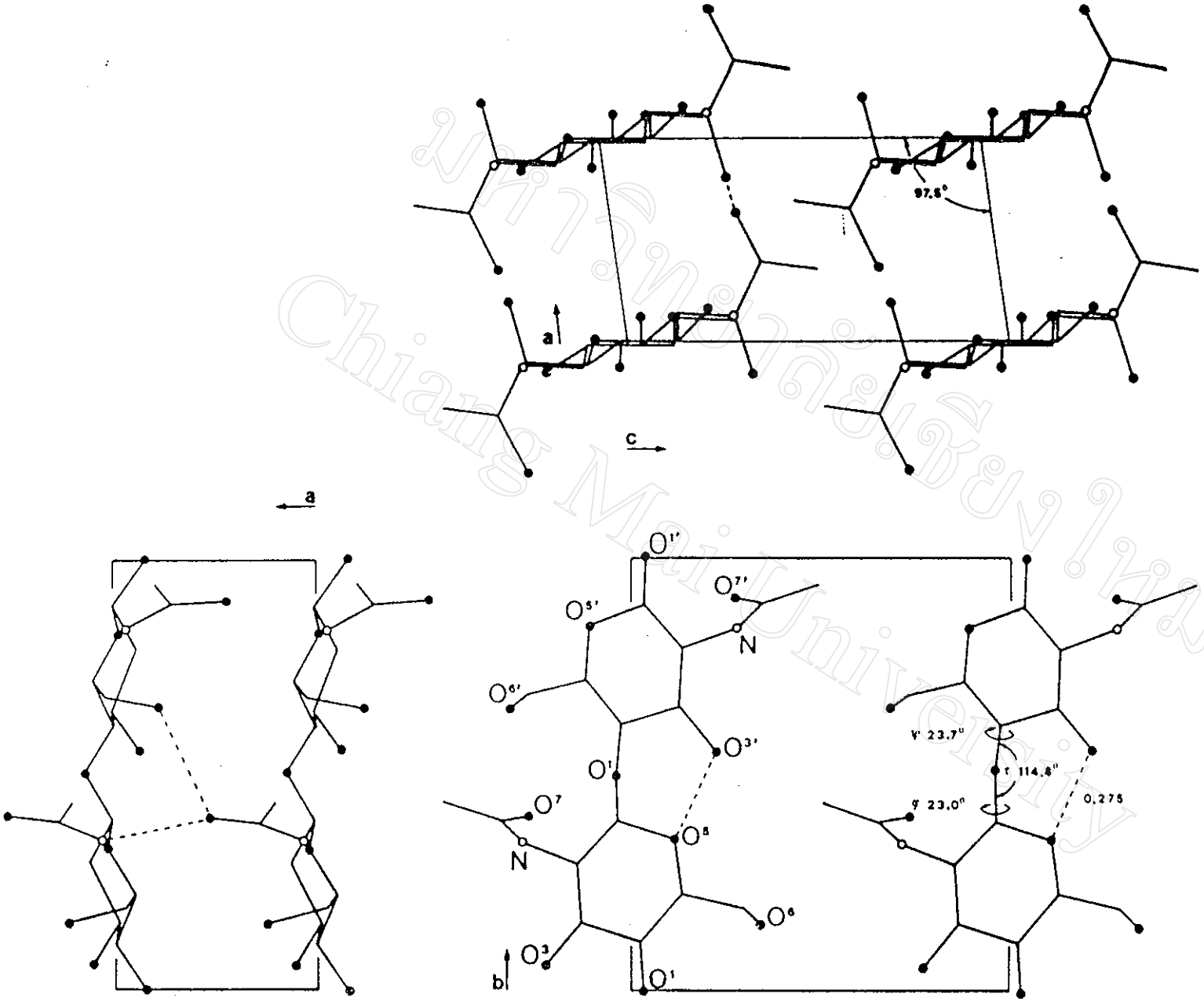

Figure 1.3: Projections of the proposed model for β-chitin [7].

forms are relevant to the different bodily functions and not to the animal grouping [6].

The α- and β-polymorphs can be distinguished by their X-ray patterns and infrared spectroscopy. For example, in its infrared spectrum, α-chitin exhibits a characteristic doublet absorption at 1656 and 1621 cm^{-1}. Other techniques such as CP-MAS, C13-NMR, FT-IR and FT-Raman spectroscopy have also been used to characterise the different polymorphs [5].

1.3 Preparation of Chitin and Chitosan

There are many minerals, pigments and proteins present in chitinous raw materials. The raw materials most abundantly available include crab shells, shrimp shells and prawn waste. Several procedures have been reported in the literature [6-11] for preparing chitin and chitosan, briefly.

In the procedure described by Stanley et al. [8], the ground shell and heads of shrimp or crab were soaked in 0.5-2N NaOH solution at room temperature overnight to remove the surface meat, washed with water and dried. The powder was then soaked in 0.5-2N HCl to remove the materials, CO_2 from the reaction may be removed by suction. For fully protein hydrolysation, powder was soaked in 2N NaOH at 80°C for hours. Residue astaxathin may be removed by soaking the powder in 1% K_2MnO_4 for hours and then 1% oxalic acid. The powder was washed and dried to yield the final chitin product. In order to convert this chitin product to chitosan, the chitin powder was deacetylated with NaOH (40-50% w/w) in 100-120°C for several hours and the reaction terminated by cooling in an ice-bath. The product was

washed several times with deionised water until the pH of the suspension reached 7. The suspended particles were collected and dried at 60-80°C for several hours. The dried powdered product was chitosan with a degree of deacetylation (DD) greater than 50%. The exact DD value needed to be determined experimentally, as described in the following section 1.4.

1.4 Degree of Deacetylation

There are several methods available for determining the degree of deacetylation (DD) of chitin to chitosan. These include infrared spectroscopy (IR), near-infrared spectroscopy (NIR), first derivative UV-spectrophotometry (1DUVS), colloidal titration, linear potentiometric titration (LPT), enzymatic determination, nuclear magnetic resonance (NMR), ninhydrin testing and circular dichroism measurements [12]. The two methods which are utilized in this work are now briefly described.

1.4.1 Titration Method [13]

Chitosan is first converted to its hydrochloride by adding concentrated HCl acid into a solution of chitosan in 10% acetic acid until the chitosan hydrochloride has completely precipitated. The precipitate is filtered off, washed with alcohol, dried, dissolved in a known volume of water and titrated with a standard solution of NaOH using phenolphthalein as indicator. The degree of deacetylation (DD) of chitosan is calculated as a percentage via the formula:

$$DD = \% \text{ Deacetylation} = (C \times V_1 \times V_2 \times MW \times 100) / 1000 \times V_3 \times W$$

C	=	concentration of NaOH (molar)
V_1	=	NaOH volume used in titration
V_2	=	made-up volume of chitosan hydrochloride solution in water
MW	=	mol. wt. of chitosan hydrochloride
V_3	=	volume of chitosan hydrochloride solution used in titration
W	=	weight of chitosan hydrochloride dissolved in V_2

1.4.2 Nuclear Magnetic Resonance [12]

In the NMR method, CP-MAS C^{13}-NMR solid state was used to measure the degree of deacetylation of chitosan sample. The degree of deacetylation of the chitosan is then calculated from the formula below:

$$\%DD = 100 \times [\ 1 - (I_{CH3} / I_{C1})]$$

%DD	=	%deacetylation
I_{CH3}	=	integral of CH_3 peak (24 ppm)
I_{C1}	=	integral of C_1 peak (105 ppm)

1.5 Solution Properties of Chitin and Chitosan

One of the major problems with chitin has been to find a suitable solvent. Although a number of strong solvents such as formic acid,

concentrated mineral acids, trichloroacetic acid, dimethylacetamide-lithium chloride, and a 40/40/20 mixture of trichloroacetic acid / chloral hydrate / dichloromethane can dissolve chitin, these solvents are not convenient to handle and, in some cases, degradation of the polymer in solution is unavoidable [14].

Whereas chitin is chemically inert and difficult to dissolve, chitosan can be easily dissolved in aqueous solutions of most organic and inorganic acids of pH<6 due to the positive charge on the C-2 atom of the glucosamine monomer [15]. In solution, chitosan behaves as a cationic polyelectrolyte with the amine group on the chitosan chain protonated in dilute acid [11].

1.6 Commercial Applications of Chitin and Chitosan

Chitin and chitosan, together with their most widely used derivatives, have the potential to be amongst the most important biomedicals for use in industries as diverse as health care, cosmetics and water treatment [16].

Chitin and chitosan have been found to have various biological effects such as biocompatibility, biodegradability, non-toxicity, activation of host defenses to prevent infection, acceleration of wound healing, and so on. Amongst the objectives expected for applications of chitin and chitosan as biomaterials are: (1) to accelerate wound healing, (2) to be useful in unhygienic circumstances, (3) to decrease treatment frequency, (4) to give comfortable and painless surface protection, (5) to simplify operations and (6) to avoid or decrease the use of antibiotics [17].

The application of chitin in cosmetics began in the year 1967 as abrasives in skin creams. However, its insolubility in common solvents tends to limit its use in finished products. Carboxymethyl chitin, on the other hand, shows characteristics of polyelectrolyte behavior in aqueous solution and use has been found in hair cosmetics. Other water-soluble chitin derivatives have also been prepared and used as cosmetic ingredients in hair shampoo [18].

There have been extensive investigations of chitin and chitosan as fibre and film formers. These fibres and films could be useful as membranes, nonwovens and wound dressings. Chitosan and chitin can be converted into fibres by dissolving the polymer in a spinnable solvent and coagulating the solution in a bath by the wet spinning process. The fibres have primarily been used for special applications such as medical textiles and as components of wound dressings [19]. Apart from their applications in the medical field, chitin and chitosan fibres have potential applications in waste water treatment where the removal of heavy metal ions by chitosan through chelation has received much attention.

1.7 Aims of This Project

The main objective of this research project is to study the preparation of chitosan monofilament fibres by the wet spinning process. This will involve (a) developing an understanding of the processing variables, both those relating to the material and the apparatus, followed by (b) manipulating these variables in order to produce fibres of uniform diameter.

Following their preparation, the chitosan fibres will be characterised and tested according to their surface appearance (scanning electron microscopy), semi-crystalline morphology (X-ray diffraction), molecular weight (dilute-solution viscometry) and mechanical properties (tensile testing). In addition, methods of improving the normally poor mechanical properties of chitosan fibres by (1) plasticisation (to increase softness and pliability) and (2) crosslinking (to increase tensile strength) will also be studied.

This project is part of a wider study of the potential biomedical applications of chitosan currently being carried out in the Biomaterials Group of the National Metal and Materials Technology Center (MTEC). The Chiang Mai Polymer Research Group, within which this project was carried out, is also MTEC's Biomedical Polymers Research Unit, one of MTEC's network of research units in designated areas of specialization.