

THE COMPOSITION OF CHITIN, CHITOSAN AND ITS DERIVATIVES IN THE CONTEXT OF PREPARATION AND USABILITY- A REVIEW

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ABSTRACT. The demand for chitosan polymer in domestic and industrial use is increasingly rising. The applications are widely used in the fields of nutrition, cosmetics, biomedical, pharmaceutical, water treatment and agriculture. Normally, the preparation of chitin comes from a bio-waste source and requires three chemical processes including demineralisation, deproteinisation, and discolouration. Meanwhile, the preparation of chitosan from chitin is through the process of deacetylation. The production of chitosan and its derivatives have covered various fields, including synthetic polymers. It has also become a medium and alternative material helping to solve many problems including being able to save time, cost and energy in the production of a material. Then, there will be a positive impact on environmental sustainability and biomedical engineering. The chitin derivatives resulting from deacetylation of chitosan are also flexible enough to be lysosomal enzymes, which can be used as carriers of active drug substances in the body system. Various efforts and research have been carried out on the development of chitosan-based polymeric materials, in particular organic polymers. Chitosan-based polymers can be used as an alternative to replace petroleum and natural gas resources. Besides, it is easy to dispose of, degrades quickly, has a short shelf life and is environmentally friendly. It is proven as many previous reports and studies on the synthesis, characteristics and use of these polymers around the world. The purpose of this review is to explain the properties, methods of preparation and use of chitin, chitosan and its derivatives.

KEYWORDS. Chitosan, chitin, deacetylation, polymer

INTRODUCTION

The increase in the world population raises concerns about the use of synthetic polymers. Awareness of the importance of preserving the environment is crucial. The general knowledge of waste disposal should be taken seriously by every user in addition to the selection of environmentally friendly synthetic materials such as biopolymers as they can decompose naturally.

Since the use of synthetic polymers creates issues for customers and the natural environment, various studies were conducted to produce environmentally friendly alternative materials. The physical properties of chitosan polymers include biodegradable, biocompatible properties, unique chemical structure, multi-dimensional properties, harmless health and environment, as well as minimal toxic

content. Besides, the life cycles of products based on chitosan can be controlled and easy waste disposal. Furthermore, it is suitable as an energy and renewable source (Nwe *et al.*, 2009).

Chitosan-based polymers are extremely beneficial to humans. In general, the use of natural polymers is categorised as environmentally friendly and has wide range of uses in the sector (Cheba, 2020). Chitosan-based polymers can also be used as an alternative to replace petroleum and natural gas resources. This is because petroleum and natural gas are limited resources. Because of the unique properties of chitosan polymers, it is not impossible that the problem is reduced due to the replacement of resources.

Besides, most of the synthetic polymer is not easily degradable, the use of toxic substances and hazardous materials with high chemical content causes disposal to take a long time. The bonds between the molecules in the synthetic polymer make it difficult for microorganisms to decompose. Furthermore, it damages flora and fauna such as aquatic life like turtles, fish and seaweed. The physical and chemical properties of synthetic polymers such as lightweight plastic bags make it easy for the wind to carry everywhere. Therefore, the availability of chitosan-based polymers that are easily decomposed makes it necessary to produce paper or plastic bags.

Referring to the issues and problems above, chitosan polymers lead to research and studies to help in reducing environmental pollution. Chitosan polymers are also very disposable, degraded quickly, have a short shelf life and are environmentally friendly.

HISTORY OF CHITIN AND CHITOSAN

Chitin was first discovered by Prof. H. Braconnot, director and scientist at French Botanical Garden. Chitin first studied in one of the botanical gardens, France, in 1811. He found chitin in mushrooms. Subsequently, the mushrooms were studied by dissolving into sulfuric acid. However, it is insoluble in sulfuric acid solution. He was interested in the study and continued his research on the compounds and fibres in mushrooms. He made several catalogs of chitin with several types of mushrooms (Oskargata, 2014).

Besides, several influential scientists continue to research the perceptions of chitin in detail. Chitin directly recognised in 1843 when a well-known writer and scientist, Lassaigue have proved that the presence of nitrogen in chitin. He planned the existence of chitin in insects and plants. One of his articles is to analyse the structure of insects and plants if the existence of chitin is found in insects and plants or vice versa (Jeuniaux, 1996). Chitin is a derivative of the Greek name, which is *tunic* or *envelope* while chiton syllables carry the meaning of nail coating (Kavitha *et al.*, 2011; Jeanes *et al.*, 1974).

Next, Prof. C. Rouget discovered the chitin derivative in 1859. He did extensive research on chitin before discovering that the compound is a chitin derivative. Moreover, In the findings of his study showed that chitosan is a fibre derived from chitin by chemical treatment and at a certain temperature (Morris *et al.*, 1977). The result of the study is published in several articles on chitin derivatives, the treatment of chitin by boiling point and the concentration of potassium hydroxide (KOH) solution in water (Jack & Paul, 1955).

Furthermore, in 1878, Ledderhose identified chitin as a compound of glucosamine and acetic acid. Research on chitin and chitosan so fascinating scientists to explore more thoroughly in the early 1900s. An example is that most of the bacteria that were tested had chitin in 1916 (Muzzarelli, 1977). In 1936, Rigby *et al.* (1936) a group of United States scientists found several bacteria have enzymes to dispose of chitin (Stephen, 1995). Rigby *et al.* (1936) also expanded their research scale to the isolation

of shrimps and crabs shells from the body, preparation of chitosan and chitosan derivatives, manufacture of chitosan-based on emulsions, films and filaments, and cellulose-based material wrappers such as wood pulp and straw (Koide, 1998). Some scientists are more interested in researching the physical and chemical properties, sources of chitin, applications and uses in various fields (Grenh *et al.*, 2002).

CHITIN AND ITS COMPOSITION

Chitin is a macromolecule composed of the sub-unit *N*-acetyl glucosamine present in the outer skeleton of crustaceans such as shrimp shell, crabs and insects (Allan & Hadwiger, 1979). Besides, the main components of chitin consist of exoskeleton of invertebrates, crustaceans, insects and cell walls of fungi and yeast (Tan *et al.*, 1996; Knorr, 1984). Chitin is a non-toxic substance and biodegradable polymer with high molecular weight (Kavitha *et al.*, 2011). Chitin is a natural mucopolysaccharide consisting of 2-acetamido-2-deoxy- β -D-glucose through the connection of β (1 \rightarrow 4).

Next, chitin can be disposed of via chitinase (Zhang *et al.*, 2011). It is the second most important organic compound besides cellulose (Brzeski, 1987; Ruiz-Herrera, 1978). Chitin is a fibre compound that consisting of an amino group of polysaccharides, a mixture of sugars and proteins, while cellulose is a polysaccharide with and without the presence of nitrogen (Kavitha *et al.*, 2011; Freepons, 1991).

Chitin often used in marine invertebrates such as insects, fungi and yeast (Austin *et al.*, 1981). However, it is not present in large groups of plants and animals. Mostly it is found in crustacean animals as recorded by Knorr (1984). Based on his research, crustaceous animals contain 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin in dry mass (Knorr, 1983; Peniston & Johnson, 1980).

The Characteristics Of Chitin

There are several physical and chemical properties of chitin such as bioavailability, biodegradability, non-toxic substances and good adsorption. Chitin is bioabsorption, anti-bacterial and durability as wound healing and has low immunogenicity properties. The low immunogenicity of chitin is caused by the presence of nitrogen in chitin (Zhang *et al.*, 2011; Freepons, 1991). Therefore, there are several studies and researches based on its use in biomedical (Jolles & Muzzarelli, 1999).

Moreover, the insolubility of chitin in the material highly resembles the solubility and low chemical reactivity properties of cellulose. This causes the interaction between the hydrogen bonds. Chitin is insoluble in water and organic solvents (Annaduzzaman, 2015). However, It is soluble in Hexafluoroisopropanol, hexafluoroacetone and chloro alcohol in the synthesis of the aqueous solution (Benavente, 2008; Ravi Kumar, 2000).

Chitin is white, hard, inelastic and a type of polysaccharides which is a nitrogen compounds (Zikaris, 1984). Next, chitin affects the crystallinity, purity and composition of the polymer chain (Rinaudo, 2006). Chitin is also high in hydrophobic properties because of high interaction between hydrogen bonds (Fernandez, 2004). The use of chitin is extensive as a support and components protection (Tan *et al.*, 1996; Knorr, 1984).

Finally, the chitin-fibril is an implicit matrix structure of calcium carbonate, phosphate and protein. The matrix structure is high in protein which it is difficult to decompose from chitin fibres after the shell of shrimp and crab is dried (Muzzarelli, 1977; Austin *et al.*, 1981). Asford *et al.* (1977)

explained that 14-27% chitin and 13-15% dry mass of shrimp and crab shells from waste materials and food waste.

STRUCTURE OF CHITIN

Chitin is a homopolymer and a chemical structure composed of poly-(164)-*N*-acetyl-D-glucosamine resembling biopolymer cellulose that is readily detectable in the environment (Annaduzzaman, 2015; Shahidi, 1995). Chitin has a high molecular weight of 1000-3000 units in *N*-acetyl-D-glucosamine (NAG) units linked by β -D(164) bonds (Steve, 2005). The empirical formula of chitin is $(C_8H_{13}O_5N)_n$ while the molecular mass is 627.5928 g / mol. **Figure 1** shows the chemical structure of chitin, chitosan and cellulose.

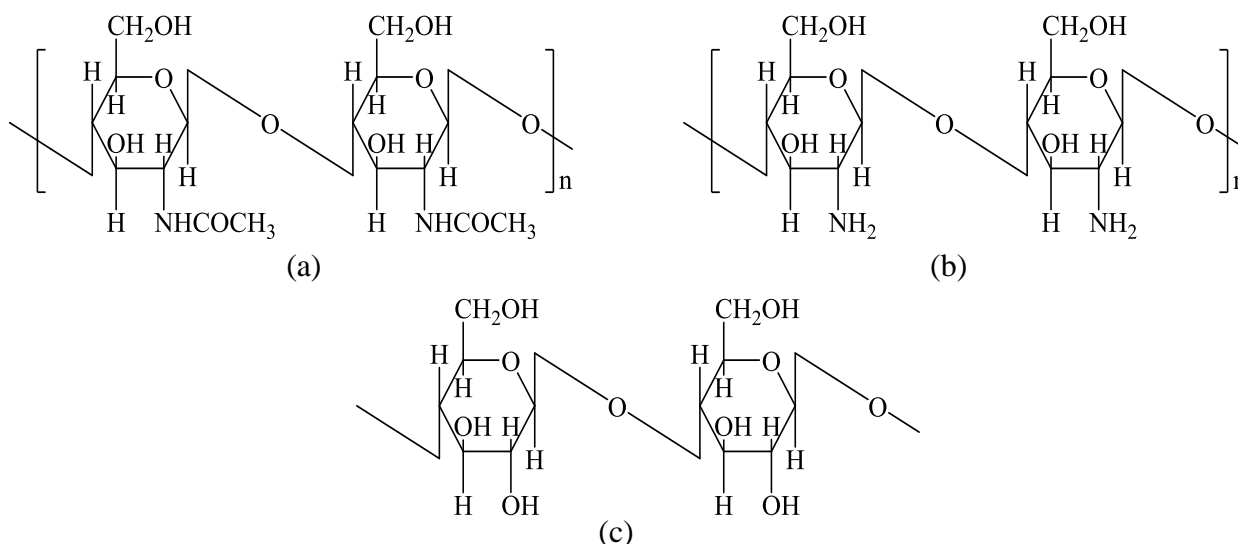


Figure 1. Chemical structure of (a) chitin, (b) chitosan and (c) cellulose.

There is a difference between chitin and cellulose in terms of functional groups between micromolecule. The difference in the structure of chitin and cellulose is the position of the hydroxyl (-OH) functional group at the C2 position which is replaced by the acetamide group. Based on **Figure 1**, chitin contains 6-7% of nitrogen. Chitin has a functional group consisting of amines, acetyl and hydroxide. Chitin is easily absorbed by metals due to the presence of large amounts of active amino group (NH_2) and the hydroxyl group (-OH) (Annaduzzaman, 2015).

There are 70-90% units of *N*-acetyl-D-glucosamine and 10-30% units of D-glucosamine in chitin are connected by (1 \rightarrow 4) β -glycosidic (Stephen, 1995). Besides, the chitin has three forms namely α , β and γ . The α -chitin form is from the shell of shrimp and crab. It is arranged in an anti-parallel. The strong hydrogen bonds in anti-parallel arrangement is very important because it provides high stability (Sikorskiet *al.*, 2009). The β -chitin form is often found on mollusc such as squid bones arranged in parallel while γ -chitin has two parallel lines, which are parallel line and an anti-parallel line (Atkins, 1985). The exchange can occur between α -chitin and β -chitin, but γ -chitin can only be converted to α -chitin by using lithium thiocyanate as processing (Schiffman & Schauer, 2009; Lavall *et al.*, 2007; Mazeau *et al.*, 2002; Rudall & Kechington, 1973).

Chitosan And Its Composition

Chitosan is a natural carbohydrate-based polymer derived from deacetylation of chitin (Steve, 2005). The development and improvement of this material are due to the material obtained is from natural ecology sources such as crabs and shrimp waste. Chitosan is a derivative of chitin produced from good quality compared to cellulose. Furthermore, the technology is now more focused on chitosan as a formula for pharmaceuticals that can be used widely (Venkatesan & Kim, 2010).

Chitosan is a biodegradable polymer that has a higher molecular weight than chitin and cellulose. Besides, it is a natural cation polymer resulting from the hydrolysis of chitin. The positive charges contained in chitosan allow it to easily combine with the negative charges of saturated fats, unsaturated fats, cholesterol, metal ions, proteins and chemically bonded molecules (Li *et al.*, 1992).

Therefore, it is used as a suitable natural material to be commercialised in accordance with its unique characteristics such as biodegradable, good absorbent, biocompatibility, ability in the formation of film and metal ions of chelate, bio-adhesive, poly-functional, hydrophilic and adsorbent (Rinaudo, 2006; Rout, 2001).

CHARACTERISTICS OF CHITOSAN

The uniqueness of chitosan characteristic is that it has its own biological, chemical and physical properties. Chitosan is anti-toxic and has a large molecular weight. In addition, chitosan is water soluble. Based on its solubility in water, it is easy and suitable to be used as a hydrolysis of chemicals or enzymes. Therefore, chitosan is suitable for use in various forms such as fibres, hydro gels, beads, sponges and membranes (Mano *et al.*, 2007).

Besides, chitosan is a derivative of chitin, which is a soluble homopolymer in dilute acids such as acetic and formic acid. It is also can form crystalline compared to chitin. This makes it easily soluble in organic solvents such as hydrochloric acid (HCl), acetic acid (CH₃COOH) and formic acid. Chitosan is easily soluble at very high concentrations of primary amine group solutions. It makes chitosan more nucleophilic and alkaline (Stephen, 1995). Chitosan is easily dissolved in weak acids than chitin. Chitosan is dissolved in aldehydes to produce immunity easily (Kurita *et al.*, 1988). When chitosan is heated, it is easily broken down than is melted. Thus, chitosan has no melting point (Zikaris, 1984). **Figure 2** show the physical appearance of chitosan produced by using shrimp shell.



Figure 2. The physical appearance of chitosan produced by using shrimp shell as raw material.

Next, chitosan is known as a weak base and undergo neutralisation reaction of alkaline compounds. In these reactions, the unpaired electron on the primary amino group in the glucosamine unit acts as a proton receiver. Thus, primary amine groups become positively charged (Tokura & Azuma, 1990). Based on **Table 1**, there are suitable organic solvents used for chitin and chitosan.

Table 1. Solvents for chitin and chitosan.

Name	Solvent	References
Chitin	Dimethyl formamide/ Lithium Chloride; Dimethyl formamide/ Lithium Chloride; Hexafluoro acetone sesquihydrate, Hexafluoroisopropanol Chloroethanol / Sulphuric acid	(Capozza, 1975) (Austin, 1975)
Chitosan	Formic acid/ Water; Acetic acid/ Water; Lactic acid/ Water; Glutamic acid/ Water	(Steve, 2005).

Finally, the chitosan is insoluble in neutral and alkaline aqueous solution. This is due to a large number of cations in the chitosan chains which permits water solubility to increase when the polarity of the compounds and the polymer-interaction value between electrostatic rejection occur (Stephen, 1995). Here is a summary of the characteristics of chitosan as shown in **Table 2**. Referring to the table, the characteristics of chitosan can be attributed to cations naturally. This is due to its uniqueness as polysaccharides and natural polymers.

Table 2. Physical properties, chemical, biological and electrolyte properties of chitosan.

Characteristic	Explanation	References
Physical and chemical	<ul style="list-style-type: none"> • Straight amino-polysaccharides with high nitrogen content; fixed D-glucosamine structure; high crystallisation; hydrophilicity. • Capacity to form bonds between hydrogen molecules; high viscosity; active groups in chemical bonding and cross-linking activities; ion insulation; producing organic salts and inorganic acids. Weak base; amino group protonation as nucleophile (pKa 6.3); insoluble in water and organic solvents; soluble in aqueous solution of dilute acid.	Crini & Pierre-Marie, 2008; Rinaudo, 2006
Poly-electrolyte (At acid pH)	<ul style="list-style-type: none"> • Biopolymer cation which has high density (single charge / balance glucosamine). • Group material agents; react with negative charges. • Absorption properties as filtration and separation. • Ability to produce films; abilities as an adhesive. • Separators in bio-molecules. 	Synowiecki & Al-Khateeb, 2003

Biology	<ul style="list-style-type: none"> • Biocompatibility (non-toxic material; biodegradable; absorbency). • Bio-activity (anti-microbial activity such as fungi, bacteria, viruses; anti-acid, anti-ulcer and anti-tumor; hypolipidemic activity; anti-coagulant blood). • Bio-adhesion. 	Struszczyk, 2002
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The Structure of Chitosan

The specific chemical name for chitosan is poly- [β - (1 \rightarrow 4) -*N*-acetyl-D-glucosamine] and is a copolymer for D-glucosamine and *N*-acetyl-D-glucosamine derived from *N*-deacetyl chitin. Chitosan is a fiber derived from chitin, a homo polymer for β (1 \rightarrow 4)-chain *N*-acetyl-D-glucosamine. The molecular mass of chitosan is 1526,454 g / mol. Chitosan is a biopolymer (Kappel& Gruber, 2020). The chemical reaction of chitosan formation in **Figure 3**.

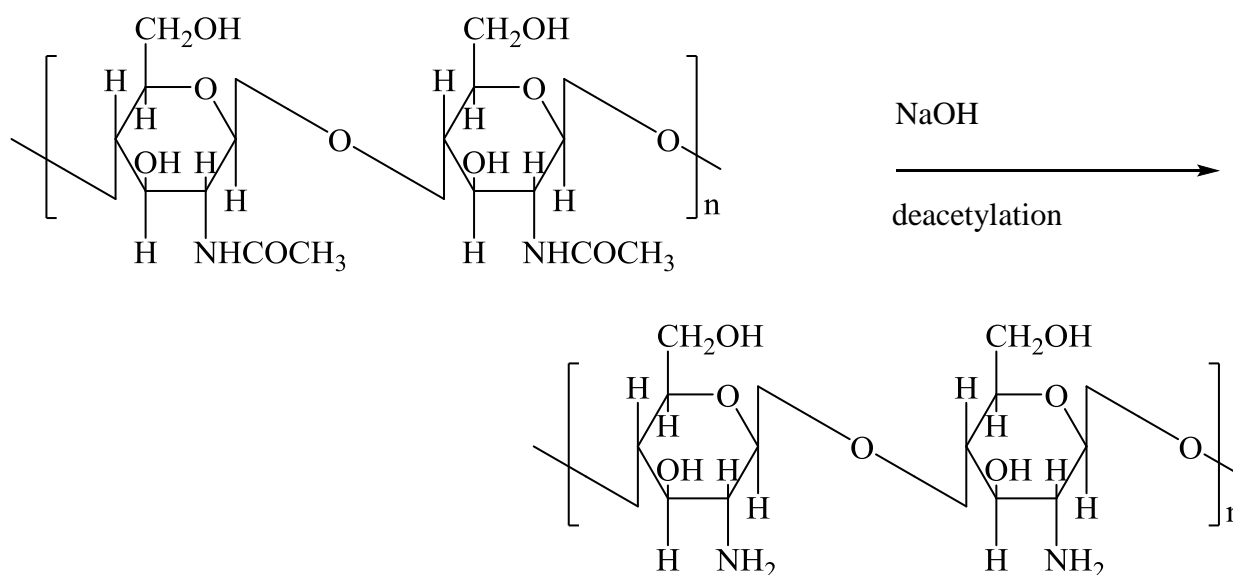


Figure 3. Preparation of chitosan from chitin is through the process of deacetylation.

Based on **Figure 3**, chitosan contains 7-9% of nitrogen. In addition, chitosan has 60-80% of the acetyl group that has been removed from chitin (Marthur & Narang, 1990). There is a free amine group in chitosan structure after deacetylation chitin occurs (No & Meyer, 1992).

Referring to the structure of chitin and chitosan in **Figure 4**, the functional groups involved are amine groups and hydroxide. Chitin and chitosan have similar chemical structures (Batista & Roberts, 1990). Chitin is a straight chain of the acetyl group of glucosamine while chitosan is a chain without the group of acetyl ($\text{CH}_3\text{-CO}$) which makes it easily soluble in dilute acid solutions (Pillai *et al.*, 2009). Thus, the difference between chitin and chitosan is the content of the acetyl group in the polymer.

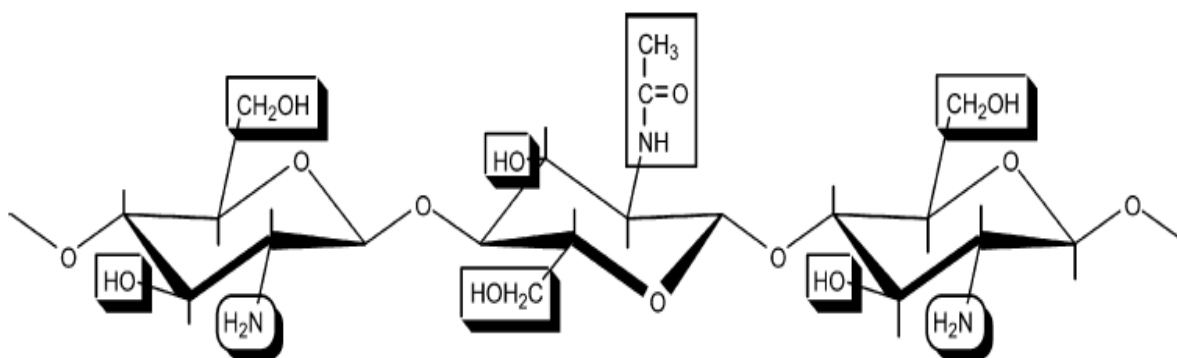


Figure 4. Illustration of the appropriate chitin and chitosan site reactions.

Chitosan contains 75-95% units of glucosamine and 5-25% units of *N*-acetylglucosamine. The molecular weight of chitosan is 106. Chitosan is easily degraded by the release of lysozyme and Magnetic Resonance Spectroscopy (NMR) (Brine *et al.*, 1977). Each glycosidic unit in the chitosan chain is chiasma and the entire unit is connected to the oxygen atom chained to the glycosidic unit at C₁ to C₄ (Stephen, 1995). All forms exhibited by chitosan are known as α conformations within cell units. It is called as orthorhombic and is arranged in anti-parallel. The conformation β is minor in chitosan and have monoclinic unit cells arranged in parallel (Gardner & Blackwell, 1975). X-ray diffraction (XRD) and NMR determine these two types of allomorphs (Takai *et al.*, 1989).

THE PARAMETER OF CHITOSAN

There are many parameters of chitosan that can be observed, but the dependency of these parameters is driven by the industry involved. Among the main chitosan parameters are the percentage solubility, the value of the deacetylation level (DD). Lastly, the colour and the percentage of chitosan moisture content in order to know the quality result of chitosan. Other chitosan parameters include intrinsic viscosity, surface tension, Flory-Huggins interaction parameters, and dielectric constant values (Darder *et al.*, 2003). In addition, the parameters used for the solvent system are the concentration of polymers, pH, the concentration of the opposite ion, the effect of temperature, the percentage of deacetylation and the molecular weight that influencing the solvent process and the viscosity of the solution (Pillai *et al.*, 2009).

Solubility

Chitin is insoluble in organic solvents while chitosan is readily soluble in dilute acid solutions at pH 6.0. Organic acids such as acetic acid, formic and lactic are examples of soluble solutions. For the most part, use 1% acetic acid solution at pH 4.0 as a reference. Chitosan is also soluble in 1% hydrochloric acid solution but insoluble in sulfuric acid and phosphoric acid. The solubility of inorganic acid chitosan is very low. Highly concentrated acetic acid and high temperatures cause chitosan to undergo polymerisation (Roberts & Domszy, 1982). The stability in chitosan solubility at pH 7.0 and above is weak. Precipitate or gelatin is more likely to be formed at high pH and the solution of chitosan produces complex poly-ions with colloidal hydro anionic. Thus, the gel formation is produced (Kurita, 1998).

The ratio between the concentrations of chitosan and acid is crucial for the production of a functional design (Mima *et al.*, 1983). As the concentration of organic solvents exceeds 50%, chitosan as an adhesive in the solution turns smooth. Several factors are emphasised in chitosan solubility such as temperature and decay time, alkali concentration, chitin removal treatment, ratio between chitin and alkali solution, and particle size (Fernandez, 2004).

However, solubility is controlled by the value of the deacetylation level and must be at least 85% completely soluble (No & Lee, 1995; Hackman, 1954). Chitosan is soluble in acidic solution, as 1% of acetic acid should be over 95%. To obtain > 95% solubility, chitosan treatment is carried out by dissolving it in 45-50% NaOH for 10-30 minutes. The result of chitosan treated with 45% NaOH for 5 minutes or 40% NaOH for 30 minutes is not fully dissolved when treat into 1% of acetic acid. Insoluble particles will be deposited in acetic acid solution (Fernandez, 2004). Based on Bough *et al.* (1978) it was stated that the 5 minute solubility reaction of chitosan with 45% NaOH was inadequate for chitin particles to completely swell. When NaOH concentrations were decreased to 40%, the response time for completely dissolved chitosan exceeded 30 minutes (No *et al.*, 2000).

Chitosan is a strong base with primary amino groups (pKa = 6.3). When the pH is lower than 6.0, the amine is protonized and positively charged, making the chitosan cationic water-soluble poly electrolyte. In the opposite, as the pH exceeds 6.0, the amines become protons, the chitosan polymer loses its charge and becomes insoluble. Characteristics of chitin and chitosan are given in **Table 3**.

Table 3. Characteristics of chitin and chitosan.

Characteristics	Chitin	Chitosan
Molecular weight (Mw)	$(1-1.03) \times 10^6$ to 2.5×10^6	10^5 to 5.0×10^3
DD	~10%	60-90
1% viscosity of the solution in 1% acetic acid	-	200-2000
Moisture content	-	6-7
Solubility	Dmac-LiCl / TCA-MC	Weak acid TCA-MC

Level of Deacetylation

The deacetylation process involves the elimination of the functional group of amines in the chitin molecule chain. The result of the process is chitosan with active level of amine (NH₂) group. The level of deacetylation is important in order to know the characteristics of physi-cochemistry and the appropriate application used (Rout, 2001). Deacetylation give effect to the biodegradable and immunology activities (Tolaimate *et al.*, 2000).

The different between chitin and chitosan are depends on the value of *N*-deacetylation (Rout, 2001). Rudall (1963) stated that in his proposal about the evident of the research that at least one out of every six or seven remaining in the chain had proportion of free amino group presented in the history chemistry. This can be classified as an assessment of the level of authentication between chitin and chitosan by determining the content of free amino groups in polysaccharides (Fernandez, 2004). Highly toxic solvents such as lithium chloride and dimethylacetamide are needed to dissolve chitin. However, chitosan is easily soluble in weak acids such as acetic acid. The advantages of chitosan is due to free

amine groups. Free amino groups have active sites that are suitable for chemical reactions (Knaul *et al.*, 1999).

The deacetylation value of chitosan is 56-99%, with an average of 80% depending on the source used either from shrimp or crab and its preparation process (No, 2000; No & Meyer, 1995). Chitin has deacetyl level of less than 10%, while chitosan is more than 75% (Fernandez, 2004). Several studies have been performed to assess the level of deacetylation, such as ninhydrin, straight-shift potentiometer titration, Infrared Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (NMR), titrimetric hydrogen bromide, near-infrared spectroscopy, and first-derived UV spectroscopy (Khan *et al.*, 2002).

IR techniques were first introduced by Moore & Robert (1978) to determine the estimated value of chitosan DD. This technique has its benefits and drawbacks. It is suitable for use because it is faster than other spectroscopy technique, does not require the purification of the sample to be examined and does not require chitosan to be dissolved in aqueous solvent solution (Baxter *et al.*, 1992). However, IR uses site lines to determine DD calculations, various controversies about DD calculations using IR techniques due to site line differences.

Besides, sample preparation, the type of instrument used and the conditions affect analysis of samples. Due to the hygroscopic nature of chitosan in its natural state and has a low sample of BOD, the IR only absorb moisture chitosan. Chitosan with high DD should be in a completely dry state (Fernandez, 2004; Khan *et al.*, 2002; Blair *et al.*, 1987).

The following are the recommended site drawings to determine the value of the deacetylation level:

- (1) Domszy & Roberts (1985), $DD = 100 - [(A_{1655} / A_{3450}) \times 100 / 1.33]$,
- (2) Sabnis & Block (1997), $DD = 97.67 - [26.486 \times (A_{1655} / A_{3450})]$,
- (3) Baxter *et al.* (1992), $DD = 100 - [(A_{1655} / A_{3450}) \times 115]$,
- (4) Rout (2001), $DD = [40.1647 \times (A_{1655} / A_{3450})]$.

Color

Pigments in the skin and crustacean shells develop in complexes together with chitin, such as 4-keto and three 4.4'-diketo- β -carotene derivatives (Rout, 2001). Chitosan powder is naturally soft. Various colors of chitosan from yellow to white. However, cellulose and starch colours are white and smooth (Fernandez, 2004).

Moisture Content

The moisture content of chitosan is determined by gravimetric techniques (Black, 1965). The mass of water is determined by the drying process of the sample until it reaches a constant weight and the sample is measured before and after the drying process. Water mass (or weight) is calculated through the difference between the weight of the sample during wet and the weight of the sample that has dried (Fernandez, 2004). This method is followed by a filtration process to isolate contaminated samples.

According to a study by Fernandez (2004), chitosan samples should be weighed before and after drying in an oven at temperature 60 °C, and placed in a dry sample for 24 hours or overnight. Chitosan samples were duplicated in aluminum metal containers to reduce contaminated samples. Moisture content is calculated through the formula as below:

$$\frac{(\text{Weight of wet chitosan powder, g} - \text{Weight of dry chitosan powder, g})}{(\text{weight of wet chitosan powder, g})} \times 100 = \% \text{ moisture content}$$

CHITOSAN DERIVATIVES

The low solubility of chitosan at pH 6.5 and above facilitates its synthesis and various characteristics are published for the production chitosan derivatives. The differences in chitin and chitosan derivatives can be seen in **Table 4**.

Table 4. The differences between chitin, chitosan and its derivatives in terms of functional group.

No.	Type of derivative	Functional group
1	<i>N</i> -Acetyl chitosan	Formyl, acetyl, propinyl, butyryl, hexanoyl, octanoyl, decanoyl, dodecanoyl, tetradecanoyl, lauroyl, myristoyl, stearoyl, palmitoyl, benzoyl, monochloroacetyl, dichloroacetyl, trifluoroacetyl, carbamoyl, Succinyl, acetoxibenzoyl.
2	<i>N</i> -Carboxyalkyl chitosan	<i>N</i> -carboxybenzyl, glicin-glucan (<i>N</i> -Carboxy-methyl chitosan), alanine glucan, phenylalanine glucan, tyroxine glucan, serin glucan, glutamic acid glucan, methionin glucan, leucin glucan.
3	<i>N</i> -Carboxyacetyl Chitosan	Anhydrite compounds such as maleic, itaconic, acetyl-tio- succinic, glutaric, cyclo-hexane 1, 2-di-carboxylic, phthalic, cis-Tetrahydrophthalic, 5-norbo- mene-2, 3-dicarboxylic, diphenic, salicylic, tri-mellitit, promellitit anhydride
4	<i>O</i> - Carboxyalkyl Chitosan	<i>o</i> -carboxymethyl, cross linked <i>o</i> -carboxymethyl
5	Sugar derivative	1-deoxy-galactic-1-yl-, 1-deoxy-glucide-1-yl-, 1-deoksimelibit-1-yl-, 1-deoxy-lactide-1-yl-, 1-deoxy-lactide-1-yl-4(2,2,6,6-tetramethylpiperidine-1-oxyl)-, 1-deoxy-6'-aldehyde- <i>o</i> - lactide -1-yl-, 1-deoxy-6'-aldehydomelibit-1-yl-, cellobit-1-yl- chitosan, products derived from ascorbic acid
6	Metal ion chelates	Palladium, copper, silver, iodine.
7	Semi synthetic chitosan resin	Methyl methacrylate chitosan copolymers, polyurea – urethane, poly (amid ester), acryl amid –maleic anhydride

(Sources: Junginer & Sadeghi, 2014; Kim, 2014; Anaraz *et al.*, 2009; Ravi Kumar, 2000; Jolles & Muzzarrelli, 1999; Hirano & Hayashi, 1992; Marthur & Narang, 1990; Johnson & Peniston, 1982; Austin *et al.*, 1981, No *et al.*, 1989).

EMULSIFICATION

Although chitosan did not initially produce emulsification, Cho *et al.* (1998) reported that the emulsification capacity of egg yolk increased when chitosan powder was added to it compared to chitosan egg yolk. At 0.5% of chitosan concentration, the capacity for emulsification agent is very good compared to 0.1 or 0.3% of chitosan concentration.

Generally, chitosan emulsification agents are more stable depends on temperature and speed changes. Factors determining the emulsification properties of chitosan are the viscosity and value of the deacetylation level. Protein solutions containing low chitosan DD are less effective than high chitosan DD. Optimum DD chitosan for sunflower oil emulsification is 81 and 89 as reported by Rout (2001) and Del Blanco *et al.* (1999).

PRODUCTION OF CHITOSAN

Chitosan is easy to form film and suitably used as a food preservative. It is used to control psychotropic pathogens in fresh or processed and packaged meat and fish stored at ambient temperature (Smith *et al.*, 1994). Referring to Charles *et al.* (1994), the main ability of chitosan in chitosan application is as a preservative coating agent in fruits. The biodegradable nature makes it one of the advantages of controlling the environment from being affected. This is because the use of plastic-based on petrochemicals leads to environmental pollution (Knorr, 1991).

N, O-carboxylethyl chitosan produces and forms a strong chitosan film because it is easily permeable to oxygen gases and carbon dioxide. Apples coated with this material can maintain their freshness for up to six months. Chitosan coating can slow down the oxidation process of bananas after peeling the skin for 30 days by maintaining the color and shape of the banana (Setha *et al.*, 2000).

CHEMICAL PROCESS IN CHITOSAN PRODUCTION

As is well known, waste from shrimp and crab shells has protein (30-40%), calcium carbonate (30-50%) and chitin (20-30%) in dry conditions (Johnson & Peniston, 1982). The following is the chemical process in chitosan production that traditionally begins with deprotein, where the separation of proteins in chitin occurs. The temperature used was 65 °C and 3.5% NaOH (w / v) for 2 hours (Fernandez, 2004).

The second step is the demeralization process. Dehydration is used to separate calcium carbonate and calcium phosphate contained in the outer shell of crustacean. 1M HCl is used for 30 minutes at room temperature. Next is discoloration, which is the removal of pigment on the chitin surface. Extraction was performed using acetone and bleached using 0.315% sodium hypochlorite solution, NaOCl (w / v) for 5 minutes at room temperature. Finally, the detoxification process is the conversion of chitin to chitosan by using 40-50% sodium hydroxide (NaOH) solution at 100 °C for 30 minutes to remove the entire acetyl group in chitin (No *et al.*, 1999; Fernandez, 2004). The following is a schematic of chitosan extraction by using deproteination, demineralisation, discoloration and deacetylation as shown in **Figure 5**.

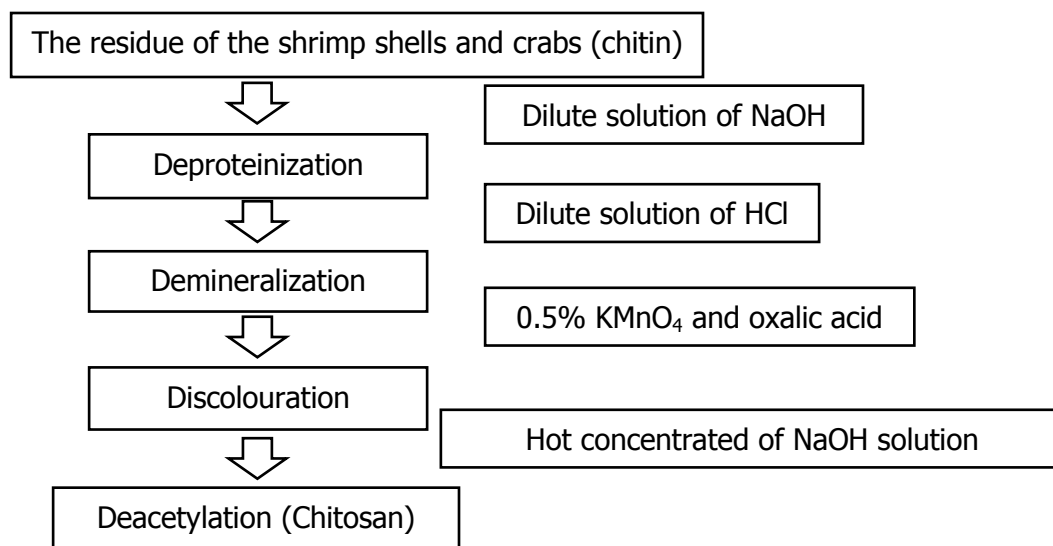


Figure 5. Schematic of chitosan extraction (*Source:* Fernandez, 2004).

CHITOSAN PREPARATION

Various methods were used for chitin deacetylation and chitosan production. The first method is to use 50% (w / v) sodium dioxide, NaOH and a temperature of 100-120 °C. Applications that have been used at room temperature and the minimisation the volume of sodium dioxide (NaOH) are more successful than previous reports and studies. The use of liquids such as acetone and tributyl ammonium (TBA) as a reaction medium along with a fixed reaction from NaOH is highly effective for the reaction of chitin-stabilising medium and particles (Junginer & Sadeghi, 2014; Kim, 2014; Fernandez, 2004; Lamarque *et al.*, 2005).

Besides, Lamarque *et al.* (2005) conducted a study using a more advanced method for the deacetylation process through pre-soaking of chitin in 15 M NaOH for 3 consecutive days at a temperature of 30 °C, chitin will swell followed by deacetylation at a temperature of 40 °C in alkaline solutions to improve swelling process. The resulting product is water-soluble at physiological pH. Next, the use of (*freeze-pump out-thaw* FPT) cycle for shortening a complicated process and reducing time. However, this method is not suitable for large-scale chitosan production (Junginer & Sadeghi, 2014).

During chitosan deacetylation, the polymer chain is degraded and the chitosan crystallisation structure becomes denatured due to severe and rough conditions during the reaction process (Regeet *et al.*, 1999). This suggests that the optimal conditions for deacetylation of chitin is by using 75% (w / v) NaOH and temperatures as high as 110°C. Based on **Figure 6**, the sources of chitin and the isolation process are one of the factors and influence the effects of chitosan quality (Galed *et al.*, 2008).

Now, chitosan has various levels of deacetylation percentage, ranging from 60% to 98% of commercially available. The levels of chitosan deacetylation can be identified through various techniques such as Infrared Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (¹H-NMR) and (¹³C-NMR), and conduct orometric titration (Kassai, 2008). The molecular weight of chitosan ranges from 50 to 2000 kDa affects the preparation of chitosan (Chenite*et al.*, 2001). Percentage of

deacetylation and average molecular weight are very important in the production of various uses of chitosan as in biochemistry and bio pharmacological fields (Tharanathan *et al.*, 2003). Chitosan is now widely produced and commercially in several countries such as India, Japan, Poland, Norway, Thailand, and Australia (Kumar, 2000). Here is a summary to produce chitosan in general as shown in **Figure 6**.

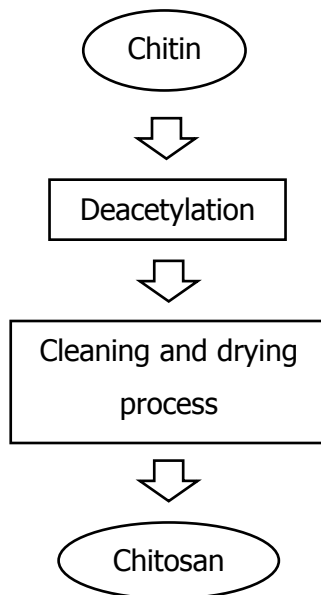


Figure 6. Schematic preparation of chitosan from chitin (*Source: Anaraz et al.*, 2009).

CHITOSAN USAGE

Chitosan has a variety of derivative each of it has its own application in various fields. There are several areas and applications of chitosan in industry including in clean water treatment systems, use in agricultural activities and additives in the preparation of cosmetics (Moorjani *et al.*, 1975; Marchessault *et al.*, 2006).

Usage In Water Treatment

In the last 30 years, chitosan is used as water purification, the positive charge of chitosan works as removing dyes, oils, grease, metal ions and fine particulate matter from the tributaries that cause turbidity in water (Roller & Covill, 1999).

Usage In Agriculture

In agriculture, chitosan is used as a filtering agent for fruits and plants. Besides, it is also used as a plant growth stimulant hormone and activates the production of yield. Chitosan also serves as an anti-severe disease and improve the bio-synthesis of phenol compounds or as secondary metabolism to speed up the immune system of plants (Uthairatanakij *et al.*, 2007).

Chitosan is often used as an organic fertiliser for growing plants and fruits thus suitable for use as a soil treatment (Crini & Pierre-Marie, 2008). Finally, chitosan is applied as a plant protection tool against any microorganisms and pests in the soil. It is particularly important as a plant defense mechanism to prevent microbial attack (Day *et al.*, 2001). It is also used in somatic carrot seeding (De

Jong *et al.*, 1993). The formation of root nodules of soybean occur quickly by using chitosan (Minami *et al.*, 1996).

Usage In Cosmetic

Chitosan is widely used in various cosmetic fields. For example, skincare, hair products and lipsticks. In skincare, hydration is one of the properties of chitosan because it can reduce the level of dehydration on the skin surface and increase skin moisture. Meanwhile, the nature of its ability in film making can help the skin become smooth. In addition, chitosan is used as a skincare formula due to its high water resistance and anti-drying properties. Furthermore, chitosan can enhance UV filter adhesive and prevent during the washing process. Its use in lipstick can also protect the dryness of the lips, moisturises the lips and help in suit the color of the lips for different types of lipstick colours. Chitosan is also used as a deodorant to prevent the reproduction of bacterial enzymes as chitosan is anti-bacterial. Finally, the use of chitosan in cosmetics is as a fragrance in perfumes and cover the smell of sweat in a long time.

Chitosan is also used as creams and lotions because it is one of the emulsifying agents. Chitosan which has both hydrophilic and hydrophobic segments are used as emulsion stabilisers through the space between the surface adsorption (Ramos *et al.*, 2003).

The Use Of Chitosan In Biotechnology And Biomedicine

Chitosan is suitable for use in biotechnology and biomedical because it is naturally cationic (Tan *et al.*, 2020). In the field of biotechnology, chitosan is used as tools of enzyme and cell immobilisation. Besides, it is used as a cell-stimulating material and matrix for chromatographic affinity. Chitosan is also known as a membrane. In pharmaceuticals, chitosan is used as a carrier to control the delivery of drugs. It is also used to form micro-capsules such as gels and drug along with anionic polymers. Finally, chitosan is used as a dermatological product to treat acne and scars on the human body (Crini & Pierre-Marie, 2008).

Due to the uniqueness of chitosan properties such as biocompatibility, biodegradability and non-toxic nature, chitosan is widely used in biomedical. For example, a derivative of chitosan is chitosan sulfate as an anti-coagulant (Junginer & Sadeghi, 2014). Besides, the properties of chitosan was used as analgesics, anti-tumor, hemostatic, hypocholesterolemic, anti-microbial and anti-oxidants. Next, chitosan can increase tissue growth, tissue regeneration, stimulates cell proliferation and artificial skin. Chitosan is very suitable for wound healing tools such as wound bandages and surgical suture threads because of its degradation properties. The use of chitosan is also as an ophthalmology which is related to eye diseases and is suitable as an eye lens (Crini & Pierre-Marie, 2008).

Several factors such as the level of deacetylation (DD) and molecular weight (Mw) gives the impact to its use in the medical industry (Kumar, 2000). The relationship between the biological properties of chitosan and its characteristics is as shown in **Table 5**.

Table 5: The relationship of biological properties of chitosan and its characteristics.

Properties	Features
Biodegradation	DD, division of the acetyl group, Mw
Biocompatibility	DD
Mucoadhesive	DD, Mw
Hemostatic	DD, Mw
Analgesic	DD

Absorption enhancer	DD
antimicrobial	Mw
Anti-cholesterolemic	DD, Mw, viscosity
Antioxidant	DD, Mw

(Sources: Junginer & Sadeghi, 2014).

Usage In Nutrition Industry

In the nutrition industry, chitosan is used as a diet and fiber food medium. In addition, chitosan, which is a hypo cholesterolemic activity, is particularly suitable for controlling appetite, reducing cholesterol and binding to mono glycerides found in the body (Shen *et al.*, 2020). Next, chitosan is used as a food preservative from microbes. Chitosan is also used as a bio-exchange to enhance the quality of food products. It is also very important in the clarification and validation of fruit juices and beverages. Finally, chitosan is particularly important in animal feed additives, emulsifying agents and color stabilisers, and the recovery of waste from waste processing (Crini & Pierre-Marie, 2008).

CONCLUSION

It is found that most synthetic polymers are materials that are not easily degraded, the use of harmful toxic substances and high chemicals cause the disposal of materials to take a long time. Therefore, with the introduction of chitosan-based polymers, all these weaknesses can be reduced and overcome. The bonds between the molecules in this chitosan help the synthetic polymer easily break down either naturally or in the presence of microorganisms. Next, the sustainability of flora and fauna such as aquatic life like turtles, fish and seaweed will be maintained and preserved for future generations. In conclusion, the above issues and problems, it is believed that chitosan polymers lead to research and studies to help in reducing the problem of environmental pollution. Chitosan polymers are also very disposable, degraded quickly, have a short shelf life and are environmentally friendly.

ACKNOWLEDGEMENTS

This research was supported by Enviro Clean Energy Sdn. Bhd. under grant (No. GL00209).

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