

FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE  
BIODEGRADABLE FILM FROM PUMPKIN STARCH

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## ABSTRACT

The purpose of the research is to fabricate difference types of composite biodegradable films from pumpkin starch, to analyze antimicrobial activities of chitosan-incorporated pumpkin starch films and to characterize the biodegradable film (pumpkin starch) in term of physical properties. In this study, the pumpkin starch is used as a raw material because of its high composition of starch. The process involve for this experiment are preparation of pumpkin flour and then the starch is isolated from the pumpkin flour by mixing with the water and settling it over night without shaking to recover the sediment as a pumpkin starch. The next process is to prepare the film by adding the polyethylene glycol (PEG) as plasticizers at a concentration of 3% and the chitosan was added in a different concentration (1.5g, 2.0g, 2.5g) as preparation of microbial film and mixing all of component. After being heated the solution, the warm mixture was casted on framed glass plates, and then dried at a room temperature for 8 hours. Then, the sample is characterized after the film is produced. The analysis shows that the sample with high concentration of chitosan has lower water solubility. The percentage of water solubility in each sample 1.5g, 2.0g and 2.5 g chitosan is 69.7%, 65.2% and 52.3% respectively. The Fourier transforms infrared spectroscopy (FTIR) result show that the similar pattern of peak at wavenumbers range  $1500\text{ cm}^{-1}$  - $1600\text{ cm}^{-1}$  with addition of chitosan the peak become narrower and weaker. Thus, the addition of chitosan can disturb the crystalline of the starch which is the main factor to determine the characteristic of biodegradable film. The antimicrobial analysis shows that the *Escherichia coli* and *Bacillus subtilis* cannot growth well at the film surface. The microorganism colonies are smaller in the film with high concentration of chitosan. The scanning electron microscope (SEM) show that the characteristic features with high concentration of chitosan has a smooth surface compare to the other sample because of the high bonding between the molecules in the sample.

## ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan pelbagai jenis filem komposit dari pati kanji labu, menganalisis kesan aktiviti antimikrob kitosan yang digunakan untuk menghasilkan filem dari pati kanji labu dan untuk mengenalpasti biodegrasi filem (pati kanji labu) dari aspek sifat fizikal. Dalam kajian ini, pati kanji labu digunakan sebagai bahan utama kerana kandungan kanji yang tinggi. Proses yang terlibat dalam eksperimen ini ialah penyediaan tepung labu dan kemudian kanji diasingkan dari tepung labu dengan mencampurkannya bersama air dan dibiarkan mendap selama semalaman tanpa menggoncangkannya untuk mendapatkan mendakan sebagai pati kanji labu. Proses seterusnya ialah penyediaan filem dengan menambahkan polyethylene glycol (PEG) sebagai agen plastic pada konsentrasi 3 % dan kitosan ditambah dengan perbezaan kandungan (1.5g, 2.0g, 2.5g) untuk menghasilkan filem antimikrob dan mencampurkan kesemua bahan. Selepas larutan dimasak, campuran yang hangat dituangkan keatas bingkai plat kaca dan dikeringkan pada suhu bilik selama 8 jam. Kemudian, sampel dianalisis selepas filem terhasil. Analisis menunjukkan sampel yang tinggi kandungan kitosan mempunyai kelarutan air yang rendah. Peratus kelarutan air untuk sampel 1.5g, sampel 2.0g dan sampel 2.5g kitosan ialah masing-masing 69.7%, 65.2% dan 52.3%. Keputusan dari Fourier transforms infrared spectroscopy (FTIR) menunjukkan terdapat pola puncak yang serupa pada jarak gelombang antara  $1500\text{ cm}^{-1}$  -  $1600\text{ cm}^{-1}$  dengan pertambahan kitosan, puncak menjadi semakin sempit dan lemah. Jadi, pertambahan kitosan akan mengganggu struktur kanji yang mana merupakan factor utama dalam menentukan karakter filem biodegrasi. Analisis antimikrob menunjukkan *Escherichia coli* dan *Bacillus subtilis* tidak boleh membiak dengan baik diatas permukaan filem. Jumlah koloni mikrob adalah sikit diatas filem yang mempunyai kandungan kitosan yang tinggi. Scanning electron microscope (SEM) menunjukkan bahawa ciri-ciri dengan kepekatan kitosan yang tinggi memiliki permukaan yang halus berbanding dengan sampel yang lain kerana ikatan antara molekul yang tinggi dalam sampel.

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**LIST OF SYMBOL**

|           |   |                       |
|-----------|---|-----------------------|
| $\alpha$  | - | alpha                 |
| $\beta$   | - | beta                  |
| $\gamma$  | - | gamma                 |
| h         | - | hour                  |
| mg        | - | miligram              |
| mM        | - | miliMolar             |
| kg        | - | kilogram              |
| mol wt    | - | molecular weight      |
| $\mu$ l   | - | microliter            |
| $\mu$ mol | - | micromole             |
| nm        | - | nanometer             |
| v/v       | - | volume per volume     |
| v/w       | - | volume per weight     |
| rpm       | - | revolution per minute |
| T         | - | Temperature           |

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background Study

For the past few years, food packaging is one of the most rapid development industries in the world. Nearly one fifth (about 3.5-4) million ton of the total plastic consumption is used as packaging. The rapid increase in production and consumption of plastics has led to the serious plastic waste problems, so white pollution, landfill depletion, due to their height volume to weight ratio and resistance to degradation (X-Ren, 2002). This pollution is caused by the raw material used for the food packaging is the petrochemical-based polymer, due to their availability in large quantities at low cost and favorable functionality characteristics, such as good tensile and tear strength, good barrier properties to O<sub>2</sub> and heat seal ability. But this material is totally non-biodegradable leading to serious ecological problems. As a result of the environmental problems caused by the massive use of synthetic material for packaging, the last decade has witnessed a notable growth in interest in developing packaging materials based on biopolymers such proteins and polysaccharides (Oscar. *et al*, .2007).

Thus, to counter the environmental waste, many of the country do a research to develop a new biodegradable packing material that can be manufacture with the utilization of environmental friendly raw materials. Among the natural polymers, starch has been considered as one of the most promising candidates for future materials because of its attractive combination of price, abundance and thermoplastic behavior, in addition to biodegradability. Starch-based materials have poor mechanical properties, however, and particularly poor elongation (around 6%) at ambient conditions (Galdeano *et al*,.2008). Plasticizers such as polyethanol glycol (PEG) are used to overcome the

brittleness of this material by incorporation with the starch. Plasticizers can reduce the intermolecular force and increase thermobility of polymer chain. The main disadvantage of biodegradable starch-based films is their hydrophilic character, which leads to low stability when these materials are submitted to different environmental conditions. (Galdeano *et al.*,2008).

In producing a film used for food packaging, the most aspect should to consider is the microbial contamination on food surface that can cause the food spoiled. The food may become contaminated with components or degradation products of plastic packaging materials (Izabela *et al.*, 2006). A microbial agent should be used to avoid the contamination by incorporation with polymeric packaging material. To fulfill the antimicrobial purpose, the antimicrobial agents must be incorporated into the food-contact layer (usually also serving as the inner heat-seal layer) of the packaging materials. Thus, the choice of the packaging materials plays an important role in this incorporation technique, which combines antimicrobials with packaging materials.

## 1.2 Problem Statement

The review on recent development of the research had been focus on biodegradable film to reduce the pollution that cause by food packaging. Usually, food packaging produced from petrochemical-based polymer due to their ability in a large quantity at low cost and favorable functionality characteristic. But this material is totally non-biodegradable leading to serious ecological problems. It will take a hundred year to degrade such as plastic and polystyrene.

There are many substances that never explore as an alternative to make biocomposite or biodegradable film such as by using starch from fruit and plant as a main component and replacing the petrochemical-based polymer. This study was done purposely the new biodegradable film from the pumpkin starch. Pumpkin is one of the fruit that contain highest starch content because of its size is big and easy to get.

### **1.3 Objectives**

The objective of this project are:

- a. To fabricate difference types of composite biodegradable films from pumpkin starch.
- b. To analyze antimicrobial activities of chitosan-incorporated pumpkin starch films
- c. To characterized the biodegradable film (pumpkin starch) in term of physical properties.

### **1.4 Scopes of Study**

There are some important tasks to be carried out in order to achieve the objective of this study. The important scope has been identified for this research in achieving the objective:

- a. To study the pumpkin flour making process
- b. To fabricate the composite biodegradable film from pumpkin using fabrication process.
- c. To analysis the character of biodegradable composite film.
- d. To study the effect of different chitosan composition on membrane performance.
- e. To analysis and compare the membrane performance

## **1.5 Significance of Study**

- a. Provide an alternative source to produce film for food packaging
- b. Produce biodegradable film based on fruit starch
- c. To reduce the landfill space and environmental pollution.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Active Food Packaging

In active packaging systems, the choice of incorporative components is often limited by the incompatibility of the component with the packaging material or by the heat liability of the component during extrusion (Lee *et al.*, 2003). Thus it is important to choose proper polymer matrix and active agents as well as other ingredients such as plasticizers. In this aspect, a novel approach can be explored for the use of biopolymer films as a competent carrier for heat liable bioactive compounds in combination with common synthetic plastic films to produce a composite structure. In addition, mechanical and barrier properties can be improved to a great extent by forming the composite films of biopolymer coatings on conventional plastics (Hong and Krochta, 2004). For obtaining fundamental data on the composite structures, however, further investigations are needed in both the optimization of coating formulations used to prepare biopolymer-coated plastic films, and the evaluation of their physical properties. This study aimed to form a variety of plasticized protein coatings on polypropylene (PP) film, and to examine their optical and tensile properties as affected by biopolymer and plasticizer types, in order to select suitable composite structures as an efficient carrier for heat-sensitive bioactive compounds applicable in active packaging systems.

#### 2.2 Biopolymers

Biopolymer-based packaging materials originated from naturally renewable resources such as polysaccharides, proteins, and lipids have become the focus of

worldwide attention in recent years since such biopolymers offer favorable environmental advantages of recyclability and reutilization compared to conventional petroleum-based synthetic polymers. Biopolymer films may also serve as gas and solute barriers and complement other types of packaging by minimizing food quality deterioration and extending the shelf life of foods. Moreover, biopolymer-based films and coatings can act as efficient vehicles for incorporating various additives including antimicrobials, antioxidants, coloring agents, and other nutrients and (Han and Gennadios., 2005).

Natural biopolymer films have limited to obtain wide industrial applications due to their disadvantages with regard to mechanical and water vapor barrier properties. Generally biopolymer films made from proteins and polysaccharides show an excellent oxygen barrier property at low to intermediate relative humidity as well as fairly good mechanical properties. However, their barrier against water vapor is poor due to their hydrophilic nature. Many research efforts have focused on improving the film properties of biopolymer films, particularly their mechanical and water vapor barrier properties, by the modification of the films (Rhim and Weller., 2000)

The biopolymers commonly used to produce film are carbohydrates, often vegetal starchy and pectin materials and proteins, vegetal and animal, in addition to the appropriate mechanical properties, the films must have also the adequate permeability to water vapors and gases.

### **2.3 Polysaccharides**

Polysaccharides are polymeric carbohydrate structures, formed of repeating units (either mono- or di-saccharides) joined together by glycosidic bonds. These structures are often linear, but may contain various degrees of branching. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Polysaccharide is an important kind of carbohydrates in nature. Depending on the structure and properties of constituent monosaccharide, polysaccharides show different

chemical and biological activities. Polysaccharides widely exist in the plants, microorganism (fungi and bacteria), algae, and animals. Together with proteins and polynucleotides, they are essential biomacromolecules in the life activities and play important roles in cell–cell communication, cell adhesion, and molecular recognition in the immune system. Polysaccharides from different sources, such as fungi and algae, have been demonstrated to possess a variety of medical activities. Due to the rich variety and availability of polysaccharides from the nature, exploitation of polysaccharides for pharmaceutical application is very promising. However, identifying polysaccharides with desired biological activity is not easy because of the separation and purification difficulties ( Cuixian *et al.* ,2009).

In recent years, some bioactive polysaccharides isolated from natural sources have attracted much attention in the field of biochemistry and pharmacology. They exhibit various biological activities affected by different chemical structures (Suarez *et al.*, 2006) reported that the immunostimulatory activity of arabinogalactans extracted from *Chlorella pyrenoidosa* cells depended on their molecular weights. The higher molecular weight arabinogalactans exhibited immunostimulatory activity, but the lower molecular weight fractions did not. Further researches show that the activities of polysaccharides are not only dependent on their chemical structures, but also are related to their chain conformations (Tao *et al.*, 2007). In general, it is interesting and important to elucidate the relation among chemical structures, chain conformations of polysaccharides and their biological activities. However, polysaccharides are usually composed of various monosaccharides linked with different glucosidic bonds. Some polysaccharides have hyperbranched structures. Moreover, polysaccharides often have high molecular weights, and tend to form aggregates in solution that can mask the behavior of individual macromolecules. In consequence, to characterize the chemical structures and chain conformations of polysaccharides is not an easy task. This article attempts to review the current structural and conformational characterization of some importantly bioactive polysaccharides isolated from natural sources (Yang and zhang., 2009).



Microbial polysaccharides are used in food, pharmaceutical, and medical applications: this wide range of usefulness derives from the great diversity in structural and functional properties. We consider the structure, properties, extraction, production, modification, and applications of a selected range of polysaccharides (Morris, 2009). Food spoilage and food poisoning are one of the most important issues facing the food industry (Sokmen *et al.*, 2004). In fact, food-borne illness is a global problem, even in developed countries. Many pathogenic microorganisms, including *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Escherichia coli*, *Zygosaccharomyces bailii* and *Candida utilis* have been identified as the causal targets of food borne diseases or food spoilage. Many researchers were challenged by the emergence of multidrug-resistant bacteria to look for food antimicrobial or the prevention of food spoilage and food poisoning.

## 2.4 Pumpkin

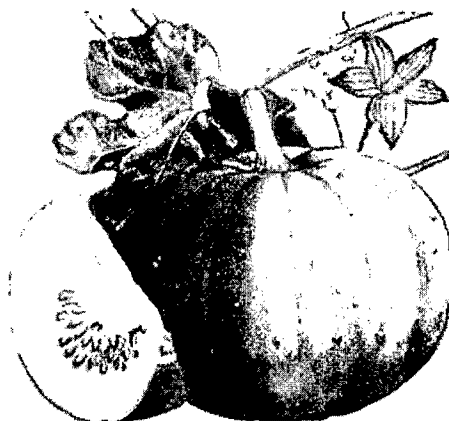
Pumpkin is a fruit of certain varieties of *cucumber pepo* or *c. moscata*, members of the family of *cucumbitaceae*. The fruit are large, generally 4-8 kg or more. Are yellowish to orange in color, and vary from oblate through globular to oblong. The rind is smooth and usually lightly furrowed or ribbed. The fruit stem is hard and woody, ridged as angled and in *C. Pepo* not flared at its point of attachment to the fruit. The very largest varieties of pumpkin are called winter squash. *C. maxima* and may weigh 34 kg or more. The pumpkin could obviously increase the levels of serum insulin, reduce the blood glucose level and improved tolerance of glucose and hence could be developed as new anti diabetic agent (Li, *et.al.*, 2005). About 18,978,328 tones of pumpkins are produced in the world in year 2005 and around 10,224 tones of Pumpkins are produced in Malaysia in year 2007 (MARDI, 2007). The five major pumpkin producing countries in the world are China, India, Ukraine, Egypt and United States (FAO, 2005). The major pumpkin producing states in Malaysia are Terengganu, Kelantan, Perak and Kedah The production of pumpkin in Malaysia as reported by Malaysian Agricultural Research and Development Institute (MARDI, 2007). Fresh pumpkins are very perishable and sensitive to microbial spoilage, even at refrigerated conditions; therefore they must be

frozen or dried. Pumpkin can be process into the flour which is it can obtain for a long period of time or has a longer shelf-life. The pumpkin has highly-desirable flavored and sweetness. Pumpkin is rich in carotene, vitamins, minerals, pectin and dietary fiber (See, 2007). Table 1 below show that the proximate composition of fresh pumpkin and pumpkin flour (See, 2007).

**Table 2.1;** Proximate composition of fresh pumpkin and pumpkin flour

| Composition (%) | Fresh Pumpkin             | Pumpkin Flour             |
|-----------------|---------------------------|---------------------------|
| Moisture        | 92.24 ± 0.01 <sup>a</sup> | 10.96 ± 0.12 <sup>b</sup> |
| Fat             | 0.15 ± 0.03 <sup>a</sup>  | 0.80 ± 0.07 <sup>b</sup>  |
| Protein         | 0.98 ± 0.06 <sup>a</sup>  | 9.65 ± 0.10 <sup>b</sup>  |
| Ash             | 0.76 ± 0.13 <sup>a</sup>  | 5.37 ± 0.14 <sup>b</sup>  |
| Crude fiber     | 0.56 ± 0.08 <sup>a</sup>  | 0.81 ± 0.07 <sup>b</sup>  |
| Carbohydrate    | 5.31 ± 0.04 <sup>a</sup>  | 72.41 ± 0.09 <sup>b</sup> |

Mean in a row with similar superscript are not significantly different at  $\alpha=0.05$   
Values are the Means ± SD and n=3 for each group

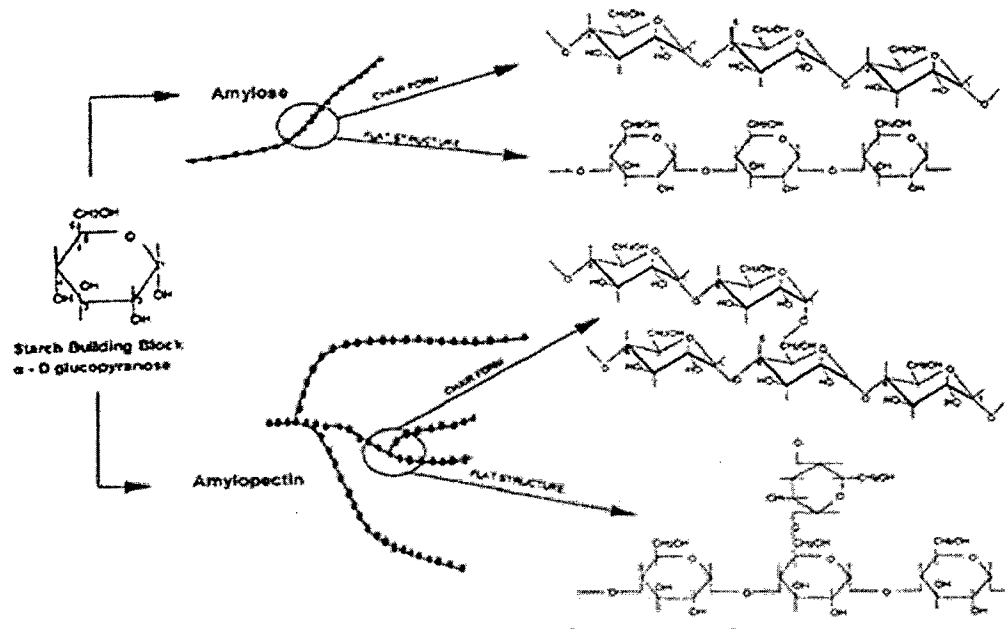


**Figure 2.1;** Pumpkin fruit

## 2.5 Resistant Starch

Starch is the major carbohydrates in the plant storages organs. Starch structures and physicochemical properties have been characterized for storage organs of many plant families. There are a four type of resistant starch. RS1 (Resistant starch type 1) is physically inaccessible starch due to the presence of intact cell walls in grains, seeds or

tubers. They are mainly found in partly milled grains, seeds and legumes. RS2 (Resistant starch type 2) is a group of the raw ungelatinized (uncooked) starches. They are classified in 3 main types: A (cereal starches, cassava starch), B (green banana and raw potato starches) and C (legume starches). RS2 is called high-amylose maize starches which are highly resistant to digestion by  $\alpha$ -amylase until gelatinized (Sozer *et al.*, 2007). RS3 (Resistant starch type 3) is the retrograded starch whereby when the starch has been cooked, cooled and stored for a periods of time such as bread, cornflakes, canned peas or beans, food products with prolonged and repeated moist heat treatment. RS4 (Resistant starch type 4) refer to the chemical starch modification or thermal treatment at high temperatures. These are derived as a result of cross bonding with chemical reagents such as ethers and esters (certain modified breads, pastas and cakes). RS may be found in both unprocessed and processed foods. Processed foods invariably undergo storage at moderate or low temperatures before consumption. Storage of foods is also a contributing factor to the changes in the available starch content of the product. The quantity of RS formed; during processing or storage depend on the severity of the processing conditions like temperature, pH, moisture, number of heating or cooking cycles adopted, condition of storage, etc. In addition, many of the processing treatments such as freezing autoclaving are also noted to have significant impact on the fermentation of RS (Kumari *et al.*, 2007).



**Figure 2.2;** Linear and Branched Starch Polymer (Murphy, 2000)

## 2.6 Chitosan

### 2.6.1 History

Chitosan was being tracked since 1811 when chitin, the derivative of chitosan was found by Professor of Natural History in France, Henri Braconnot. According to historians, while Braconnot conducted research on mushrooms, he isolated what was later to be called chitin.

After twenty years later the same compound was found in insect and plant structure and later called as 'chitin'. Further research was conducted by Lanssaigne in 1843, as the result he discover the presence of nitrogen in chitin. Chitosan emerged in the scene when C. Rouget was experimenting with chitin in 1859. Rouget observed that the chitin structure could be manipulated to become soluble. Then, in 1878 Ledderhose was identified chitin to be made of glucosamine and acetic acid. It was not actually until 1894 that Hoppe-Seyler named the tailored chitin, chitosan.

During the early 20th century, several researches took chitosan as their subject of study. They then found the other sources of chitin, which is crab shells and fungi. It was the work of Rammelberg in the 1930s that led to the confirmation on the identity of chitosan from these sources. It was also noted that by hydrolyzing chitin in several ways, it was determined by experts that chitin is a polysaccharide of glucosamine. During the 1950s, the use of x-ray analysis had advanced the study of chitin or chitosan in fungi. However, it is only the most advanced technologies that proved the most reliable in accepting the existence of chitin as well as cellulose in the cell walls. The first book on chitosan was published 140 years after the initial observation of Braconnot, and that was in 1951.

During the early 1960s, chitosan was examined for its ability to bind with the red blood cells. That time also, the substance was considered as a hemostatic agent. Then, for the past three decades, chitosan has been used at water purification plants for detoxifying water. It is spread over the surface where it absorbs greases, oils, and other potential toxins

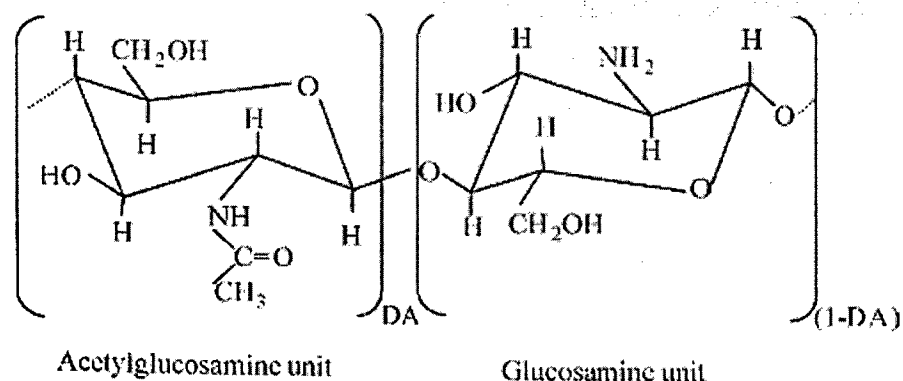
### **2.6.2 Introduction**

Chitosan [poly (1, 4- $\beta$ -D-glucopyranosamine)], an abundant natural biopolymer, is produced by the deacetylation of chitin obtained from the shells of crustaceans. Chitin and chitosan are available bio-resources, particularly in crab shells or crustacean skin. Both chitin and chitosan have been applied in various field, including wastewater treatment, artificial dialysis membranes, biodegradable plastic and diet control (T. Takahashi *et al.*, 2007) it is a polycationic polymer that has one amino group and two hydroxyl groups in the repeating hexosaminid residue. Chitosan has great potential as a biomaterial because of its bio-compatible properties. It is hydrophilic, non-antigenic and has a low toxicity toward mammalian cells. In addition, chitosan is known to facilitate drug delivery across cellular barriers and transiently open the tight junctions between epithelial cells. Chitin and chitosan are aminoglucopyrans composed of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues. These polysaccharides

are renewable resources which are currently also being explored intensively for their applications in pharmaceutical, cosmetics, biomedical, biotechnological, agricultural and food industries. The industrial preparation of chitin uses the shells of crabs, shrimps, prawns, and lobsters coming from the peeling machines in canning factories.

### 2.6.3 Production of chitosan

The commercial method of preparation of chitin from shrimp shell involves strong acid and alkali treatment to remove the minerals and proteins, respectively. However, the use of these chemicals causes depolymerisation of the product and therefore affects properties such as molecular weight, viscosity and degree of deacetylation. These chemical treatment methods bring about hazardous environmental problems like disposal of wastewater. The cost of the chemicals is another drawback of this approach. To overcome the problems of chemical treatments, different microorganisms and proteolytic enzymes were used to remove the proteins and mineral content. During fermentation with microbes, deprotenisation takes place by the activity of proteases in the microorganisms and demineralization by the acid produced by the microorganisms during fermentation. *Bacillus subtilis* and *Bacillus firmus* is the widely used bacteria for this purpose. (Theruvathil K. Sini, 2007).



**Figure 2.3;** Chitosan Structure (Guibal, 2005)

## 2.7 Polyethylene glycol (PEG)

### 2.7.1 Background

Every year, millions of tons of PEG are manufactured worldwide and most of them reach conventional sewage disposal systems after industrial utilization. From the last three decades, concern has been expressed about the fate of these polymers in the environment and several studies have been performed on their biodegradability (Huang *et al.*, 2005).

Polyethylene glycols (PEGs) are polymers of ethylene oxide with the generalised formula  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$ , and “n” indicating the average number of oxyethylene groups. The synthetic name of PEG is “(oxy-1,2-ethanediyl)- $\alpha$ -hydro- $\omega$ -hydroxy”. Polyethylene glycol is produced by interaction of calculated amount of ethylene oxide with water, ethylene glycol or ethylene glycol oligomers (Claudia, 2005). The reaction is catalyzed by acidic or basic catalysts. Ethylene glycol and its oligomers are preferable as a starting material than water, because of it allows to obtain polymer with narrow molecular weight distribution (low polydispersity). Polymer chain length depends on the ratio of reactants. PEGs and PEG derivatives do not represent definite chemical entities, but are mixtures of compounds with varying polymer chain lengths. The average number or the molecular weight of the polymer chain is often indicated in the generic name of the specific substance, as, for instance, in PEG-8 which is equivalent to PEG-400 (Claudia, 2005).

Polyethylene glycols (PEGs,  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$ ) are an important group of non-ionic synthetic water-soluble polymers of ethylene oxide. These compounds are widely used in the production of pharmaceuticals, cosmetics, lubricants and antifreeze for automobile radiators, in the conservation treatment of ancient waterlogged wood, and in the manufacture of non-ionic surfactants (Huang *et al.*, 2005). Products made from monofunctional polyethylene glycols find uses as lubricants, foam regulators, defoaming agents, emulsifiers, thickeners, superabsorbent polymers, stabilizers in emulsion and suspension polymerization, concrete superplasticizers and textile

auxiliaries. Ultrapure M-PEGs are used for coupling to biologically active molecules such as proteins, antibody fragments, peptides and small molecules (Bhajendra *et al.*, 2009). PEGs and PEG derivatives also used as humectants, solvents, binders, emulsion stabilizers, and viscosity increasing agents in a wide variety of personal care products (Claudia, 2005).

Polyethylene glycols are water soluble non-ionic synthetic polyethers of ethylene oxide and are used in a wide range of applications. The annual production of PEGs is millions of tons worldwide. After industrial utilization, a high proportion of them enter conventional wastewater treatment plants (WWTPs) (Huang *et al.*, 2005). In general, water-soluble plastics are discarded via the sewage system after use and can reach the aquatic environment after wastewater treatment. Low molecular PEGs occur as metabolites of the biodegradation of polyethoxylated surfactants such as alcohol ethoxylates (Marcomini *et al.*, 2000) and PEG 550 (number indicates average MW) were found in river water and seawater between 0.5 and 68 mg/L (Jonkers and de Voogt, 2003).

The biodegradability of PEG by environmental bacteria was found to depend on the polymerization degree of the polyether molecule. PEGs with molecular weight lower than 4000 were biodegraded by many species whereas PEGs with molecular weight higher than 4000 were significantly more recalcitrant. In fact, microbial attack mainly depended on the cell wall structure, which was less or more hydrophobic and made the substrate accessible to the microbial cells. Likewise, in Gram-positive actinomycetes, mycolic acids in external membrane explain the degradation capacities towards high-molecular weight PEGs or tetrahydrofurane (Kohlweyer *et al.*, 2000).

### **2.7.2 Methods of manufacture and composition**

Polyethylene glycol is produced by interaction of calculated amount of ethylene oxide with water, ethylene glycol or ethylene glycol oligomers. The reaction is catalyzed by acidic or basic catalysts. Ethylene glycol and its oligomers are preferable as a starting material than water, because of it allows to obtain polymer with narrow molecular