Chapter I

General Background

1. Introduction

Chitin is the second most plentiful natural polymer. Currently, chitin and chitosan are manufactured commercially in large scale from the outer shell of Crustaceans. (shrimp and crab) Within the vast amounts of shellfish waste now possible, applications requiring large amounts of chitin and chitosan are now possible.

As characteristic features for using as an industrial material, chitin and chitosan are:

1. a main component of biomass such as the shells of crab, shrimp and krill.
2. natural resources biologically reproducible
3. biodegradable and do not pollute natural environment
4. biocompatible not only in animals but also plant tissues
5. biopolymers (aminopolysaccharide)
6. almost non-toxic (LD_{50} 16 g/kg body weight in mice)
7. biologically functional
8. changeable in the molecular conformation
9. able to manufacture into gels, beads, fibers, colloids, films, etc.
10. have amino and hydroxyl groups chemically modifiable

Many of these attributes, make them attractive biopolymer for biomedical applications. Chitin and especially chitosan are being evaluated in a

number of pharmaceutical applications. They can be used as vehicle in sustained release preparations, as disintegrant in fast release tablets, as liposome stabilizer, and others.

Chitin and chitosan have been demonstrated to be used as disintegrants both in direct compression technique (Bruscato, et al., 1978; Sawayanagi, Numbu, and Nagai, 1982; Adusumilli and Bolton, 1990) and in wet granulation technique (Bruscato, et al., 1978; and Parichat Chomto, 1992). "The application of chitin and chitosan as disintegrants in paracetamol tablet" was studied by Parichat Chomto (1992). The results showed that chitosan had disintegrating property better than of chitin. Moreover, chitin or chitosan from different sources (Thailand and Japan) had different disintegrating properties. This discrepancy may be due to the differences in manufacturing processes of chitin, and the deacetylation variables during chitosan production. In deacetylation process, there are many factors -- concentration of alkali used, reaction time and temperature, and atmospheric condition -- affecting characteristics of chitosan products such as degree of deacetylation and molecular weight. In this study, some of these variables were studied and the properties of chitosan products were evaluated.

Key to any successful manufacturing process is quality control. This study may suggest the requirements for chitosan product for using as a disintegrant.

2. Objectives of the Study

The aims of this study are:

a. to investigate the characteristics of chitosan products, producing by different conditions, such as degree of deacetylation, molecular weight, viscosity in acid solution, rate of water uptake, and others.

b. to evaluate the relation between the characteristics of chitosan studied in a. and its tablet-disintegrating properties.

c. to find out the tendency for suitable characteristics of chitosan for using as a tablet-disintegrant.

3. Literature Review

The name "chitin" comes from the Greek word "chiton" , meaning a coat of mail. Chitin was apparently first used by Odier in 1823. The late 1930s and early 1940s saw considerable interest in chitin and its derivatives but production costs and shortage of waste raw material probably caused loss of this early interest. Substantially increased raw material supply and improved technology again aroused interest in the 1970s, and commercial and experimental quantities were made available.

Sources of Chitin

After cellulose, the most plentiful natural polymer is chitin. The distribution of chitin and its quantitative importance in living being is now well known. Chitin is synthesized by some unicellular organisms. For instance, diatoms, chrysoflagellates and protozoa, especially ciliates. Chitin is a cell-wall constituent of most fungi, molds and yeasts. Chitin is also present in cuticular or exoskeletal structures of most Invertebrates, and Echinoderms. The amount of chitin with respect to total dry weight is the highest in Crustaceans, mainly Decapods. Crab and shrimp shells contain approximately 15 - 20 and 15 - 30 % chitin on a dry weight basis, respectively. This observation may explain the use of Crustacean shells as main source of chitin by most chemical industries.

The chitin content in various origin wastes is given in table 1.

Table 1 Characteristic composition of chitinaceous wastes.

Origin of waste	Dry-weight composition (%)		
	Inorganic	Protein/Fats	Chitin
Shellfish	25 - 50	25 - 50	14 - 35
Krill	24	61	7
Clams/Oysters	85 - 90	negligible	3 - 6
Squid	negligible	76 - 95	1 - 2
Fungi	negligible	25 - 50	10 - 25
Insects	negligible	60 - 80	0 - 8

Production of Chitin and Chitosan

Chitin and chitosan prepared from crab and shrimp shells are now commercial products in many countries such as United State, Maxico, Norway, and Japan. The preferred species of various countries are different.

In Japan where chitosan was produced industrially for the first time in the world in 1971, chitin and chitosan have been produced by 15 companies in 1986. A main source of chitin is from crab shells. Commercial chitin and chitosan vary in quality with each of these companies. They supply these products in the forms of flakes, powders, beads, fibers, sheets, and films. The total capacity of an essential annual production of chitin by these Japanese companies is about 2,000 tons. (In 1986, they produced 1,270 tons of chitin.)

Protan, with corporate headquarters in Drammen, Norway, produces and markets fine chemicals from natural resources including chitosan processed from both crab and shrimp shells. A series of the purified chitosans is now being

marketed under the name of ***Protasan***. They have several characteristic fundamental properties such as thickening, gelling, flocculating, and film forming.

In USA, chitin is made from both crab and shrimp shells by many companies such as Jan Dekker International and Index Chemical Co.

Molecular Structure of Chitin and Chitosan

Similar to cellulose, chitin is linear chained molecules of β-(1→4) linked glycans. The repeating unit in chain is 2-Acetamido-2-deoxy-D-glucose. (N-Acetyl-D-glucosamine) The degree of polymerization of the polymer is also similar to that of cellulose with chains of 1000 - 3000 basic units, with a molecular weight of several hundred thousand. Chitin ($C_8H_{13}NO_5$) is composed of 47.3% carbon, 6.5% hydrogen, 6.9% nitrogen, and 3.4% oxygen.

Chitosan is an inhomogeneous mixture of that repeating unit with the deacetylated form, (1→4)-2-Amino-2-deoxy-D-glucose. (Glucosamine) The term "chitosan" is preferred when the nitrogen content is higher than 7% by weight, and it is generally used for artificially deacetylated chitin. The molecular weight of chitosan will depend on the processing condition and more grades within the range 10,000 - 1,000,000 Daltons will be available. When chitin is completely deacetylated, it is called "chitan". The molecular structures of chitin and chitosan are depicted in Figure 1 and 2.

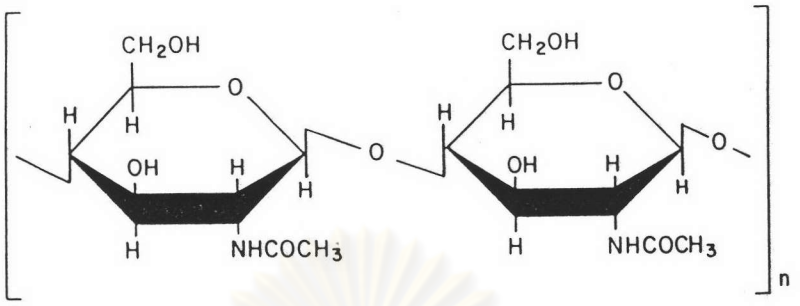

Figure 1 N-Acetyl-D-glucosamine repeating unit.

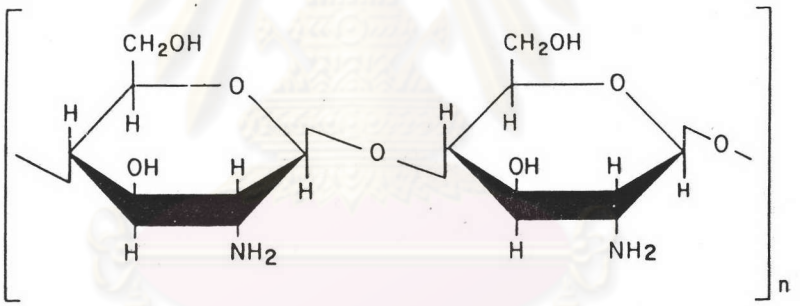

Figure 2 D-Glucosamine repeating unit.

Chitin Manufacturing Procedure

Several procedures have been developed through the years to prepare chitin and the manufacture of chitin has been the subject of a number of patent specification and papers in the technical press. Some methods have been review in "Chitin" by Muzzarelli (1977).

The key steps in the extraction of chitin from the shells are the removal of proteins and minerals such as Calcium carbonate and Calcium phosphate by treatment with alkali and acid. First, proteins are removed from ground shell by treatment with Sodium hydroxide. Minerals are extracted with Hydrochloric acid. After rinsing, the chitin is dried as a flake material. The steps in this process are shown below.

Raw material : Crab / Shrimp shells

⇓ grind

⇓ deproteinize with NaOH

⇓ demineralize with HCl

⇓ dry

Chitin

Some different processes and auxiliary treatments in chitin manufacturing process have also been reported such as in the following.

- Ethylene diamine and Formaldehyde have been used in recovery chitin .

- The purification of chitin (from King crab shells) has been accomplished by decalcifying with Ethylene diamine and digesting with proteolytic enzymes (to remove protein), Dodecyl benzenesulfonate and Dimethyl formamide.

- Pink coloration in chitin has been removed by mild oxidation with Sodium metabisulfite or Hydrogen peroxide or Sulfuric acid in contact for 6 hours at room temperature, or 0.02% Potassium permanganate at 60°C.

Deacetylation of Chitin

Amide linkages are more difficult to cleave under basic conditions than ester groups. Under vigorous basic conditions, acetamido groups adjacent to *cis* related hydroxyl groups may undergo N-deacetylation, but *trans* related analogs are much more resistant.

Chitin is converted to chitosan by deacetylation with concentrated alkali at high temperature. Variations in the reagent used and its concentration, as well as time and temperature of the treatment, determine the quality and performance of the product. The manufacture of chitosan is documented in many literature, and some other methods have been extensively reviewed by Muzzarelli (1977).

The variables in deacetylated conditions of chitin and their influences on the characteristics of chitosan products were studied and could be summarized as follows:

1. **Reagent** (type, concentration, and chitin-reagent ratio)

Strong alkali used in deacetylation of chitin is Sodium or Potassium hydroxide, but Sodium hydroxide is more widely used. The Sodium hydroxide concentration was varied from 35 - 50 %. In the process using Potassium hydroxide, the concentration was sometimes equal to 55%(w/w). The chitin-reagent ratio was extremely varied. Lusena & Rose (1953) treated one part of chitin with 100 parts of 55% Potassium hydroxide whereas Bough, et al. (1978) reacted one part of chitin with 5 parts of 50% Sodium hydroxide.

Lusena & Rose found that decreasing the alkali concentration increased the time required to obtain soluble chitosans and products were less viscous. Increasing the alkali concentration to saturation had little effect on deacetylation and viscosity.

2. Size of chitin particles

Lusena & Rose (1953) concluded that the size of chitin particle within the range 20-40, 40-60, and 60-80 mesh had no effect on deacetylation and viscosity.

Bough, et al. (1978) studied about the effects of particle size of the starting materials on the composition and viscosity of chitosan preparations. By this study, these batches of shrimp hulls having maximum particle size of 1, 2, and 6.4 mm was produced. The results indicated that grinding to 1 mm produced a higher-viscosity and slightly higher molecular-weight when identical manufacturing conditions were employed. In preparing chitosan solution, insoluble particles were found in the solution of 6.4 mm chitosan product. It was postulated that shrimp hulls ground to a 6.4 mm particle size could be deproteinated and demineralized as well as those with small particle sizes, but in deacetylation, this sample only the outer layers of the particles could be converted to soluble chitosan. The inner portions remained unreacted, insoluble and tended to precipitate. Thus, a large particle size requires longer swelling time and results in a slower deacetylation rate. However, the over particle size reduction causes degradation of molecular chain.

3. Reaction temperature

The reaction temperature used in almost studied was so high, usually at 100 -120°C, sometimes at 140 -150°C, and in some cases up to 170 -

180°C. However, the lower temperature, 50-60°C, was used in some deacetylation experiments.

Lusena and Rose (1953) stated that increasing temperature increased percentage deacetylation and reduced molecular size. The alkali treatment should be carried out at low temperature as possible to avoid degradation of the molecular chain.

4. Reaction time

The reaction time used depend on the degree of deacetylation of chitosan product required and also depend on alkali concentration and reaction temperature. Under strong condition, 50% NaOH at 145 -150°C, chitosan is obtained within 5 -15 minutes. (Bough, et al., 1978) By using the same concentration but at 100°C, the time required to obtain 80% deacetylation chitosan is about 5 hours. (Wu and Bough, 1978) In later case, using more than 5 hours did not deacetylate significantly and only degrades the molecular chain, because increasing time reduced molecular size. Some attempts, the alkali treatment and washing in water two or more times, are prepared to obtain chitosan products which are 90-95% deacetylated, (Mima, et al.,1983) without serious degradation of the molecular chain.

5. Exclusion of air

Exclusion of air was investigated as a variable in the manufacturing of chitosans. Deacetylation in an atmosphere of nitrogen yielded chitosans of higher viscosity and molecular weight distribution than deacetylation in air. (Lusena and Rose, 1953) Because oxygen is catalyst in hydrolysis-degradation of molecular chain. In the study of Domard and Rinaudo (1983), thiophenol was used to trap oxygen thus preventing degradation.

Characteristics of Chitin

Chitin, a stable, leathery solid, is grayish-white in color. It is thermal stable to about 260°C, where it decomposes.

1. Chitin polymorphism

Chitin occurs in three polymorphic forms that differ in the arrangement of the molecular chains within the crystal cell. α-chitin is highly compacted, most crystalline polymorphic form where the chains are arranged in an anti-parallel fashion; β-chitin is the form when the chains are parallel; and γ-chitin is the form where two chains are "up" to every one "down".

The most abundant polymorphic form is α-chitin that is formed in the arthropod cuticles and in certain fungi. β-chitin exists in a crystalline hydrate that accounts for its lower stability since water can penetrate between the chain of the lattice. Also γ-chitin can be transform into α-chitin.

Blackwell, Minke, and Gardner (1978) determined the structures of α-chitin and β-chitin by X-rays diffraction. They stated that the presence and absence of intersheet (intermolecular) hydrogen bonding in α-chitin and β-chitin accounts for the inability of α-chitin to swell in water and the ease of β-chitin to swell in water to produce hydrates.

2. Solubility

Chitin is insoluble in organic solvent and will resist being dissolved by acids and bases. Chitin dissolves in concentrated mineral acid, Formic acid (anhydrous) , Hexafluoroisopropanol, Hexafluoroacetone, and 1,2-Chloroalcohol.

Characteristics of Chitosan

The main characteristics of a chitosan sample are the degree of deacetylation and its molecular weight. The others interested properties such as solubility, viscosity, rheology, and polyelectrolyte complex formation, are also described here.

1. The degree of deacetylation

Chitosan is a polysaccharide obtained by partial or complete deacetylation of chitin, and its molecular structure and properties are largely affected by the degree of deacetylation. For this reason, it is needed to determined exactly the degree of deacetylation. The mole fraction of deacetylated units defined as the degree of deacetylation will usually range from 70-90%. A simple and reliable method for the determination of this parameter has been sought over many decades. Some methods can be briefly mentioned here:

1.1 Colloidal titration (Amino residue analysis)

First, chitosan hydrochloride is prepared by addition an excess of concentrated Hydrochloric acid. After that, titration of ammonium cation is performed by using negative colloid solution, Potassium salt of Polyvinyl sulfate (Terayama, 1952), or by using Sodium hydroxide or Silver nitrate. (Hayes, 1978) The end point is detected by indicators.

1.2 Infrared spectrometry

The ratio of the absorbance of the 1655 cm^{-1} band to that of the CH stretching band at 2867 cm^{-1} is used to estimate CONH content of chitosan with excellent accuracy by Miya, et al.. (1980) This method will be limited to the samples deacetylated by > 90% per glucosamine residue.

1.3 Gas chromatography

This method proposed by Muzzarelli, et al. (1980) is based on the measurement of the retention time of methanol in a chitin/chitosan column that is proportional to the degree of acetylation.

1.4 Thermal analysis

The determination of the percentage of acetyl groups is performed by using thermogravimetric analysis with the empirical calibration technique. This method was proposed by Alonso, Peniche-Covas, and Nieto (1983) They stated that this method compared satisfactory with the one that makes use of IR spectrometry and was more rapid and simple than the chemical methods.

1.5 First derivative ultraviolet spectrophotometry

The degree of acetylation of chitosan can be determined in acetic acid solution (~ 0.01 M) containing 1 gram dry chitosan per liter by first derivative ultraviolet spectrophotometry at 199 nm. This method is simple, more precise and faster than the infrared method. (Muzzarelli, 1985)

1.6 ^{1}H-NMR spectroscopy

This is novel method to determine the degree of deacetylation proposed by Hirai, Odam, and Nakajima (1991)

1.7 Elemental analysis

This method determines carbon/nitrogen ratio.

1.8 Mass spectrometry

Mass spectra provide information on degradation temperature and polymer identification and, with further work, will permit the

determination of the degree of acetylation in a polymer and possibly the $-NH_2$ / $-NHCOCH_3$ ratio. (Hayes, 1978)

2. Molecular weight

The chitosan product consists of a mixture of different polymer sizes. (The average molecular weight of chitosan typically ranges from about 50,000 to 4 million.) The range of sizes or polydispersity of the molecular weight distribution is influenced by variables such as time, temperature, concentration, and atmospheric conditions employed in the deacetylation reaction. The molecular weight of a polymer has been considered to be one of the most important characteristics affecting functionally of the polymer. Thus the methods for determination of the molecular weight of chitosan samples have been developed, but the average molecular weight of chitosan is certainly the most difficult parameter to obtain with precision.

Some molecular weight determination methods of chitosan were summarized and compared by Rinaudo and Domard (1989). The list of these methods is given here:

1. Measurement of the intrinsic viscosity (Muzzarelli, 1977)
2. Membrane osmometry (Domard and Rinaudo, 1983)
3. Gel permeation chromatography (Mima, et al., 1983)
4. High performance liquid chromatography (Wu, et al., 1976)
5. Static light scattering measurement (Domard and Rinaudo, 1983)
6. Laser light scattering measurement (Muzzarelli, Lough and Emanuelli, 1987)

3. Solubility

The term "chitosan" may be consider as referring to a family of polymers derived from chitin that has been deacetylated to provide sufficient free amino groups to render the polymer soluble in certain aqueous acid system. The exact degree of deacetylation required to render a polymer soluble is not readily determined, and it undoubtedly varied with such factors as polymer molecular weight, temperature, and concentration and nature of the acid species. In general, solubilization begins at about 60%, usually about 75% deacetylation depending on the molecular weight of chitosan formed. Chitosan samples 75% or more deacetylation dissolve readily in dilute organic acids to give clear, homogeneous and viscous solutions.

For practical purposes, chitosan is insoluble in sulfuric acid and phosphoric acid, while a certain solubility exists for other mineral acids like hydrochloric acid, nitric acid and perchloric acid. Compared with the more common organic acids, the solubility in inorganic acids seems more limited concerning the concentration ratio chitosan / acid. The solubility of chitosan in some organic acid is up to 50% such as acetic acid, lactic acid, formic acid, and propionic acid. (The standard solvent commonly used for solution property measurement is acetic acid.)

4. Viscosity

The viscosity of chitosan is related to the average molecular weight affected by many factors in deacetylation process. Not only the molecular weight but the viscosity of chitosan solution is a function of its concentration, and the particular acid and its concentration used as a solvent as well.

Filar and Wirich (1978) defined the molecular weight ranges of chitosan in terms of solution viscosity. These viscosity types were selected as representative of readily be produced on a commercial scale from shrimp shell. The viscosity ranges are:

High : > 1000 cps., 1% polymer in 1% acetic acid

Medium : 100 -250 cps., 1% polymer in 1% acetic acid

Low : 25 - 70 cps., 2% polymer in 2% acetic acid

5. Rheology

Due to the high molecular weight and the linear unbranched structure of the molecules, chitosan is an excellent viscosifier in an acid environment. It behaves as a pseudoplastic material showing decreasing viscosity at increased shear.

6. Polyelectrolyte complex formation (Ionotropic gellation)

Reacting chitosan with a controlled amount of a multivalent anion will result in a cross-linking between the chain molecules. The network formed has the ability to keep large amounts of water, can be done in holding as much as 95% or more. This cross-linking can be done in acid, neutral, or basic environments, depending on the method applied. Several gelling "counterions" are available of which some are listed in the following.

1. Low molecular weight counterions
   - Pyrophosphate
   - Tripolyphosphate
   - Tetrapolyphosphate
   - Octapolyphosphate
   - Hexametaphosphate

- $(Fe(CN)_6)^{-4}$/ $(Fe(CN)_6)^{-3}$

2. High molecular weight counterions
   - Alginate
   - Kappa carrageenan
   - Poly-1-hydroxy-1-sulfonate-propene-2
   - Polyaldehyro-carbonic acid
3. Hydrophobic counterions
   - Octylsulphate
   - Laurylsulphate-hexadecylsulphate-cetylstearylsulphate

Some specifications for chitin and chitosan were raised by Muzzarelli (1985) and given in Table 2.

Table 2 Some specifications for chitin and chitosan.

Specification	Description
Moisture	From 2 to 10% under laboratory conditions.
Nitrogen	Usually from 6 to 7.0% in chitin. From 7.0 to 8.4% in chitosans.
Degree of deacetylation	Usually ~10% in chitin, 60% in chitosan and between 90-100% in fully deacetylated chitosans.
Ashes at 900°C	Usually lower than 1.0%.
Viscosity of solutions in 1% acetic acid (for Chitosans only)	From 200 to 3000 cps., degraded chitosans have lower viscosities.
Molecular weight	Native chitins > $1x10^6$, commercial chitins and chitosans $1\text{-}5x10^5$, polydispersity is rarely indicated.
Titrations	With Potassium polyvinlysulfonate and by alkalimetry ; moisture content and pH of the waters from which chitosans were isolated should be taken into account.
Dissociation constant, K_a	Between 6.0 and 7.0; most often 6.3
X-rays diffraction data	Typical peaks at 8° 58' -10° 26' and 19°58' - 20°00'
Carotenoids	chitins and chitosans may contain caratenoids: otherwise, it should be indicated whether they have been extracted or bleached.
Amino acids	Glycine, serine and aspartic acid may be present.
Transition metals	With the exception of ion, normally below a total of 5.0 μg/g; typically, for crab chitosans: V 0.12, Cr 0.04, Mn 0.09, Ni 1.3, Cu 1.03, Ag 0.02, Cd 0.22, Hg 0.025, Pb 0.15 μg/g

Chemical Modification of Chitin and Chitosan

A large number of chitin and chitosan derivatives have been proposed and studied. The modifications include many reactions and some reactions described by Muzzarelli (1977). The derivatives of chitin and chitosan are given here:

1. Alkali chitin
2. Salt form e.g. Chitosan citrate
3. Polyelectrolyte complex
4. Metal chelate
5. Sulfated, Phosphated or Nitrated derivative
6. Deoxyhalo derivative
7. Depolymerized derivative
8. Sugar derivative
9. N-Arylidene or N-Alkylidene derivative (Schiff's base)
10. N-Acyl derivative
11. N-Alkyl derivative
12. N-Carboxyalkyl (aryl) derivative
13. O-Carboxy or O-Hydroxy-alkyl derivative
14. O-Acyl derivative
15. O-Sulfonyl derivative

The attempts to find practical use of modified chitin and chitosan have been made for many years. Thus their physical properties and potential uses are now published in a large amount of literature. Some applications of these derivatives were summarized by Hirano. (1989) Although a lot of derivatives were prepared but only a few of them could be used in pharmaceutical and cosmetic industries.

Derivatives of Chitin and Chitosan Used for Pharmaceutical and Cosmetic Applications

Some of chitin and chitosan derivatives are potentially, but not practically, used in pharmaceutical and cosmetic industries. The list of them are given here:

1. **O-Carboxy or O-Hydroxy-alkyl derivatives** (except Carboxy-methyl chitin) : water-soluble derivatives, use as emulsifiers, moisture retainers, or ingredient of skin care products

2. **O-Acyl derivatives** : use as emulsifiers

3. **N-Acyl derivatives** : use as eroding materials for slow-release drug delivery system

4. **Sugar derivatives** : use as viscosity builders

5. **Chitosan citrate** : use as a matrix for controlled release formulation. (Adusumilli and Bolton, 1991)

In many countries, some chitin and chitosan derivatives were patented (mentioned by Lang and Clausen,1989) but it had no evidence to indicate the practical uses of them. Some of derivatives are given here:

1. **Glycol chitosan sulfate:** It is used for stabilizing cosmetic and pharmaceutical emulsions. (Patented by Shiseido in a Japanese Patent, JP59/ 139310 A2, 1984)

2. **Glycol chitin ether, Carboxymethyl chitin ether**, and/or **Chitin sulfate**: It is used in cosmetic preparations, e.g. skin lotions. (Patented by Ichiman in JP59/ 106409 A2, 1984)

3. Anionic chitosan derivatives prepared by the treatment of chitosan with unsaturated anhydrides, e.g., Maleic acid anhydride and subsequent

treatment of the product with primary or secondary amines. These products are used in cosmetic preparations. They are used for preventing the hair from looking oily. (Recent patent applications by Grollier, et al. in DE 3713099, 1987 and DE 3044738, 1980)

The derivatives that have been put into the practical applications are given here:

1. **Carboxymethyl chitin** (CM-chitin) is essentially similar to that of hyaluronic acid produced in our skin. CM-chitin was patented as "CHITIN LIQUID" by Ichiman Farukosu Inc. (Gifu), and this is now utilized as an ingredient of skin care products in several cosmetic companies.

2. **N-Carboxybutyl chitosan** (Evalsan) is a product of Jan Dekker described as a fully biocompatible, substantive humectant. It is an amphoteric biopolymer, with a prevailing cationic character and a molecular weight of about 600,000 Daltons. One out of these repeating units of this polymer carries a carboxybutyl constituent. This polymer is water-soluble and can be used as film-forming polymer, stabilizer for liposomes, viscosifying agent with the ability to chelate metal ions. It has been highly recommended for use in skin-conditioning milks, creams and gels, aftershaves, and oral hygiene products. In various applications, it has been at levels of 2.5 -5% by weight.

3. **Quaternised hydroxypropyl chitosan** (Lexquat CH) is a product of Inolex, which is soluble in water, ethanol, isopropanol, propylene glycol and water/ethanol 1:1 mixtures. It is stable and functional over a pH range from 2 to 12 and is completely compatible with all ionic classes of surfactants. It has been shown to exhibit complete salt tolerance. But it can not be used to thicken systems by salt addition. It is use as an ingredient in shampoo and bath preparation.

Application of Chitin, Chitosan and Derivatives.

Commercial uses and potential applications of chitin, chitosan and their derivatives are in many fields as follows.

- Biotechnology uses
- Medical uses
- personal care uses
- Agricultural uses
- Food uses
- Clarification and waste management

The applications of chitin, chitosan and their derivatives to personal care, medical, and biotechnology uses are given in Table 3.

Table 3 Main applications for chitin, chitosan and their derivatives.

Application	Function
1. Immobilize enzyme / living cells	Gel immobilization matrix, increase stability, compatible with phosphates
2. Personal care products	
hair care	substantive to hair and skin
skin care	form clear protective coating, moisture retention
viscosifier	build viscosity in amphoteric/nonionic shampoo
cosmetics	viscosity building, coating, moisture retention, non-allergenic
3. Medical	
lower cholesterol	anticholesteric
wound care	accelerate wound healing
eye bandages	forms tough protective, coating, biodegradation
drug delivery	bioerodable, non-toxic
contact lens	crosslinked to give porous, "grindable" lens material, non-allergenic
dental	bioadhesive
absorbable surtures	biodegradable, accelerate wound healing
orthopedic	temporary bioengineering material
4. Biotechnology	
immobilized enzymes	complexes with proteins
immobilized living cells	forms gel matrix (e.g. beads)
encapsulated cells	replace polylysine in algin bead process
filtration	membrane can be cast, film
recover valuable protein	complexes with protein, flocculate
chromatography	support enzymes/cell stabilizers

Pharmaceutical Applications

Although a large number of chitin and chitosan derivatives have been produced, only chitin and chitosan have been studies for using as excipient in pharmaceutical preparations.

Some of pharmaceutical dosage forms with chitosan were prepared and investigated in clinical use by Knapczyk,et al. (1989). These dosage forms are nystatine lozenge, clotrimazole and nystatine vaginal tablet, clotrimazole and nystatine dry powder for gel preparations (New commercial form). All forms have been approved by patients.

The studies of potential application of chitin and chitosan for pharmaceutical preparations are briefly mentioned as follows:

1. Applications of chitin and chitosan to conventional tablets.

1.1 In direct compression technique

Bruscato, et al. (1978) proposed the highly beneficial disintegration properties of chitin in directly compressed tablets containing about 2-20% by weight of chitin. Therefore the application of chitin as a disintegrant was patented in United state (U.S. Patent 4,086,335)

After that, Sawayanagi, Numbu, and Nagai (1982) studied the fluidity and compressibility of combined powders of lactose or potato starch or mannitol with chitin and chitosan, and disintegration properties of tablets made from these powders. Moreover, the lubricity also studied by measuring the ejection force. It was found that the fluidity of combined powders with chitin and chitosan was greater than that of the powder with crystalline cellulose, the tablets obtained had good hardness and passed disintegration test of JP X. Thus chitin

and chitosan may be suitable for using as a diluent with friction-lowering properties in direct compression process.

In addition, Nigalaye, Adusumilli and Bolton (1990), found that tablets prepared with a chitosan concentration of less than 33% were fast releasing. Chitosan used in a concentration of about 10% acted as a disintegrant.

1.2 In wet granulation technique

Wet granulation tablet formulation was also studied by Bruscato, et al. (1978). The results exhibited the same disintegration property as in directly compressed tablets.

The application of chitin and chitosan as disintegrants in Paracetamol tablet was studied by Parichat Chomto (1992). The paracetamol tablets containing chitin and chitosan as disintegrants were granulated with PVP K30 binding solution. The disintegration time of these tablets were longer than those of sodium starch glycolate and crosscarmellose sodium but shorter than those of corn starch and microcrystalline cellulose at the same concentration and compressional force. In addition, it was found that the tablets containing chitosan had shorter disintegration time than those of chitin.

2. Enhancement of dissolution of poorly soluble drugs

2.1 Chitin and chitosan

Sawayanagi, Nambu, and Nagai (1982, 1983) studied the dissolution behaviors of poorly soluble drugs -- griseofulvin, phenytoin, phenobarbital, prednisolone, flufenamic acid and indomethacin -- from ground mixtures with chitin and chitosan. It was found that the dissolution rate of drugs from ground mixtures were greater than that from physical mixtures and the ground mixtures with chitosan showed the great dissolution compared with those

ground mixtures with chitosan showed the great dissolution compared with those with chitin and crystalline cellulose. In addition, the X-ray diffraction patterns and differential scanning calorimeter indicated a lowering of the degree of crystallinity of the ground mixtures. These results indicate that chitin and chitosan are useful for an enhancement of dissolution properties of poorly soluble drugs.

2.2 Low-molecular chitosan (LM chitosan)

The hydrolysis product of chitosan calls low molecular chitosan that is extremely soluble in water. (more than 50%(w/v))

Shiraishi, et al. (1989) studied the dissolution behaviors of acid, basic and neutral drugs -- phenytoin, diazepam, betamethasone, prednisolone, and digoxin -- from kneaded mixtures with LM chitosan in comparison with that of the drugs alone. In aqueous solution, no interaction of any drug with LM chitosan was observed but drug crystals were changed. It was found that the contact angle of kneaded mixtures was reduced in comparison with that of the corresponding drug powder and physical mixtures. Thereby, the enhanced dissolution rate of kneaded mixtures may be due to improvement of wettability and to changes of the crystallinity, microcrystal size and shape.

3. Applications of chitin and chitosan to oral controlled release preparations

Chitin and especially chitosan can be used effectively in oral sustained release dosage forms. Although many of the studies have developed novel applications for chitin and chitosan in theory, few have been practically feasible from an industrial standpoint. Reports that have been published about these applications are briefly mentioned here:

3.1 Controlled release gel system

Miyazaki, Ishi and Nadai (1981) proposed the use of chitin and chitosan as drug carriers. Dried gel of chitin and chitosan containing indomethacin or papaverine hydrochloride was prepared. Sustained release of drugs from the dried was obtained, and in the case of chitosan gel, drug were released at a constant rate. (zero-order)

3.2 Controlled release matrix systems

3.2.1 In direct compression technique

Preparations of direct compression matrix tablets of both water soluble and water insoluble drugs were prepared by using chitosan as a vehicle. In the case of water soluble drugs, propanolol hydrochloride tablets (prepared by Sawayanagi, Nambu, and Nagai, 1982) and chlorpheniramine maleate tablets (prepared by Brine, 1989) were prepared, and zero-order and pseudo-zero-order drug release were obtained, respectively.

In another case of poorly soluble drug, a hydrocolloidal matrix system containing theophylline and chitosan was prepared and evaluated by Nigalaye, Adusumilli, and Bolton.(1990) It was found that chitosan when used alone in a tablet formulation, did not impart sustained release properties at low concentration (10%). When it was used in concentration of 50% of tablet weight, a non-erosion type matrix system was formed. In order to produce a 24-hours sustained release tablets, the combination with both carbomer-934P and citric acid was needed.

3.2.2 In wet granulation technique

In the case of matrix tablets produced by wet granulation, only water soluble drug was performed. Aspirin was a model drug in this case. Prolonged release tablets of aspirin with chitosan were first prepared by Kawashima, et al. (1985) that the granules were dried in a fluidized bed dryer. After that, Brine (1989) prepared in the similar fashion but dried in vacuum oven. Wet granulation formulation for aspirin was modified and optimized in the later study and zero-order drug release was obtained.

3.2.3 By cross-linking procedure

Spherical agglomerates (beads) of water-insoluble drugs were prepared by using polyelectrolyte complex formation properties of chitosan. Pellets of griseofluvin, ibuprofen, indomethacin, sulfadiazine were prepared by dispersing each drug in solution of chitosan and dropping these dispersions to solutions of the counterion Tripolyphosphate or Calcium chloride. The droplets instantaneously formed gelled spheres by ionotropic gellation. These spherical beads had narrow size distribution, good flow property and low friability. In addition, they showed pH-dependent swelling, disintegration and dissolution behavior. (Bodmeier and Paeratabul, 1989; and Bodmeier, Oh, and Parmar, 1989)

Not only spherical agglomerates but granules (cylindrical in shape) having swelling floating properties also prepared by cross-linking procedure. Hou, et al. (1985) prepared indomethacin granules by this technique and studied release rate in beagle dogs. (Miyazagi, Hou, et al., 1988) The results indicated that the chitosan granules were superior to conventional capsules.

This technique was documented in United State Patent as a method to prepare prolong release of macromolecules such as peptide hormones e.g., growth hormone by Cardinal and Curatolo, (1990)

Cross-linked chitosan microspheres is novel preparation for using as a matrix for the controlled release system developed by Thanoo, Sunny and Jayakrishnan (1991). Microspheres were prepared by the glutaraldehyde cross-linking of an aqueous acetic acid dispersion of chitosan in paraffin oil using dioctylsulfosuccinate as the stabilizing agent. Drug-loaded microspheres were also prepared in a similar fashion by incorporating drugs (theophylline, aspirin, or griseofulvin) into the chitosan solution. Drug incorporation efficiencies exceeding 80% could be achieved for these drugs. It was observed that the drug release rate were influenced by the cross-linking density, particle size and initial drug loading in the microspheres. For the controlled release system, these parameters can be manipulated to obtain a nearly zero-order release from the matrix.

3.3 Coated granules for controlled release preparations

The preparation of theophylline granules coated with a polyelectrolyte complex of Sodium tripolyphosphate and chitosan was developed by Kawashima, et al. (1985) Initially granules containing theophylline and Sodium tripolyphosphate were prepared. After that, granules were coated with the film of Sodium tripolyphosphate and chitosan. During the coating process, it was assumed that Sodium tripolyphosphate in the granules dissolved and moved to the outer surface. On the surface, the Sodium tripolyphosphate reacted with chitosan in the coating solution, resulting in the formation of an insoluble polyelectrolyte complex film. The drug release pattern of the coated granules follows zero-order kinetics.

3.4 Film preparation

Film dosage forms for oral administration have many advantages over conventional formulation as follows: (1) good drug stability (2) less scattering of drug powder (3) simple preparation method. Thus film preparation using chitosan as a film-forming polymer was first studied by Kanke, et al. (1989) In this study, three types of chitosan films containing prednisolone were prepared and drug release from the film was studied in vitro. Three types of films are given here:

- A monolayer type (ML) prepared by evaporating the solvent from a drug/Chitosan mixture
- A double layer type (DL) prepared by sticking together two ML films, one of which contained a drug.
- N-Ac film is DL film with one of the ML film N-acetylated with Acetic anhydride and stuck onto the other ML film.

Release of the drug from N-Ac film was found to follow zero-order kinetics.

Another study about film dosage form was carried out by Miyazaki, Yanmaguchi, and Takada (1990) By using diazepam as a model drugs, a film composed of a 1:0.5 drug-chitosan mixture was obtained and might be an effective dosage form that is equivalent to the commercial tablet dosage forms. (Release characteristics were evaluated both in vitro and in vivo.)

3.5 Buoyant sustained release dosage form

Sustained release intragastric "floating" dosage forms based on chitosan were developed by Machida, et al. (1989) Both chitosan granules, prepared by deacidification and had internal cavities, and laminated preparation prepared by laminating chitosan granules between chitosan membrane without

drug, were buoyant and provided sustained release of drug (prednisolone). The release properties were controlled by changing the composition of granules and/or the properties of chitosan membrane. In addition, in an absorption study using beagle dogs, sustained drug absorption from these preparations was obtained.

4. Use of chitosan as a bioadhesive polymer

An incorporation of bioadhesive polymers in the formulation has been considered as a useful method to localize the dosage forms at a specific site and to prolong a drug release from the formulation. Some bioadhesive polymers now used such as hydroxypropyl cellulose and carboxyvinyl polymers (polyacrylic acid derivatives), although have strong adhesive forces to mucous membrane, it has been the interpolymer complex formations, These complexes affected bioadhesive force of the tablet and drug release from the tablet.

Takayama, et al. (1990) investigated bioadhesive properties of chitosan and Sodium hyaluronate in order to find out suitable polymers by using a lyophilized porcine dermis as a model of mucous membrane. The results suggested that the tablets containing both chitosan and Sodium hyaluronate have strong adhesive forces, and the release rate of brilliant blue (as a model of water soluble drug) from chitosan-Sodium hyaluronate tablets was greatly affected by the change of the polymer mixing ratio. As this results, chitosan can be used as bioadhesive polymer if it is combined with Sodium hyaluronate in proper ratio.

Mucoadhesive properties of chitosan were also studied (Lehr, et al., 1992) by measuring the force of detachment for swollen polymer films from pig intestinal mucosa in a saline medium. Surprisingly, hydroxypropyl- and

carboxymethyl-cellulose showed almost no mucoadhesion, whereas the cationic polymer, chitosan, was fairly mucoadhesive in comparison to polycarbophil as a reference substance.

5. Use of chitosan as a liposome stabilizer

Chitosan and some derivatives such as N-Carboxy chitin can be applied to a suspension of liposomes to form a coating of the phospholipid membrane, so that acts as a cryoprotecter and prolongs the shelf-life of the liposome preparation.

6. Use of chitosan as a protective substance or shield

Chitosan has gel-forming properties in the low pH range, and antacid and anti-ulcer activities that may prevent or weaken drug irritation to the stomach Knapczyk, et al. (1989) proposed that chitosan exhibit biomedical properties of drug, gastroprotective effect, depending on the dose. The study indicated that chitosan protected the gastric mucosa against acute damage induced by absolute ethanol.

Another application of chitosan is to shield or protect sensitive drugs from the deactivating enzymes and pH in the stomach. In this case, film coating techniques are used.

7. Use of chitin as emulsion stabilizers

Magdassi and Neiroukh (1990) studied the use of solid chitin particles for the stabilization of mineral oil (paraffin oil) in water emulsions. It was found that stable emulsions were obtained at a concentration of 2.5%(w/w) chitin, when the water phase contained no surfactant. In the presence of 0.005%(w/w) Tween 80, stable emulsions were obtained at 0.5%(w/w) chitin. From zeta

potential measurement, it was concluded that the stabilization of the emulsion did not result from changes in the electrical properties of emulsion droplets.

8. Enhancement of target-organ-drug concentration

Magnetic microspheres containing chemotherapeutic agents have shown the ability to enhance greatly target-organ-drug concentration compared to conventional mode of drug administrations. Thus, Hassan, Parish and Gallo (1992) prepared magnetic chitosan microspheres (MCM) containing the anticancer drug, oxantrazole, by using a combined emulsion/polymer cross-linking/solvent evaporation technique. By optimization procedure, optimal formulation -- being of a submicron-size, high drug entrapment and low surface associated drug -- could be obtained. However, in vivo investigations will be performed in the future.