CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Chitin

Chitin is the second most abundant polysaccharides in nature and is found in the shell of crustacean and in the cell wall of fungi and algae. It is found commercially in the waste products of the marine food processing industry. (Choi *et al.*, 2002) It mainly consists of the aminosugar N-acetylglucosamine — β-(1,4)-2–deoxy–D–glucopyraose polysaccharides. It is also known to be one of natural hetero-polysaccharides with biocompatibility, biodegradability and low toxicity. Polymer are isolated from crab and shrimp shells have α-crystallographic structure where main chains arrange in an anti-parallel fashion with strong intermolecular hydrogen bonding. Chitin which is obtained from squid pens has β-crystallographic structure where chitin arrange in a parallel fashion with weak interaction force. (Tolaimate *et al.*, 2000)

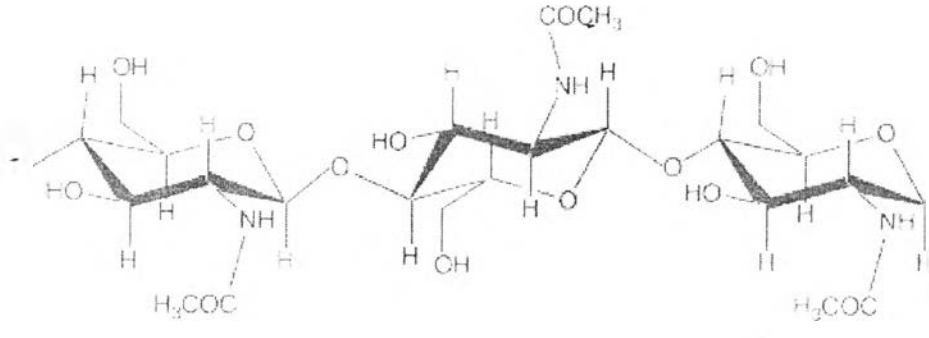

Figure 2.1 Chemical structure of chitin. (Jayakumar *et al.*, 2011).

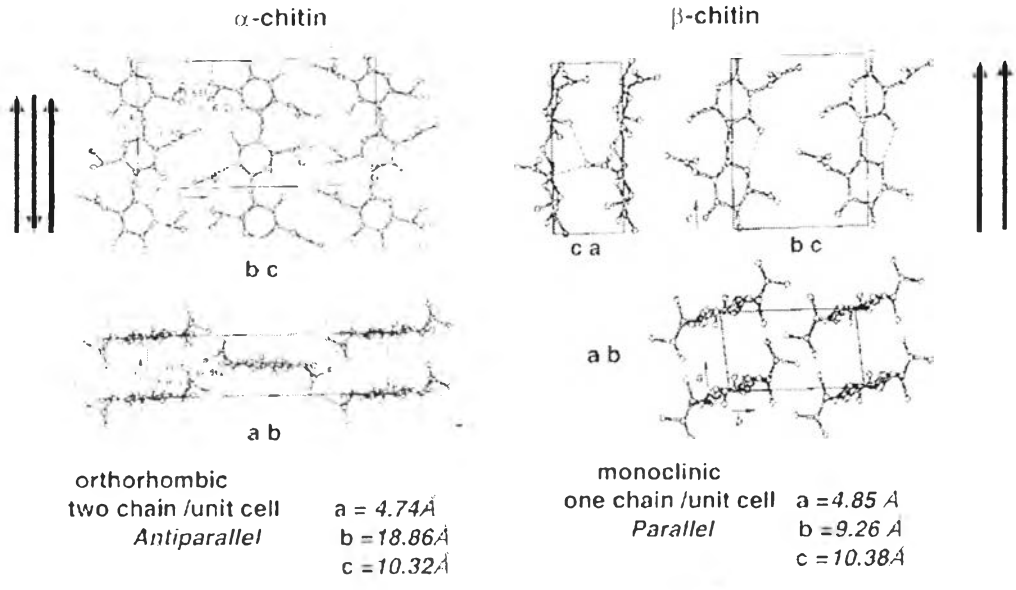

Figure 2.2 Crystalline structure of chitin (Tamura *et al.*, 2006).

However, chitin is highly insoluble in general solvents due to its rigid crystalline structure of hydrogen bonding through the acetamide group and hydrogen bonds, as the chitin molecule consists of N-acetylglucosamine residues, including the acetamide group at the C-2 position of glucosamine, the secondary hydroxyl group at C-3 and the primary hydroxyl group at the C-6 positions. (Tamura *et al.*, 2006) Several research papers have been reported about dissolution chitin in difference types of solvent which were sulfuric acid, formic acid, LiCl-DMAc but those solvent are toxic and caused to decrease the molecular weight of chitin. In 2006, Tamura *et al.*, found that $CaCl_2$.MeOH system acts as a good and nontoxic solvent combination with chitin. The amount of calcium ions and water are main factor affecting the dissolution of chitin in calcium solvent. It has also found that chitin hydrogels can be prepared using this calcium solvent system. (Tamura *et al.*, 2011) The calcium solvent destroy the crystalline structure of chitin in either hydrous or anhydrous condition and it can regulate the distribution of N-acetyl glucosamine and glucosamine between amorphous and crystalline region. Recently, the modification of chitin into chitin hydrogel for biomedical using calcium solvent have been investigated because

chitin hydrogel can be prepared as membrane scaffolds , wound dressing and other medical application.

2.1.2 Chitin Hydrogel

Due to, the poor solubility of chitin in common solvent, leaded to low reactivity, the chemical modification of chitins were studied. In 2006, Tamura *et al.*, prepared the chitin hydrogel under mild conditions by dissolving in Calcium chloride-dihydrate saturated methanol. The result showed that the solubility of chitin in the solvent depended on the degree of N-acetylation and the molecular weight of chitin as shown in figure 2.3

The chemical modification chitin , chitin hydrogel, a random coil structure chitin , lead to the decreasing of the crystalline structure by interrupting between H-bonding of polymer chains and increasing in the chemical reactivity because of the formation of chitin-calcium ion complex between the polymer chain, resulting in the disruption of hydrogen bond formation. The calcium ions which is used to dissolve chitin will form complex between the acetamide group and after adding water into the chitin solution , the water molecule will exchange with calcium ion and the chitin hydrogel will be formed.

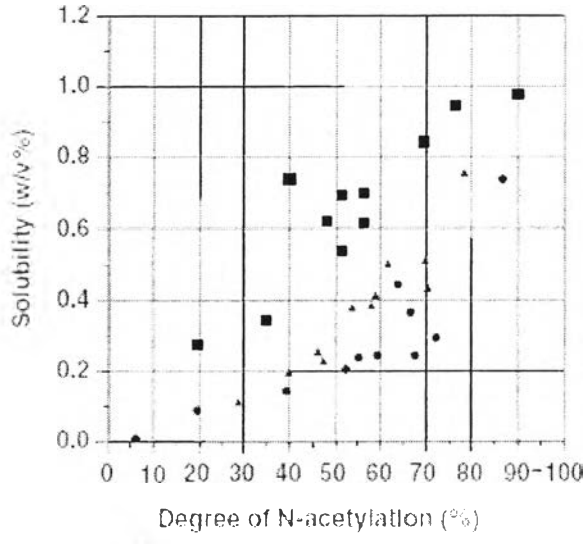

Figure 2.3 Dependence of the solubility of chitin on the degree of acetylation (DA) and on the molecular weight of the chitin itself. Solid square, 1.2×10^4, solid triangle, 4.0×10^4, solid circle, 1.6×10^5. (Tamura *et al.*, 2006).

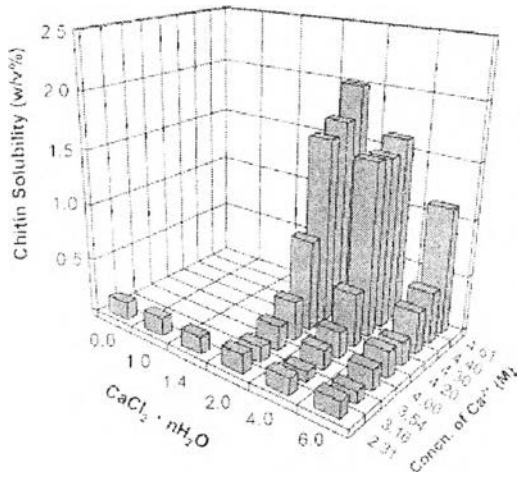

Figure 2.4 Dependence of chitin solubility on water content and calcium ion concentration (Tamura *et al.*, 2006).

In addition, the amount of water of calcium ions are influence to the dissolution of chitin in calcium chloride dehydrate-saturated methanol. As the results, when increased the concentration of calcium ions and water in calcium dehydrated saturated methanol , the percent of chitin solubility increased as showed in figure 2.4

2.2 Deacetylation by Alkaline Solution

Chitosan is come from the partial deacetylation of chitin. The degree of deacetylation (DD) is determined the behavior of chitosan if DD is higher than 0.5 chitosan becomes soluble in acidic aqueous solutions and it behaves as a cationic polyelectrolyte. Deacetylation of chitin can be done by treating with alkaline solution. The preparation of alkialine solution for chitosan were studied in two processes. (Tolaimate *et al.*, 2003) The first one was Broussignac method using the mixture of solid potassium hydroxide (KOH) (50 w/w%), 96 % ethanol (25 w/w%) and monoethylene glycol (25 w/w%) which is nearly anhydrous reaction medium. Ethanol and monoethylene glycol were first mixed and then added solid potassium hydroxide in small portion under stirring , the temperature of the solution will increase up to 90 C ^{o}due to the exothermic of the reagent. Then chitin was filled to the reagent and alcohol was distrilled return back to the reactor. The treatment was continued for required duration, and after filtration washed chitosan with water neutral water to neutral pH and dried at room temperature in air stream. Chitin was used in these reaction for 500 mg and suspended in 30 ml the mixed solvent.

Another method was Kurita process using the sodium hydroxide solution (50% w/v). Chitin was suspended for 500 mg in aqueous sodium hydroxide solution washed with distrilled water to neutral pH, then with methanol, and lastly with acetone. Chitosan was dried in an oven at 50 C^{o} during 12 hours. $NaBH_4$ was added during the deacetylation process to prevent polymer degradation.

Many literature reviews have reported on the *N*-deacetylation reaction, various alkaline methods have been proposed by different authors, most involving the use of sodium or potassium hydroxide solutions or enzymatic deacetylation. In the chemical processes of chitin deacetylation, the distribution of viscosity average mo-

lar weight is influenced by various parameters, such as: time, temperature, concentration and relation of alkali/chitin solution utilized in the deacetylation reaction (Moura *et al.*, 2011) The conditions of deacetylation were varied in order to obtain the best degree of deacetylation (DD) because the higher percent of DD , the more properties of chitosan so it is important to find the optimal condition including temperature;concentration of alkaline solution, to converse chitin to chitosan because chitosan have many advantages in bioactivity Also, the factor that effect the amount of chitosan is depend on the source of chitin because difference kind of source difference in the chemical structure, the crystallinity and others component – protein, calcium carbonate ($CaCO_3$), mineral, which make the difference in amount of chitin and chitosan and the properties.

Table 2.1 The deacetylation conditions obtained from literature reviews

Alkaline solution	Temperature (C^0)	Degree of deacetylation (%DD)	Reference
50% KOH in ethanol and monoethyleneglycol	120	90-96	Tolaimate *et al.*, 2003
40% KOH in ethanol and monoethyleneglycol	80	75-83	Broussignac *et al.* 1968
40–50% NaOH aqueous solution *(Microwave technique)*	150	> 90%	Lertwattanaseri *et al.*.2009
40% NaOH aqueous solution *(Ultrasonic radiation)*	60 – 100	40 – 95%	Zhang *et al.*.2007
40% NaOH aqueous solution	80	70 - 99 %	Kurita *et al.*,2001

2.2.1 Degree of Deacetylation

The term "degree of deacetylation or %DD" has been used to report the percent of D-glucosamine units presence in the polymer chain of chitin and chitosan. The properties of chitosan are largely affected by the degree of deacetylation (D.D.), when the degree of deacetylation (%DD) of chitin reaches more than 50%, it becomes soluble in aqueous acidic media and is called chitosan.

Table 2.2 Determination of degree of deacetylation by using FTIR

%DD	Absorbance Ratio	Equation	Reference
Low DD	A1550 / A2878	%DD = 101- [35.71.(A1550 / (2878)]	Sannan *et al.* (1977)
45 - 100	A1655 / A3450	%DD = 100 - [(A1655 / A3450) .115]	Baxter *et al.* (1991)
70 - 95	A1655 / A3450	%DD = 97.67 - [26.486.(A1655 / A3450)]	Sanbnis *et al.* (1997)
> 90	A1655 / A2867	Comparison of calibration curve	Miya *et al.* (1979)

Figure 2.5 showed the baseline of chitin sample which was used to calculated following the equation of Sannan *et al.*(1997) This equation is appropriated with the low level of N-acetylation.

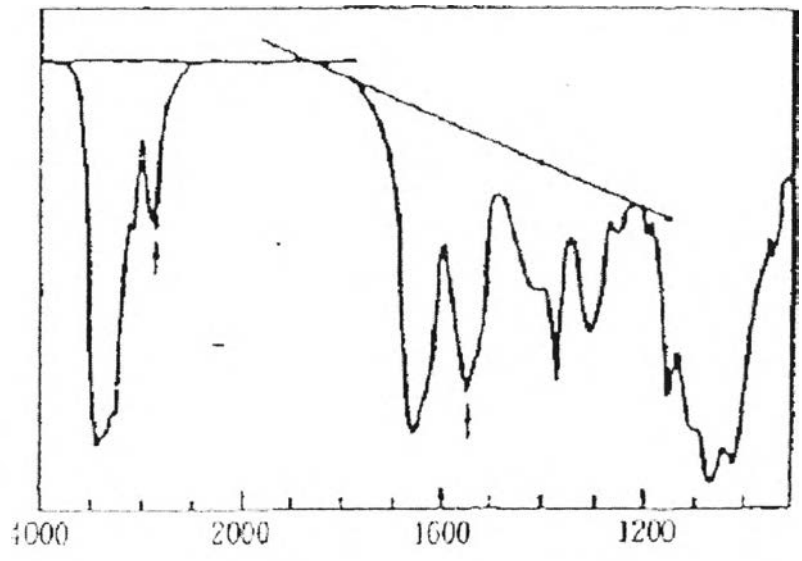

Figure 2.5 IR spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1550 /A2878. (Sannan *et al.*1977).

The baseline of calculating (Baxter *et al.*1991) method was showed in figure 2.6 which was used for the percent of degree of deacetylation of 45-100% . The figure showed the absorbance for the ratio A1655/A3450.

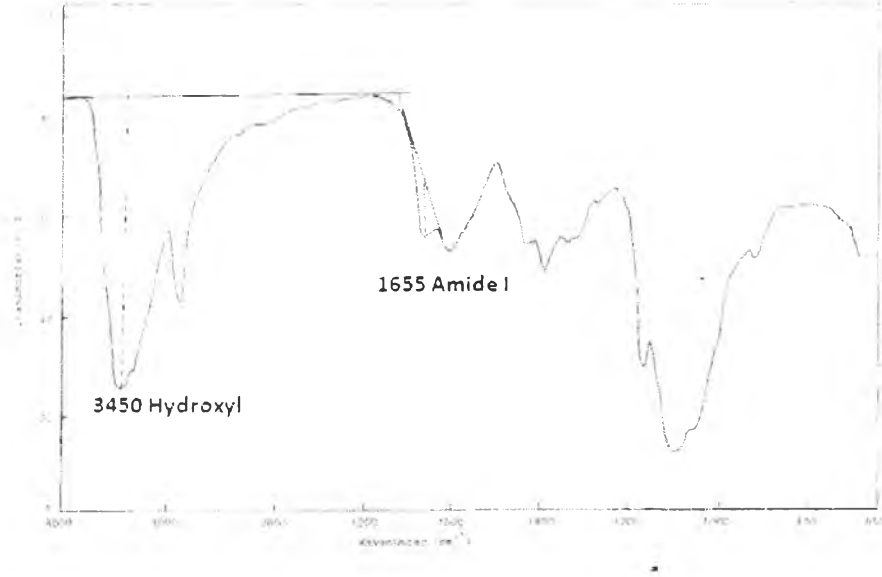

Figure 2.6 IR spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1655 /A3450. Baxter *et al.*(1991).

For the percent of degree of deacetylation in the range of 70-95%, was calculated following Sabnis *et al.* (1997). the I.R. spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1655 /A3450, as shown in Figure 2.7.

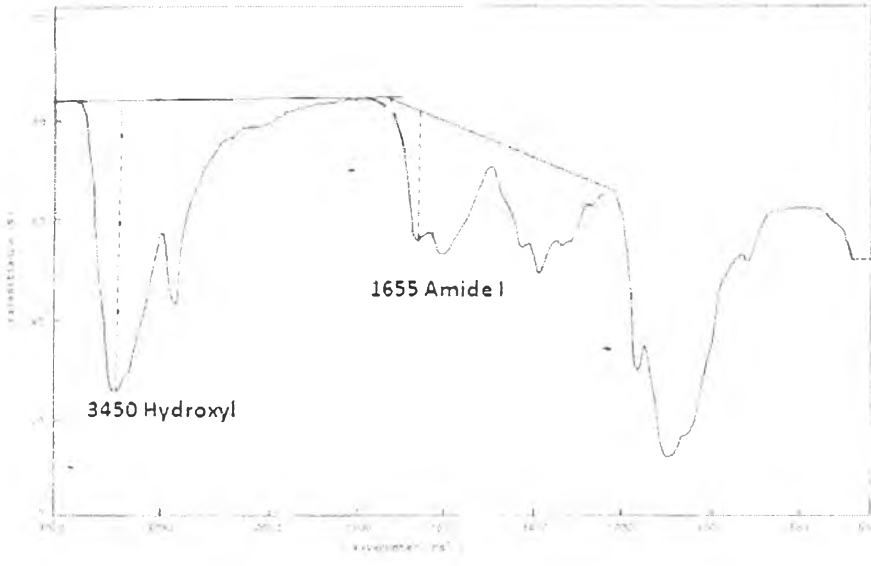

Figure 2.7 IR spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1655 /A3450. Sabnis *et al.* (1997).

2.3 Chitosan

Chitosan, a (1.4)-linked 2-amido-2-deoxy-β-d-glucan,is the N-deacetylated derivative of chitin. It is natural, non toxic, copolymer of glucosamine and N-acetylglucosamine prepared from chitin by deacetylation, which in turn, is a major component of the shells of crustaceans. It has been demonstrated by a number of researchers that chitosan has a great potential for a wide range of uses due to its biodegradability, biocompatibility, non toxicity, antimicrobial activity and versatile chemical, physical properties. etc.

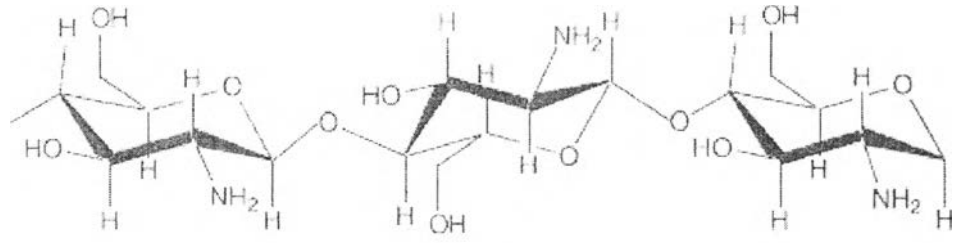

Figure 2.8 Chemical structure of chitosan (Jayakumar *et al.*, 2011).

2.4 Acid Hydrolysis

Method for preparing low molecular weight chitosan and chito-oligosaccharides are separated into enzymic and chemical hydrolyses. Enzymic method gives a high yield of chito-oligosaccharides (COs) but it is not effective in preparing products with molecular weight greater than chito-heptamer and the specific enzyme, is too expensive to be commercialized for the production of LMWC/COs. enzymatically hydrolyzed chitosan preparations for biochemical and food purposes is the undesirable level of pyrogenicity caused by the presence of protein admixtures. (Prashanth *et al.*, 2005)

Acid hydrolysis is the process which degraded the chain of chitosan by breaking down β 1,4-glycosidic bonds. It is still attractive mainly due to its cost effectiveness compared to the enzymatic hydrolysis. (Ajavakoma *et al.*, 2012) Acid hydrolysis was introduce to depolymerize the chiotosan. Various of acids have been used for hydrolysis — lactic acid, hydrocholic acid, nitrous acid, phosphoric acid, hydrogen fluoride, oxalic acid, sulfuric acid and etc. The acid-catalyzed hydrolysis of chitin involves two main acid-catalyzed reactions, that is, the hydrolysis of the glycosidic linkage (depolymerization) and the N-acetyl linkage (de-*N*-acetylation). (Varum *et al.*, 2001).

The figure 2.9 below illustrated the rate mechanism of *O*-glycosidic linkages (depolymerization) and the *N*-acetyl linkage (*N*-deacetylation) by hydrocholic (HCl) hydrolysis in chitosan. (Varum *et al.*, 2001) They reported that the hydrolysis can occurred in two processes , depolymerization and *N*-deacetylation. The experi-

mental was done in boths dilute and concentrated acid hydrolysis. The result found that in dilute HCl, the rate of hydrolysis of the glycosidic linkage was equal to the rate of de-*N*-acetylation, while in concentrate HCl the glycosidic linkages was hydrolysed faster than the *N*-acetyl linkage 10 times. It can be assumed, the hydrolysis of the glycosidic linkages is S_N2 reaction and it is a rate determining step from addition of water to the carbonium ion while S_N1 reaction is hydrolysis of the glycosidic linkages, is the formation of carbonium ion.

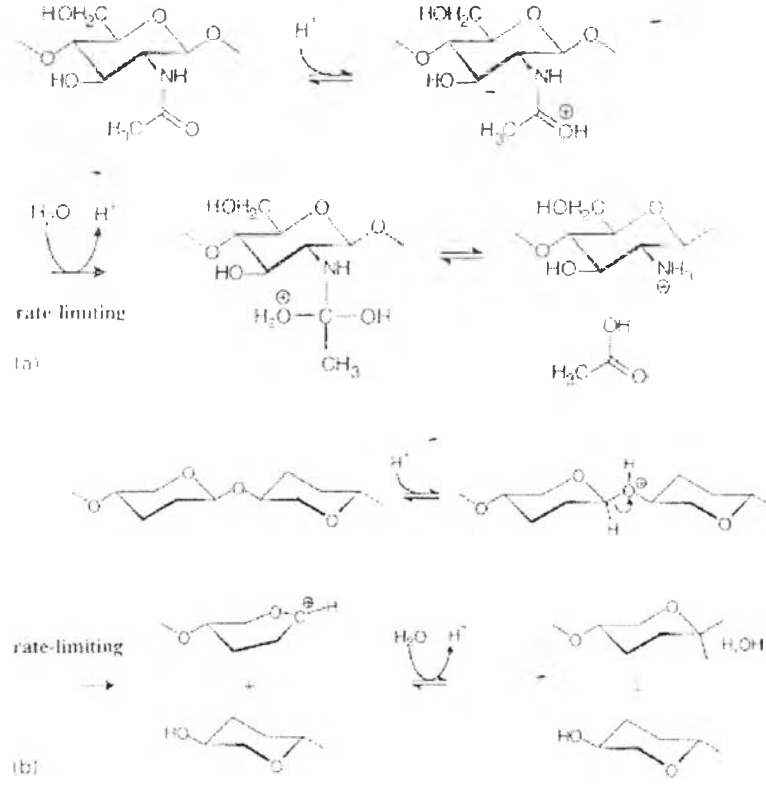

Figure 2.9 showing the reaction mechanism for the acid-catalysed hydrolysis of the *N*-acetyl linkage chitosan (a) Hydrolysis of the *N*-acetyl linkage (S_N2 reaction). (b) The most accepted mechanism for hydrolysis reaction of the glycosidic linkage (S_N1).

In 1993, Hasegawa *et al.* prepared the low molecular weight chitosan in homogenous hydrolysis using the concentration of phosphoric 85% at room temperature for 1-6 weeks. The degree of polymerization (DP) was 17 and 7 which mean two type of chitoan were produced . The lower DP was assumed to be useful as model compounds and bio-active of chitosans. In addition, phosphoric hydrolysis effect not only in depolymerization but also in deacetylation of chitin.

In 2004, Il'ina *et al.*, studied on the effects of the main chitosan characteristics included molecular weight and degree of acetylation on its hydrolysis in lactic acid. Two type of chitosans, high molecular weight and low molecular weight were hydrolyzed in 1% lactic acid solution for 180 days at 8,22,37 C^{o}. The rate of acid hydrolysis are depended on several parameters, the concentrations of the catalyst (lactic acid) , substrate (chitosan), the temperature, the duration of reaction and the degree of acetylation chitosan sample. The viscosity of high molecular weight chitosan was decreased by 90% over 180 days and in case of low molecular weight chitosan was decreased by 51%. This phenomena can be explained by the orientation of molecule, the more molecular weight , the more unoreintation of the molecule so the more amorphous region can be hydrolyzed by acid more than crystal region. Also, the result demonstrated that the higher the degree of acetylation, the quicker of hydrolysis. The decrease in chitosan molecular weight and viscosity are related to the duration of acid hydrolysis. The longer hydrolysis , the lower viscosity and molecular weight.

Recently in 2012, Ajavakom *et al.*, investigated on the hydrolysis of α-chitin in concentrated hydrochloric acid assisted microwave and ultrasonic wave in order to improve the reaction rate and selectivity. The microwave can be accelerated the reaction and generated glucosamine hydrochloride (GlcN.HCl). This method was processed in12 minutes which faster than the conventional method 90 minutes. Sonication brought about the dissolution of chitin in HCl solution at 20 C^{o} that gave a method for selective acid hydrolysis in chitin at low temperature to produce *N*-acetyl glucosamine (GlcNAc).

2.5. Plasma

2.5.1 Solution Plasma Processing (SPP)

Solution- phase plasma or solution plasma (SPP) is a new technique which is used in liquid-phase plasma Recently, SPP has been used in many potential application fields such as water treatment, surface modifications, nanomaterial synthesis, sterilization, recycling of rare metals, and decomposition of toxic compounds. The variety of plasmas can be generate choosing the combinations of solvents and solutes in solutions. SPP can be used liquid nitrogen, supercritical fluids aqueous and nonaqueous solutions etc. Generation of active chemical species such as hydroxyl radical , hydroperoxyl radical, free electron, superoxide anion, atomic oxygen anion, highly electron and uv radiation. These reactive species and physical conditions have been shown to efficiently decompose many organic compounds such as phenol and poly chlorinated biphenyls with high rates of reaction (Watthanaphanit *et al.*, 2013) and has a high reaction rate under lower-temperature with greater chemical reaction. The molecular in liquid phase is higher than gas phase.(Takai, 2008)

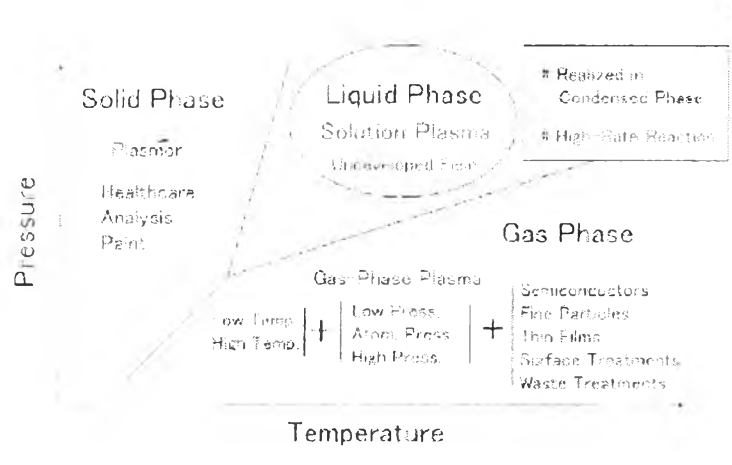

Figure 2.10 Showing three categories of plasma corresponding to the pressure–temperature relationship of three phases.(Takai *et al.* 2008).

The structure of the solution plasma is described in figure. 2.10 which the plasma is generate in the center and surround by the gas phase, the liquid phase also cover the the gas phase. So, there are two interfaces: plasma/gas and gas/liquid.

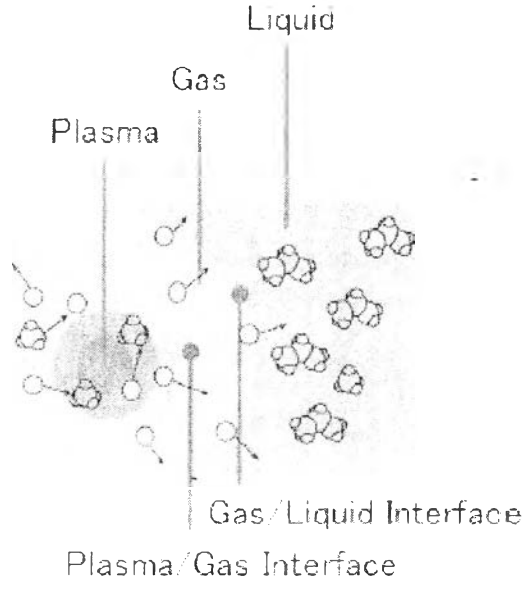

Figure 2.11 Solution plasma reaction model (Takai *et al.* 2008).

2.5.2 Solution Plasma Set up

The solution plasma system was set up as shown in figure 2.11 The pulsed electric discharge was generated between two needle electrodes, made of tungsten, using a high frequency bipolar pulsed DC power supply. The two electrodes, of which the distance is 0.2 mm, are set inside a glass reactor where polymer solution is filled. Once the power is applied, the plasma was generated.

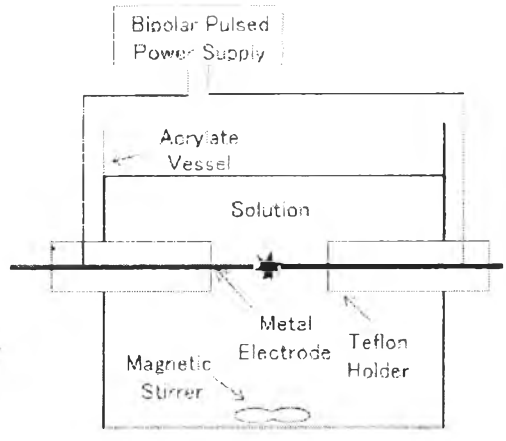

Figure 2.12 Solution plasma experimental set up (Takai *et al.* 2008)

There are many reports have demonstrated on the depolymerization of polymer by solution plasma. In 2012, Prasertsung *et al.* studied that solution plasma system can prepare low molecular weight chitosan (LMWC) and chitooligosaccharide (COS) because it generated active species such as hydroxyl radical ($OH^{\bullet}$) from figure 2.13 which broke β-1-4 glycosidic linkage, showed in figure 2.14 and 2.15. The degradation process of chitosan has effect only on the molecular weight but not in the structure reported by FT-IR. Also, GPC showed that the viscosity and apparent molecular weight of plasma-treated chitosan was decrease when increase plasma treatment time but after 180 minutes, there has no influence on both viscosity and apparent molecular weight. Solution plasma can obtain chitooligosaccharide with degree of polymerization of 2-8 in TOF-MS results.

$$O^{\bullet} + H_2O \rightarrow 2OH^{\bullet}$$

Figure 2.13 Showing creation of hydroxyl radical from solution plasma (Prasertsung *et al.* 2012).

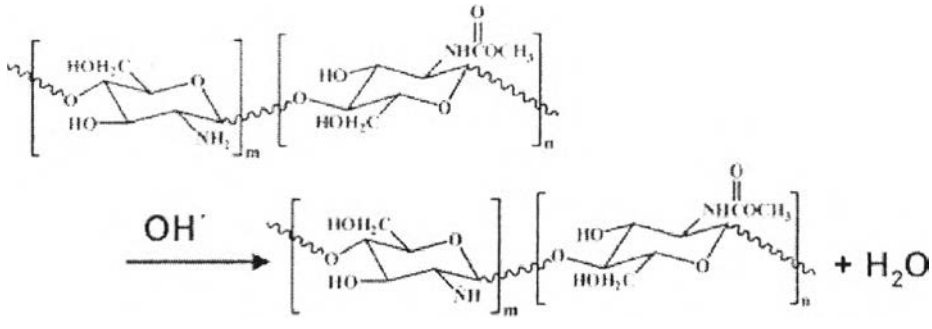

Figure 2.14 Degradation mechanism of chitosan by solution plasma process (Prasertsung *et al.* 2012).

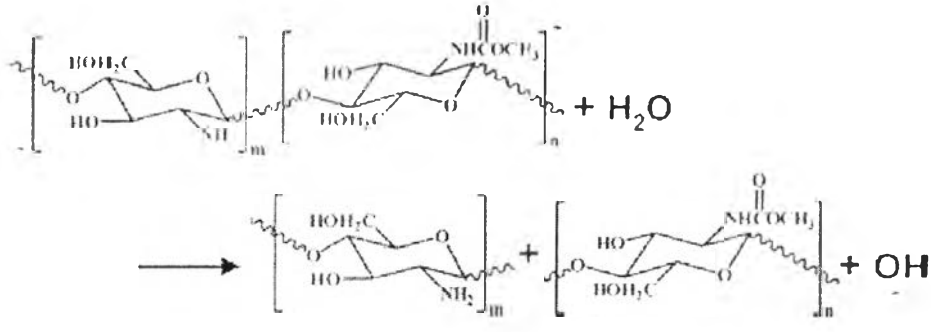

Figure 2.15 Degradation mechanism of chitosan by solution plasma process (Prasertsung *et al.* 2012).

Later year, 2013, The studied on the effect of polymer concentration on the depolymerization of sodium alginate by solution plasma have been investigated by Anyarat *et.al.* They reported that the plasma in liquid environment can lead to the scission of polymer chains of sodium alginate due to, the generation of reactive species occurred. The results supported that the molecular weight and the viscosity decreased after treating with solution plasma. Also, the research experimented on the concentration of sodium alginate solution at 0.2, 0.5, 0.9% w/v , they found that when increasing the concentration, the applied voltage decrease because of the higher concentration was helpful to generate plasma. The degree of entanglement of polymer chain had an effect on the voltage, also because of the more entanglement , the more friction between the chain can accelerate the breakdown so it will need less

voltage. This process is simple and can produce low molecular weight sodium alginate.

In 2011, Saito *et al.*, synthesized ZnO nanoflower using solution plasma. They examined on the effect of the applied voltage and the concentration of the electrolyte on the morphology of the ZnO. The applied an electric voltage were varied from 42 to 200 V to the electrodes of both Zn and platinum in a K_2CO_3 solution with different concentrations ranging from 0.01 to 5.00 M, and then observed the products by using a SEM and TEM. From the results showed that the solution plasma had produced spherical nanoparticle as show in figure.2.16 The surface cathode melt, first and then the solidification occurred in the solution ,after that the spherical nanoparticle was created. However, the flower-like products can formed another mechanism, which was not solidification after melting but nucleation and crystal growth. The flower-like structures have been produced through a hydrothermal synthesis and solution routes. The mechanism of the ZnO nanoflower was in Fig.14. On the surface of Zn,the $[Zn(OH)_4]_2-$ ions were formed and the product ZnO reacted with the hydroxide ions and water to produce the $[Zn(OH)_4]_2-$ ions. After that, $[Zn(OH)_4]_2-$ ions transferred from the electrode surface to the area of low temperature.Finally, solid ZnO crystallized with a decrease in temperature via reaction.

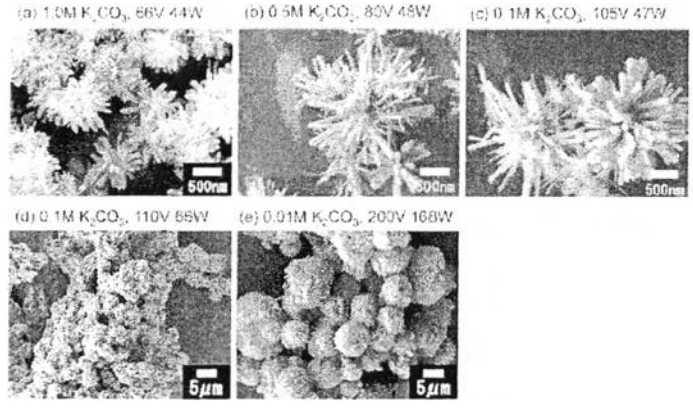

Figure 2.16 SEM images of products obtained under different condition of K_2CO_3 concentration and charged electric voltage. High K_2CO_3 concentration and low electric voltage induced the production of ZnO nanoflower (Saito *et al.* 2011).

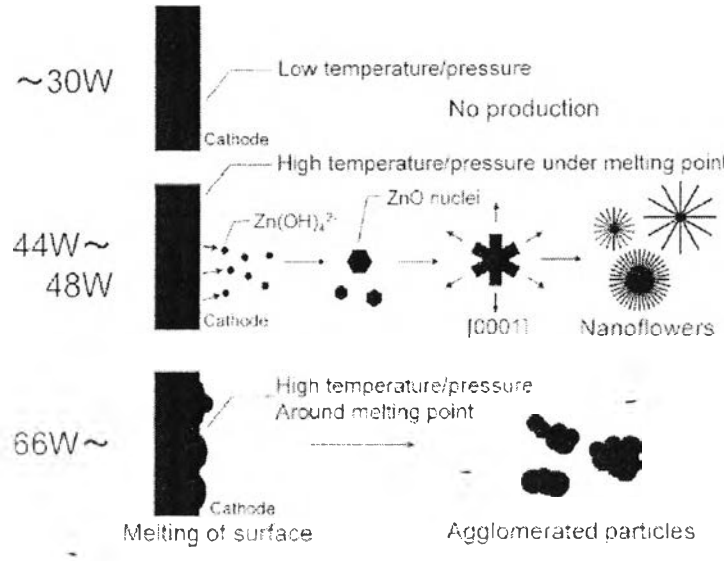

Figure 2.17 Images of ZnO synthesis via solution glow discharge (Sugiarto *et al.* 2000).

Sugiarto *et al.*, 2000, invented the degradation of organic compounds in water. The active species were created by the electrical discharge and played an important role in degrading organic compounds. Three types of electrical discharge formed by the needle-plate electrode system were used in this experimental. The degradation rate of phenol was affected by electrical discharged type and oxygen gas bubbling. The addition of small amount of hydrogen peroxide increased the degradation rate of phenol. The spark with streamer discharge was effective for degrade phenol completely.