CHAPTER II
LITERATURE REVIEW

2.1 Chitin

2.2.1 Structure and properties of chitin

Chitin is the second most abundant natural polysaccharide in the world next to cellulose and is a high-molecular weight linear polymer of *N*-acetyl-D-glucosamine (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose) units linked by β-D (1→4) bonds (Dutta *et al.,* 2004) The structure of chitin shows in fig. 1. Chitin is a high molecular weight biopolymer found predominantly in exoskeleton shells of arthropods as well as the internal flexible backbone of cephalopods. Chitin is known to be non-toxic, odorless, biocompatible with living tissues, and biodegradable (Kumar *et al.,* 2000).

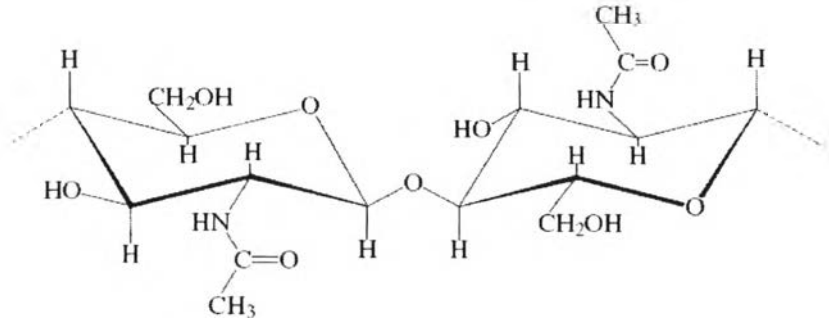

Figure 2.1 Structure of chitin. (Dutta *et al.,* 2004)

Chitin is the main component in the shells of crustaceans and classified into α-, β- and γ- chitin. α-Chitin has a structure of antiparallel chains such as shrimp shell and crab shell whereas β-chitin has intrasheet hydrogen-bonding by parallel chains such as squid pen. However, γ-chitin has a parallel and antiparallel structure, that is a combination of α-chitin and β-chitin (Yen *et al.,* 2009). β-Chitin has more open structure (parallel chain alignment) than γ-chitin (antiparallel chain

alignment). The resulting β-Chitin is much weaker intermolecular hydrogen bonding of the main chain. It, therefore, shows higher solubility, swelling, and reactivity than γ-chitin (Methacanon *et al.,* 2009). γ-Chitin has been proposed to form a much tighter crystalline structure than β-chitin. The crystalline structure of chitin shows Figure 2.2 In generally, chitin is a highly insoluble in general solvents and low chemical reactivity. By reason of its high rigid crystalline structure, which is based on the hydrogen bonding through the acetamide group and hydrogen bonds (Tamura *et al.,* 2010).

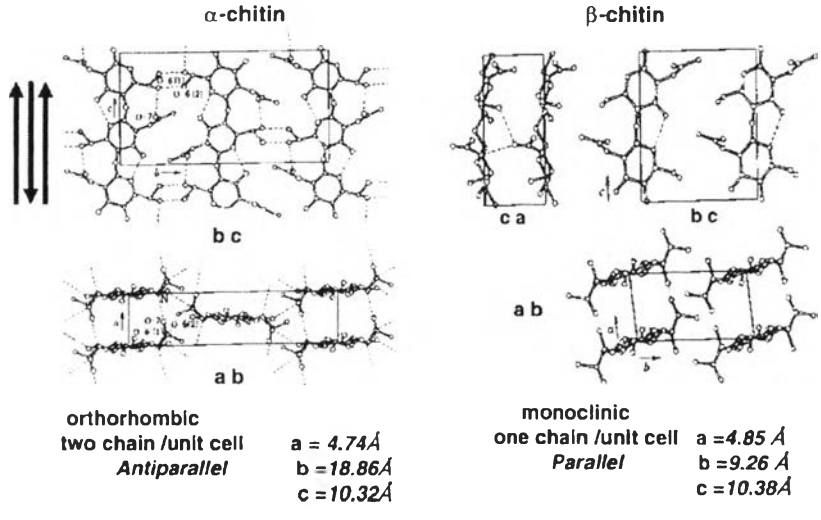

Figure. 2.2 Crystalline structure of chitin. (Tamura *et al.,* 2006).

Yen *et al.* (2009) prepared the crab chitosan by alkaline N-deacetylation of crab chitin for 60, 90 and 120 min and the yields were 30.0-32.2% with that of chitosan C120 being the highest. The degree of N-deacetylation of chitosans (83.393.3%) increased but the average molecular weight (483526 kDa) decreased with the prolonged reaction time. Crab chitosans showed lower lightness and WI values than purified chitin, chito- sans CC and CS but higher than crude chitin. With the prolonged reaction time, the nitrogen (8.99.5%), carbon (42.245.2%) and hydrogen contents (7.98.6%) in chitosans prepared consistently increased whereas N/C ratios remained the same (0.21). Crab chitosans prepared showed a melting endothermic peak at 152.3159.2 C. Three chitosans showed similar

microfibrillar crystalline structure and two crystalline reflections at 2h = 8.89.0 and 18.919.1. Overall, the characteristics of three crab chitosans were unique and differed from those of chitosan CC and CS as evidenced by the element analysis, differential scanning calorimetry, scanning electron microscopy and X-ray diffraction patterns.

Methacanon *et al.* (2003) studied that Chitin was extracted from squid pens and its heterogeneous alkaline deacetylation was performed using various conditions. The reactionfollowed the pseudo-first-order kinetics during an initial period. The influences of alkaline concentration, temperature, time and chitin to solution ratio on the N-deacetylation were investigated. The degree of deacetylation (DD) increased mainly with increasing temperature, NaOH concentration and time. The effect of the chitin to solution ratio was insignificant. In the temperature range of 40–100 8C, the apparent rate constant and the activation energy of the reaction ranged from 1.0 to 2.4 and from 5.4 to 11.9 kcal/mol, respectively. The linear regression analysis was performed to predict the optimum conditions for 90% DD chitosan product.

2.1.2 Chitin hydrogel

Tamura *et al.* (2006) studied the solubility of chitin in calcium chloride dihydrate-saturated methanol was found todepend both on the degree of N-acetylation and the molecular weight of the chitin (Figure 2.3). Several N-acetylated chitosans were obtained in a homogeneous system using chitosans with a molecular weight of 1.2×10^4, $4.0\times10^4$and 1.6×10^5 respectively, where the degree of deacetylation was controlled by the amount of acetic anhydride added (Hirano and Ohe 1975). The limited solubility of higher molecular weight chitin seems to be a property of its high viscosity despite the fast dissolution of N-acetylated chitosan. Chitosan with high degree of deacetylation was insoluble in calcium chloride-saturated methanol, thus, fiber spinning using the solvent was successful under themilder condition (Tamura *et al.*, 2004). However,the existence of water molecules was also found to be important to the dissolution of chitin (Figure 2.4). The observation was supported by the fact that Ca–MeOH prepared by anhydrous calcium chloride does not dissolve chitin at all. The concentration of calcium was also a very important factor for the dissolution of chitin.These results suggest that many factors contribute to the dissolution process of chitin. One possible manner to

estimate the chitin molecular conformation seems to be the arbitral viscosity equation which uses standard chitins of various molecular weights (prepared by N-acetylation of chitosans for which the molecular weights are known). According to this arbitral viscosity equation, chitin seems to have a partially random coil structure.

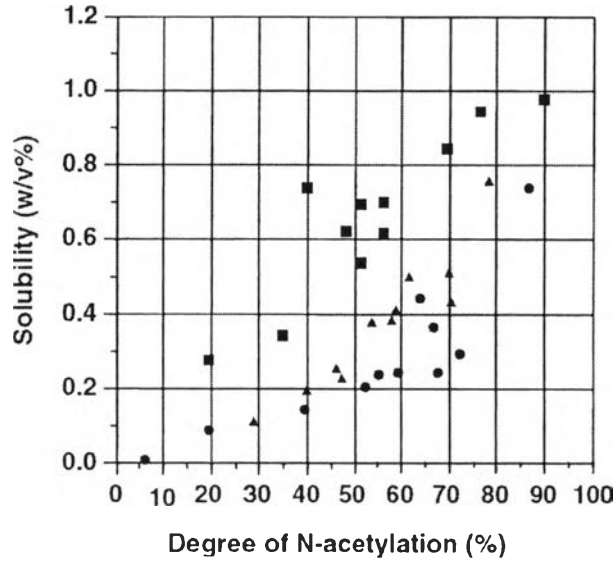

Figure 2.3 Dependence of the solubility of chitin on the degree of acetylation (DA) and on the molecular weight of the chitin itself. Solid square, 1.2×10^4, solid triangle, 4.0×10^4, solid circle,1.6×10^5.

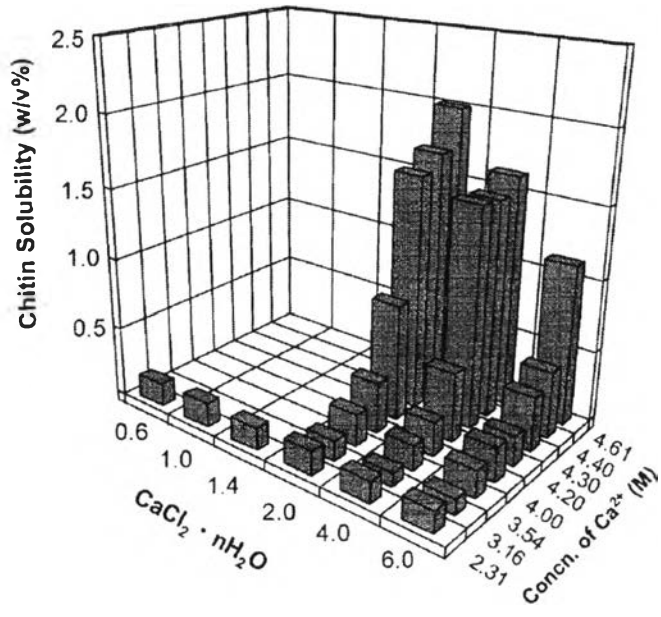

Figure 2.4 Dependence of chitin solubility on water content and calcium ion concentration.

An approach to increase the chemical reactivity of chitin is to destroy the crystalline structure of chitin by dissolving chitin in calcium chloride-saturated methanol. The product in the form of chitin hydrogel with higher chemical reactivity than native chitin is obtained. It has been proposed that calcium chloride-saturated methanol can dissolve chitin because of the formation of chitin-calcium ion complex, resulting in the disruption of hydrogen bond formation. By dissolving chitin in calcium chloride-saturated methanol solvent system, it has been proposed that calcium ions will form complex with chitin at acetamide group. After adding water into chitin solution, the exchange between water molecule and calcium ions occurs and chitin hydrogel will be obtained.

Shimojoh *et al.* (2011) studied on chitin hydrogel and reported on its improved chemical reactivity. It was found that chitin hydrogel is an amorphous form of chitin. The resulting chitin hydrogel exhibited higher chemical reactivity as evidenced by adsorption of copper (II) and acetylation reaction

2.1.2.1 Absorption of copper (II) ions by using chitin hydrogel in comparison with native chitin

Figure 2.5 shows the absorption of copper (II) ions by using chitin hydrogel in comparison with native chitin. It was found that adsorption of copper (II) ions by chitin hydrogel was more facile, especially in the early stage of absorption, but the difference became smaller on prolonged absorption time.

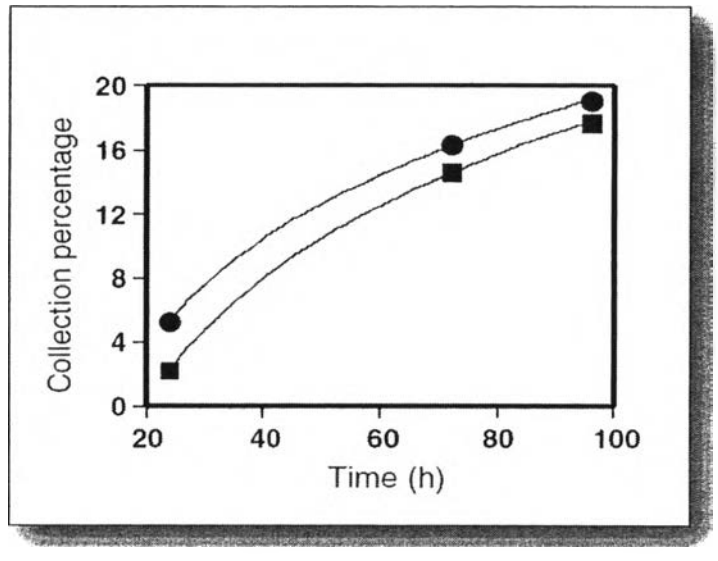

Figure 2.5 The absorption of copper (II) ions by using chitin hydrogel(●) in comparison with native chitin (■).

2.1.2.2 Acetylation reaction

For acetylation reaction of chitin hydrogel using acetic anhydride in pyridine, the chitin hydrogel showed a sharp rise in the degree of acetylation with the increasing of swelling time. The results is shown in Figure 2.6

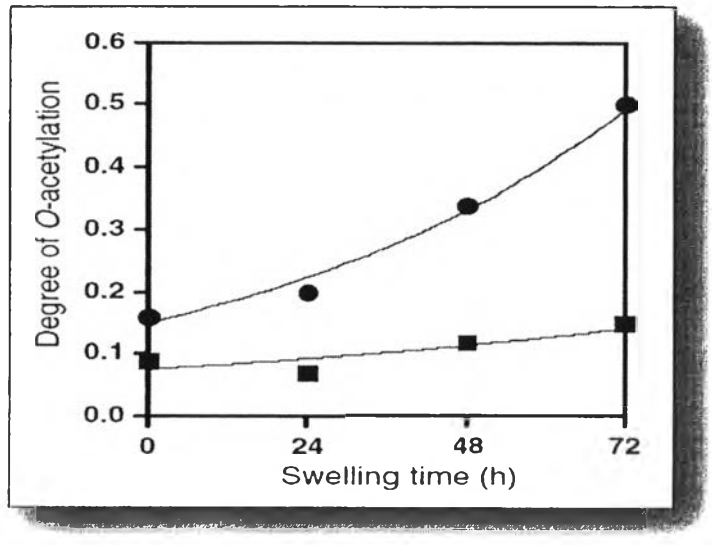

Figure 2.6 Acetylation reaction of chitin hydrogel (●) and native chitin (■).

2.2 Deacetylation

Chitosan is a natural, non-toxic, biodegradable copolymer of (1.4-β-)-2-amino-2-deoxy-D-glucopiranose and (1.4-β-)-2-acetamido-2-deoxy-D-glucopiranose or a homopolymer of (1.4-β-)-2-amino-2-deoxy-D-glucopiranose. Partially deacetylation of chitin shows in Figure 2.7. It is usually obtained from chitin through chemical deacetylation carried out under heterogeneous and homogeneous conditions.

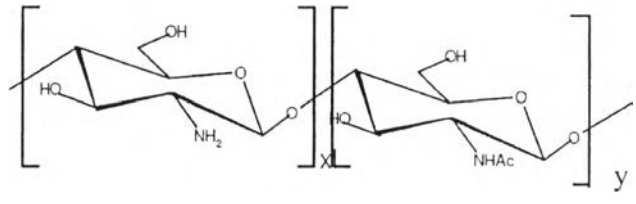

Figure 2.7 Partially deacetylation of chitin. (Pajak and Szumilewicz, 2006).

2.2.1 Heterogeneous conditions

Due to its biological activity and functional features, there are applications for chitosan in numerous fields (Rinaudo et al. 2006). The chemical structure and degree of polymerization influence the biological and functional properties of the polymer (Chae *et al.* 2005).

The chemical and physical properties of chitosan can be modified by controlling the molecular weight of the raw material and the length of time and temperature of deacetylation (Wojtas *et al.*, 2006). Chitosan with low degrees of polymerization and varied degrees of deacetylation is desirable. In order to obtain this, chitin has to be subjected to degradation prior to the hydrolysis of amide bonds (Wojtas *et al.*, 2007). Chitin molecular weight should be reduced in a controlled manner without effecting substantial changes in the chemical structure of the raw

material. The method used should also not be harmful to the environment (Pajak and Szumilewicz, 2006).

Studies conducted thus far have shown that this can be achieved by degrading chitin with hydrogen peroxide in a microwave field (Wojtas et al. 2007). The molecular weight of the chitin obtained differs from that of the standard material, but there are no significant differences in the chemical structure of the polymers. This method meets the requirements of Green Chemistry thanks to the raw materials used (hydrogen peroxide and water) and the short process period (30min). The question is raised, however, if simultaneously subjecting chitin to hydrogen peroxide and a microwave field can cause changes in the structure order of the polymer. Such changes could influence the chitin deacetylation results including reaction yield and degree of polymerization in comparison to chitin that is not subjected to degradation. There is a lack of information in the literature on the impact chitin degradation with hydrogen peroxide in a microwave field has on the course and results of deacetylation

Pajak and Szumilewicz (2006) reported that two-step procedure of obtaining chitosan with tailored properties has been elaborated. In the first step chitin from the shells of krill and common shrimp was subjected simultaneously to a microwave radiation field and hydrogen peroxide. The effect of this kind of chitin treatment was obtaining introductory degraded chitin. In the second step alkaline hydrolysis of the degraded chitin was carried out. The susceptibility of the amide bonds of degraded chitin to alkaline hydrolysis was evaluated by estimating the deacetylation degree of the chitosans obtained from standard and degraded chitin. It was found, that subjecting chitin initially to a microwave field and hydrogen peroxide under the conditions presented in this paper did not substantially alter the susceptibility of the chitin amide bonds to further alkaline hydrolysis as compared with standard chitin. The introductory reduction of chitin polymerization degree with hydrogen peroxide in a microwave field permitted obtaining chitosan with low molecular weights without disadvantageous alterations in their chemical structure. It was also determined that the yield of chitosan depends on the initial degree of polymerization of the chitin.

In general, the high values of degree of deacetylation can be achieved only at high temperature and using high concentrations of alkali solutions. These data are shown in Table 2.1

Table 2.1 Chitosan production obtained from literature review

Alkaline/solvent	Temperature (°C)	Degree of Deacetylation	Reference
40% NaOH aqueous solution	80	70 - 99 %	Kurita *et al.*,2001
40–50% NaOH aqueous solution	100-160	95	Horton *et al.*,2000
40% KOH in ethanol and monoethyleneglycol	80	75 - 83 %	Broussignac *et al.* 1968
50% KOH in ethanol and monoethyleneglycol	120	95 - 98.5%	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

2.2.2 Homogeneous conditions

Deacetylation under homogeneous conditions is to dissolve chitin in an alkali at intermediate concentrations through freezing and thawing, which results in the formation of alkaline chitin solutions (ACSs). Like cellulose, chitin is swollen in alkalis. The degree of swelling increases with a decrease in temperature. Previous studies showed that repeated freezing and thawing of a chitin suspension in 8% NaOH lead to the formation of an ACS . This process is not accompanied by deacetylation of the polymer. Dissolving of chitin in alkalis may proceed in one stage. Swelling and dissolving of chitin in alkalis lead to the activation of this polymer, which becomes amorphous. It may be suggested that deacetylation of cryoactivated chitin occurs under moderate conditions and the rate of this process is similar in various regions of the polymer molecule. This method allows obtaining of chitin with a regular distribution of acetamide groups and free amino groups along the chain. Swelling and dissolving of chitin in alkali during freezing and thawing depend on the physical state, method of isolation, and dispersion of particles. Immediately after isolation, wet chitin has a greater solubilityand forms more homogeneous solutions than dry chitin. The homogeneity of the ACS depends directly on the degree of polymer disintegration

Nemtsev *et al.*, (2002) isolated enzymatically from Antarctic krill shells was dissolved in aqueous NaOH by freezing and thawing to create homogeneous conditions. Deacetylation was performed at room temperature or under heating.The degree of deacetylation, molecular weight, and dynamic viscosity of solutions were estimated in chitosan samples. Deacetylation of chitin under homogeneous conditions was optimized. Chitosans with molecular weights of 180–220 and 250–300 kDa were obtained from the chitins of Antarctic krill and northern shrimp, respectively.

2.2.3 Chitosan

Chemical modification of chitin is an approach to obtain chitin derivatives with higher chemical reactivity and better solubility. Chitosan is one of the most studied chitin derivatives and chitosan is derived from chitin by deacetylation reaction. The number of D-glucosamine units in chitosan is higher than the number of N-acetyl-D-glucosamine units (m>n).

When consider structural composition, chitin has the number of *N*-acetyl-D-glucosamine unit higher than the number of D-glucosamine unit whereas chitosan has the number of D-glucosamine unit higher than the number of *N*-acetyl-D-glucosamine unit. Chitin has relatively higher molecular weight and crystallinity than chitosan. While chitin does not dissolve in common solvents, chitosan is soluble in aqueous organic acids. Antimicrobial activity is a dominant biological activity of chitosan but this property of chitin is relatively low. These data are shown in Table 2.2.

Table 2.2 The different between chitin and chitosan

Structure and Properties	Chitin	Chitosan	reference
Ratio of *N*-acetyl-D-glucosamine to D-glucosamine	The number of *N*-acetyl-D-glucosamine unit is higher (Low value of %DD)	The number of D-glucosamine unit is higher (High value of %DD)	Kurita *et al.*, 2001
Molecular weight	Relatively higher	Relatively lower	Methacanon *et al.*,2003.
Crystalline structure	High rigid crystalline structure	Depend on physical state	Kurita *et al.*, 2001
Solubility	Not soluble in common solvents	Soluble in aqueous organic acids, e.g. acetic	Kurita *et al.*, 2001
Antimicrobial activity	Relatively lower	Relatively higher	Aranaza *et al.*,2009

2.2.3 Degree of deacetylation

The properties of chitosan are largely affected by the degree of deacetylation (D.D.), which is one of the most basic structural parameters for chitosan When the degree of deacetylation (%DD) of chitin reaches more than 50%, it becomes soluble in aqueous acidic media and is called chitosan

Table 2.3 Determination of degree of deacetylation by using FTIR

%DD	Absorbance Ratio	Equation	Reference
Low DD	A_{1550} / A_{2878}	$\%DD = 101 - [\ 35.71.(A_{1550} / A_{2878})\ ]$	Sannan *et* al. (1977)
45 - 100	A_{1655} / A_{3450}	$\%DD = 100 - [(A_{1655} / A_{3450}) .115]$	Baxter *et* al. (1991)
70 - 95	A_{1655} / A_{3450}	$\%DD = 97.67 - [\ 26.486.(A_{1655} / A_{3450})\ ]$	Sanbnis *et* al. (1997)
> 90	A_{1655} / A_{2867}	Comparison of calibration curve	Miya *et* al. (1979)

The degree of deacetylation of the chitin samples was calculated using baseline which was proposed by Sannan *et al.*(1977) and is shown in Figure 2.8. This method will be very inaccurate at low levels of N-acetylation.

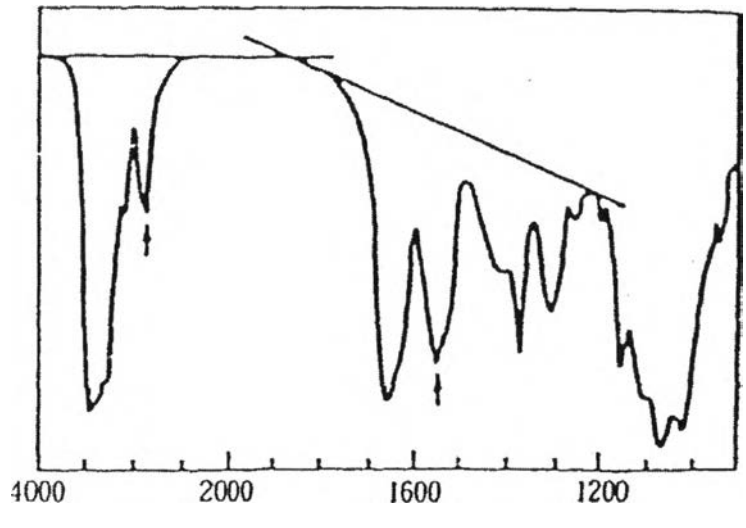

Figure 2.8. I.R. spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1550 /A2878. Sannan *et al.*(1977).

Figure 2.9 indicated the I.R. spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1655 /A3450. The degree of deacetylation of the chitosan samples was calculated using baseline which was proposed by Baxter *et al.*(1991) For DD appoximate range 45 - 100 % .

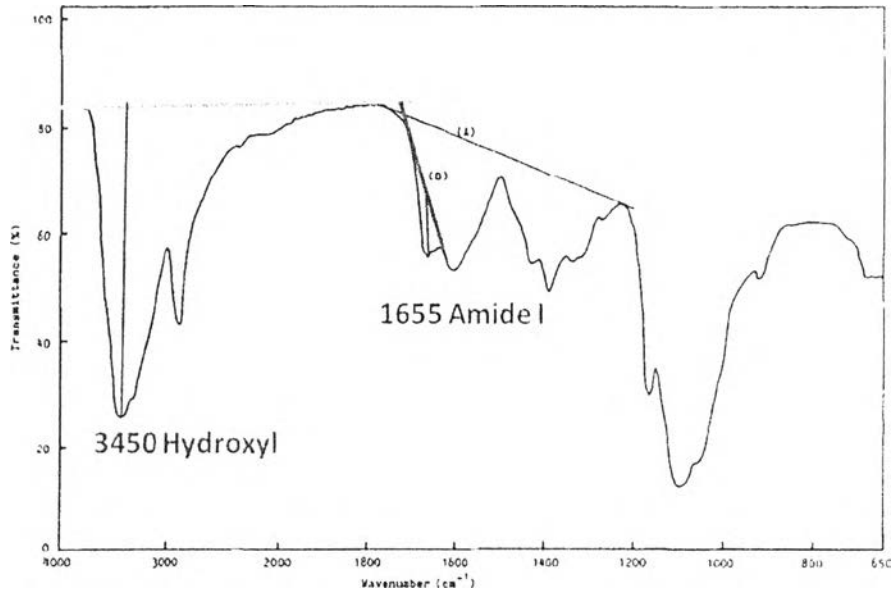

Figure 2.9 I.R. spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1655 /A3450. Baxter *et al.*(1991).

The degree of deacetylation of the chitosan samples was calculated using baseline which was proposed by Sabnis *et al.* (1997). For DD appoximate range 70 - 95 % and 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 2.10.

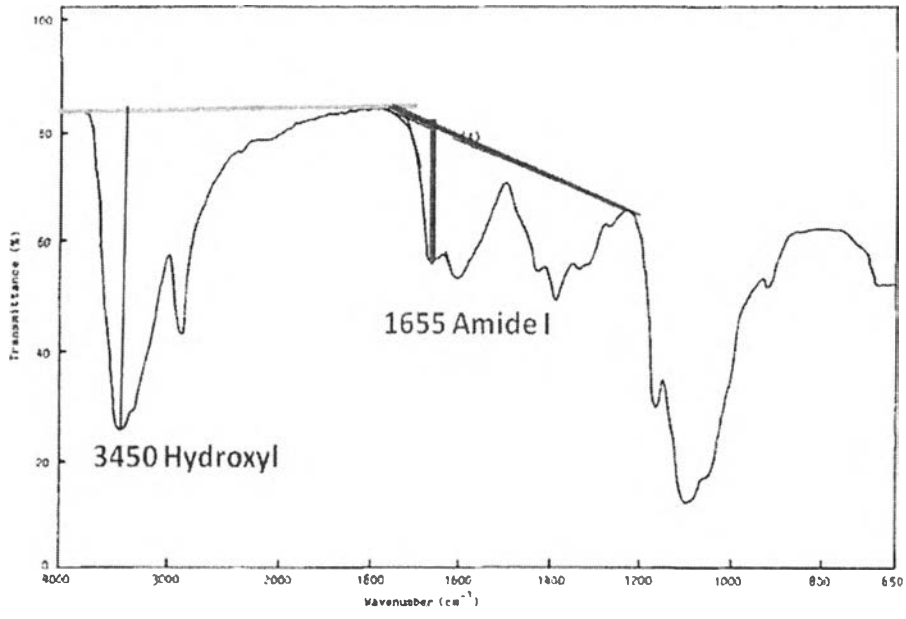

Figure 2.10 I.R. spectrum of chitosan showing the baselines for calculating the amide I band absorbance for the ratio A1655 /A3450. Sabnis *et al.* (1997).

2.3 Solution plasma processing (SPP)

Plasma is a useful tool for promoting chemical reactions because plasma is composed of electrons, ions, radicals, and neutrals which are in either fundamental or excited states.

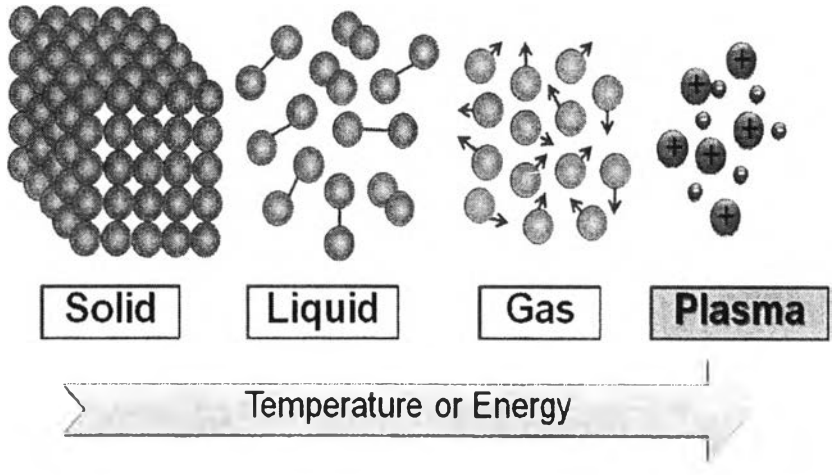

Figure 2.11 Medel of changing state to from plama.

Solution plasma, glow discharge in the liquid phase, provides extremely rapid reactions using activated chemical species and radicals under pressure. The emission center of plasma is located in the gas phase surrounded by the liquid phase, as shown in Figure 2.12. The solution plasma has attracted a great attention because of its applicability to industrial material processing.

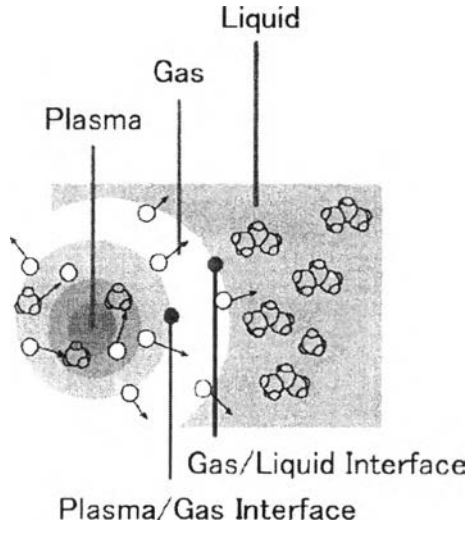

Figure 2.12 Model of the solution plasma. (Takai *et al.* 2008).

Solution plasma is a plasma system that generates plasma in liquid. (Takai,2008). This system is able to produce highly active species such as hydroxyl radical, hydroperoxyl radical, free electron, superoxide anion, and atomic oxygen anion.

Potocky *et al.* (2009) reported the various active species are generated in water under plasma discharge. It was found that various active species are generated in water under plasma discharge. These active species plays an important role in chemical reactions.

Saito *et. al.* (2012) reported synthesis of ZnO nanoflowers by solution plasma. Since the IR-loss is concentrated at the cathode/solution interface, the solution near the cathode is heated to the boiling point. The gas-layer is generated at the surface of the cathode. The plasma sheath is located in the gas phase surrounded by the liquid phase and water 2 molecule generated $2OH^{\cdot}$,as shown in Figure 2.13.

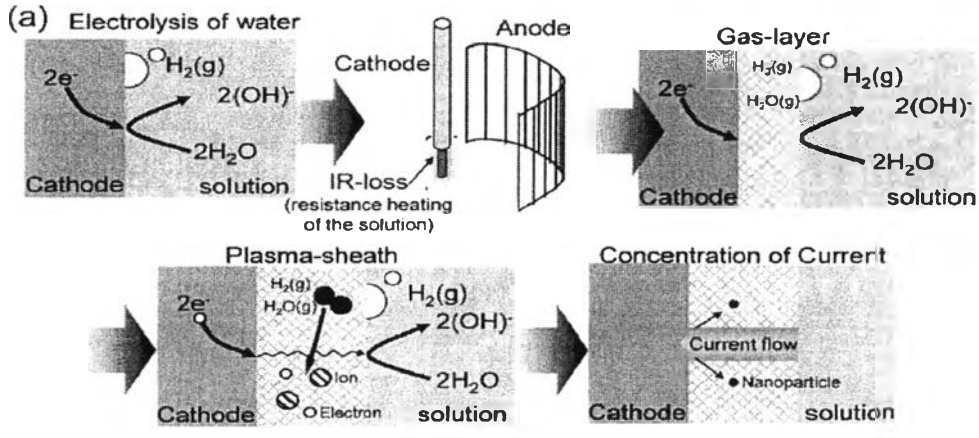

Figure 2.13 Model of synthesis of ZnO nanoflowers by solution plasma.

$[Zn(OH)4]^{2-}$ ions were formed around the electrode; the surface of the Zn electrode formed ZnO by the steam corrosion at a high temperature. The possible formation mechanism of the ZnO nanoflowers was demostrated in Figure 2.14.

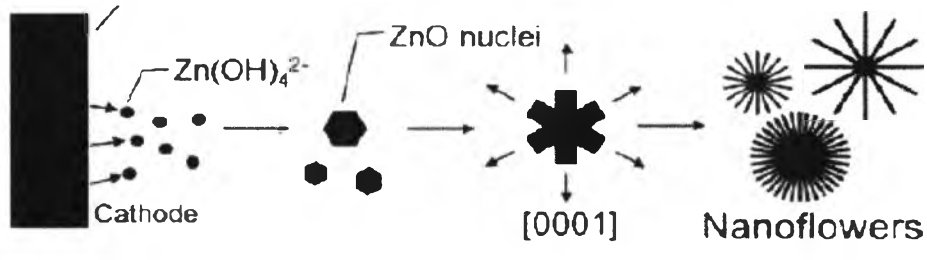

Figure 2.14 The possible formation mechanism of the ZnO nanoflowers.