

CHAPTER IV
SOLUTION PLASMA PROCESS FOR
DEACETYLATION AND DEPOLYMERIZATION OF CHITIN HYDROGEL

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4.1 Abstract

In this study, solution plasma process (SPP), a plasma generated in a liquid phase, was applied in depolymerization and deacetylation reactions of chitin hydrogel to form chitosan. Chitin hydrogel, an amorphous form of chitin, was depolymerized by SPP in 1% acetic acid solution for 0 h, 1 h, 3 h, and 5 h in order to reduce its molecular weight before being subjected to deacetylation reaction to form chitosan. The effect of depolymerization prior to deacetylation on the degree of deacetylation (DD) of the deacetylated products was examined. Deacetylation reaction was performed by applying SPP in different concentrations of KOH in MeOH. The concentrations of KOH in MeOH were varied to be 0%, 7%, and 10%. Deacetylation reaction via SPP was done repeatedly up to 5 cycles (one hour for each cycle to achieve the highest values of %DD). The effects of KOH concentration and plasma treatment time on %DD, molecular weight, chemical structure, and crystallinity of the deacetylated products were investigated by FTIR, GPC, and XRD. The results showed that %DD determined by FTIR increased with an increasing of the KOH concentration and plasma treatment time. In addition, the depolymerization of chitin hydrogel prior to deacetylation resulted in the higher values of %DD and %solubility of the deacetylated products.

Keywords: Chitin hydrogel, Solution plasma process, Deacetylation, Depolymerization, Chitosan

4.2 Introduction

Chitin is a polysaccharide of a linear copolymer of $\beta(1\rightarrow4)$ -linked 2-amino-2-deoxy-D-glucan and 2-acetamido-2-deoxy-D-glucan (Einbu *et al.*, 2008). The main sources of raw materials for the production of chitin- are cuticles of various crustaceans, principally crabs and shrimps (Percot *et al.*, 2002). Chitosan is the most important derivative of chitin due to the unique properties of amino group in chitosan structure. In general, chitosan is obtained from deacetylation reaction under strong alkaline and high temperature. An example of solvent systems used for deacetylation reaction of chitin is 40%-50% KOH in ethanol/monoethyleneglycol (Broussignac *et al.*, 1968). Chitin and chitosan have been applied to many biomedical applications because of their biocompatibility, biodegradability and low toxicity (Varum *et al.*, 2001, Tamura *et al.*, 2011). However, due to the rigid and high crystalline structure of chitin, low chemical reactivity and low solubility are the important drawbacks of chitin. According to these drawbacks, chitin hydrogel, an amorphous form of chitin, was used in order to increase the reactivity by destroying the rigid crystalline structure of chitin. Chitin hydrogel was prepared by dissolving chitin in calcium chloride dihydrate-saturated methanol, followed by precipitation in an excess amount of distilled water (Tamura *et al.*, 2011). Solution plasma process (SPP) is a liquid-phase plasma system (Takai, 2008) which has been applied in several applications such as synthesis of metal nano-particles (Saito *et al.*, 2011) decomposition of organic compounds (Sugiarto *et al.*, 2001) and depolymerization (Watthanaphanit *et al.*, 2013). During reaction, SPP generates many highly reactive species such as hydroxyl radicals, hydroperoxy radicals, free electrons, superoxide anions which are able to stimulate the chemical reactions (Prasertsung *et al.*, 2012). In this study, chitosan was prepared by deacetylation reaction of chitin hydrogel using low concentrations of alkaline solution (1%, 7% and 10%w/v of KOH/MeOH) with the aid of solution plasma technique. The depolymerization of chitin hydrogel using SPP prior to deacetylation was performed in order to decrease molecular weight of chitin and consequently obtain high degree of deacetylation as well as better

solubility. The chemical structure, crystallinity and degree of deacetylation of the deacetylated products were examined.

4.3 Experimental

4.3.1. Materials

Shrimp shell (*Pandalus borealis*) was kindly provided by Surapon Foods Public Co., Ltd. (Thailand). Potassium hydroxide (KOH, analytical grade, Sigma Aldrich), glacial acetic acid (CH₃COOH, analytical grade, J.T. Baker), Anhydrous sodium hydroxide pellets (NaOH, analytical grade, Univar), hydrochloric acid (HCl, analytical grade, ACI Lab Scan), methanol (CH₃OH, analytical grade, ACI Lab Scan) were used as received.

4.3.2. Preparation of Chitin

The shrimp shells were cleaned and dried under sunlight for 3-5 days to remove the pigment and then grinded into small pieces. Shrimp shell chips were decalcification by immersion in 1N HCl solution for 2 days with occasional stirring by changing the acid solution daily. Deproteinization was done by boiling the decalcified chitin in 4 %w/v of NaOH solution at 80-90 °C for 4 h.

4.3.3. Preparation of chitin hydrogel

4.3.3.1 Preparation of calcium chloride-saturated methanol

The calcium chloride dihydrate (850 g) was suspended in methanol (1 l) and refluxed for 30 min to a near state of dissolution, followed by standing overnight at room temperature before filtration in order to remove insoluble salt.

4.3.3.2 Preparation of chitin solution

The α -Chitin powder (20 g) was suspended in calcium chloride-saturated methanol (1 l) and refluxed for several hours with stirring until chitin is completely dissolved.

4.3.3.3 Preparation of chitin hydrogel

To obtain chitin hydrogel, a large amount of distilled water was added to 1 l of chitin solution with vigorous stirring, after that the precipitated product was filtrated and centrifuged, followed by dialyzing against distilled water

for several days to remove the calcium ions and methanol. The chitin hydrogel was kept in a refrigerator before use.

4.3.4. Deacetylation of chitin hydrogel by using solution plasma

Chitin hydrogel (15 g of wet weight) was suspended in different concentrations of KOH in MeOH (0%, 7%, 10%). The suspension was then subjected to solution plasma to conduct the deacetylation reaction. The frequency, voltage and pulse width for solution plasma treatment were 15.0 kHz, 150 KV and 2 s, respectively. The deacetylation reaction in KOH/MeOH via solution plasma was performed repeatedly up to 5 cycles and the plasma treatment time for each cycle was 1 h. The deacetylation of chitin by solution plasma was done in comparison with the conventional heat treatment.

4.3.5. Depolymerization of chitin hydrogel by using solution plasma

Acetic acid solution (1% v/v) was used to depolymerize the chitin hydrogel by using solution plasma process. The frequency, voltage and pulse width for solution plasma treatment were 15.0 kHz, 120 kV and 2s, respectively. After that the depolymerized products was precipitated in MeOH and washed until neutral before being subjected to deacetylation. The reaction times for depolymerization were varied to be 0h, 1 h, 3 h and 5 h.

4.3.6 Characterization

The chemical structures of chitin and chitosan were determined by using FTIR spectroscopy (Thermo Nicolet Nexus, 670) at the wavenumber ranging from 4000 to 400 cm^{-1} . The degree of deacetylation (%DD) was calculated by using Baxter's equation (Baxter, Dillon, Taylor, & Roberts, 1992) as follows:

$$\text{DD (\%)} = 100 - [(A_{1655} / A_{3450}) \times 115] \quad (1)$$

where A_{1655} is the absorbance at 1655 cm^{-1} of the amide-I band as a measurement of the N-acetyl group content and A_{3450} is the absorbance at 3450 cm^{-1} of the hydroxyl band as an internal standard.

The crystallinity of chitin hydrogel and plasma-treated chitin hydrogel products was identified by wide angle X-ray diffraction (WAXD) analysis (Bruker AXS, D8 advance) operated with the use of Cu $K\alpha$ as an X-ray source and scanned from $2\theta=5^\circ$ to $2\theta=50^\circ$.

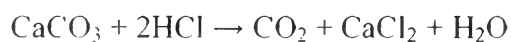
GPC was used to determine the weight-average molecular weight (M_w), number-average molecular weight (M_n) and molecular weight dispersion (M_w/M_n) of obtained product from deacetylation and depolymerization reaction. The obtained products were purified before using. The chitosan products were dissolve in acetate buffer at pH 5.5, a mixture of 0.1 CH_3COOH and 0.2 CH_3COONa , and filtrated through nylon 66 membrane with the pore size of 0.45 μm (Millipore, USA) before injection into the GPC instrument (Waters. Water 600E) equipped with an refractive index (RI) detector using an ultrahydrogel linear column. Flow rate of mobile fate was set constant at 0.6 mL min^{-1} . The volumn of injection sample was 20 μL and chitosan concentration of 2.5 mg/mL at at 30 $^\circ\text{C}$.

4.4 Results and discussion

4.4.1 Characterization

4.4.1.1 *Yield of production*

The raw shrimp shells was isolated by deproteinization and demineralization to obtain chitin product. These two processes are used to eliminate some inorganic and organic component from the raw material. The deproteinization was carried in aqueous NaOH at room temperature using 1 M HCl. It was observe that the emission of CO₂ gas because the acid reacts with the calcium carbonate according to the following simple equation. (Percot *et al.*, 2003)



As the result has shown that from 100 % of dry weight of shrimp shells, after decalcified and demineralized, the yields of chitin is 22.87% as reported in Table 6.1.

Table 4.1 Percent yield of chitin

Samples	Dry weight (%)
Dry shrimp shell	100.00
Decalcification product	30.48
Deproteinization product	22.87
Chitin	22.87

In table 4.2 demonstrated the percent yield of chitin hydrogel which was obtained from chitin solution by adding chitin solution in the excess amount of water.

Table 4.2 Percent yield of chitin hydrogel

Samples	Weight (g)
Chitin Solution	100.00
Chitin Hydrogel	52.53*

* Average weight of 3-experiments with SD = 1.907258

Chitin was dissolved in calcium chloride dehydrate saturated methanol and the large excess of water was added to form chitin hydrogel. The water and chitin content of chitin hydrogel were 96.73% and 3.27 %, respectively. The water content of the chitin hydrogel could be controlled by centrifugation.(Tamura *et al.*,2006) The content of chitin and water in chitin hydrogel was represented in Table 6.2

Table 4.3 Percent of chitin and water contents in chitin hydrogel

Samples	Amount (%)
Chitin hydrogel	100.00
Water	96.73**
Chitin	3.27***

** Average weight of 3 experiments with SD = 0.30848

*** Average weight of 3 experiments with SD =0.30848

4.4.1.2 Chitin Hydrogel Formation

The dissolving chitin in calcium chloride-saturated methanol and precipitation The chitin-calcium ion complex was performed during dissolving chitin in calcium chloride methanol process. The calcium ion complex disrupted the hydrogen bonds between the polymer chain and physical interact with the functional group of chitin chains and the formation of chitin calcium ion complex was occurred.(Tamura *et al.*, 2006)

4.4.1.3 Characterization of Shrimp Shells, Chitin Powder and Chitin Hydrogel

Figure 4.1 showed the FTIR-spectra of shrimp shell powder and chitin powder . The FTIR-spectra of shrimp shell powder was contained of impurity from proteins, minerals, lipids and pigment. They all have to be quantitatively removed to achieve the high purity necessary for biological applications. (Percot *et al.*,2003) After decalcification in acid solution and deproteinization in strong alkaline solution, the chitin was extracted . Figure 4.1 showed the FT-IR spectra of chitin powder. The characteristic peaks of chitin were at wavenumber 3450, 2878, 1655 and 1550 cm^{-1} which refer to hydroxyl group, C-H band ,amide I and amide II band. The purity level of chitin was followed by the evaluation of the calcium and protein contents as a function of the reaction time.

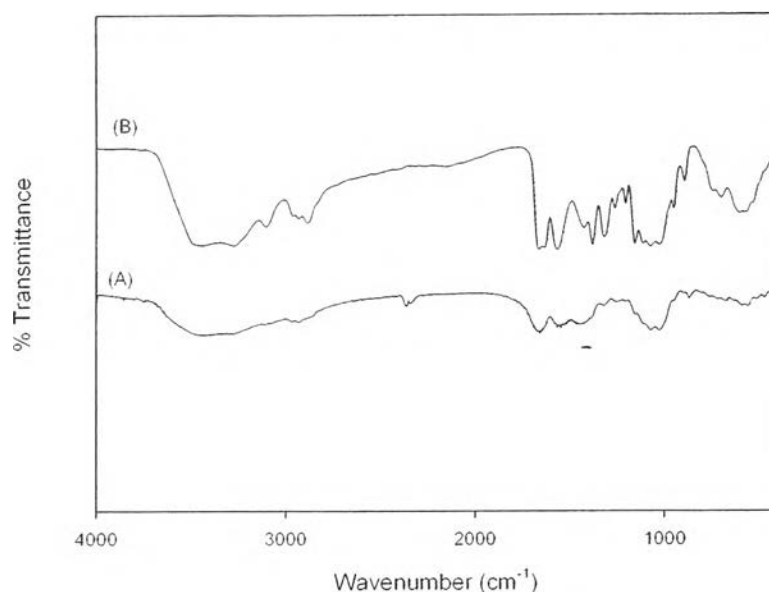


Figure 4.1 FT-IR spectra of shrimp shells powder (a) chitin powder (b)

4.4.1.4 Characterization of Chitin Powder and Chitin Hydrogel

The FT-IR spectra of chitin powder and chitin hydrogel were shown in figure 4.2, demonstrated the characteristic peaks of chitin at 3450, 2878, 1650, 1550 and 1152 cm^{-1} , which corresponded to the hydroxyl group, C-H band, amide I, amide II. The degree of deacetylation (%DD) was determined following Sannan *et al.* (1977). The %DD of chitin powder and chitin hydrogel were 35.39% and 37.36%, respectively.

Sannan method

$$\%DD = 101 - \left[35.71 \cdot \frac{(A_{1550})}{(A_{2878})} \right] \quad (1)$$

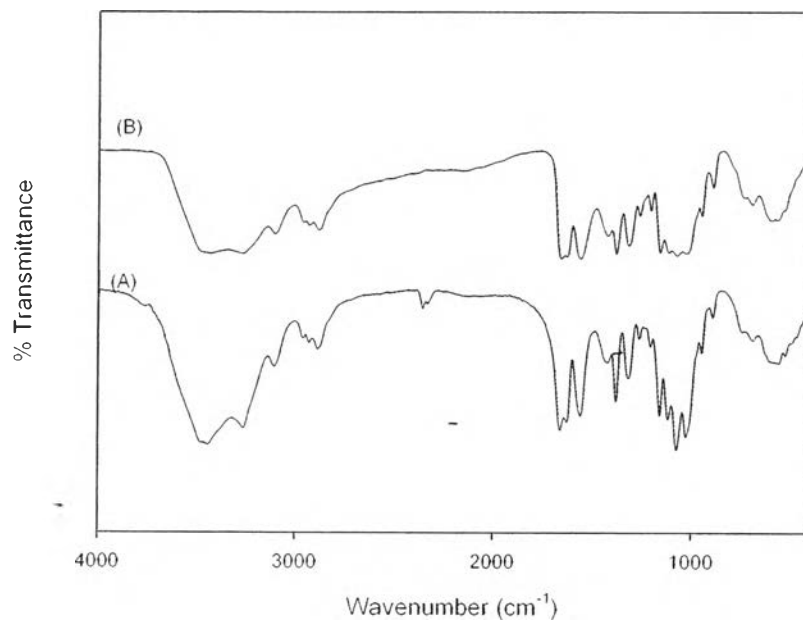


Figure 4.2 FT-IR spectra of chitin powder (a) chitin hydrogel (b)

4.4.3.5 X-ray Diffraction (XRD)

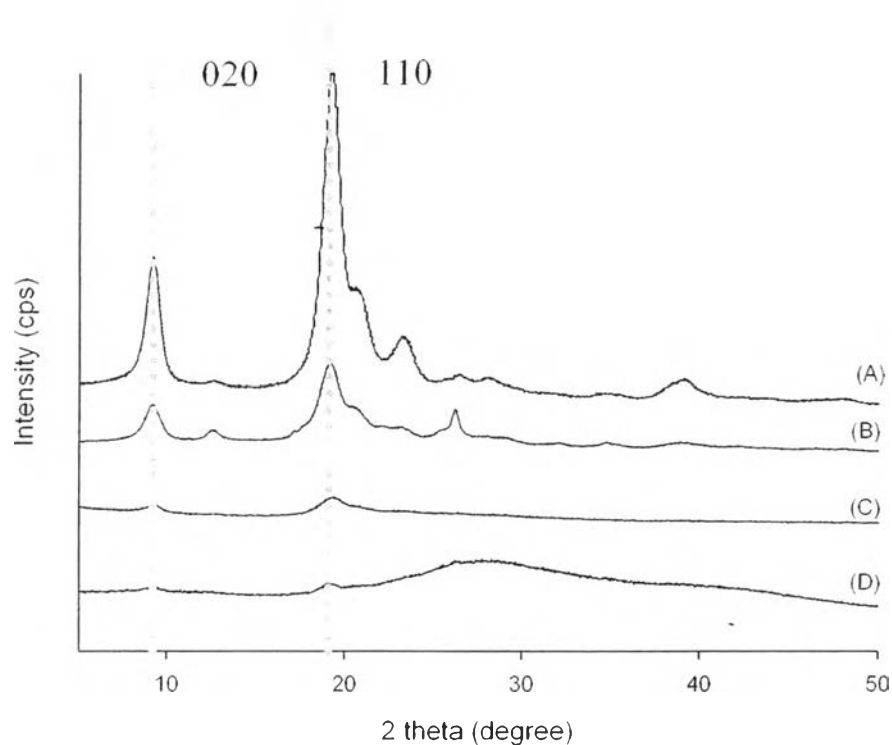
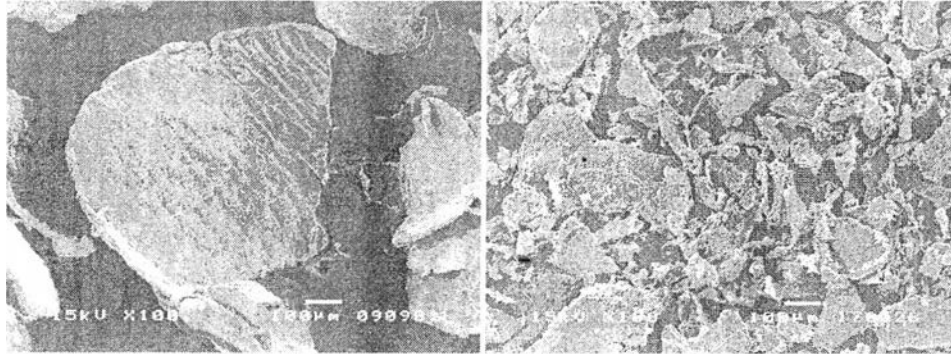


Figure 4.3 XRD spectra of (a) chitin flake (b) chitin powder (c) dried chitin hydrogel, and (d) wet chitin hydrogel.

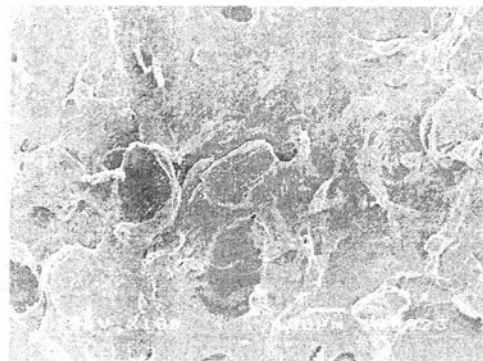
The XRD spectra of α -chitin flake, powder, dried chitin hydrogel and wet chitin hydrogel are shown in figure 4.3. The XRD result shows the characteristic peaks of α -chitin corresponding to the 020 and 110 plane (Tamura *et al.*, 2006). The XRD spectrum of chitin flake showed the sharpest and narrowest peaks indicating the crystalline structure of chitin whereas the wet chitin hydrogel had a broad peak revealing its amorphous structure. After drying of chitin hydrogel, the reformation of crystalline structure was observed in the XRD spectrum of the dried chitin hydrogel.

4.4.3.5 Morphology of chitin



(a) Chitin flake

(b) Chitin powder



(c) Air dried chitin hydrogel

Figure 4.4 SEM images of morphology of differences type of chitin (a) chitin flake (b)chitin powder (c) Air dried chitin hydrogel.

SEM images in figure 4.4 showed the morphology of difference type of chitin, chitin flake ,chitin powder and chitin hydrogel. The results showed that the structure of chitin have changed due to the physical, chemical modification and crystallinity.

4.5 Deacetylation of Chitin Hydrogel by Solution Plasma

4.5.1 The Effect of KOH Concentration on Degree of Deacetylation

Chitin hydrogel was performed by suspending chitin hydrogel in KOH/MeOH solutions containing NaBH₄ 0.1g/100 mL before being subjected to Solution Plasma (SPP). The reaction time for each cycle was 1 hour and the KOH/MeOH solution was changed every cycle in order to achieve high value of %DD. From the initial degree of deacetylation (%DD) of chitin hydrogel is 37.36% and deacetylation reaction from 1 to 5 cycle of treatment times in different concentration of KOH 0%, 7% and 10%. The operating condition as follows: the frequency of 15.0 Hz, primary voltage of 2.24 kV, pulse width of 2 μs, and electrode gap of 1 mm.

As the result from figure 4.5 indicated that the degree of deacetylation has been increased as the time, temperature and concentration increased. The %DD of deacetylated chitin hydrogel using SPP of 0%, 7% ,10% was 55.31 ,68.83 and 74.44 .respectively in 5 cycle of treatment times because of the reactive species generated in the system more when increase the concentration. The degree of deacetylation (%DD) was determined by FTIR following the equation of Baxter *et al.* (1991) . The equations are used depend on the properties of the product.

Baxter method

$$\%DD = 100 - \left[115 \cdot \frac{(A_{1655})}{(A_{3450})} \right] \quad (2)$$

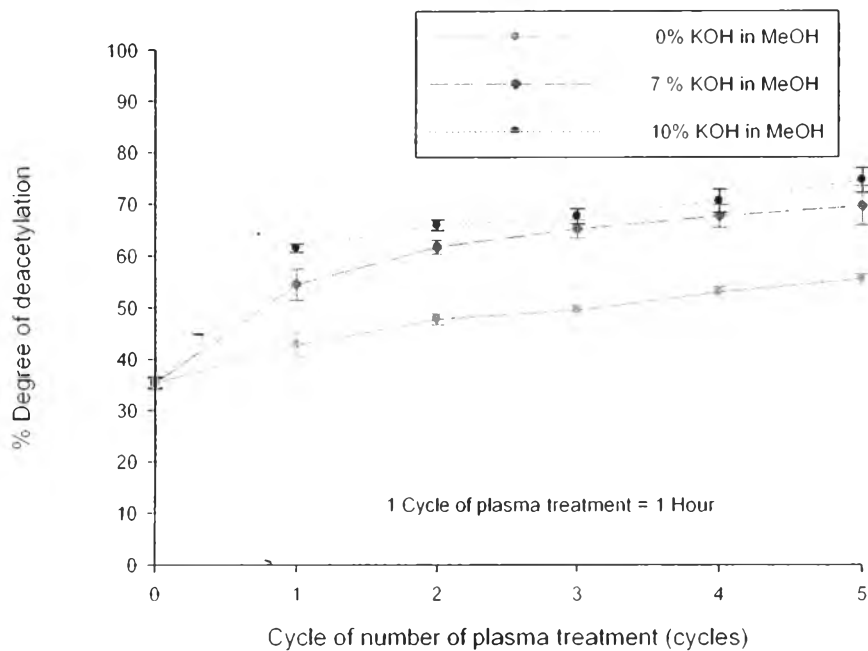


Figure 4.5 The effect of KOH on the degree of deacetylation.

4.5.2 The Effect of Solubility of Chitin Hydrogel on the Degree of Deacetylation for Deacetylation Reaction

In figure 4.6 showed the solubility of plasma-treated chitin hydrogel in 1% v/v acetic acid solution in (A) 7% KOH (B) 10% KOH concentration. The results indicated the percent solubility of deacetylated chitin hydrogel increased with the increasing of number of cycle of deacetylation and the concentration of alkaline solution. The deacetylated chitin hydrogel with plasma treatment can convert to chitosan due to the solubility in acetic solution.

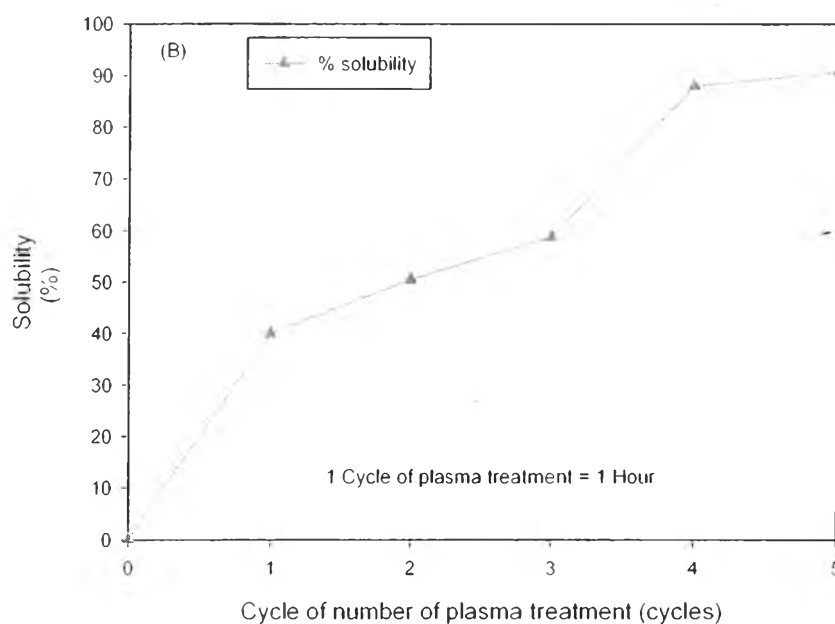
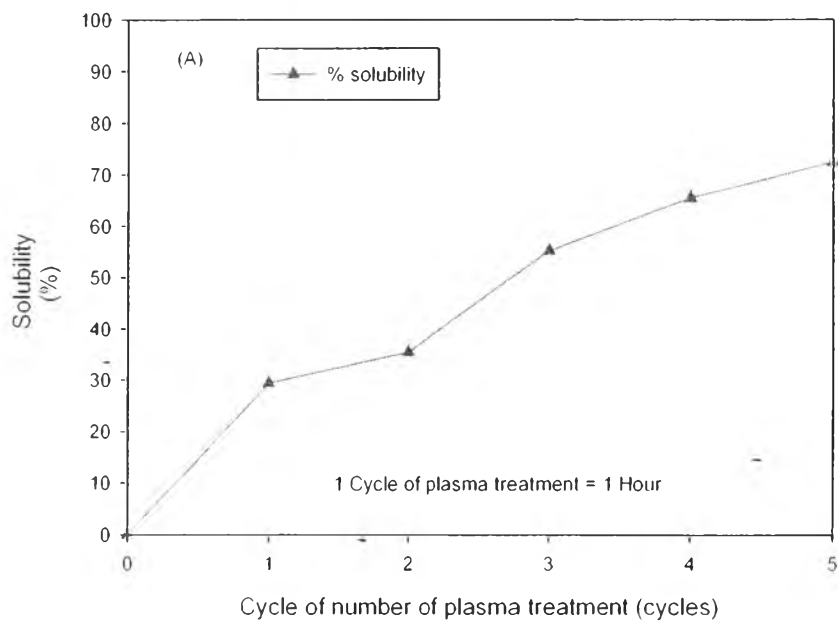


Figure 4.6 Solubility of plasma-treated chitin hydrogel in 1% acetic solution with (a) 7% KOH (b) 10% KOH concentration

4.5.3 Fourier transformed infrared spectroscopy (FTIR)

As shown in figure 4.7, the FTIR spectra of chitin hydrogel showed the characteristics peak at 3450 cm^{-1} which referred to the hydroxyl groups of chitin, 1650 cm^{-1} which referred to carbonyl of acetamide groups. 1573 cm^{-1} which referred to amine group. After deacetylation, the success of the conversion of chitin structure to chitosan structure was confirmed by the characteristics peak at 1655 cm^{-1} reduced compared with 3450 cm^{-1} .

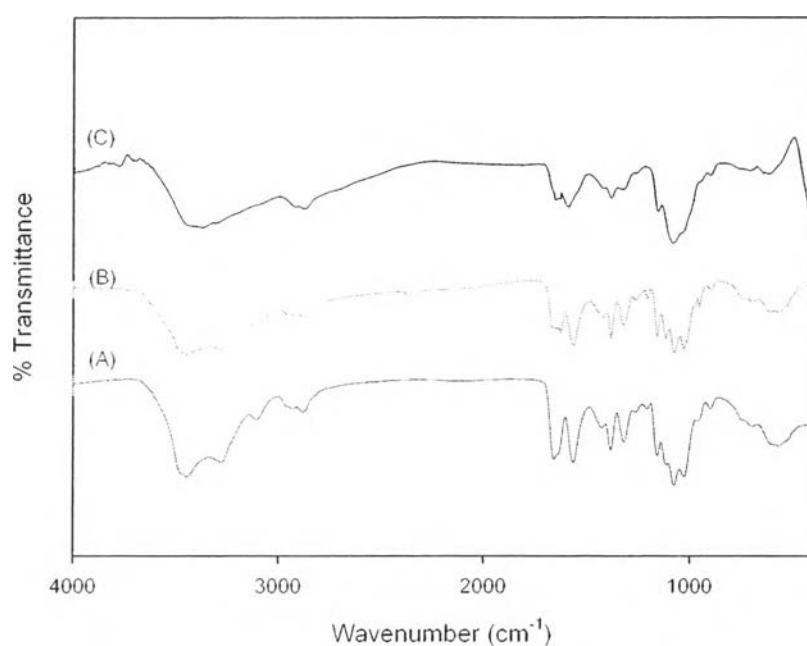


Figure 4.7 The effect of KOH concentration on degree of deacetylation (a) 0% KOH (b) 7% KOH (c) 10% KOH concentration.

4.5.4 The Effect of Amount of Chitin Hydrogel in Alkaline Solution for Deacetylation Reaction

In this study, the effect of amount of chitin hydrogel in alkaline solution for deacetylation reaction using solution plasma were studied. In figure 4.8, demonstrated the three difference amount of chitin hydrogel in alkaline solution, 10 g, 20g and 30g, the number of cycle for deacetylation was 1, and the temperature was 64 C°. The results showed that in the %DD and % solubility were increased respectively as the amount of chitin hydrogel decrease because when decrease the amount of chitin hydrogel, the ratio of reaction of hydroxide anion which came from KOH solution and many reactive species, OH•, H• and H₂O₂ (Prasertsung *et al.*, 2012) generated by solution plasma, increased. The amount of chitin hydrogel of 10 g in 100 mL KOH solution was gave the highest degree of deacetylation in 1 cycle of plasma treatment, due to, the highest the interaction ratio between hydroxide anion and chitin hydrogel solution.

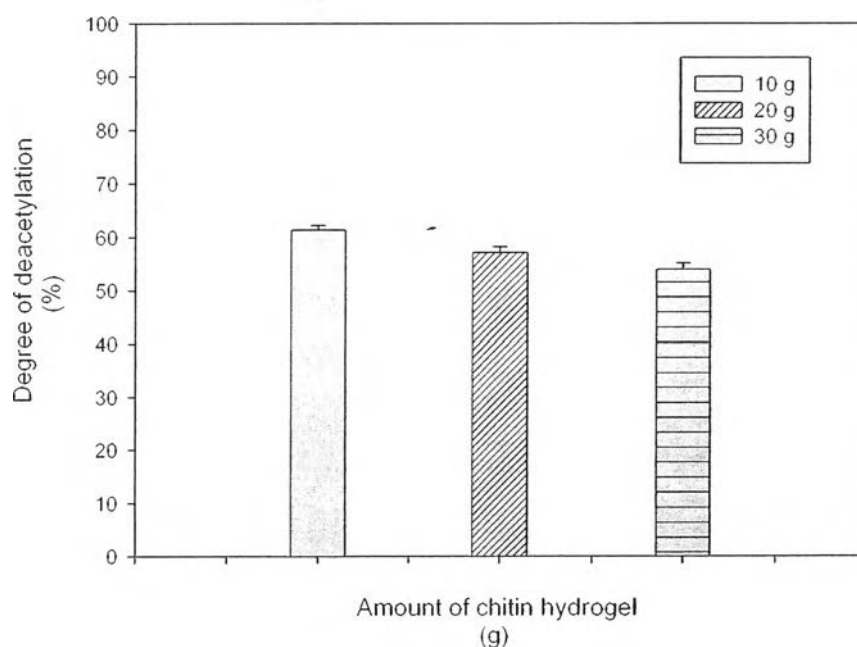


Figure 4.8 The effect of amount of chitin hydrogel in KOH solution on the percent of degree of deacetylation.

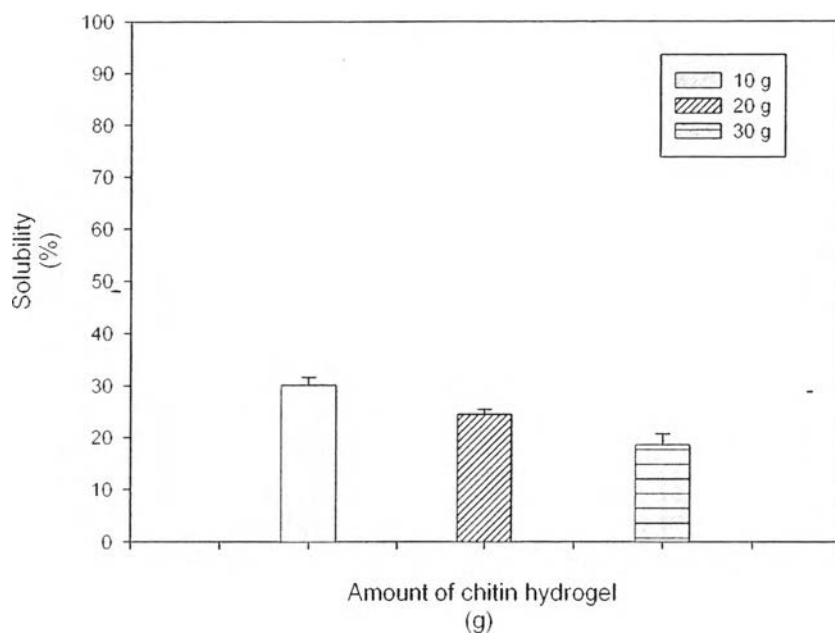


Figure 4.9 The effect of amount of chitin hydrogel in KOH solution on the percent of solubility.

4.5.5 The Comparison of Degree of Deacetylation between Conventional Heat Treatment and Solution Plasma Technique

The % DD calculated from FTIR was shown in figure 4.10. The results showed that the deacetylation of chitin hydrogel increased with an increase of alkali concentration. The % DD obtained from solution plasma treatment was higher than that of the conventional heat treatment. During SPP, many reactive species such as $\text{OH}\cdot$, $\text{H}\cdot$ and H_2O_2 was generated from the association, dissociation and recombination processes of the solution (Prasertsung *et al.*, 2012) containing KOH, MeOH and H_2O which might help in deacetylation reaction while conventional heat treatment only produced OH radicals obtained from the alkali solution. Thus, the deacetylation via solution plasma technique is more effective than the conventional heat treatment method.

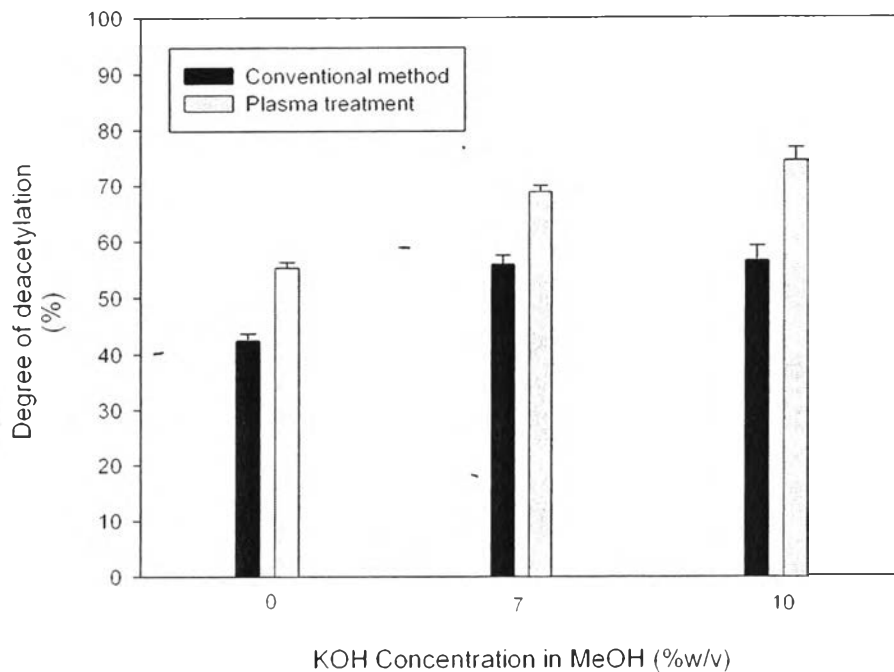


Figure 4.10. Comparison the % DD between solution plasma treatment and conventional heat treatment at 0%,7% and 10% KOH/MeOH.

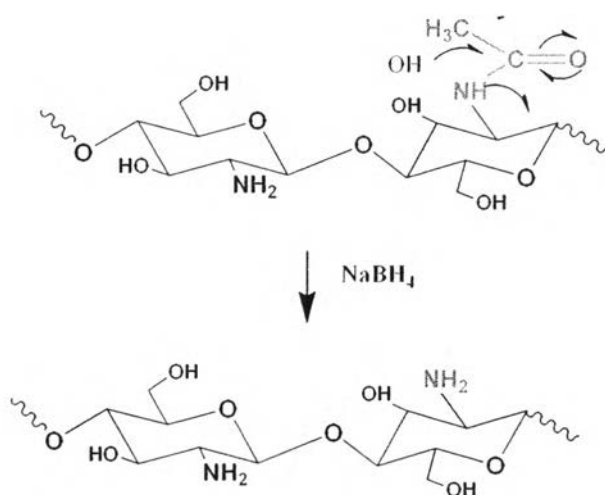


Figure 4.11. Mechanism of deacetylation reaction of chitin hydrogel by using solution plasma treatment in KOH concentration.

Table 4.4 Comparison the % DD between solution plasma treatment and conventional heat treatment at 0%,7% and 10% KOH/MeOH

Conc. KOH in MeOH	% DD		Number of cycles of alkaline treatment	Temperture (C ⁰)	% increment of DD
	Plasma treatment	Conventional heat treatment			
0 %	55.310±0.9914	42.4933±1.2513	5	64	12.82
7%	68.83.50±0.84	55.56±1.6749	5	64	13.64
10%	74.44 ±2.4015	56.53 ±2.6687	5	64	17.91

The difference % increasing of DD between conventional heat treatment and solution plasma technique were increased respectively as the increase of concentration of alkaline treatment because the amount of reactive species were more generate in higher concentration of alkaline and the more chemical interaction were occurred in the reaction.

4.5.6 Comparison between the Yield of Acid Soluble Fraction Yield of Obtained Product between Conventional Method and Solution Plasma Technique

The yields of acid soluble fraction plasma-treated chitin hydrogel has higher than the conventional method after the deacetylation reaction because in plasma system, generated both heat and reactive species (Prasertsung *et al.*, 2012) which degraded the polymer chain of chitin hydrogel. However, the deacetylation by conventional method, provided only heat so it gave a higher yield of obtained product and when increasing the treatment time, the yields of acid soluble fractions of both method has increased .due to, the temperature and the amount of reactive species increased as shown in figure 4.12.

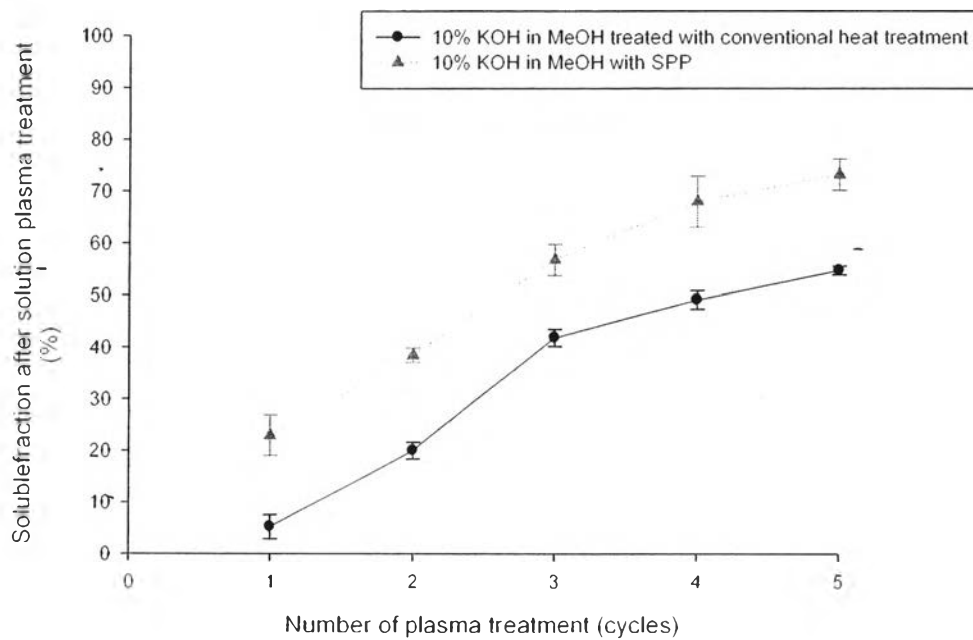


Figure 4.12 comparison between the acetic soluble fraction after plasma treated chitin hydrogel and conventional heat treatment with 10% KOH/MeOH

The yield of plasma-treated chitin hydrogel after deacetylated of chitin hydrogel in 10% KOH/MeOH from 1 to 5 cycles of plasma treatment were decrease with the increasing of number of cycle of deacetylation reaction which was presented in figure 4.13.

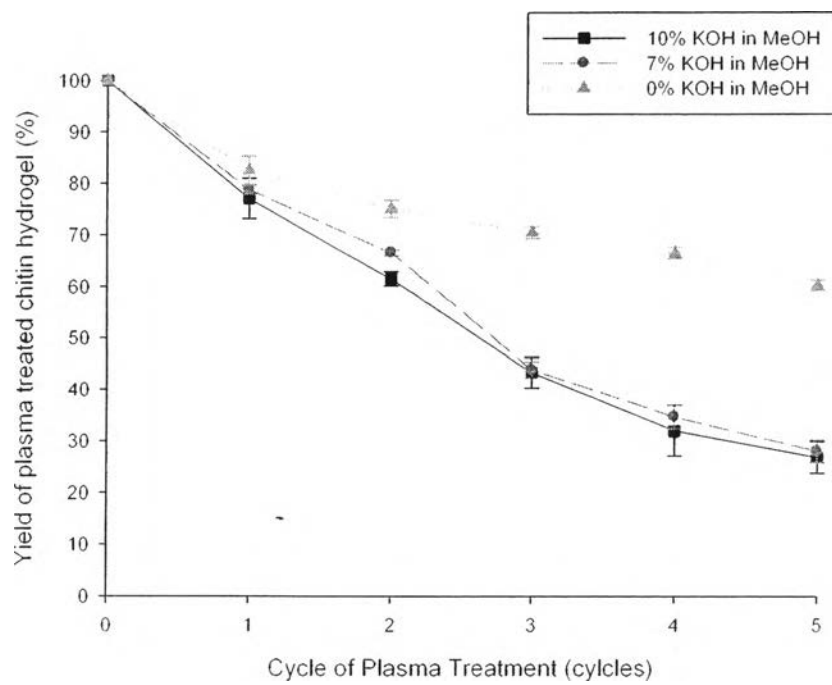


Figure 4.13 the effect of % yield of plasma treated chitin hydrogel with 0% .7% , 10% KOH/MeOH.

4.5 Depolymerization of Chitin Hydrogel by Solution Plasma

Chitin hydrogel was depolymerized by SPP in 1% acetic acid solution for 0h, 3h and 5h in order to reduce its molecular weight before subjected to deacetylation reaction to Solution Plasma (SPP) to form chitosan. The operating condition as follows: the frequency of 15.0 Hz, primary voltage of 1.92 kV, pulse width of 2 μ s, and electrode gap of 1 mm and the reaction temperature was 69-72 C^o.

4.5.1 Morphology of Depolymerized Chitin Hydrogel via SPP

In figure 4.14 showed the morphology of 5 h depolymerized chitin hydrogel via SPP. After depolymerization of chitin hydrogel in 1 % v/v acetic solution for 5 h, the morphology of depolymerized chitin hydrogel was in fibrous form.

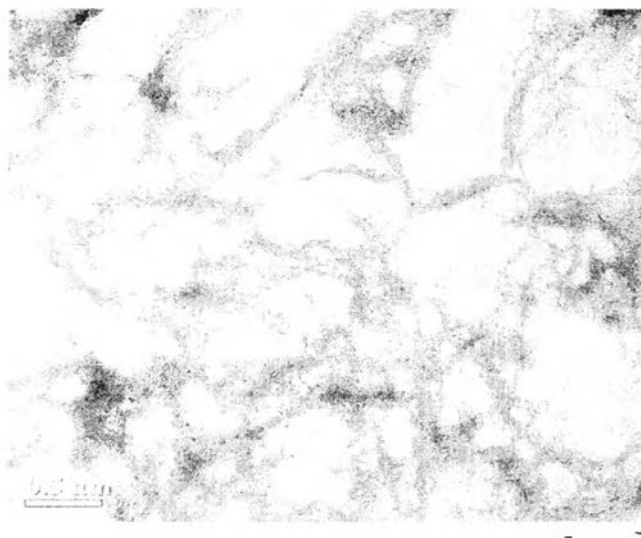


Figure 4.14 TEM images of morphology of 5 h depolymerized chitin hydrogel via SPP.

4.5.2 The Effect of Time of Depolymerization on the Degree of Deacetylation

Figure 4.15 showed the effect of time on the degree of depolymerization of chitin hydrogel in 1 % acetic acid solution from 0, 3 and 5 hours using solution plasma technique. From the results indicated that the depolymerization reaction effect on the degree of deacetylation of initial chitin hydrogel because of the increasing of time, the increasing of degree of deacetylation. In the depolymerization reaction using solution plasma generated active species such as the hydroxyl radical, hydroxide anion and hydrogen radicals which came from the solution and the active species reacted on the functional groups on the polymer chain of chitin hydrogel in the plasma reactor.

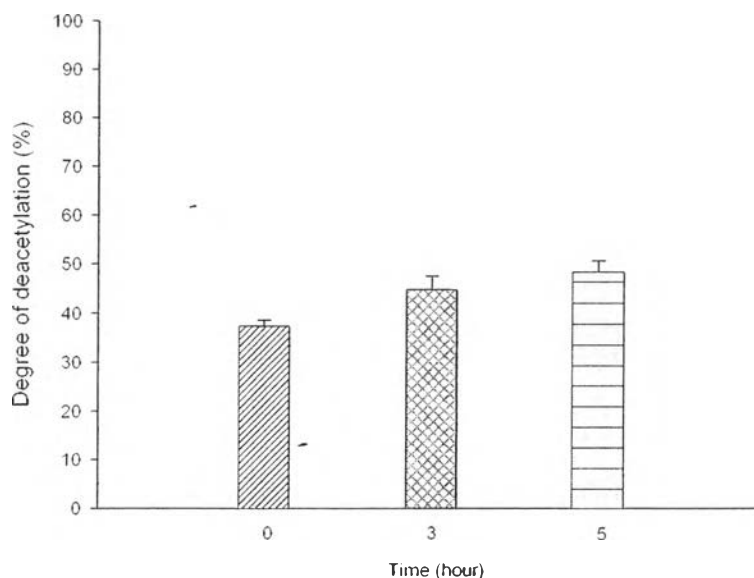


Figure 4.15 the effect of time of depolymerization of chitin hydrogel using solution plasma on the degree of deacetylation.

4.6.2 The Effect of Type of Chitin on the Degree of Deacetylation

Chitin powder, chitin hydrogel, depolymerized chitin hydrogel for 3 h and 5 h were deacetylated in 10 % KOH /MeOH solution in order to compare the effect of crystalline structure on the degree of deacetylation without deacetylation reaction. The results from figure 4.16 demonstrated that the degree of deacetylation of chitin powder, depolymerized chitin hydrogel with 1% v/v acetic acid solution in 0h, 3h and 5h, were increase as the decreasing of crystalline structure. The rigid crystalline structure effected the deacetylation because the surface area of interaction increase as the crystalline increase.

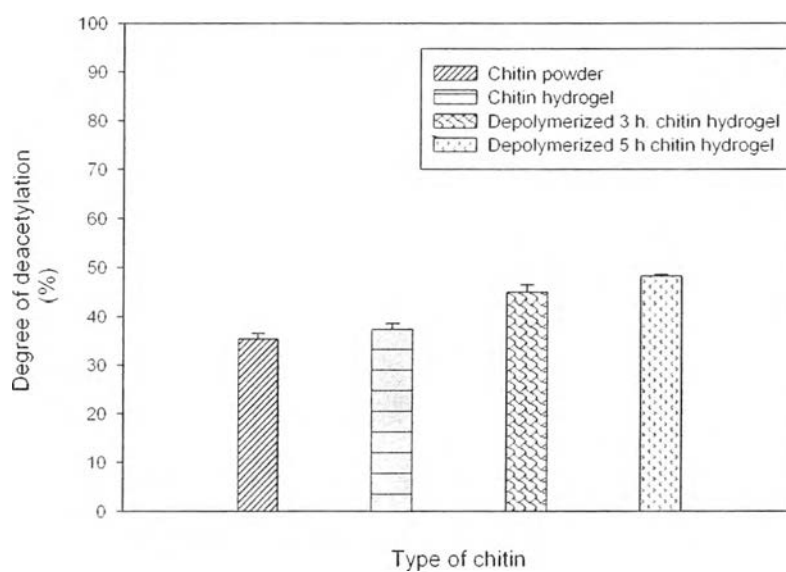


Figure 4.16 the effect of type of chitin hydrogel using solution plasma on the degree of deacetylation.

4.6.3 The Effect of Type of Chitin on the Degree of Deacetylation

In figure 4.17 showed the effect of type of chitin on the degree of deacetylation, the chitin powder, chitin hydrogel and depolymerized chitin hydrogel in 1% acetic acid solution for 3 and 5 hours using solution plasma were deacetylated in 10 % KOH/MeOH from 1 to 5 cycles and study the degree of deacetylation. As the results demonstrated that the 5 hours of depolymerized chitin hydrogel after deacetylated for 5 cycles gave the highest of degree of deacetylation and the chitin powder after deacetylated for 5 cycles gave the lowest of degree of deacetylation because of the crystallinity structure and the molecular weight of chitin effect the %DD.

Table 4.7 showed the type of samples

	Type of samples
A	Chitin powder
B	Chitin hydrogel
C	3 hours depolymerized chitin hydrogel
D	5 hours depolymerized chitin hydrogel

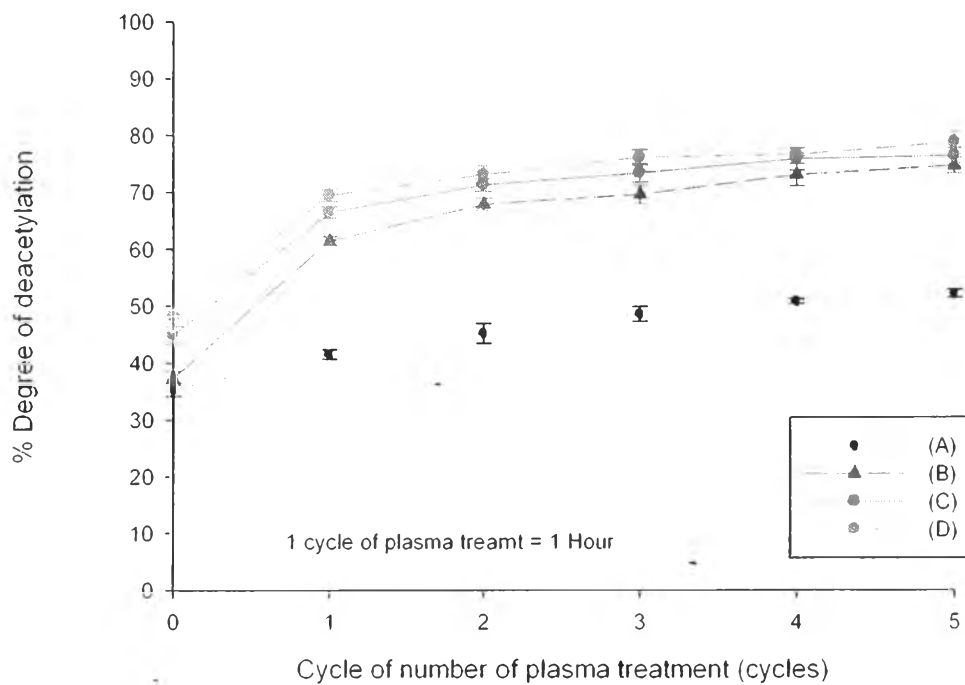


Figure 4.17 the effect type of chitin on the degree of deacetylation.

4.6.5 Fourier transformed infrared spectroscopy (FTIR)

From the FTIR-spectra in figure 4.18, showed the chemical structure of depolymerized and deacetylated of chitin hydrogel in difference conditions, the results demonstrated that the peak at 3450 cm^{-1} which is refer to OH stretching, became broader and move to a lower frequency with increasing %DD, indicating an increase in the disordered structure. (Zhang *et al.*, 2005) and the transmittance at wavenumber of 1655 cm^{-1} which refer of carbonyl in acetyl group, decreased with the increasing of time of depolymerization.

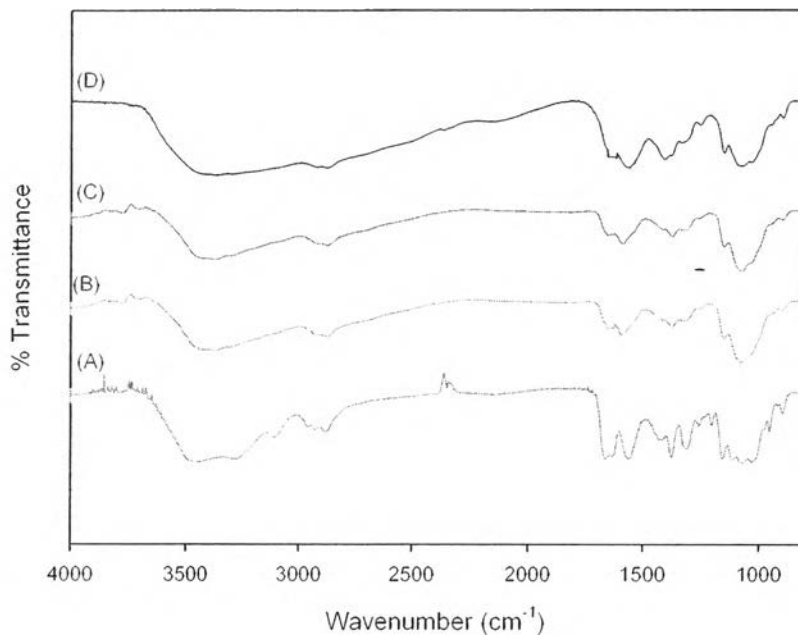


Figure 4.18 FTIR spectra the effect type of deacetylated chitin in 5 cycles of plasma treatment on the degree of deacetylation (a) chitin powder (b) chitin hydrogel (c) 3h depolymerized chitin hydrogel (d) 5 h depolymerized chitin hydrogel.

4.6.6 X-ray Diffraction (XRD)

XRD spectra of different types of chitin: chitin powder, chitin hydrogel, depolymerized chitin hydrogel in 3h and 5 h, using solution plasma of were shown in figure 4.19. The results demonstrated that the sharpness of plasma treated chitin powder was the highest but for the chitin hydrogel and depolymerized chitin hydrogel gave the less sharpness peak because the maximum peak of intensity at 020 reflection decreased with the increase of DD, and moved to a higher angle, and the second maximum peak of intensity at 110 reflection also decreased with the increase of DD (Zhang *et al.*, 2005)

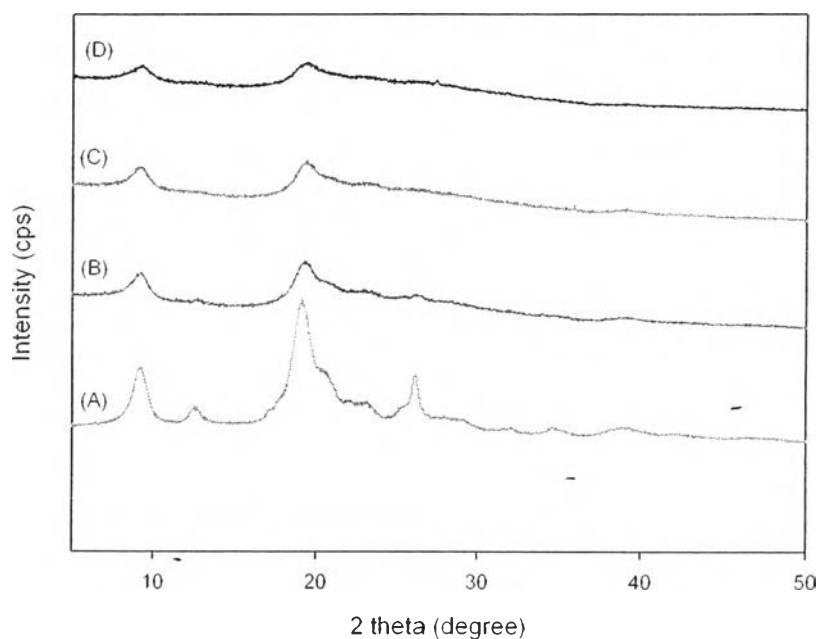


Figure 4.19 XRD spectra showed the effect type of deacetylated chitin in 5 cycles of plasma treatment on the degree of deacetylation (a) chitin powder (b) chitin hydrogel (c) 3h depolymerized chitin hydrogel (d) 5 h depolymerized chitin hydrogel

4.6.7 The Effect of Type of Chitins on the Solubility

Figure 4.20 showed the solubility of plasma-treated chitin in different conditions: chitin hydrogel, 3h depolymerized chitin hydrogel, and 5 h depolymerized chitin hydrogel. The results indicated that the depolymerized chitin hydrogel before being subjected to deacetylation showed higher solubility than the deacetylated chitin hydrogel because the initial molecular weight of the chitin hydrogel was reduced.

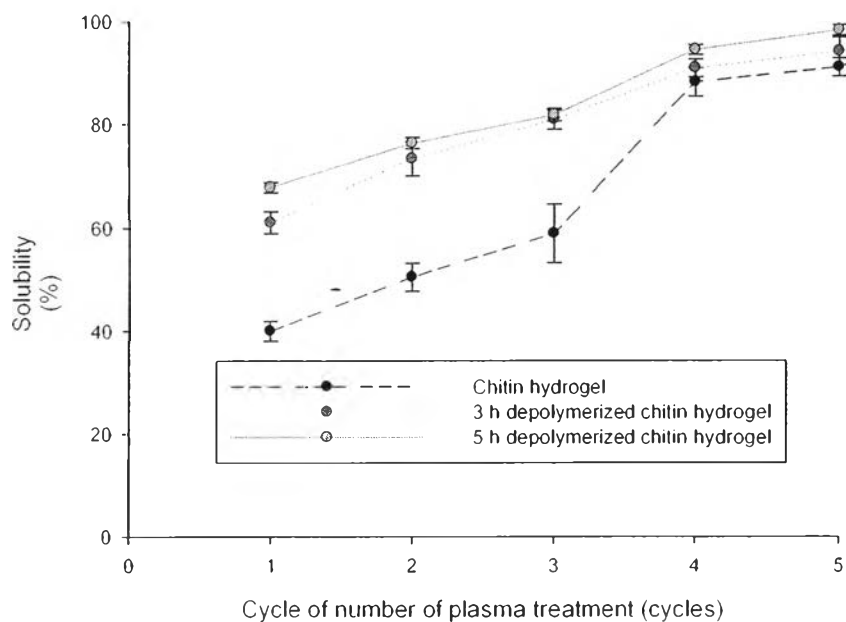


Figure 4.20 the effect type of chitin on the degree of deacetylation on solubility.

4.6.8 Comparison between Chitosan Production Obtained from Solution Plasma, from Literature Reviews and Conventional Method on the Degree of Deacetylation

Table 4.6 showed the %DD which came from difference conditions. The results from literatures reviews demonstrated that the high degree of deacetylation could be achieved by high alkaline concentration and high temperature which became environmental problem in the process of neutralization by acid.

To decrease of using alkaline concentration, solution plasma was introduced to deacetylate and depolymerized. From table 4.6 indicated that these technique could increase the %DD with a low temperarue and alkaline concentration and the depolymerization process before subjected to deacetylation could improve the %DD due to the reducing of initial molecular weight.

Table 4.6 Comparison between chitosan production obtained from solution plasma, from literature reviews and conventional method on the degree of deacetylation

Alkaline solution	Temperature (C⁰)	Degree of deacetylation (%DD)	Reference
50% KOH in ethanol and monoethyleneglycol	120	90-96	Tolaimate <i>et al.</i> , 2003
40% KOH in ethanol and monoethylene glycol	80	75-83	Broussignac <i>et al.</i> , 1968
10% KOH in methanol with chitin hydrogel (conventional method)	64	55.31	-
10% KOH in methanol with chitin hydrogel (Solution plasma treated)	64	74.44	-
10% KOH in methanol with depolymerization of chitin hydrogel (Solution plasma treated)	64	78.51	-

4.7 Gel Permeation Chromatography (GPC)

4.7.1 Comparison the Compared the Molecular Weight between Deacetylation with and without Reducing Initial Molecular Weight of Chitin Hydrogel via SPP

Figure 4.21 and table 4.7 compared the molecular weight between deacetylation with and without reducing initial molecular weight of chitin hydrogel via SPP. As the results showed that the reducing initial molecular weight of chitin hydrogel by using solution plasma in 1% acetic acid solution for 5 h and then deacetylated in 10% w/v KOH/MeOH solution via SPP for 5 cycles gave the lower molecular weight of obtained products than process of deacetylation of chitin hydrogel in 10% w/v in KOH/MeOH for 5 cycles because the degradation of polymer chains of obtained products in 1% v/v acetic acid solution were performed. In addition to deacetylation reaction; the main chains of polymer were scissored at glycosidics bond due to the OH^- and OH^\cdot from the alkaline solution as showed in figure 4.22

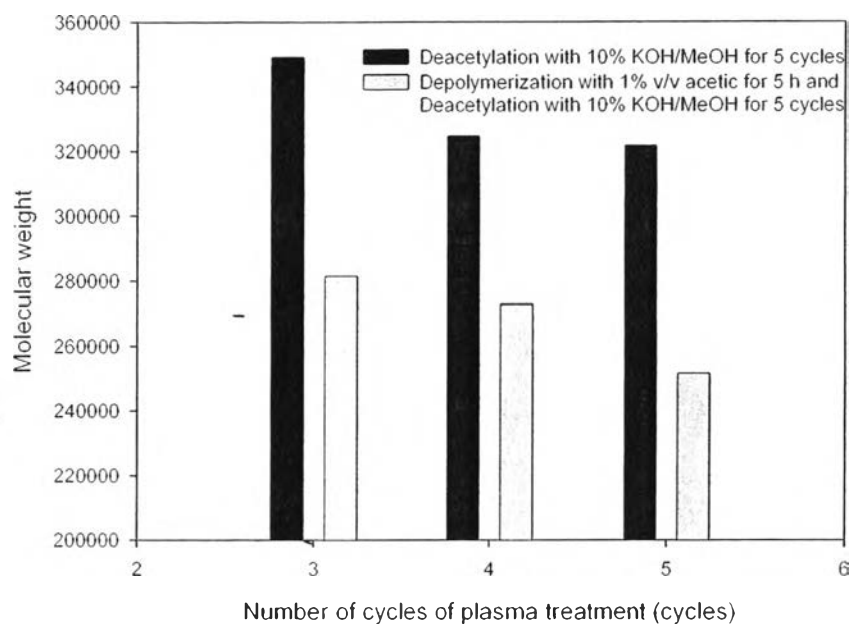


Figure 4.21 Comparison the compared the molecular weight of obtained products between process of deacetylation with 10% KOH/MeOH for 5 cycles and process of dpolymerization with 1 %v/v acetic acid solution for 5 h and deacetylation with 10 % w/v KOH/MeOH chitin hydrogel via SPP.

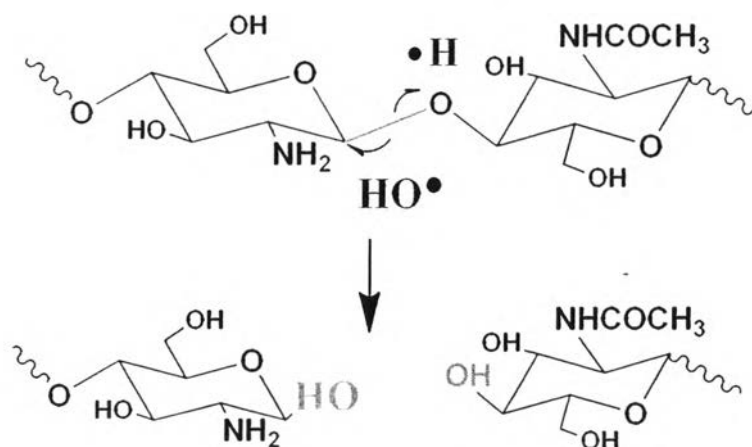


Figure 4.22 Degradation of chitin hydrogel via SPP of alkaline depolymerization.

Table 4.7 represented the molecular weight of plasma-treated chitin hydrogel with deacetylation in 10% w/v KOH/MeOH solution at 3,4 and 5 cycles. As the results demonstrated that the molecular weight of obtained products were decreased from 340,124, 340,124 and 321.624, respectively.

Table 4.7 The molecular weight of deacetylation chitin hydrogel in 10% w/v KOH/MeOH with different times by using solution plasma

Conditions	Treated chitin hydrogel 3 hours	Treated chitin hydrogel 4 hours	Treated chitin hydrogel 5 hours
M_w	340,124	324,709	321.624
M_n	116.703	124.551	128.701
PDI	2.91	2.61	2.50

Table 4.8 represented the molecular weight of plasma-treated chitin hydrogel with depolymerization in 1 % v/v acetic solution and then deacetylated in 10% w/v KOH/MeOH solution at 3,4 and 5 cycles. As the results demonstrated that the molecular weight of obtained products were decreased from 281.454, 272.856 and 251.567, respectively.

Table 4.8 The molecular weight of depolymerization chitin hydrogel in 1%v/v acetic solution and deacetylation in 10% w/v KOH/MeOH with different times by using solution plasma

Conditions	Treated chitin hydrogel 3 hours	Treated chitin hydrogel 4 hours	Treated chitin hydrogel 5 hours
M_w	281.454	272.856	251.567
M_n	117.292	128.705	123.631
PDI	2.40	2.12	2.03

4.7.2 Effect of The Initial Weight on the Degree of Deacetylation

Table 4.10 and 4.11 showed the effect of the initial weight of chitin hydrogel on the degree of deacetylation. As the results indicated that the decreasing of the initial molecular weight of chitin hydrogel, the degree of deacetylation were decreased from 3,4 and 5 cycles. The reducing initial molecular weight of chitin hydrogel before deacetylation process gave a higher %DD than the process of deacetylation of chitin hydrogel. The reduction of the initial molecular weight of chitin hydrogel by depolymerization with SPP under 1% acidic solution for 5 h. before being subjected to the deacetylation with SPP could improve the % DD of the obtained chitosan products.

Table 4.10 The effect of molecular weight of deacetylation chitin hydrogel on the degree of deacetylation without reducing the initial molecular weight with different times by using solution plasma

Number of cycles of deacetylation reaction	Molecular weight	Degree of deacetylation (%DD)
3	340.124	73.28
4	324.709	75.64
5	321.624	76.16

Table 4.11 The effect of molecular weight of deacetylation chitin hydrogel on the degree of deacetylation without reducing the initial molecular weight with different times by using solution plasma

Number of cycles of deacetylation reaction	Molecular weight	Degree of deacetylation (%DD)
3	281,454	75.98
4	272,856	76.42
5	251,567	78.51

4.8 Conclusion

Solution plasma process (SPP) was applied in deacetylation and depolymerization reactions of chitin hydrogel to obtain chitosan with high values of % DD. Compared with the conventional heat treatment, SPP treatment was an effective process to increase the % DD by using the low alkali concentrations. Using SPP, the % DD increased with the increasing of KOH concentrations in MeOH as well as the increasing of the cycles of plasma treatment time. The reduction of the initial molecular weight of chitin hydrogel by depolymerization with SPP under mild acidic condition before being subjected to the deacetylation with SPP could improve the % DD of the obtained chitosan products.

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