การดัดแปรไคโตแซนเพื่อใช้กำจัดสีย้อมแอนไอออนิกจากน้ำเสีย

นายต่อศักดิ์ กิตติกรณ์

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MODIFICATION OF CHITOSAN FOR ANIONIC DYES REMOVAL FROM WASTE WATER

Mr. Thorsak Kittikorn

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Applied Polymer Science and Textile Technology

Department of Material Science

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นายต่อศักดิ์ กิตติกรณ์: การดัดแปรไคโตแซนเพื่อใช้กำจัดสีย้อมแอนไอออนิกจากน้ำเสีย. (MODIFICATION OF CHITOSAN FOR ANIONIC DYES REMOVAL FROM WASTE WATER) อ. ที่ปรึกษา : ผศ.ดร. กาวี ศรีกูลกิจ, อ.ที่ปรึกษาร่วม : ดร. อศิรา เฟื่องฟูชาติ, 98 หน้า. ISBN 974-13-0796-9.

ได้นำไคโตแซนมาดัดแปรด้วยเทคนิคการกราฟท์โคพอลิเมอร์ไรเซชันด้วยอะคริลาไมด์มอนอเมอร์ หลังจากนั้นได้ทำการดัดแปรต่อด้วยเทคนิคฮอฟแมนเดกเกรเดชัน(Hofmann degradation) เพื่อเปลี่ยนหมู่อะคริลาไมด์ให้เป็นหมู่อะมิโนอิสระและหมู่คาร์บอกซิลิก ซึ่งสามารถพิสูจน์โครงสร้างเคมีด้วยเทคนิคอินฟาเรดสเปคโตสโคปี และโปรตอนเอ็นเอ็มอาร์ โดยผลิตภัณฑ์สุดท้ายที่ได้คือ ไวนิลเอมีนโคอะคริลิคเอซิดโคไคโตแซน

จากเทคนิคการไตเตรชันพบว่าไคโตแซนที่ผ่านการดัดแปรพบว่ามีจำนวนหมู่อะมิโนอิสระเพิ่มขึ้นซึ่งคาดว่าจะช่วยทำให้ไคโตแซนมีประสิทธิภาพในการดูดจับสีย้อมและธาตุโลหะหนักได้ดีขึ้น หลังจากนั้นจึงได้นำไคโตแซนดัดแปรมาทดสอบการจับสีแอนไอออนิก พบว่าประสิทธิภาพการจับสีแอนไอออนิกด้วยไคโตแซนดัดแปรสามารถจับสีย้อมได้เพิ่มสูงขึ้นตามปริมาณจำนวนหมู่อะมิโนเพิ่มขึ้น แต่อย่างไรก็ตามประสิทธิภาพการจับสีย้อมของไคโตแซนดัดแปรกลับต่ำกว่าไคโตแซนก่อนทำการดัดแปร ทั้งนี้เนื่องจากว่าไคโตแซนก่อนการดัดแปรมีน้ำหนักโมเลกุลสูงกว่าไคโตแซนที่ผ่านการดัดแปรซึ่งมีน้ำหนักโมเลกุลลดลงอย่างมากเนื่องจากโครงสร้างของไคโตแซนถูกทำลายในขณะที่ทำฮอฟแมนเดกเกรเดชัน แสดงให้เห็นว่าน้ำหนักโมเลกุลของไคโตแซนมีอิทธิพลต่อการจับสีย้อมมากกว่าปริมาณของหมู่อะมิโนอิสระที่เพิ่มขึ้น สำหรับประสิทธิภาพการจับโลหะหนักของไคโตแซนดัดแปร พบว่าประสิทธิภาพการจับนิกเกิลของไคโตแซนดัดแปรจะดีกว่าไคโตแซนก่อนการดัดแปร แสดงให้เห็นว่าความสามารถในการจับโลหะหนักของไคโตแซนขึ้นอยู่กับจำนวนหมู่อะมิโนอิสระและหมู่คาร์บอกซิลิกมากกว่าน้ำหนักโมเลกุลของไคโตแซน

ภาควิชาวัสดุศาสตร์ ลายมือชื่อนิสิต.....

สาขาวิชาวิทยาศาสตร์พอลิเมอร์ประยุกต์ฯ ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2543 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4172292023 : MAJOR APPLIED POLYMER SCIENCE AND TEXTILE TECHNOLOGY

KEY WORD: chitosan / acrylamide / Hofmann degradation / vinylamine / anionic dye

THORSAK KITTIKORN : MODIFICATION OF CHITOSAN FOR ANIONIC DYES REMOVAL FROM WASTE WATER. THESIS ADVISOR : ASSIST. PROF. KAWEE SRIKULKIT Ph.D., THESIS COADVISOR : ASIRA FUONGFUCHAT Ph.D., 98 pp.

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Chitosan was modified by graft copolymerization using acrylamide monomer. The resultant modified chitosan was further subjected to Hofmann degradation in order to convert acrylamide group into free amino groups and carboxylic groups. Infrared spectroscopy and ^{1}H NMR were employed to identify the chemical structures of acrylamide grafted chitosan and Hofmann treated graft chitosan. As a result, the final product was proven to be the vinylamine-co-acrylic acid-co-chitosan.

The result of potentiometric titration showed that the final product contained an increase in amount of free amino groups, which was expected to enhance the performance of removal capacity of anionic dye and heavy metal ion. The assessment of removal capacity of anionic dyes by modified chitosan indicated that an increase in free amino groups of modified chitosan led to an increase in dye removal capacity. However, its dye removal capacity was much lower than those of virgin chitosan. It was found that the molecular weight of the modified chitosan was significantly lower than that of virgin chitosan due to the degradation of chitosan backbone during Hofmann degradation treatment. This indicated that the molecular weight of chitosan played an important role in anionic dye removal. In the case of heavy metal removal, the results showed that modified chitosan exhibited better performance than virgin chitosan, indicating that the presence of free amino groups and carboxylic groups dominated the formation of chitosan-metal complex not the molecular weight of chitosan itself.

Department Materials Science
Field of study Applied Polymer Science
Academic year 2000

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CHAPTER I

INTRODUCTION

Chitosan is the product obtained from N-deacetylation of chitin in strong alkaline condition. The latter, poly-β(1-4)-N-acetyl-D-glucosamine, is intractable and abundant naturally occuring polysaccharide forming part of shell of crustacea such as shrimp, crap and insects. Because of amino groups in the structure, chitosan has been applied in many fields such as water treatment, cosmetic, textile finishing, medicine application, or food nutrition.

The modification of natural polymers is a promising method for preparation of new materials. This enables one to introduce special properties and enlarge the field of potential applications of those biopolymers. Among the diverse modifications that are possible to achieve, grafting of synthetic polymer is a convenient method.

Graft copolymerization is always the method to modify chitosan because it has the functional groups, both hydroxyl and amino, that ease to be activated, giving free radical, by redox initiator such as potassium persulate and cesium ammonium nitrate.

In this work, we studied the modification of chitosan by graft copolymerization with acrylamide monomer using potassium persulphate as redox initiator. Combined effect of principal reaction variables for grafting reaction was carried out in homogeneous phase under acetic condition. Thereafter, the polyacrylamide that grafted on chitosan was converted to vinylamine by Hofmann Degradation Process in order to investigate changes produced to the properties of products and to compare this with that of unmodified chitosan. Vinylamine grafted chitosan as a final product was tested anionic dye flocculation and compared with virgin chitosan.

CHAPTER II

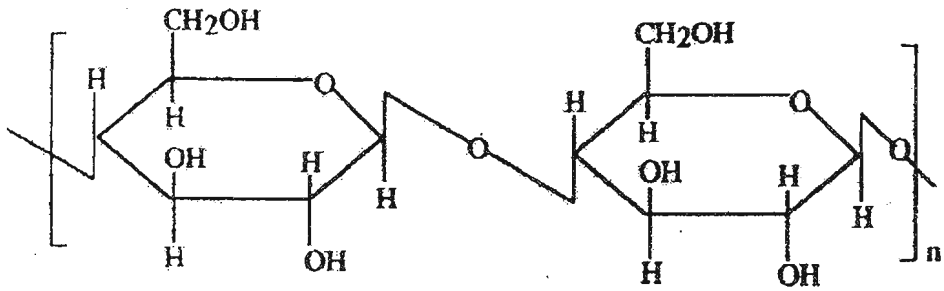
LITERATURE SURVEY

Chitosan is the natural polyelectrolyte. It's a polysaccharide obtained by deacetylating of chitin which is the major constituent of exoskeleton of crustaceous water animals. Normally, the deacetyltion of chitin is carried out by treating chitin with 40-50% NaOH at high temperature such as at temperature of 100 ^{0}C. The main driving force in development of new application for chitosan lies in the fact that the polysaccharide is not only naturally abundant but also nontoxic and biodegradable. Unlike oil and coal , chitosan is obtained from a naturally regenerating resources eg. crab and shrimp shell.

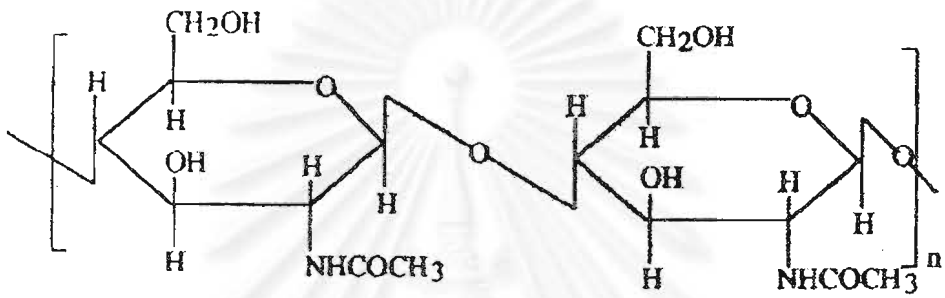
2.1 Physicochemical properties of chitosan[1,2]

Chitosan is a collective name given to a group of deacetylated of chitin. The only difference between chitin and chitosan is the degree of deacetylation. Generally, the reaction of deacetylation of chitin in alkaline condition cannot reach completely even under harsh treatment. Normally, degree of deacetylation is found to be in the range of 70-95% depending on the treatment method. According to Muzzarelli's ' review[3], the technique of Holowitz, for example, chitin treated with potassium hydroxide for 30 minutes at 180°C can remove acetyl groups as high as 95%.

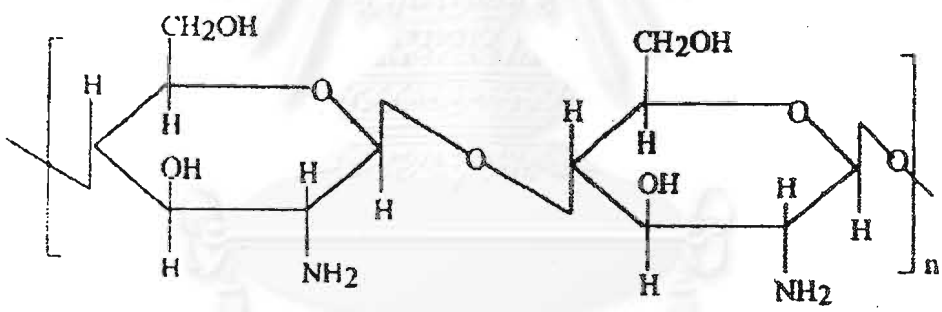
The molecular arrangement of chitin have two structure models; α-chitin that tightly arranged in antiparallel and β-chitin that is in parallel form. An analysis of X-ray diffraction of chitin and chitosan reveals the structure resemblance. So, it suggests that chitosan has a structure similar to α-chitin.



Cellulose



Chitin



Chitosan

Figure 2.1 Structure of cellulose, chitin and chitosan.

Commercial chitosan contains 5-30% of the acetyl group depending on the origin of chitin and condition of processing. The quality and properties of chitosan products such as purity, viscosity, deacetylation, molecular weights, and polymorphous structure may vary widely because many factors in the manufacturing process can influence the characteristics of the final product. For example, there was found that the adsorption ability of chitosan for metal ions depends on the hydrolysis process. A homogeneous hydrolysis

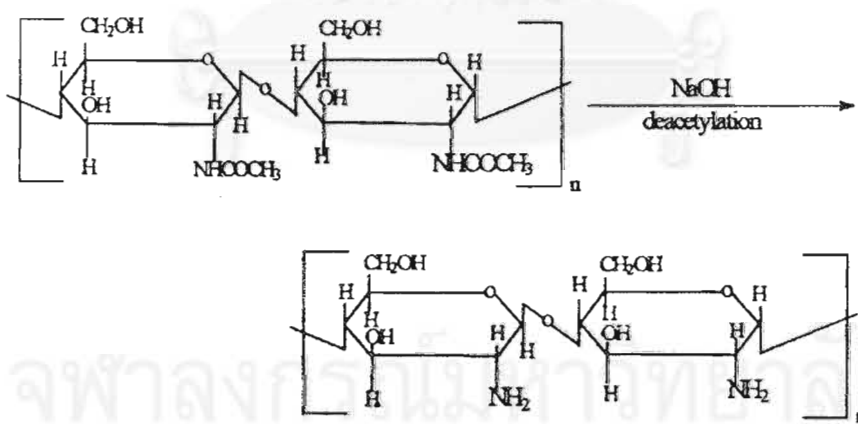
process could give a chitosan exhibiting higher adsoption rate than chitosan product prepared from heterogeneous hydrolysis process, resulting from the difference in crystalline structure of chitosan. Furthermore, there are found that the highest viscosity and highest molecular weight could be obtained by grinding the shrimp hulls to 1 mm prior to treatment, using alkali deproteination, purging nitrogen into the reaction vessel and increasing the deacetylation time.

The degree of deacetylation is one of the most important chemical characteristics of chitosan. It is used to determine the free amino content in the polysaccharide. The degree of deacetylation of chitosan can be measured by many methods such as infrared spectroscopy, titration, gas chromatography, and dye adsorption. Based on these methods, Muzzalli[3] suggested that first derivative ultraviolet spectophotometry at 199 nm was probably the best method for nondestructively and accurately determining the degree of deacetylation of chitosan. Namely, the N-acetylglucosamine absorbance readings were linearly dependent on concentration and were not influenced by the presence of acetic acid. Another method for analyzing the degree of deacetylation in chitosan is dye adsorption. In acid conditions, there was a 1:1 stoichiometry for interaction of free amino in chitosan with sulfonic acid groups on dye ions which there was found the dyeing of C.I. Acid Orange 7 was the most rapid method to determine the degree of deacetylation in chitosan.

The molecular weight of native chitin is larger than 1,000,000 while commercial chitosan has the molecular weight varies in from 10,000 to 1,000,000 and sometimes could be obtained to 1,200,000 molecular weight with 79-91% deacetylation. During the manufacturing process, harsh condition can lead to degradation of chitosan. For example, under Horowitz method[3],

after 30 minutes treatment at 180 °C, the chain length of chitosan was degraded and found twenty repeating unit only. In generally, there are many factors of degradation effect on chitosan. For example, dissolved oxygen can slowly degrade on chitosan. Thermal degradation of chitosan takes place at the temperature over 280°C. On the other hand, shear stress or hydrodynamic force can result in break down the long chain chitosan molecules to critical length but it does not affect to molecular weight distribution. There are many processes that provide a relatively narrow molecular weight distribution; for example, treating chitosan solution with 0.05% ClO_2 produced average molecular weight of only 60,000 with narrow molecular weight distribution. Treating

chitosan solution with enzyme such as papin, cellulase, and acid protease is another method that gives an average molecular weight of 36,000 with narrow molecular weight distribution.



Scheme 1.

Scheme 2.1 Deacetylation of chitin

The molecular weight of chitosan can be determined by methods such as chromatography, light scattering, and viscometry which is the most simple

and rapid method. Although, the molecular weight of chitosan was not always directly related to viscosity because the effect of colloid particle but still the technique is widely used to determine molecular weight of chitosan.

The viscosity of chitosan in solution is influenced by many factors such as the degree of deacetylation, molecular weight, concentration, ionic strength, pH, and temperature. In general, as the temperature rise, the viscosity of polymer solution decreases. However, pH-dependence of viscosity depends on the kind of acid used. In acetic acid condition, the viscosity of chitosan tends to increase when pH decreases; but in HCl condition, the viscosity decreases when pH decreases because shielding of chlorine. Ionic strength is also the important factor affecting viscosity of chitosan solution. Namely, in high ionic strength system, polymer tends to form coil due to electrostatic interactions between poly ion and counterions then the intrisic viscosity of polymer solution decreases.

Chitosan is insoluble in water, alkali, and organic solvents but can be dissolved in most solutions of organic acids when the pH of solution is less than 6. Acetic and formic acids are the most widely used to dissolve chitosan. Some dilute inorganic acids such as, nitric acid, hydrochloric acid, perchloric acid, and phosphoric acid, can be used to dissolve chitosan under stirring and warming.

The chain conformation of chitosan reflects; to some extent, the 1,2 helical structure of parent polymer units. The chitosan dissolves readily in an acid medium such as aqueous acetic acid giving a solution of very high viscosity which depends on the deacetylation of origin chitin. The chitosan can be dissolved in water at neutral pH when it has about 50% acetyl groups because of its amorphous structure. The ability to dissolve in water decreases when increasing crystalline structure. There are many methods to improve

water soluble ability of chitosan; for example, preparing the water soluble chitosan salt by evaporate aqueous chitosan solution to have concentration of 10% and spray drying at 175°C.

2.2 Application of chitosan

Chitosan has been commercially produced since 1970s. The major applications of chitosan were in sludge dewatering, food processing such as, removing the dye in juice and sugar[5], and metal ion chelating.[7] In present, chitosan is applied in cosmetic field[4], such as additive in shampoo for increasing the strength of hair due to interaction between amide group of protein and amine of chitosan. It can also be used as drug carriers by encapsulating[6], feed additive, and coating seed, semipermeable membranes in artificial kidney, pharmaceutics as a fatty controller by binding properties of chitosan to anionic ion of fatty acid. Furthermore, it is prepared as the film to heal wound because O_2 and CO_2 can flow through the chitosan film. Contact lens is one of applications from chitosan because swelling ability and O_2/CO_2 permeablility of chitosan. Low cost and a large quantity of raw material source driving forces to study for new application of chitosan nowadays.

2.3 Application in wastewater treatment

Another interesting and attractive application of chitosan is that free amino groups on backbone chain are attributed to a good coagulant and flocculent due to regular structure and high charge density of chitosan. The amino groups of chitosan can interact to negatively charged substances such as, protein, solid, anionic dye, and polymer. The nitrogen in amino groups of chitosan acts as a electron donor to coordinate with metal ions[26], allowing chitosan to be used for chelating harmful metal ions [8] such as copper, lead,

mercury, nickel and uranium from waste water. There are many factors to increase the complex efficiency with metal ions such as high deacetylation of chitosan, period of treatment, the amorphous structure, the particle size of chitosan, and affinity for water complex efficiency.

2.3.1 Color removal from textile mill effluents[9]

1) Sorption of dyes

Chitosan is used to remove various dye stuffs from textile effluent. Due to its unique molecular structure, chitosan has an extremely high affinity for many classes of dyes, including disperse, direct, reactive, acid, vat, sulfur, and naphtol dyes. The rate of diffusion of dyes in chitosan is similar to that in cellulose. Chitosan has a low affinity only for basic dyes. Chitosan is versatile in sorbing metals and surfactants, as well as to derivatization to attract basic dyes and other moeities (e.g., protein from food processing plant).

The sorption of dyes by chitosan is exothermic. An increase in temperature leads to an increase in dye sorption rate, but diminishes total sorption capacity. However, this effect is small and wastewater temperature variations do not significantly affect the overall decolorization performance. Also, the wastewater pH may be an important factor in the sorption of certain dyes onto chitosan because, at low pH, chitosan's free amino groups are protonated, causing them to attract anionic dyes. Contact time or, inversely, flux(wastewater flow per unit cross-sectional area) affects sorption in complex manner in a fixed-bed designed reactor system due to contact time, bed penetration and boundary layer effects. At high flux, the diversion of liquid into larger channels around particle and turbulent flow occurs. In general, a low flux tends to give more complete contaminant removal. For almost all the treatment strategies, a major factor which has not yet been adequately

characterized is the effect of typical wastewater contaminants on decolorization efficiency. In typical dyeing systems, it is well known that certain additives such as salts and surfactants can either accelerate or retard dye sorption process. The extreme variability of textile wastewater must be taken into account in the design of any decolorization system.

Finally, a factor which significantly increases the sorption rate is the loading thermodynamics, which indicates whether a reaction is favoured. As loading increases, the driving forces for sorption decrease, leading to an ultimate saturation beyond which further sorption is not possible.

2) Dye-binding properties of chitin and chitosan

The dye-binding properties of chitin and chitosan was examined by weighting 0.5 or 2.0 g chitin or chitosan particle in centrifuge tube, adding 20 g of aqueous dye solution (5 to 49 mg dye/l), and then shaking the closed centrifuged for 35 min at 4500 x g; the supernatant was decanted and the water uptake of chitin and chitosan was determined. The absorbance of the supernatant was measured at 505 nm using decolorized water as a blank. The weight of the supernatant was used as the basis for the calculation of total amount of dye bound or released. pH adjustment was carried out by using either 10 ml of commercial buffer solution or by adding 0.1 M HCl to the slurry of 0.5 g chitin/chitosan and 10 ml of dye solution. After stirring for 15 mins, the pH was readjusted and deionized water added. Chitosan form gel at pH value below 5.5 and no dye-binding measurement could be obtained.

Dye concentrations had no marked effect on the water uptake but correlated significantly with the dye-binding capacity of chitin and chitosan. The effect of pH on dye-binding capacity of chitin and chitosan was also studied. A decline in the dye-binding capacity above pH 7.0 was observed. Within the pH range 2.0-7.0, the dye-binding capacity of chitin was shown to

be stable, while chitosan formed gels below pH 5.5 and could not be evaluated.

Kim[10] studied the effect of dye and metal sorption capacity on chitosan and summarized that the rate and dye sorption capacity depend on the amount of amino groups of chitosan and pH. Namely, dye sorption capacity increases with increasing of deacetylation of chitin, but decreases with increasing of pH. This is mainly due to increase quaternary amine groups ($-NH^{+}_{3}$) of chitin with a high deacetylation and low pH. The sorption of metal is same as the dye sorption, it increases due to the increasing of amino groups. Therefore, the controlling of deacetylation of chitin to maximum efficiency can be achieved in removal of dye and metal ions from textile effuent due to the high charge density of amino groups.

2.4 Modification of chitosan

Water-soluble chitosan form is one of chitosan derivatives that is prepared to replace virgin chitosan when use of acid substance is underdesirable such as cosmetic, medicine product or food.

The modification of chitosan by ionizing the side groups on chitosan backbone is the ready method to improve the water soluble ability of chitosan. The presence of two different groups, NH_2 on C_2 and OH on C_3, C_6 makes the chitosan attractive and ease of subsequent derivatization reaction. The products of reaction can be cationic, anionic, or ampholytic chitosans. There are many reactions to modify chitosan as follows:

1) The quaternization of NH_2 group to the NR^{+}_{3} group by reaction with CH_3I under inorganic base condition. The reaction product containing a degree of quaternization of 25% is found to be water soluble with irrespective of pH value.

2) The carboxylation of OH group of chitosan improves the soluble property or binding property of chitosan.
3) The sulfation of chitosan on N and O atoms could be achieved by reaction with $ClSO_3H$
4) O-Phosphatation in the C_3 and C_6 position of chitin and chitosan could occur by reacting with P_2O_5 in methanesulfonic acid. The chitosan phosphate derivative shows the increased metal cation-binding capacity property.
5) Etherification of chitosan can be performed at NH_2 and OH groups with conventional reagent and can be used to synthesize anionic and cationic ether such as carboxymethyl chitosan by reaction with $ClCH_2COOH$ in alkaline condition.
6) The graft copolymerization of vinyl monomers onto chitosan.

The graft copolymerization of vinyl monomers onto chitosan was widely studied in order to obtain new type of tailored hybrid materials composed of natural polymer and synthetic polymer. The properties of graft copolymer such as, solubility, multifunctionality, low reactivity and flocculation ability or binding property would be controlled by characteristic of side chain of graft polymer, the molecule structure, chain length, and the number of repeating units.

2.5 Functionalization of chitosan by graft copolymerization technique

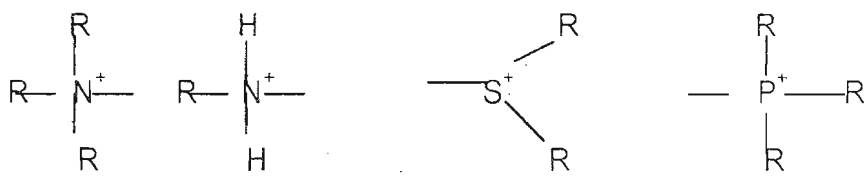
Chitosan is often used as the flocculent because cationic groups on the chains can form complex with anionic species. So, grafting the electrolyte monomer such as, acrylonitrile, acrylamide[23], acrylic acid[11], acrylate, vinylpyridiene[12] and other monomers was used to improve binding properties to ion species. Furthermore, modified chitosan containing polyanions is suitable for wide pH range applications. Similar, grafting the cationic polymers

on chitosan increases the cationic charge density on copolymer that improves binding property to anionic species.

Thidib Tripathy[13] improved flocculent by grafted acrylamide on alginate for remove metal ion. Acrylamide grafted alginate showed higher efficiency than virgin alginate because the branch polymer that grafted on main chain can approach to absorb ion in system more than polymer chain only. So, it eases to form bridge between ions and flocculent.

Cationic polymers are a class of polyelectrolytes[14]that derive their unique properties from the density and distribution of positive charge along the back bone chains as well as molecular weight. Chain conformation and solubility depend on the extent of ionization and interaction with water. Cationic functional groups can strongly interact with suspended, negative charged particle or oil droplets which are useful and used in waste treatment application.

Water-soluble polymers containing cationic charge can be classified into three main categories; ammonium (amines and quaternaries), sulfonium, and phosphonium quaternaries, which are shown below:



Ammonium Sulfonium Phosphosnium

R = alcyl group

Ammonium polymer such as cationic or quaternary polyacrylamide, ammonium polymer, polyamines, polyimines including polyvinylammonium

polymer(polyamines and polyquaternary ammonium salts), these polymer are the commercially significant cationic polymers. Acrylamide monomer can be homopolymerized or copolymerized with cationic polymer to yield water-soluble polymers with positive electrical charges.

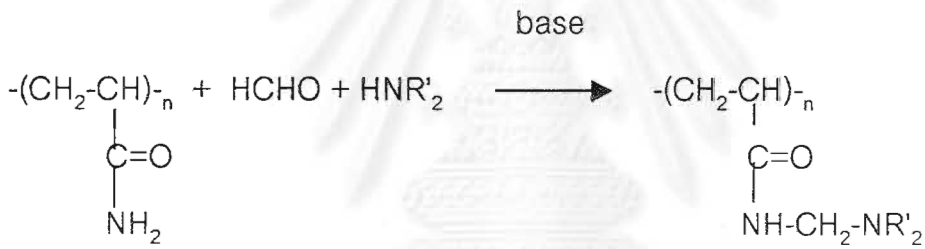
Cationic polyacrylamide can be prepared using techniques analogous to those for synthesizing nonionic or anionic. Polymerizations based on free radical mechanisms can be conducted in aqueous solution, mixed solvent solution, suspension, and water-in oil-dispersions or inverse emulsions. The free radical initiators can be water or oil soluble including persulphates, peroxides, azo compounds, and oxidation reduction pairs.

Solution polymerizations involve either water or mixed solvents. Molecular weight can be controlled by initiator, temperature, and monomer concentration. The polymer can be isolated by drying or precipitation using non solvent such as methanol and acetone, which remove unreacted monomer and others impurities. In mixed solvent such as water and tertialy butyl alcohol, polymer chains grow until they precipitate. In this type of polymerization, molecular weight is controlled by cosolvent choice, temperature, and initiator. Polymers prepared from mixed solvents generally have lower molecular weights and narrow molecular weight distributions, and form uniform solutions without gels and fish eyes. In addition, the lower solution viscosity make isolation processes polymer by fluid bed, tray, or drum drying relatively easy.

Inverse emulsion polymerization involves dispersing an aqueous solution of the water soluble monomers in a hydrocarbon solvent containing one or more emulsifiers. Because monomer concentrations in dispersed phase can be very high, the resulting polymers generally have extremely high molecular weights. However, because the polymer remains in the dispersed

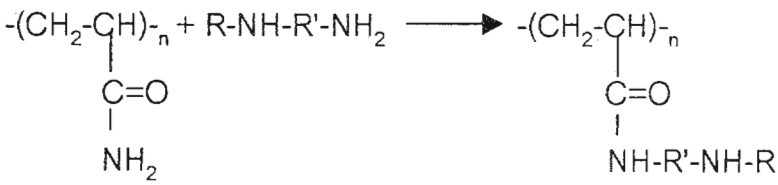
phase, the emulsion viscosity remains relatively low. The high polymer concentration and low emulsion viscosity provide a significant advantage in handling and dilution for subsequent use.

Cationic polyacrylamides can be prepared by postpolymerization functionalization of a polyacrylamide. In Mannich-type reaction[15], polyacrylamide reacts with formaldehyde to form N-methylol groups, which can be treated with a dialkylamine, such as dimethylamine, to yield pendent amine groups. The synthesis of a model cationic polymer based on the Mannich reaction of polyacrylamide has been described by following equations

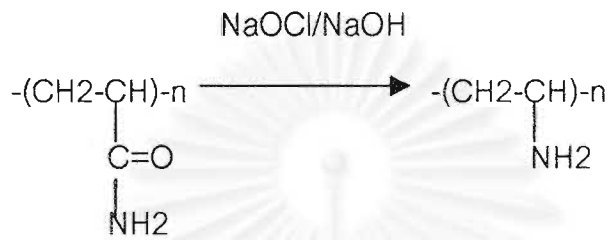


Scheme 2.2 Mannich-type reaction

These amine groups can be subsequently quaternized. Another approach for introducing amino groups into polyacrylamide is by reaction with polyamines as follows:



Hofmann degradation[15,16,17] is another technique to modify amino groups on polyacrylamide to vinylamine by treated with sodium hypochlorite under sodium hydroxide condition, as follows.



Scheme 2.3 Hofmann degradation reaction

Cationic polyacrylamide have many applications, such as water treatment, petroleum exploration and production, mineral processing and recovery, and paper making.

Polyelectrolytes are applied in water treatment because of their superiority in solid-liquid and liquid-liquid seperation and deposit control. Polymer charge type and density, along with molecular weight, are the dominant parameters that control performance. Polymers function to coagulate and/or flocculate contaminants in water. Coagulation involves charge destabilization by neutralization of anionic charge on suspended solids or oil in water by the cationic functionality. These polymers also compete with other inorganic coagulants such as aluminum(alum) and iron salts.

Another mechanism that can occur simultaneously with coagulation or charge destabilization is flocculation. This involves the physical formation of large size agglomerates or aggregates due to polymer bridging or entrapment of discrete particles and is mainly dependent on polymer molecular weight. Polymer bridging is complex. Polymer chains adsorb on particulate surfaces at one or more sites along the polymer chain. The remainder of the polymer

may remain extended into the solution and adsorb on available surface sites of other particulates, thus creating a bridge between surface. If the extended polymer cannot find vacant sites on the surface of the particulates, no bridging will occur. So, there is an optimum degree of coverage or extent of polymer adsorption at which rate of aggregation will be maximum.

Because polymer bridging is an adsorption phenomenon, the optimum dose will generally be proportional to the concentration of particulates. An increase in molecular weight are advantageous because of the increase in polymer size and thus the potential extent of bridging. Solution properties such as pH and ionic content affect the polymer configuration in solution and at the interface. High ionic strength tends to cause polymers to coil, thus decreasing their radius of gyration or length of extention. So, the potential bridging of polymer decreases.

By employing an appropriately designed cationic polymer usually at a treatment level of about 10-100 ppm, the seperation of solids or oil from water can be accelerated from days or even months to minutes.

2.5.1 Grafting copolymerization on chitosan by redox initiator[27]

A redox reaction in which one or more electron is transferred between reactant species per molecule reacted can be a particularly useful mode of initiation. There is also the advantage that most of the redox reactants in common usage are soluble in water. For example, one redox reaction which persulphate and thiosulphate are used frequently to initiate emulsion polymerizations can be written, follow as

$$S_2O_8^{2-} + S_2O_3^{2-} \longrightarrow S_2O_3^{\cdot-} + SO_4^{2-} + SO_4^{\cdot-}$$

It is the sulphate radical ion ($SO_4^{\cdot-}$) which is the active initiator. Another redox initiation system involves the reaction of ceric (Ce(IV)) ion, added in the

form of ceric ammonium nitrate, with isopropanol. The result of this reaction is summarized in general terms by stating that the reaction of Ce(IV) with the alcohol forms a complex, which subsequently decomposes to yield an organic radical, the active initiating species, together with Ce(III) and H^+ . This system has, for example, been used to initiate the polymerization of methylmethacrylate in its aqueous solution at temperature around 310 K

The OH and NH_2 groups on chitosan can be activated to free radical and initiate graft polymerization if other monomers are present.

Redox initiator is always used in graft copolymerization in polysaccharide families because of hydroxyl groups on backbone chains are easily activated to free radical. Cesium ammonium nitrate and potassium persulphate are the redox initiators for graft polymer on polysaccharide such as cellulose, chitin, and chitosan, because it can produce free radical at hydroxyl groups on backbone chains, as follows.

Reaction of potassium persulphate as a redox

$$K_2S_2O_8 \longrightarrow S_2O_8^{2-} \longrightarrow 2SO_4^{\cdot-}$$

$$S_2O_8^{2-} + \text{Rcell-OH} \longrightarrow \text{Rcell-O}^{\cdot} + SO_4^{\cdot-} + HSO_4^-$$

Reaction of Cesium ammonium nitrate as a redox

$$Ce^{4+} + \text{Rcell-OH} \longrightarrow \text{Rcell-O}^{\cdot} + Ce^{3+} + H^+$$

Cerium ion is also a suitable redox initiator for graft polyacrylamide and poly(acrylic acid) on chitosan. The derivative chitosan having poly(acrylic acid) on side chain shows high flocculation ability in both acid and basic conditions because of zwitterionic characteristics of derivative.

Keisuke Kurita[18] used cerium(IV) as the redox initiator at 60°C for grafting copolymer of acrylamide and acrylic acid on to chitin for application in water absorbents, chelating agents, and ion exchangers. It shows the suitable initiators for chitin because it gives scarce amounts of homopolymer.

Hamit Caner[8] used cerium(IV) as the redox initiator for grafting 4-vinylpyridine onto chitosan under homogenous condition in 1% acetic acid solution. Good percent grafting was obtained. However, it was difficult to separate cerium(IV) initiator because of insoluble complex between chitosan and cerium(IV) ion formed during graft polymerization.

M. Yazdani-Pedram[19] modified chitosan by grafting of vinyl pyrrolidone under homogenous reaction by using potassium persulphate($K_2S_2O_8$) as a redox initiator to study the effect of complex formation of chitosan with copper ion. Vinyl pyrrolidone grafted chitosan was test to form complex with copper ion and showed that the percent grafting 0% and 269% have as same as the efficiency at 18 mg of Cu^{2+} to 1 g of vinyl pyrrolidone grafted chitosan. It showed the vinyl pyrrolidone on chitosan can be complex with copper ion is similar to chitosan.

Aly Sayed Aly[11] used potassium persulphate to graft acrylonitrile and acrylic acid monomers onto chitin. Acylonitrile grafted chitin was reacted with hydroxyl amine hydrochloride, as well as, sodium hydroxide in order to obtain chitin-(amidoxime-co-acrylonitrile) and chitin-(acrylate-co-acrylamide) graft copolymer, respectively. They were used in waste water treatments for adsorption of heavy metal ions such as, Cu^{2+}, Cd^{2+}, Cr^{3+}, and Fe^{3+}as well as acid and basic dyes. Chitin-(acrylate-co-acrylamide) showed higher adsorption amounts of 1430 ppm of Cu^{2+} to 1 g chitin derivative for metal ion cations due to NH_2 and COONa groups on side chains while acrylonitrile grafted chitin showed higher adsorption acidic dye that depend on time treatment. For basic dye treatment by chitin derivative, acrylic acid grafted chitin and chitin-(acrylate-co-acrylamide) is the best performance to remove basic dye due to carboxylic groups.

Yong-Beom Kim[25] had developed the new amphoteric flocculent by grafting monomer such as acrylamide, acrylic acid, maleic acid, and citraconic acid, on to chitosan with ceric ammonium nitrate as redox initiator. The new amphoteric flocculent that has anionic and cationic groups on the chains have flocculation efficiency to metal ion such as Pb^{2+}, Cd^{2+}, or Cu^{2+} more than chitosan in pH range 2.5 – 9.5. Especially, under base condition it shows high flocculation efficiency because carboxylic was ionized to carboxylate ion that is hard base. So, it eases to form complex with metal ion.

P. Ghosh[20] grafted acrylamide on cotton celluiose by using potassium persulphate as a redox initiator for improve the hydrophilic, dyeability, and tenacity properties of cotton cellulose. The factors of graft copolymerization was varied. Optimal conditions for graft copolymerization was the temperature of 70°C and pH value of 3-4 in acetic acid medium. Various properties of acrylamide grafted cellulose were improved.

2.5.2 Effect of variables onto grafted copolymerization using a redox initiator

M. Yazdani[19] and K.L. Shantha [21] studied the effect of variables on grafted copolymerization and summarized as follows:

2.5.2.1 Effect of redox initiator concentration.

M. Yazdani[19] and K.L. Shantha [21] studied the effect of redox initiator concentration to graft copolymerization on chitosan. They observed that the percent grafted and grafting efficiency increased to critical value at certain initiator concentration and decreased, thereafter as Figure 2.2. At lower concentration of redox initiator, the initiator will be completely employed to produce free radical on

chitosan backbone. However, at higher initiator concentration, active site for grafting may be higher but at the same time, some of initiator may be consumed for termination reaction, hence decreasing percent grafting. The total percent conversion also increased in similar manner. This may be attributed to the increased termination reaction taking place after the completion of grafting reaction.

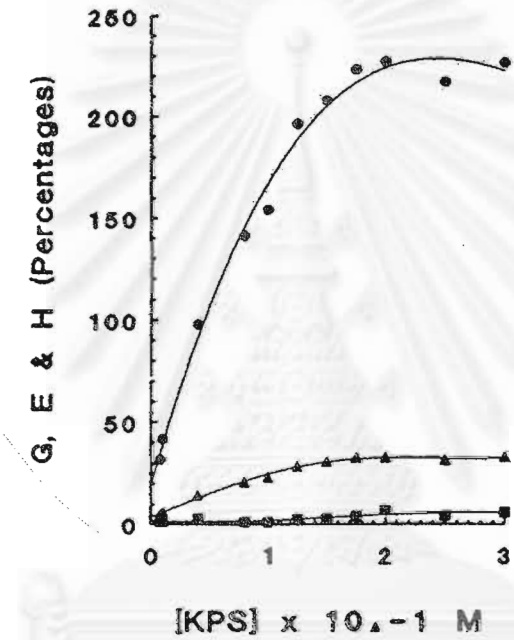


Figure 2.2 Effect of initiator concentration on grafting reaction:
●, % Grafting percentage: ■, % Homopolymer percentage;
▲, % Efficiency percentage.
Reaction condition: chitosan 0.3 g; vinylpyrrolidone 2ml;
120 minutes, 60 °C.

2.5.2.2 Effect of monomer concentration

Similar to initiator concentration effect, Figure 2.3, show that the percent grafting and grafting efficiency increase up to a certain extent of increase in monomer concentration. Thereafter, the reduction in the grafting efficiency and percent grafting were found with the increase concentration of monomer which may be attributed to the limited number of active centres available for grafting on the backbone and more monomer units competing for the same site. There was a decrease in percent conversion with an increase in monomer concentration which inidicates that the termination reaction will be more favourable after the saturation of grafting sites.

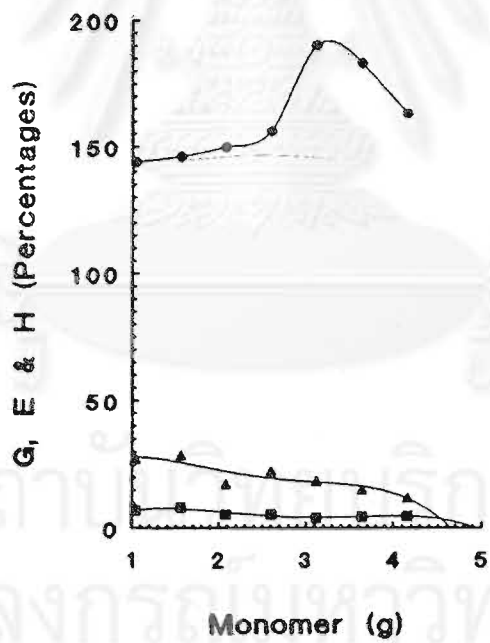


Figure 2.3 Effect of monomer concentration on grafting

●, % Grafting percentage; ■, % Homopolymer percentage;

▲, % Efficiency percentage.

Reaction condition: chitosan 0.3 g; $K_2S_2O_8$ 0.15 M;

120 minutes; 60 °C.

2.5.2.3 Effect of temperature

From Figure 2.4 shown that the percent grafting increased sharply with a increasing in temperature up to 70^{0} C and was decreased afterwards. This phenomena may be a result of the side effects such as viscosity, decomposition of initiator, generation of chitosan macroradical, terminal and chain transfer reaction.

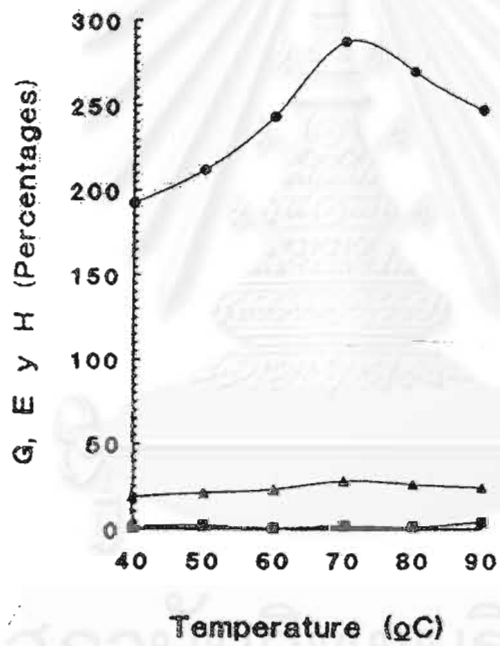


Figure 2.4 Effect of reaction temperature on grafting

●, % Grafting percentage; ■, % Homopolymer percentage;

▲, % Efficiency percentage.

Reaction condition: chitosan 0.3 g; vinylpyrrolidone 2ml;

$K_2S_2O_8$ 0.15 M; 120 minutes.

2.5.2.4 Effect of reaction time

Like above, the percent grafting tended to increase up to critical percent grafting due to reaction time and was decreased afterwards as Figure 2.5.

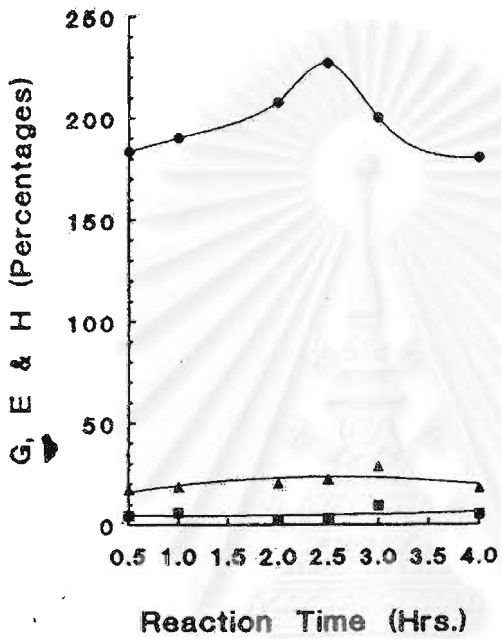


Figure 2.5 Effect of reaction time on grafting

●, % Grafting percentage; ■, % Homopolymer percentage;

▲, % Efficiency percentage.

Reaction condition: chitosan 0.3 g; vinylpyrrolidone 2ml;

$K_2S_2O_8$ 0.15 M; 60 °C

2.5.2.5 Effect of pH

Effect of pH on graft copolymerization of acrylamide on cellulose using $K_2S_2O_8$ was reported[20]. At pH 7, the percent grafting and grafting efficiency was significantly low but conversion was high. Change to slightly lower or higher of pH 7 led to some increase in percent grafting, grafting efficiency, and percent conversion. However, at lower pH, it was likely to cause notable

or relatively severe hydrolytic degradation and hence weakening of cellulosic fiber strength. The best pH values were found to be between pH 3 and pH 4.

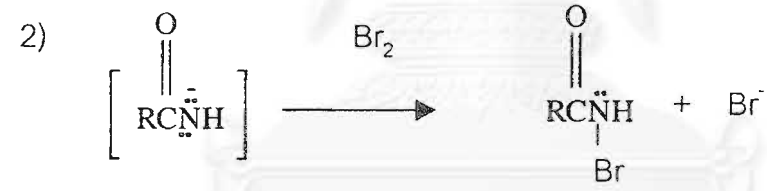
2.6 The Amide-Amine Conversion by Hofmann Degradation.

Mechanism step of Hofmann Degradation[28] could be divide in to several, steps as follows:

Machanism step of Hofmann Degradation

1. Bromination of N

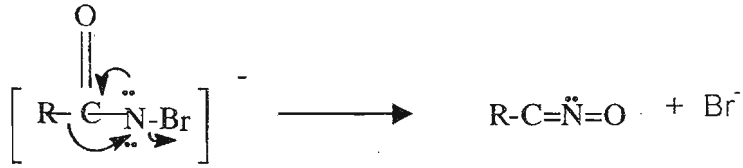
v



1. Extraction of H^+ by OH^-

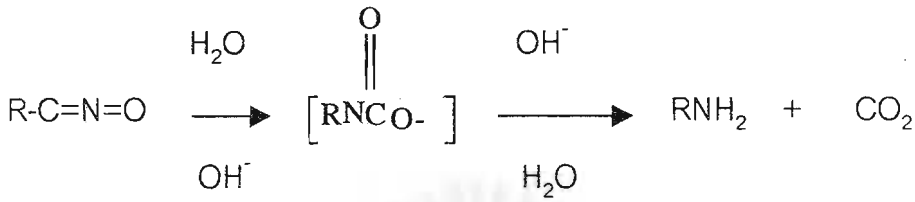
Proton is losses from N and unstable anion N is formed

3. Displacement of Br^-



The product of rearrangement is isocyanate.

4. Hydrolysis of the isocyanate



Scheme 2.4 mechanism of Hofmann degradation

Because in aqueous base condition, isocyanate is not stable then it undergoes hydrolysis to yield amine and carbonate ion.

The use of Hofmann degradation for converting amide compounds into free amines was widely studied since it is a simple method. In addition, there is, at present, no direct method of introduction of free amino group into polymer backbone.

Y. Yamamoto and M. V. Sevton[17] prepared amino group functionalized acrylamide copolymer by Hofmann degradation technique. The polyacrylmide of the poly(acrylamide-co-methyl methacrylate) was converted to polyvinylamine by Hofmann degradation. The final product was the poly (acrylamide-co-methyl methacrylate-co-vinylamine) of improved hydrophilic property. In Hofmann degradation process, poly(acrylamide-co-methyl methacrylate) was dissolved in 8 ml of 50% aqueous dioxane and cooled to zero degree. Various amounts of cooled sodium hypochlorite (0.67 M) that was used instead of Br_2 was added to the polymer solution and stirred. After 5 minutes, 21 ml of cooled 7.6 M aqueous NaOH solution was added to mixture solution. The reaction time was from time 1 to 6.5 hours at zero degree. After the reaction, excess $NaHSO_4$ was added to polymer solution in order to reduce the residual chlorine, followed by addition of 2M HCl solution

for neutralization. The polymer was separated by centrifugation and washed in distilled water.

In this research, the incorporation of free amine groups on backbone chain of chitosan will be attempted by graft copolymerization with acrylamide monomer using potassium persulphate as a redox initiator. Then amide group is converted to free amine group by Hofmann degradation process. The increase in free amine groups is believed to play the role in binding with anionic dyes. As a result, the modified chitosan may be applied as flocculating agent for color removal from textile waste water.The carboxylic acid and primary amine[15,16,17] produced after Hofmann degradation were characterized by FT-IR and potential titration. The increase in NaOCl as well as decreasing NaOH concentrations which have an effect on the performance of Hofmann degradation reaction will be investigated. Finally, the performance of modified chitosan as dye removal will be assessed.

CHAPTER III

EXPERIMENTAL

3.1 Materials and chemicals

Chemical name	Source	Chemical structure
Chitosan with 92% Deacetylation	Seafresh, Thailand	$C_6H_9O_4NH_2$
Acrylamide monomer	Fluka Chemika, switzerland	CH_2=CH-$CONH_2$
Potassium persulphate	May & Baker LTD, Dagenham, England	$K_2S_2O_8$
Sodium hypochlorite, Cl_2 8.4%	APS Ajax Finechem, Netherlands	NaOCl
Acetone	Siam chemical, Thailand	$(CH_3)_2CO$
Methanol	Siam chemical, Thailand	CH_3OH

3.2 Equipment

- 3-necked round bottom flask
- Thermometer
- Magnetic stirrer, Framo-Geratetechnik, model M21/1

Instruments:

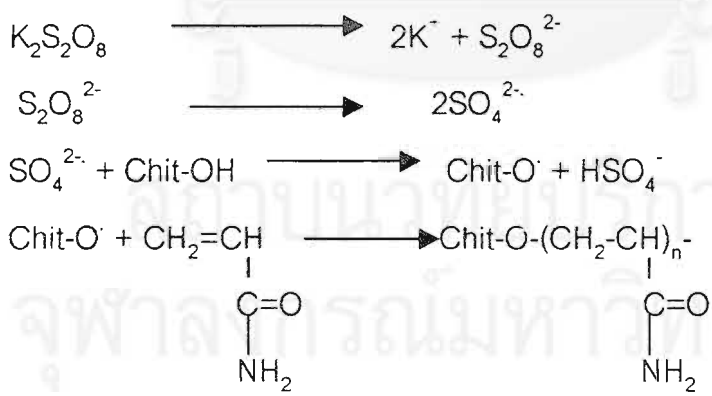
1) Fourier-transform infrared spectrophotometer, Nicolet impact 400D
2) Nuclear magnetic resonance spectrometer, JEOL JNM-A300
3) pH meter, Denver Instrument, model 215
4) Differential scanning calorimeter, Netzsch DSC 500
5) GPC PL 110
6) UV - Visible spectrophotometer, Jenway LTD, model 6405

7) Atomic absorption, Varian AA 1275 series

3.3 Graft Copolymerization of Acrylamide onto Chitosan

Graft copolymerization of acrylamide in homogeneous system was carried out. The chitosan stock solution of 100 ml(1% w/v) was added to 250 ml three necked round bottom flask. The mixture was purged with nitrogen gas in order to remove dissolved oxygen. There after, 3 g of acrylamide and potassium persulphate which varied to 7.4 mmole, 12.96, and 18.5, in 10 ml water was added. While stirring, the solution was heated to 60 °C and maintained at this temperature under nitrogen atmosphere for 120 min.

After that, homopolymer was extracted by methanol 2 times 300 ml each. The grafted chitosan which was soluble in methanol was dried at 55°C to remove methanol. Finally, acrylamide grafted chitosan was re-dissolved and precipitated by acetone, filtered and dried in dessicator. The graft yield was calculated. The reaction may be written as follows:

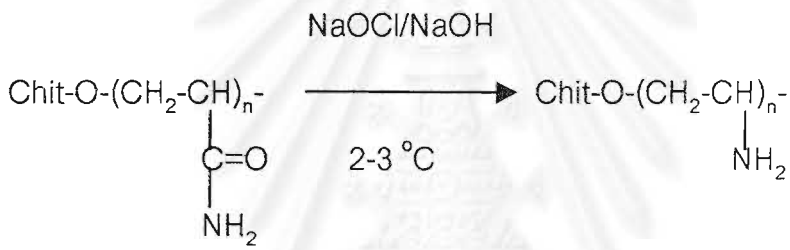


Scheme 3.1 graft copolymerization by redox initiator

3.4 Hofmann Degradation Reaction of acrylamide grafted chitosan

Acrylamide grafted chitosan 0.5 g was dissolved in 30 ml deionized water. Cooled ice was added to solution to lower the temperature to 2-3 °C.

Then 2 ml sodium hydroxide(10% w/v) was added. The required amount of sodium hypochlorite solution (density:1.2) was added. The mixture was continuously stirred while the reaction temperature was kept between 2-3 °C in thermostat bath. After reaching 60 minutes, the reaction was stopped and acetone was added to precipitate the product of Hofmann degradation reaction. The precipitate was washed several times using acetone. The obtained sample was dried in dessicator before measuring yield of reaction. The reaction involved may be written as follows:



Scheme 3.2 Hofmann degradation reaction.

3.4.1 Effect of factors on Hofmann degradation reaction

In Hofmann degradation reaction, mole of NaOCl, time, and temperature were varied to study the conversion of amide group to vinylamine and acrylic acid. The experimental procedure was employed in similar manner as described in section 3.4.

3.5 Characterization

3.5.1 Infrared (*IR*) spectroscopy.

3.5.1.1 Principle

Infrared spectroscopy is one of the vibrational spectroscopy technique that can be used to identifying and detailing information on polymer structure.

Vibrational spectroscopy is concerned with the detection of transitions between energy levels in molecules which result from vibrations of the interatomic bonds. The vibrational frequencies are shown to be characteristic of particular functional groups in molecules. They are sensitive to molecular environment, chain conformations and morphology.

At room temperature most molecules exist in their ground vibrational states and in order to excite them to higher vibrational states energy must be absorbed. Infrared spectroscopy are used to detect changes in vibrational energy states. When molecular vibrations result in a change in the bond dipole moment, as a consequence of change in the electron distribution in the bond, it is possible to stimulate transitions between energy levels by interaction with EM radiation of the appropriate frequency. In effect, when the vibrating dipole is in phase with the electric vector of incident radiation, the vibrations are enhanced and the energy is transfered from the incident radiation to the molecule. It is the detection of this energy absorption which constitutes IR spectroscopy. In practice, the spectral transitions are detected by scanning through the frequency whilst continuously monitoring the transmitted light intensity. The energies of molecular vibrations of interest for analytical work correspond to EM wavelengths in range 4000-400 cm^{-1}.

3.5.1.2 Method of analysis

KBr disk sample technique was used to analyse. The sample as a dry particle 1-2% is ground to powder with KBr. Then compress it in mould to obtained transparent disk. FT-IR spectra was taken by Nicolet 400D using parameters as follows:

Wavelength range 4000-400 cm^{-1}

Resolution scanning 4 cm^{-1}

Scan number 32 seconds

Figure 3.1 FT-IR spectroscopy

3.5.2 Potentiometric Titration

3.5.2.1 Principle

It is the method to determine ion species in the solution. In this research, free amine and carboxylic acid can be dissociated in different pH as follows:

From Henderson-Hasselbalch Equation

$$NH_3^+ \Leftrightarrow NH_2 + H^+$$

$$Ka = [H^+][NH_2]/[NH_3^+]$$

$$\log Ka = \log [H^+] + \log ([NH_2]/[NH_3^+])$$

$$pH = pKa + \log([NH_2]/[NH_3^+])$$

The pKa of free amine is about 9[17]. So, at pH higher than 9, NH_3^+ is dissociated to NH_2 and can be found by inflection point.

Similar to carboxylic acid

$$COOH \Leftrightarrow COO^- + H^+$$

$$Ka = [H^+][COO^-]/[COOH]$$

$$\log Ka = \log [H^+] + \log ([COO^-]/[COOH])$$

$$pH = pKa + \log ([COO^-]/[COOH])$$

pKa of carboxylic is about 6[17]. So, at pH higher than 6, COOH is dissociated to COO^-.

Figure 3.2 pH meter

3.5.2.2 Method of analysis

The amount of primary amine was quantitatively measured by potentiometric titration 50 mg of acrylamide grafted chitosan and vinylamine-co-acrylic acid grafted chitosan samples were dissolved in deionized water (50 ml). The pH value was adjusted to pH 11 using 1 ml of 0.2 M NaOH. The solution was titrated with standard 0.01 M HCl using 50 ml burette and pH value was measured after 1 ml addition by a pH meter equipped with combination electrode(Denver model 200). HCl solution was added until the pH was about 2.5. Measurements were done at room temperature.

3.5.3 Proton NMR Spectroscopy

3.5.3.1 Principle

Nuclear magnetic resonance (NMR) spectroscopy is an important method for materials characterization and for the study of polymer structure and property relationships. The NMR signals in virtually all modern NMR spectrometers are obtained by combination of pulsed NMR and fourier transformation. Common tools for all NMR spectrometers are high field magnetic, a source of radio frequency (rf), and a computer.

In the absence of rf pulses, the nuclear magnetic moments tend to align with magnetic field. The application of rf pulses rotates the magnetisation into the xy plane, where it rotates, or processes, at a rate that depends on the magnetic environment. This rotation is detected as the oscillating signal known as a free induction decay and converted into a frequency spectrum by fourier transformation.

3.5.3.2 Methof of analysis

Chemical structure of chitosan, acrylamide grafted chitosan and vinylamine-co-acrylic acid grafted chitosan was analysis by proton NMR with D_2O.

3.5.4 Gel permeation chromatography

3.5.4.1 Principle

Gel permeation chromatography is the method to determine average molecular weights and molecular weight distributions of polymers by calculate from size distribution chromatogram. GPC technique is a form of liquid chromatography in which the molecules are seperated according to their molecular size. The procedure involves injecting a dilute solution of polydisperse polymer into a continuous of flow of solvent passing through a column containing tightly packed microporous gel particles. The gel has particle sizes in the range 5-10μm with have the pore size in range 0.5-10^5 nm, which correspond to effective size range of polymer molecules. Separation of molecules occurs by penetration of different sized molecules into pores; small molecules are able to permeate more easily through the pore than large size molecule. So, small size molecule requires longer elution time and large size molecular requires shorter elution time. So, elution time is inversely proportional to molecular size and capable to determine unknown molecular weights polymer by calculate from elution time relate with known molecular weights polymer.

3.5.4.2 Practice for analysis

Gel Permeation Chromatography, PL-GPC 110

Eluent: 0.5 M acetic acid and 0.5 M sodium acetate(acetate buffer),

pH 3, Flow rate 0.6 ml/min

Inject volume 20 μL, Temperature: 30 °C

Column set: Ultralinear Hydrogel

Molecular weight range: 1,000 – 20,000,000

Polymer standard: Polysaccharide(Pullulan: Mw 700 – 1,600,000)

Calibration method: Polysaccharide standard calibration

Flow maker: 5% w/v glecerol

Sample preparation: Sample was dissolved in acetate buffer and filtered using nylon membrane(pore size 0.45 μm) before injected into the column

3.5.5 Differential Scanning Calorimetry

3.5.5.1 Principle

It is the method to determine thermal properties of material by heating on material and detecting the heat current. Heating current or watts of electric of sample was detected by compare with reference. Signal was presented in watts or endothermic or exothermic that depends on tool setting.

3.5.5.2 Method of analysis

Thermal analyses of test samples were carried out by DSC. Using DSC (Netzsch DSC 500) under N_2 atmosphere maintaining a heating rate of 10 °C and 20 °C per minutes and sample weight of about 20 mg. Temperature ranging from 30 °C to 250 °C was employed to study the glass transition temperature(Tg) and melting temperature(Tm).

Figure 3.3 differential scanning calorimeter

3.5.6 Evaluation of dye removal

3.5.6.1 Principle

Dye exhaustion or amount of dye absorbed by flocculent is usually defined in terms of difference between the initial and final dye concentrations. UV-VIS spectroscopy is the most commonly used technique for quantitative determination of dye concentration. Usually the Beer-Lambert law is employed to determine the concentration of dye in solution from a measurement of absorbance at wavelength of maximum absorption of dye.

$$\text{Absorbance} = \log(I_o/I) = acl$$

Where I_o = intensity of light incident on optical cell holding the dye Solution

I = intensity of light transmitted through the cell

a = absorptivity or extinction coefficient(L /g.cm) of dye

c = concentration of dye solution in the cell(g/l)

l = path length through the cell(cm)

Nylosan green N-GL, the acid dye type which has the chemical structure show in Figure 3.5; was used as a testing dye. A 50 mg sample of grafted chitosan was dissolved in 8 ml dyestuff solution(0.1%w/v) and the pH value was adjusted to pH 2 using HCl. The mixture was stirred to obtain homogeneous mixing for 24 hours. The insoluble polymer-dye complex was filtered-out and the remaining solution volume was made-up to 100 ml. The UV/VIS spectrophotometer was used to measure the residual color. The amount of precipitated dye was calculated based on the calibration curve illustrated in Figure E.1 (appendic E.)

Table 3.1, the characteristic of dye

C.I. Number	Commercial Name	Chemical Class	Molecular weight	λ max (nm)
Acid green 25	Nylosan green N-GL	Anthraquinone	700	639

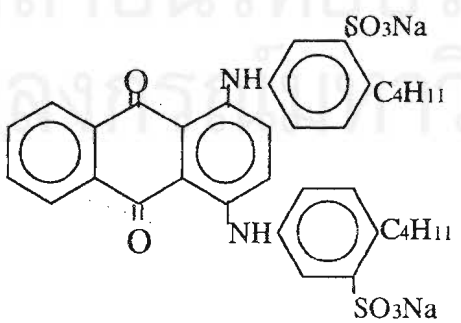


Figure 3.4 chemical structure of Nylosan green

Figure 3.5 UV/Visible spectrophotometer

3.5.7 Determination of Heavy Metal Removal by Atomic Absorption Technique

3.5.7.1 Principle

Atomic absorption is the quantitative method to analyse metal ion in solution. When burning metal ion with fuel gas, metal ion is excited to the exciting state. However, it releases energy to ground state in the analysed. Emitted light is a direct ratio of mole of metal ion. So, it can be analyse metal ion by detecting light emitted from a burner.

3.5.7.2 Method to analysis

The sample(50 mg) was dissolved in deionized water(5 ml). For the virgin chitosan, acetic acid solution(1%v/v) was employed. nickel sulfate solution(40 g/L) was then added to the sample solution. Insoluble complex was removed. The remaining solution was diluted by 1,000 times before

subjecting to the atomic absorption analysis(Varian AA 1275 series) using the following factors:

Oxidant: air, flow rate 38 cm^3/sec

Fuel: acetylene, flow rate 18 cm^3/sec

Wavelength: 232 nm

Figure 3.6 atomic adsorption

CHAPTER IV

RESULT AND DISCUSSION

4.1 Gravimetric analysis of Grafted Chitosan

In this study, the effect of initiator concentration was investigated, while keeping the temperature and time of graft copolymerization constant at 60 °C, and 120 minutes, respectively.

Table 4.1 The dependence of graft yield on $K_2S_2O_8$ concentration 60 °C, 120 minutes

Chitosan	$K_2S_2O_8$ (mmol)	Acrylamide monomer	Graft yield of Chitosan(g)
1 g	7.4	3 g	1.20
1 g	12.96	3 g	1.68
1 g	18.5	3 g	1.38

As can be seen from table 4.1 , graft yield increased with an increase in intiator concentration up to 12.96 mmol, and then leveled off when further increasing in initiator concentration. Initially, the increase in the initiator concentration produced more radical sites on chitosan backbone, leading to an increase in graft yield. At higher initiator concentrations, excessive radicals reacted with each other or terminated the growth chains, resulting in shortened chain length of the grafts. Beyond the certain amount of initiator, therefore, the grafting efficiency was not expected to increase.

4.2 FT-IR Analysis of Grafted Chitosan

The existence of grafting was confirmed by the difference between FT-IR spectra of virgin chitosan and grafted chitosan as shown in Figure 4.2. As can be seen, marked difference between two spectra could be observed, illustrating the successful graft copolymerization using only persulphate as redox initiator. It was thought that acrylamide was covalently bonded to chitosan backbone through ether linkage (C-O-C) which is characterized by the presence of absorption band at 1100 cm^{-1} . In addition, the strong band at 621 cm^{-1} is due to the vibration out of plane bending from amide group.

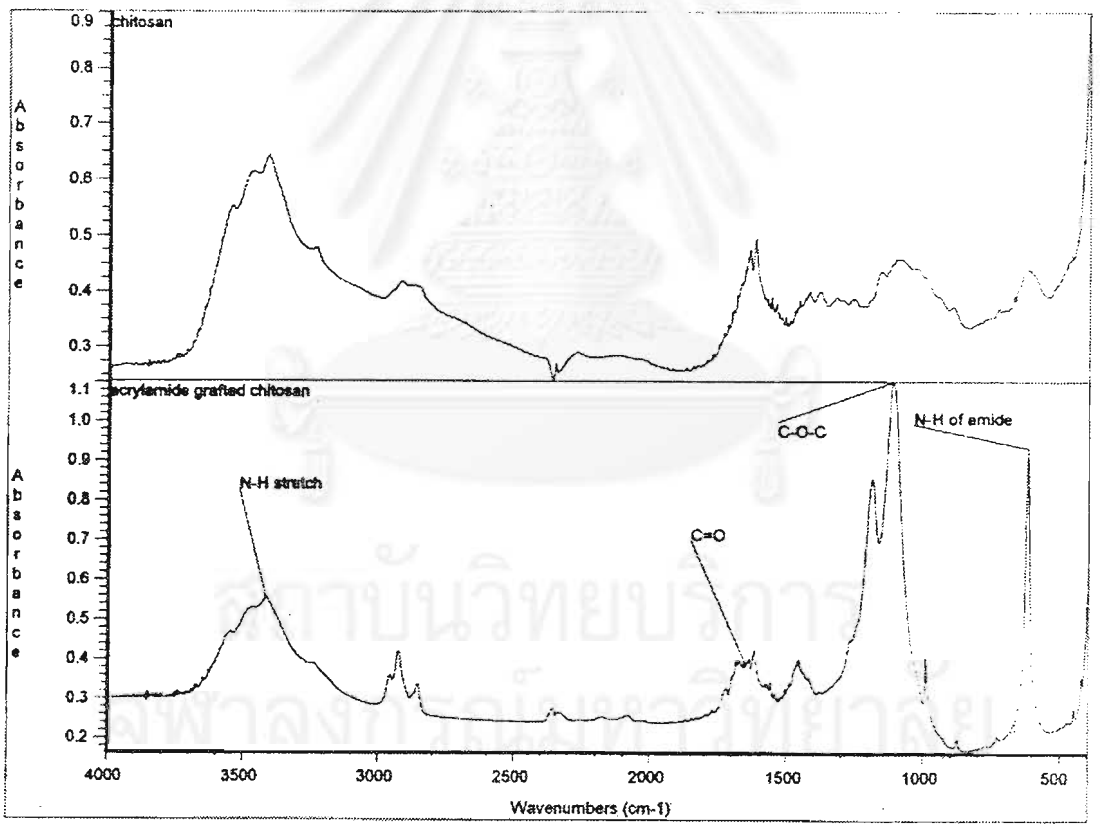


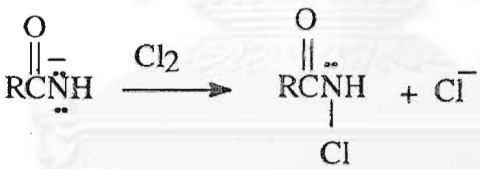
Figure 4.1 FT-IR spectra of virgin chitosan and acrylamide grafted chitosan

4.2.1 Hofmann Degradation reaction on acrylamide grafted chitosan

Polyacrylamide grafted chitosan obtained was subjected to Hofmann degradation in order to convert the amide groups into free amino groups. Hofmann degradation is simple and very useful indirect method of synthesizing primary amine, especially the synthesis of polyvinylamine due to no report on direct synthesizing method.

Hofmann degradation involves two intermediate steps; the action of bromine or chlorine on acid amide and subsequent alkaline hydrolysis. The mechanism can be written as follows:

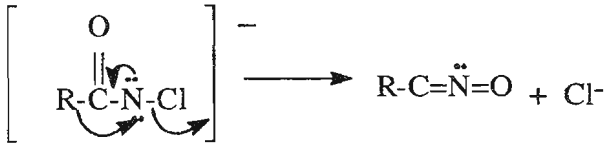
1. Chlorination of N



2. Extraction of H^+ by OH^-

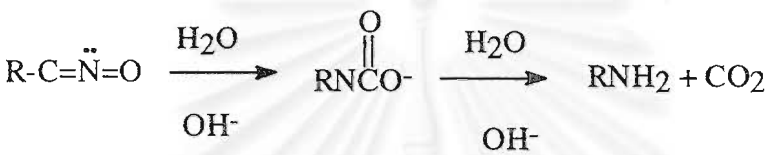
Proton is lost from N and unstable anion is formed.

3. Displacement of Cl^-



The product of rearrangement is isocyanate.

4. Hydrolysis of the isocyanate



Scheme 4.1 machanism of Hofmann degradation

addition, carboxylic acid regarded as side reaction's product always occurs during Hofmann degradation as a result of hydrolysis of original amide group. In this study acrylamide grafted product of chitosan with 1.20 g/1 g chitosan was selected to carried out the study on Hofmann degradation. FT-IR analysis was investigated to follow the Hofmann reaction. The result spectra of acrylamide – grafted chitosan after Hofmann degradation are show in Figure 4.3. Compared to untreated, the results indicate that Hofmann reaction of grafted chitosan introduced two functional groups which exhibited absorption band at 1450 cm^{-1} and 887 cm^{-1}. These bands could be assigned to the characteristic absorption of the carboxylate group(COO^- stretching asymmetry) and the primary amine (N-H wagging). In addition, the intensity of amide peak 621 cm^{-1} notably decreases when compared to ether linkage band 1100 cm^{-1} which was unchanged during reaction. The presence of these NH wagging and COO^- stretching cleary confirms that the pendant amide units on grafted chitosan was successfully converted into free amino groups and carboxylic groups by Hofmann degradation reaction. This is the

great advantage of Hofmann degradation reaction in that two functional groups could be introduced into chitosan by one step and simplest method.

The intensity ratios of total amide+amine(NH stretch 3400 cm^{-1})/ primary amine (NH wagging 887 cm^{-1}) peak with respect to various sodium hypochlorite concentrations were calculated and shown in Table 4.2 It was found that there was significantly different in intensity ratio, indicating that the concentration of sodium hypochlorite above 0.0322 mole grafted chitosan has great effect on amide – amine conversion.

Table 4.2 Effect of NaOCl concentration on the product of Hofmann Degradation treated grafted chitosan

Graft yield of chitosan (g)	Mole of NaOCl	Absorbance ratio 1100 cm^{-1} / 3400 cm^{-1} (ether/amino)	Absorbance ratio 887 cm^{-1} / 3400 cm^{-1} (N-H wagging of amine/N-Hstretch)
1.20	-	2	-
	0.0322	1.89	0.94
	0.0644	1.32	0.75
	0.0966	1.32	0.75

However, when compare C-O-C/NH stretch (amide+amine) peak ratio of each NaOCl concentration, ether linkage tends to gradually decrease with an increase in NaOCl concentration. As expected grafted chitosan could be degraded under Hofmann degradation reaction that can be reduced this effect by decreasing NaOCl.

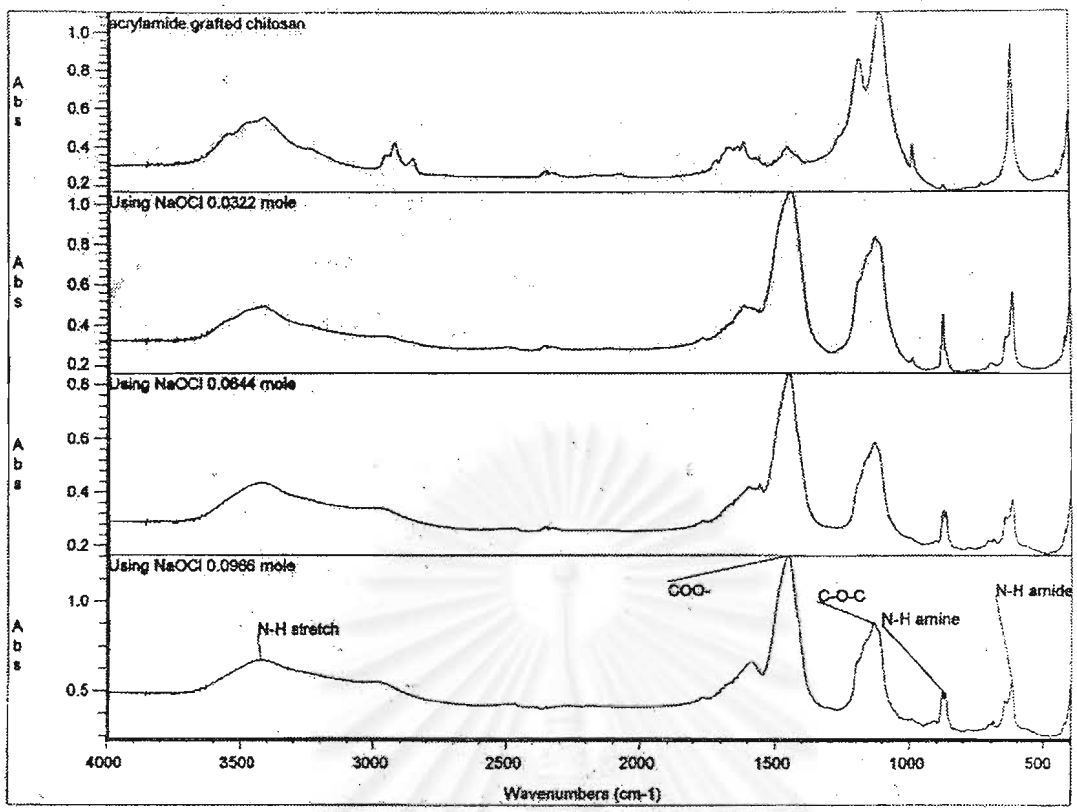


Figure 4.2 FT-IR spectra of acrylamide grafted chitosan, Hofmann Treated grafted chitosan at various NaOCl concentration.

4.3 Potentiometric Titration analysis

Potentiometric titration of acrylamide grafted chitosan and Hofmann treated grafted chitosan was carried out. The titration curves are shown in Figure 4.4. The titration curve of acrylamide grafted chitosan shows only one break point above pH 9 due to protonation of free amine group of virgin chitosan. The amount of free amine groups of virgin chitosan calculated at equilibrium is found to be 0.00015 mole. The titration curves of Hofmann treated acrylamide grafted chitosan show main inflection point at pH 9 and 6 which indicate the end points of protonation of primary amine and carboxylic acid, respectively. In all cases, the amounts of acid at equivalent point of protonated amine are higher than that of acrylamide grafted chitosan. Certainly, the increase in amount of free amine groups was introduced by

Hofmann reaction. The increased amount of free amine groups was determined by the difference between the amounts of acid used at equivalent points of Hofmann treated chitosan and blank. The effect of NaOCl/NaOH ratio on amide-amine conversion was studied. From Figure 4.4, the results show that varying the mixture of NaOCl/NaOH at different ratio brings about little change in the amount of increased primary amine.

The another inflection point at pH 6 was from the protonation of carboxylate anion. The formation of carboxylic acid pendent groups was due to the alkaline hydrolysis of original amide group. This reaction was considered as a side reaction but, in this case, was desirable. Since, the function of carboxylic group is well known as a chelating group for heavy metal ions. From titration curves, it can be seen that an increase in amount of NaOCl decreased the conversion of amide groups to carboxylic acid groups. Therefore, by varying NaOCl/NaOH ratio, the balance between primary amine and carboxylic acid formation could be controlled. These finding results was in good agreement with previous works.[17]

From FT-IR and potentiometric titration results, it leads to conclusion that the product of Hofmann degradation of polyacrylamide grafted chitosan was the copolymer which consisted of three repeating units; remaining unreacted acrylamide, vinylamine and acrylic acid. The chemical structure of modified chitosan may be written as follows:

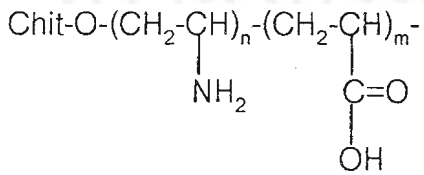


Figure 4.3 structure of vinylamine-co-acrylic acid grafted chitosan

4.3.1 Effect of NaOCl to NaOH ratio on Hofmann reaction

From Table 4.3, when decreasing NaOCl/NaOH mole ratio from 19.5/1 to 6.5/1, there was not different in an increase in free amine groups. Therefore, increasing the mole ratio of NaOCl/NaOH have not effect to increase the free amine. However, side effect due to the hydrolysis of acrylamide to carboxylic groups could occur under Hofmann degradation. The results showed that at 6.5/1 NaOCl/NaOH ratio also produces the highest carboxylic groups when compared to other ratios.

Figure 4.4 Titration curve of Hofmann treated grafted chitosan: effect of NaOCl/NaOH mole ratio, 2-3 °C, 60 minutes, using 1.2 g graft yield of acrylamide grafted chitosan.

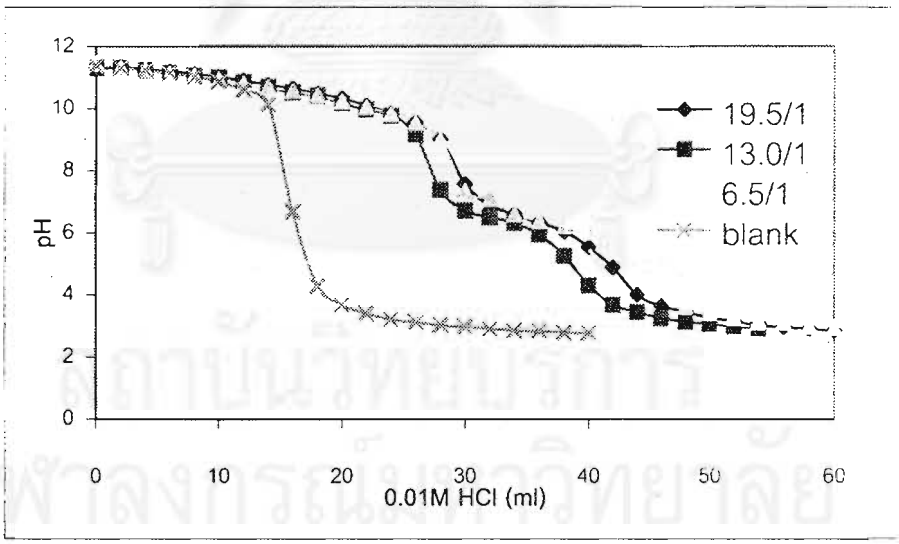


Table 4.3 Effect of NaOCl/NaOH on the amount of free amine and Carboxylic acid produced.

NaOCl/NaOH mole ratio	Free amine(mole)	Carboxylic acid(mole)
6.5/1	1.25×10^{-4}	1.35×10^{-4}
13/1	1.0×10^{-4}	1.13×10^{-4}
19.5/1	1.25×10^{-4}	1.17×10^{-4}

4.3.2 Effect of time on Hofmann reaction

Titration curve of Hofmann treated grafted chitosan at various reaction times is shown in Figure 4.5. The calculated amounts of free amine and carboxylic acid are given in Table 4.4.

The insignificant difference in the titration profiles at various reaction times indicates that the Hofmann degradation reaction was very fast. This leads to believe that the effective amide-amine conversion was obtained earily at the beginning of reaction. Disadvantageously, longer reaction time was not preferable since it allowed the side reactions such as hydrolysis and depolymerization of chitosan predominant. Hence, as low as 15 minutes of reaction time might be enough to achieve good yield of conversion.

Figure 4.5 Titration curve of Hofmann treated grafted chitosan at Various reaction time, 2-3 °C, NaOCl/NaOH 6.5/1 Using 1.50 g graft yield acrylamide grafted chitosan.

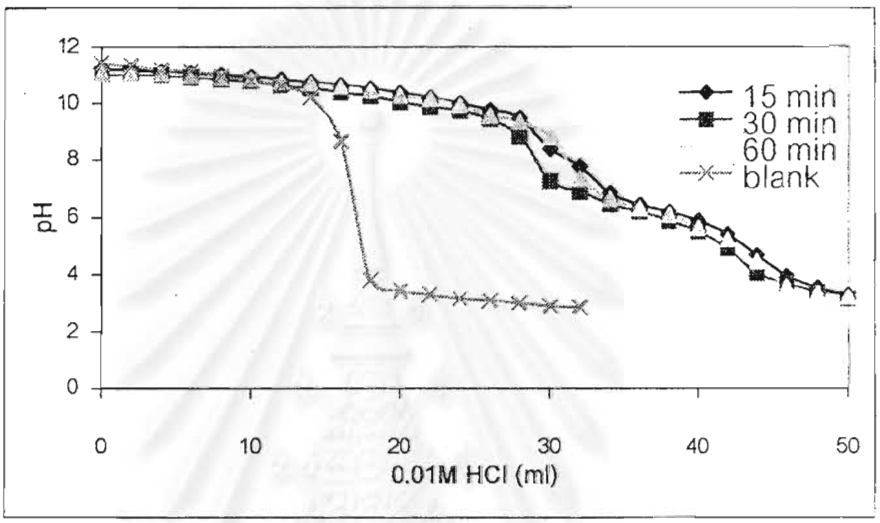


Table 4.4 Effect of time on the generation of free amine and carboxylic acid

Time(minutes)	Amount of free amine (mole)	Amount of carboxylic acid (mole)
15	1.3×10^{-4}	1.34×10^{-4}
30	1.08×10^{-4}	1.48×10^{-4}
60	1.46×10^{-4}	1.27×10^{-4}

4.3.3 Effect of temperature on Hofmann reaction

Hofmann degradation reaction of grafted chitosan was carried out at 2-3 °C and 30 °C for 15 minutes. The amide – amine conversion was quantitatively measured by potentiometric titration. The results was graphically

illustrated in Figure 4.6 and presented in Table 4.5. It is clearly seen that there is no difference in the titration profile, indicating that temperature had no effect on Hofmann degradation reaction. This may imply that the rate of reaction was so fast that the degree of conversion was independent with temperature. However, it is recommended to keep reaction temperature low to minimize degradation.

Figure 4.6 Effect of temperature on the amide – amine conversion Using 1.60 g graft yieldacrylamide grafted chitosan , NaOCl/NaOH 3.25/1, 15 minutes.

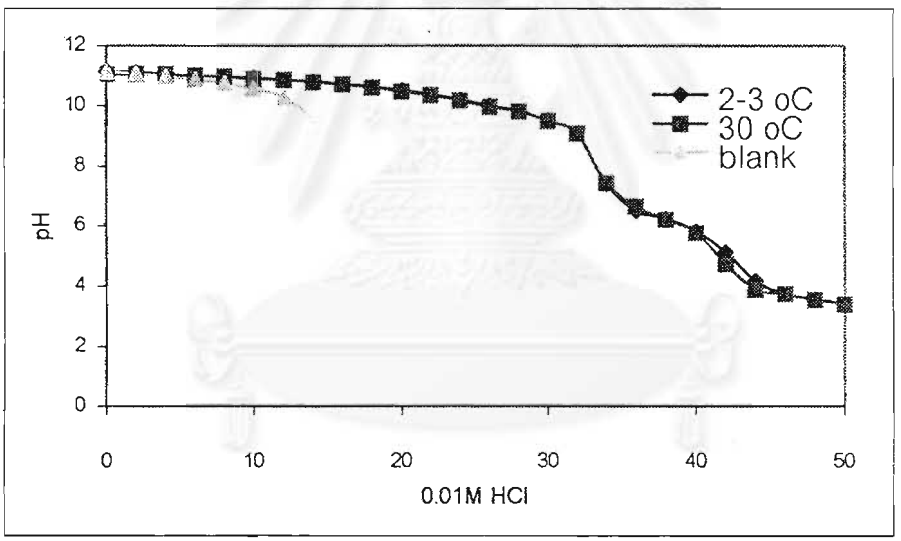


Table 4.5 Dependence of amount of free amine and carboxylic acid groups On temperature, 1.6 g graft yield

Temperature(°C)	Amount of free amine (mole)	Amount of carboxylic acid(mole)
2-3	1.76×10^{-4}	7.33×10^{-5}
30	1.76×10^{-4}	7.33×10^{-5}

4.4 Proton NMR

The spectrum of virgin chitosan, acrylamide grafted chitosan, and vinylmine-co-acrylic acid-co-chitosan was shown in Table 4.6

Table 4.6 ^{1}H-NMR spectrum of polymer[15]

Polymer	-C*H_2-	C*H-(COOH) or C*H-($CONH_2$)	C*H-NH_2
CS	-	-	3.8 and 3.2
CS-1	1.9-1.8	2.5	3.8
CS-2	1.4	2.0	3.8

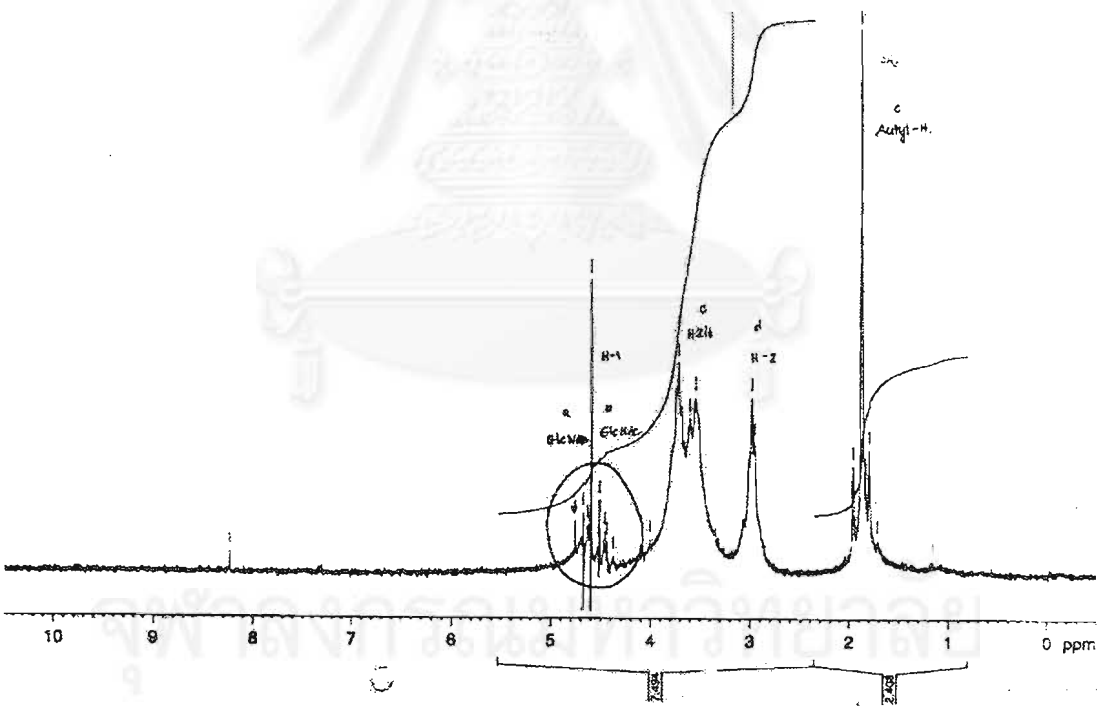


Figure 4.7 ^{1}HMR of Chitosan

^{1}H NMR spectra(JEOL 500) for virgin chitosan(CS), acrylamide grafted chitosan(CS-1), and vinylamine-co-acrylic acid-co-chitosan(CS-2) are shown

in Figure 4.7, 4.8, and 4.9, respectively. The important signals are summarized in Table 4.6. The characteristic of virgin chitosan is identified by the signal of acetyl proton at 1.8 ppm and amino proton at 3.8 and 3.2 ppm, respectively. For the CS-1, the evidence of successful grafted polymerization of acrylamide is confirmed by the presence of strong peak at 2.5 ppm which could be assiged to signals of polyacrylamide and about 1.9-1.8 ppm of methylene groups of polyacrylamide backbone.

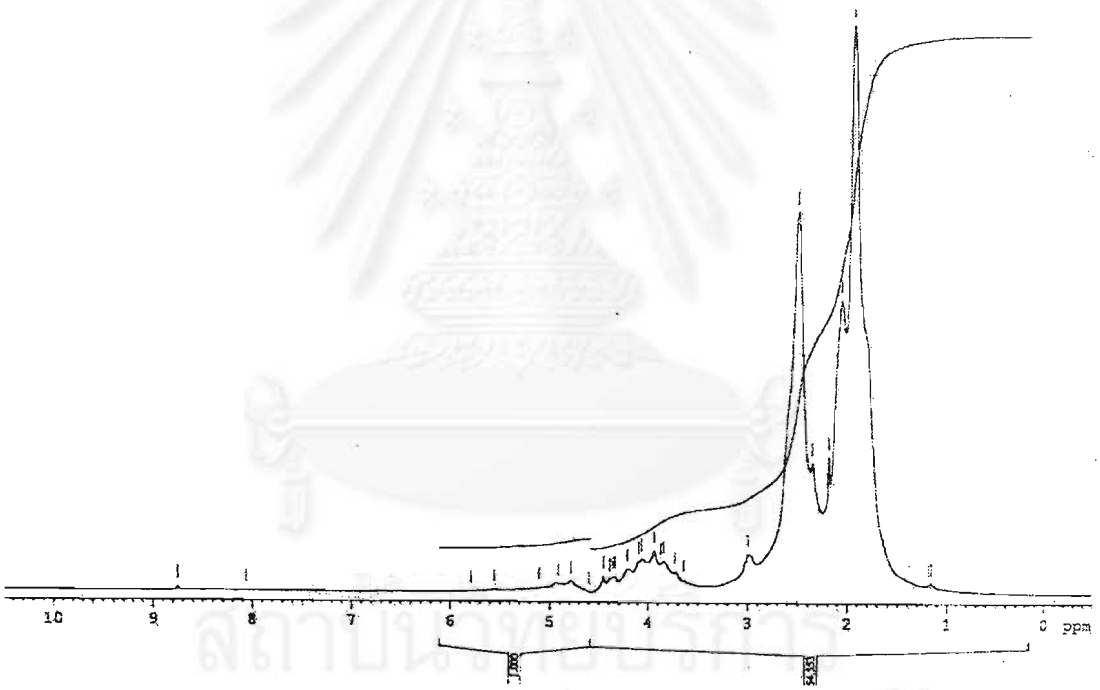


Figure 4.8 ^{1}HMR of Acrylamide grafted chitosan

The notable peaks of Hofmann treated grafted chitosan are found at 3.8 and 2.0 ppm which corresponds to the characteristic of vinylamine and acrylic acid[15]. NMR evidence clearly confirms that grafted polymerization did occur

and the resultant grafted chitosan was chemically converted to vinylamine-co-acrylic acid- co-chitosan by Hofmann reaction.

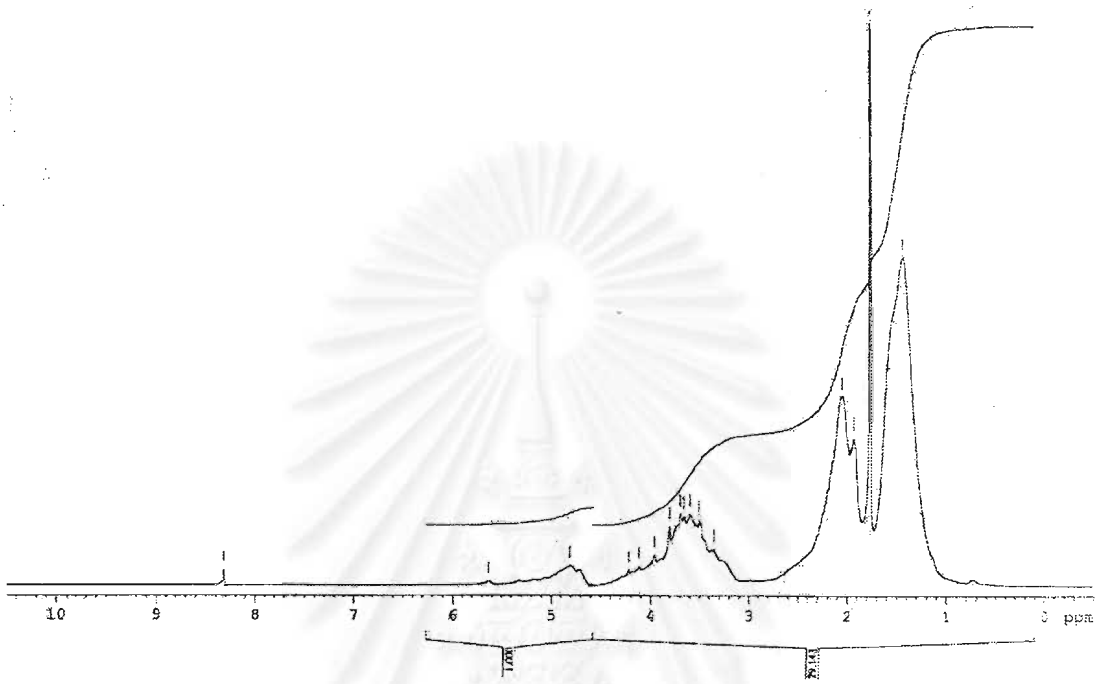


Figure 4.9 ^{1}HMR of Vinylamine-co-acrylic acid-co-chitosan

4.5 Thermal analysis

DSC thermogram of grafted chitosan was measured using Netzsch. The result is shown in Figure 4.10. It is clearly seen that the presence of grafted polyacrylamide is confirmed by strong endothermic peak[28] at 191.5 °C which is the melting point of polyacrylamide. On the other hand, melting point of chitosan does not show up due to its degradation at temperature above 200 °C before melting.

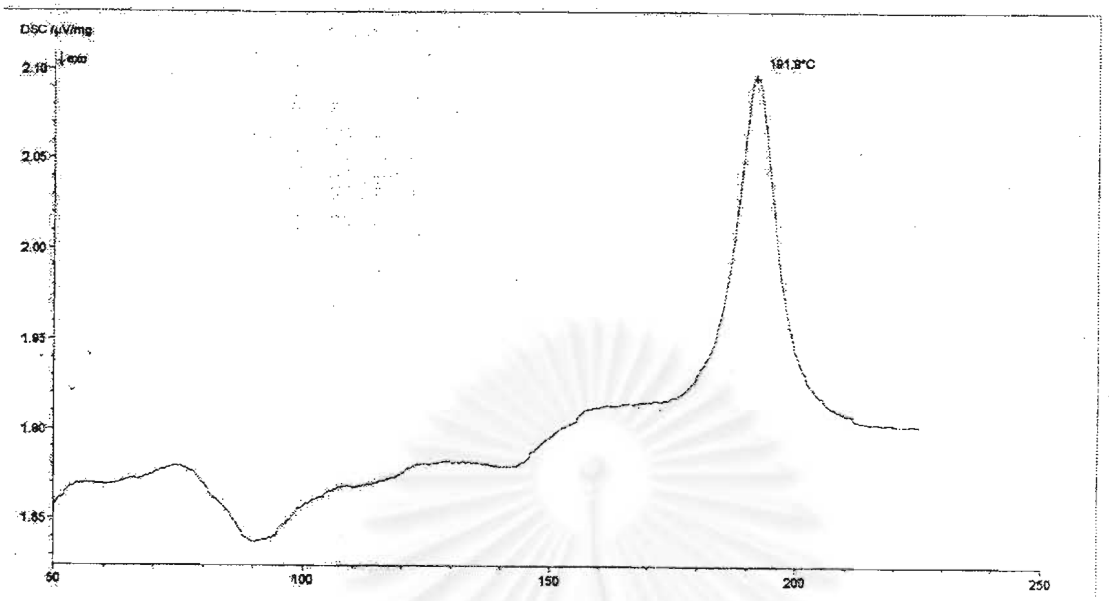


Figure 4.10 Acylamide grafted chitosan

4.6 Gel permeation chromatography

The molecular weight of chitosan after being modified was determined by GPC and the results are shown in Table 4.7. The results show that the weight average molecular weight of grafted chitosan was reduced substantially to about 2,000 - 3,000 from 800,000 of chitosan after Hofmann degradation. This implied that sodium hypochlorite the oxidizing agent in nature, readily broken down the polymer chain of chitosan. So, the molecular weight changes can be occurred under $K_2S_2O_8$ and NaOCl/NaOH conditions because of oxidation can be occurred under these conditions.

Table 4.7 The molecular weight of grafted chitosan after Hofmann degradation reaction: 2-3 °C; 60 minutes GPC column: ultralinear hydrogel, deionized water as mobile phase, 30 °C

Graft yield (g)	$K_2S_2O_8$ (mole)	NaOCl (mole)/NaOH 0.005 mole	Weight average molecular weight
1.20	7.4	0.0322	2,401
1.68	12.96	0.0322	3,988
	12.96	0.0644	2,408
	12.96	0.0966	3,669
1.38	18.5	0.0322	2,225
Virgin chitosan	-	-	800,000

Therefore, effect of NaOCl on degradation of chitosan without grafting was studied.The results from Table 4.8 clearly prove the degradation of chitosan backbone by sodium hypochlorite under Hofmann degradation reaction. This finding was truly the drawback of this reserch since it was not expected. However, under Hofmann degradation pr ocess, sodium hypochlorite may exhibit two roles: converting pendent amide to vinylamine and depolymerizing chitosan into low molecular weight chitosan oligomer. Therfore, it may not be interesting in exploiting the Hofmann reaction for functionalization of chitosan.

Table 4.8 The effect of sodium hypochlorite on the depolymerization of chitosan, 2-3 °C, 60 minutes, NaOH 0.0005 M
GPC column: ultralinear hydrogel, acetate buffer as mobile phase

NaOCl (mole)	Weight average molecular weight
None	800,000
0.00322	140,000
0.00644	100,000
0.00966	50,000

4.7 Evaluation of Flocculatability of Modified Chitosan

Chitosan itself is a natural polycationic polymer that, in its protonated form, is capable of forming insoluble complex with anionic dyes, the major dye classes containing in textile waste water. The capacity of the use of chitosan for removing textile dyes was reported[29]. The main mechanism of dye-chitosan complex formation is electrostatic interaction between positive charges on chitosan and anionic groups of dye molecules. Therfore, by introducing additional free amine groups into chitosan backbone, the performance of modified chitosan for dye removal will be enhanced, leading to reduce amount of virgin chitosan base for graft copolymerization requirement.

UV/VIS spectrophotometer was used to evaluate the removal of color by modified chitosan. A adding the of modified chitosan to color solution(Nylosan green 25, 1 g/L) followed by acidifying to pH 2, the precipitation of dyed particle occurred immediately, indicating the formation of insoluble dye-chitosan complex. The transmittance of color solution before and after

chitosan addition was measured. As can be seen, a significant decrease in transmittance after chitosan addition was obtained. The amount of precipitation dye was calculated based on difference between transmittance value before and after chitosan addition. Standard curve of dye concentration related with the absorbance is showed in Figure D.1(appendix D). The results are shown in Table 4.9

Table 4.9 The relationship between NaOCl concentration and dye removal capacity (dye concentration 1 g/L)
Dye acid green 25: 8 mg, pH 2

Sample	NaOCl (mole)	Free amine produced (mole)	Carboxylic Produced (mole)	Dye removal/ 50 mg of grafted chitosan
1.20 g graft yield	0.0322	1.25×10^{-4}	1.35×10^{-4}	4.67 mg
	0.0644	1.0×10^{-4}	1.13×10^{-4}	2.61 mg
	0.0966	1.25×10^{-4}	1.17×10^{-4}	2.46 mg
1.68 g *graft yield	0.0322	1.08×10^{-4}	1.30×10^{-4}	4.89 mg
	0.0644	9.14×10^{-4}	8.6×10^{-5}	3.34 mg
	0.0966	7.09×10^{-4}	9.7×10^{-5}	1.70 mg
Acrylamide grafted chitosan	0	0	0	0.5 mg
Virgin chitosan (Mw 800,000)	0	2.875×10^{-4}	0	8.0 mg
Virgin chitosan (Mw 50,000)	0.00966	2.875×10^{-4}	0	2.0 mg

* Figure B.1, appendix B

The results show that the molecular weight of chitosan dominates the flocculation capability of chitosan; the bigger the molecular weight the better flocculation capability. As stated earlier, the Hofmann reaction could introduce more free amine groups into chitosan. However, its molecular weight was decreased substantially. Therefore, comparison of performance of grafted chitosan should be based on the similar molecular weight of chitosan.

Taken chitosan of Mw 50,000 as an example, virgin chitosan alone could remove the anionic dye from the solution but at lowest capacity when compared with the modified chitosan. The color removal capacity of modified chitosan is dependent on the amount of increased free amine groups which was controlled by Hofmann reaction condition, especially NaOCl/NaOH ratio. The effect of increased amount of free amine groups calculated from titration curves, on color removal capability, are presented in Table 4.9. As expected, a decrease in amount of additional free amine groups on chitosan results in a decrease in the color removal capacity. It is important that in order to achieve the optimum performance of modified chitosan the highest amide-amine conversion must be obtained. In practice, however, it is difficult to achieve full conversion due to the competitive hydrolysis reaction as well as the presence of unreacted acrylamide. The latter perhaps was caused by the relatively low reactivity of typical polymer reaction compared to small molecule. Therefore, the reaction conditions such as time, temperature, and NaOCl and NaOH concentration need further investigation.

4.8 Efficiency of grafted chitosan on removal nickel ion

Table 4.10, showed the efficiency of vinylamine-co-acrylic acid grafted chitosan with different free amine groups in removing nickel ion. The results

showed that the removal of nickel ion was dependent on the ratio of free amine and carboxylic groups on the modified chitosan.

Table 4.10 nickel removal by grafted chitosan and virgin chitosan

Grafted chitosan: test at pH 8

Chitosan: test at pH 4

Nickel concentration 200 mg/10 ml

Sample	Amount of free amine(mole)	Amount of carboxylic acid (mole)	nickel ion removal
CS-2 no 1	1.0×10^{-4}	1.13×10^{-4}	107 mg
CS-2 no 2	1.5×10^{-4}	8.33×10^{-5}	116 mg
CS-2 no 3	1.76×10^{-4}	7.33×10^{-5}	119 mg
Virgin chitosan Mw 800,000	2.875×10^{-4}	0	87.5 mg
Virgin chitosan Mw 50,000	2.875×10^{-4}	0	89.4 mg

CS-2: vinylamine grafted chitosan

No 1: 1.68 g graft yield; NaOCl/NaOH 6.5/1; 2-3 °C; 60 minutes

No 2: 1.50 g graft yield; NaOCl/NaOH 6.5/1; 2-3 °C; 15 minutes

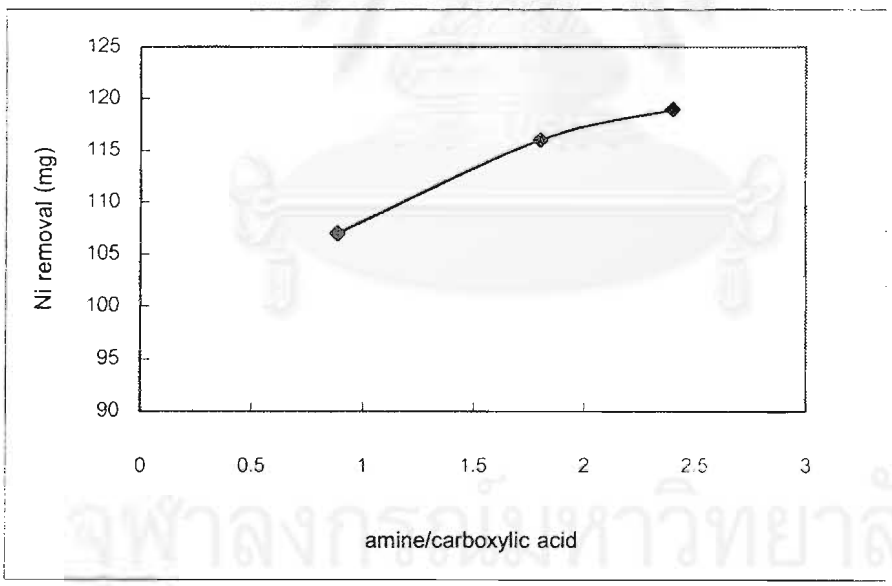
No 3: 1.50 g graft yield; NaOCl/NaOH 3.25/1; 2-3 °C; 15 minutes

It can be seen that, grafted chitosan exhibited better efficiency in removing nickel ion than virgin chitosan. This is because modified chitosan contained amine and carboxylic groups were known to be capable of excellent chelating groups to form insoluble complex with heavy metal[13,16]. In

the case of virgin chitosan, its ability of heavy metal ion removal is lower because of the absence of carboxylic groups in chitosan structure.

Figure 4.11 showed the relationship between nickel removal and amine/carboxylic acid ratio. It shows that nickel ion removal ability tends to increase with an increase in amine to carboxylic acid ratio, which may indicate that amine groups play an important role in removing heavy metal ion when compared with carboxylic groups.

Figure 4.11 amine/carboxylic acid ratio relate with nickel removal



CHAPTER V

CONCLUSIONS

The grafting acrylamide on chitosan by using potassium persulphate as a redox initiator was successful. FT-IR evidence showed that polyacrylamide was bonded to chitosan through ether linkage at 1100 cm^{-1}. In addition, the N-H out of plane of amide group at 621 cm^{-1} showed up strongly from the spectrum of polyacrylamide grafted chitosan. The amide pendant groups of polyacrylamide was then chemically converted to free amine groups by Hofmann degradation technique, yielding the polyvinylamine grafted chitosan. Again, FT-IR evindence illustrated the presence of vinylamine groups at 1580 cm^{-1}, 887 cm^{-1} , corresponding to N-H bending and N-H wagging of free amine groups, respectively. From FT-IR results, the carboxylic acid groups was found as a by product due to the hydrolysis of amide pendant group by alkali.

The quantitative measurement of free amine group was determined by potentiometric titration. The results showed that high point of amide -amine conversion was observed at NaOCl concentration 0.0322 mole and then leveled off with further increase in NaOCl concentration. Mean while, varying temperature between 2-3 $^{\circ}C$ and 30 $^{\circ}C$ had no significant difference in the degree of conversion.

The disadvantage of Hofmann degradation of grafted chitosan was that the depolymerization severely occurred during treatment even at low temperature, due to the strong oxidizing characteristic of NaOCl. As a result, the performance of rich amine content modified chitosan with low molecular weight could be greatly reduced, compared to the high molecular weight virgin chitosan and have the great effect on dye flocculation.

The efficiency of free amine-rich chitosan as a flocculating agent for color removal was evaluated. The results showed that the capacity of dye removal of modified chitosan was greatly dependent on its molecular weight as well as the amount of free amine groups. Therefore, the virgin chitosan was found to exhibit the highest capacity of anionic dye removal due to its high molecular weight. On the other hand, the modified chitosan was degraded substantially during Hofmann treatment. Hence, its color removal capacity was greatly reduced. However, the results showed that at equivalent molecular weights modified chitosan could remove more dyes from the solution when compared to the depolymerized chitosan. This proves that the introduction of free amino groups into chitosan backbone enhanced the capacity of color removal by ionic-ionic complexation formation.

However, the method of Hofmann degradation technique results in serious draw back in the introduction of free amine groups into chitosan. In order to increase the capacity of color removal of chitosan, it is important that the corporation of free amine group must be achieved without causing the depolymerization of chitosan backbone.

CHAPTER VI

RECOMMENDATION

1) Hofmann degradation technique not recommended further study on the introduction of free amine groups into chitosan due to the serious problem of chitosan depolymerization.
2) Graft polymerization with formamide type monomer such as poly(*N*-vinylformamide)is quite interesting since formamide pendant group could be easily hydrolyzed into free amine group at relatively mild condition.
3) The mechanism of free amine-rich chitosan in color removal should be intensively investigated.
4) The preparation of porous membrane from free amine-rich chitosan is interesting with an aim to exploit this friendly environment of material.

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Appendices

Appendix A

FT-IR spectrum

Chitosan

Functional groups	Wave number (cm^{-1})
N-H stretch of amino	3,400
C=O carbonyl	1,640

Acrylamide grafted chitosan

Functional groups	Wave number (cm^{-1})
N-H stretch of amino	3,400
C=O carbonyl of amide	1,640
C-O-C ether linkage	1,100
N-H out of plane of amide	621

Vinylamine-co-acrylic acid grafted chitosan

(Hofmann degradation treated)

Functional groups	Wave number (cm^{-1})
N-H stretch of amino	3,400
C-O-C ether linkage	1,100
N-H out of plane of amide	621
N-H wagging of amine	887
COO- stretch of carboxylate	1,454

Figure A.1 chitosan

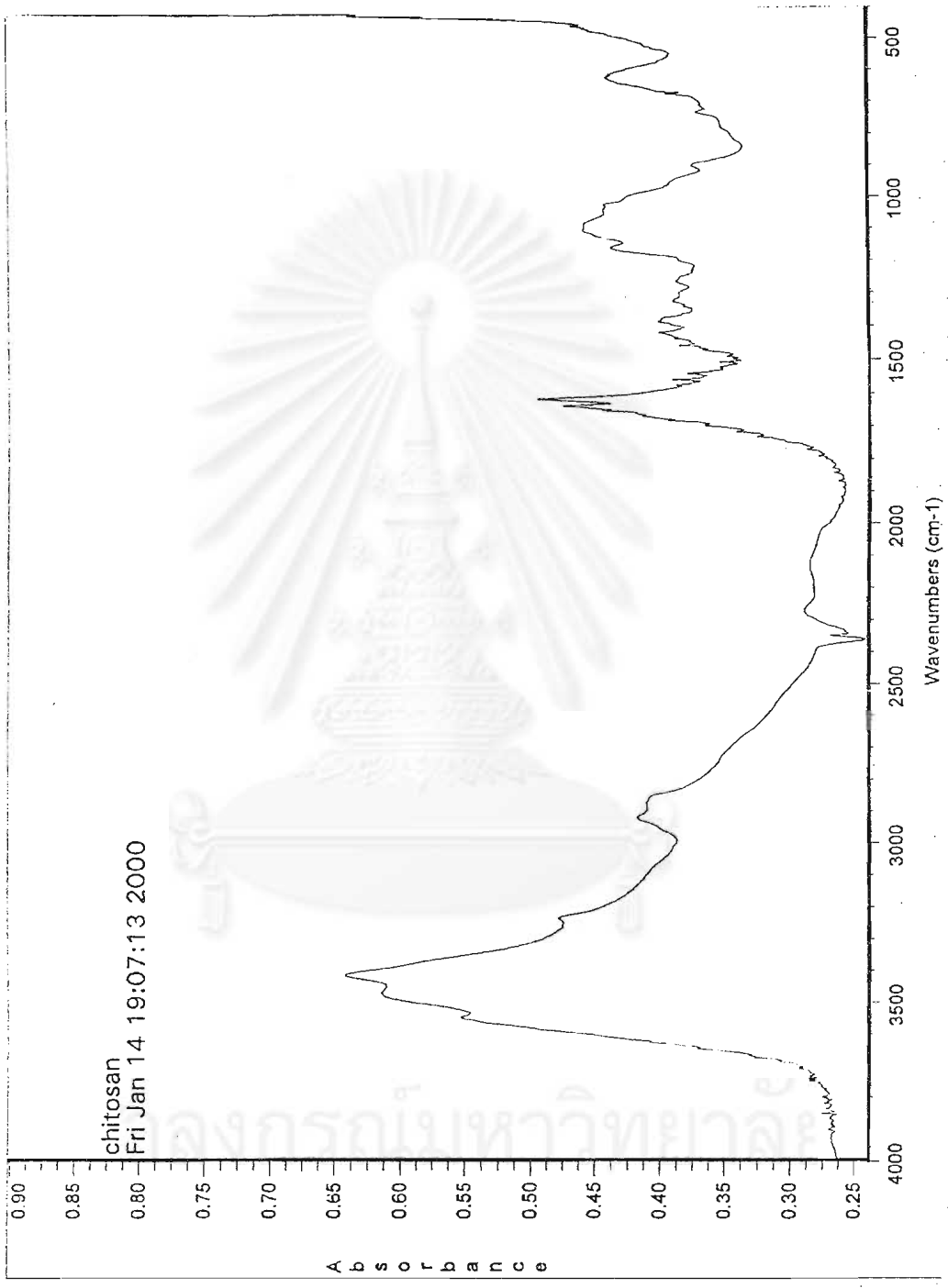


Figure A.2 1.2 g graft yield, acrylamide grafted chitosan

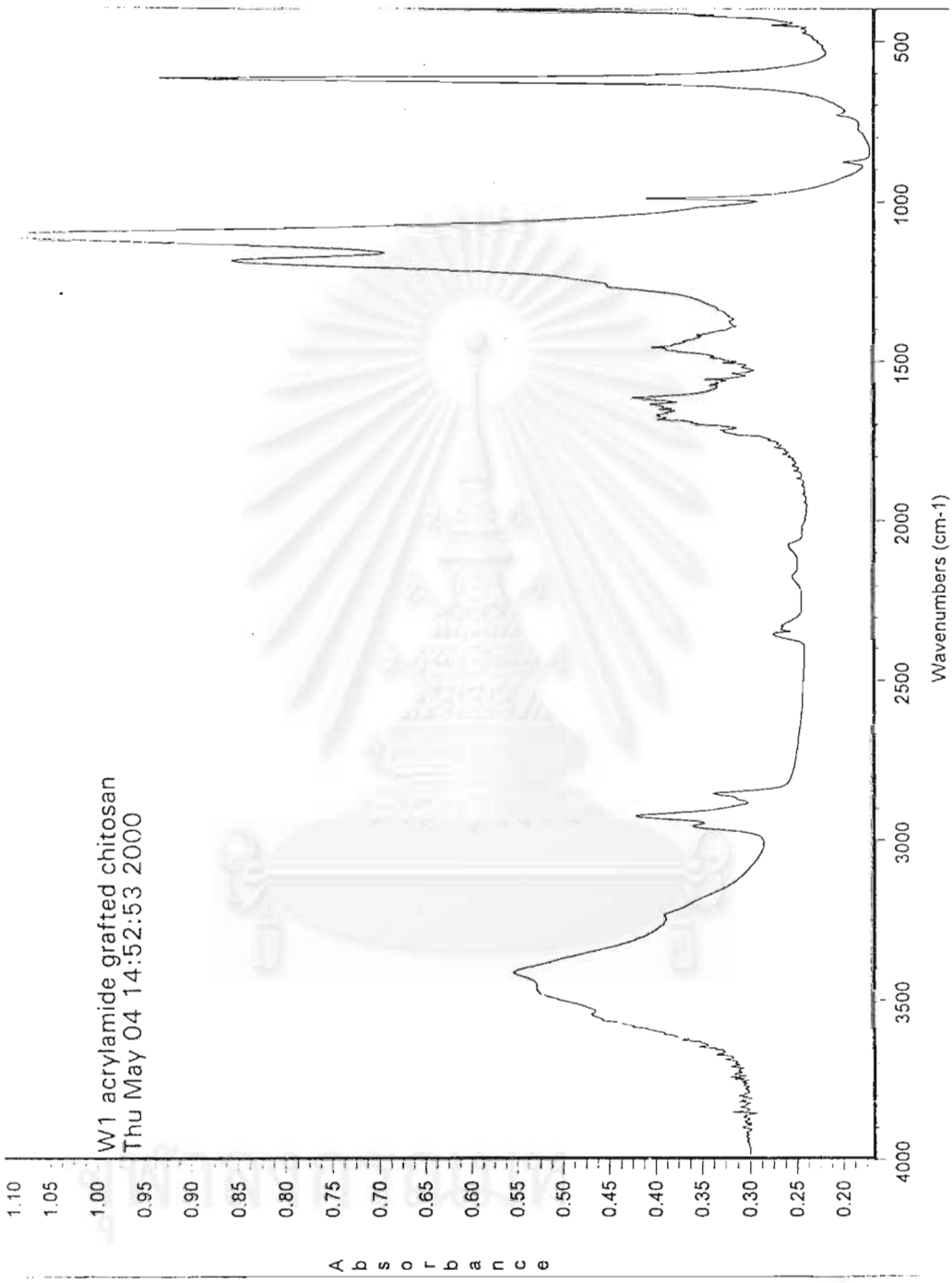


Figure A.3 1.2 g graft yield, acrylamide grafted chitosan

Hofmann degradation: NaOCl/NaOH 6.5/1 mole ratio

2-3 °C, 120 minutes

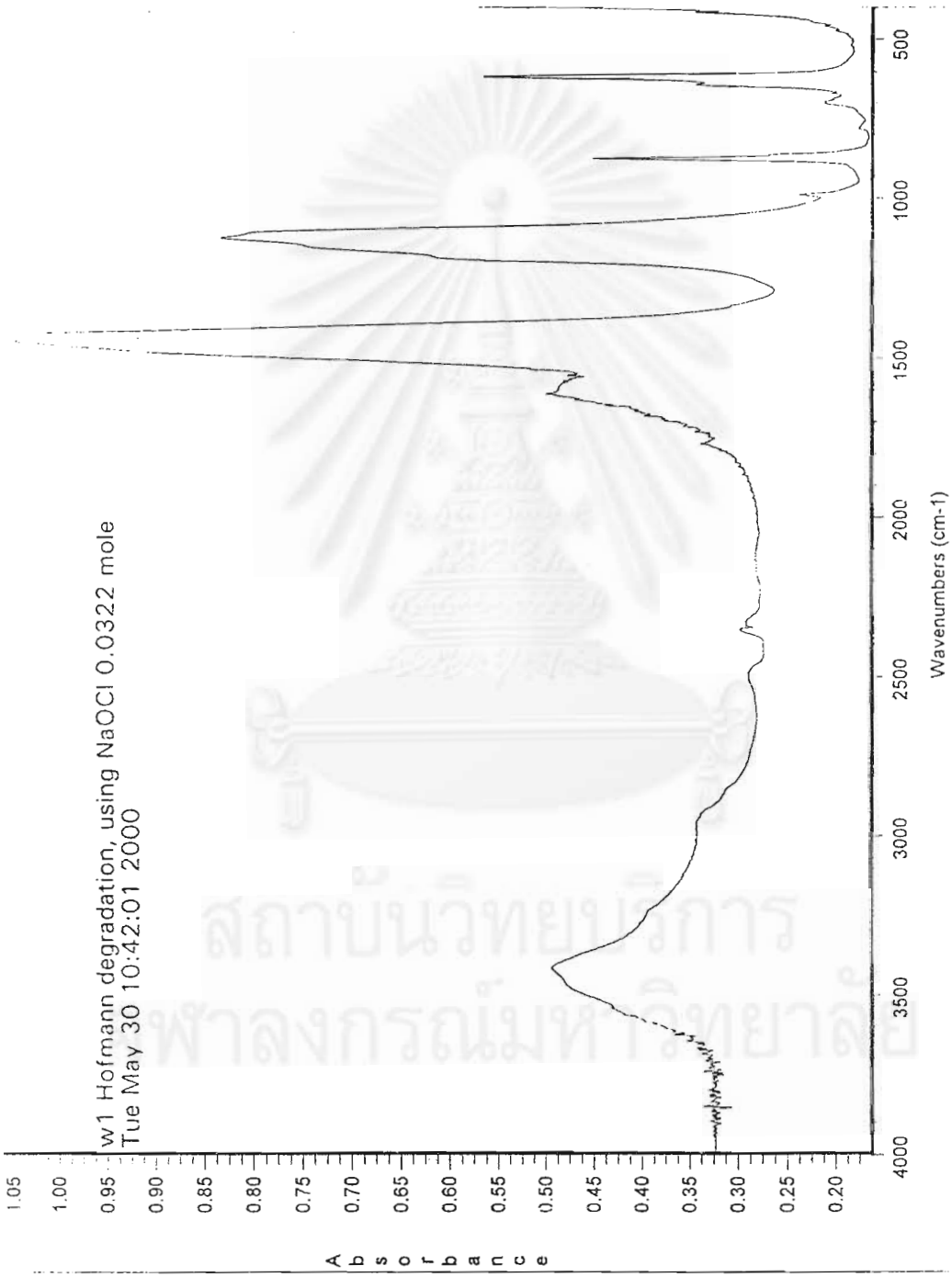


Figure A.4 1.2 g graft yield, acrylamide grafted chitosan

Hofmann degradation: NaOCl/NaOH 13/1 mole ratio

2-3 °C, 120 minutes

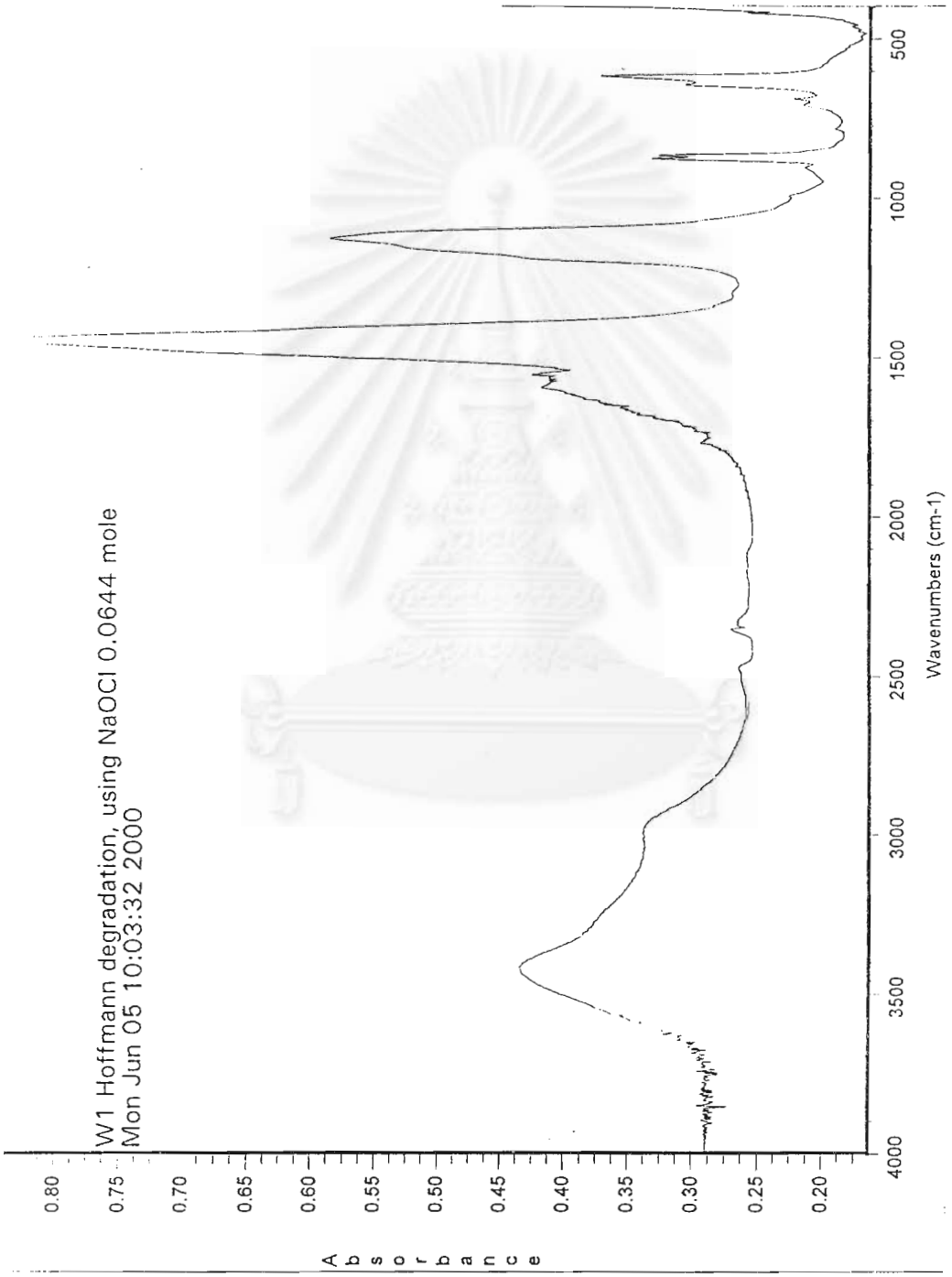


Figure A.5 1.2 g graft yield, acrylamide grafted chitosan

Hofmann degradation: NaOCl/NaOH 19.5/1 mole ratio

2-3 °C, 120 minutes

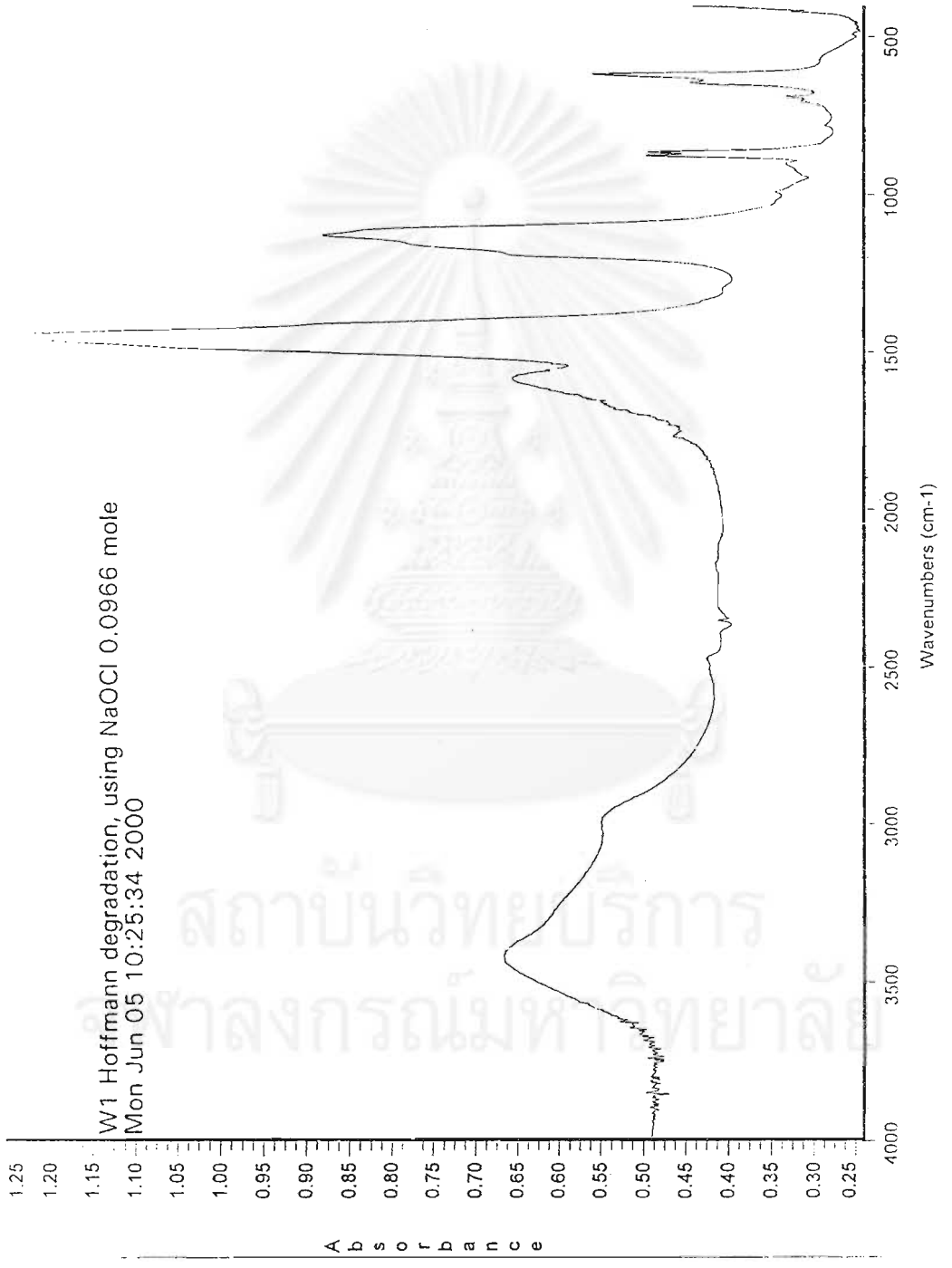


Figure A.6 1.68 g graft yield, acrylamide grafted chitosan

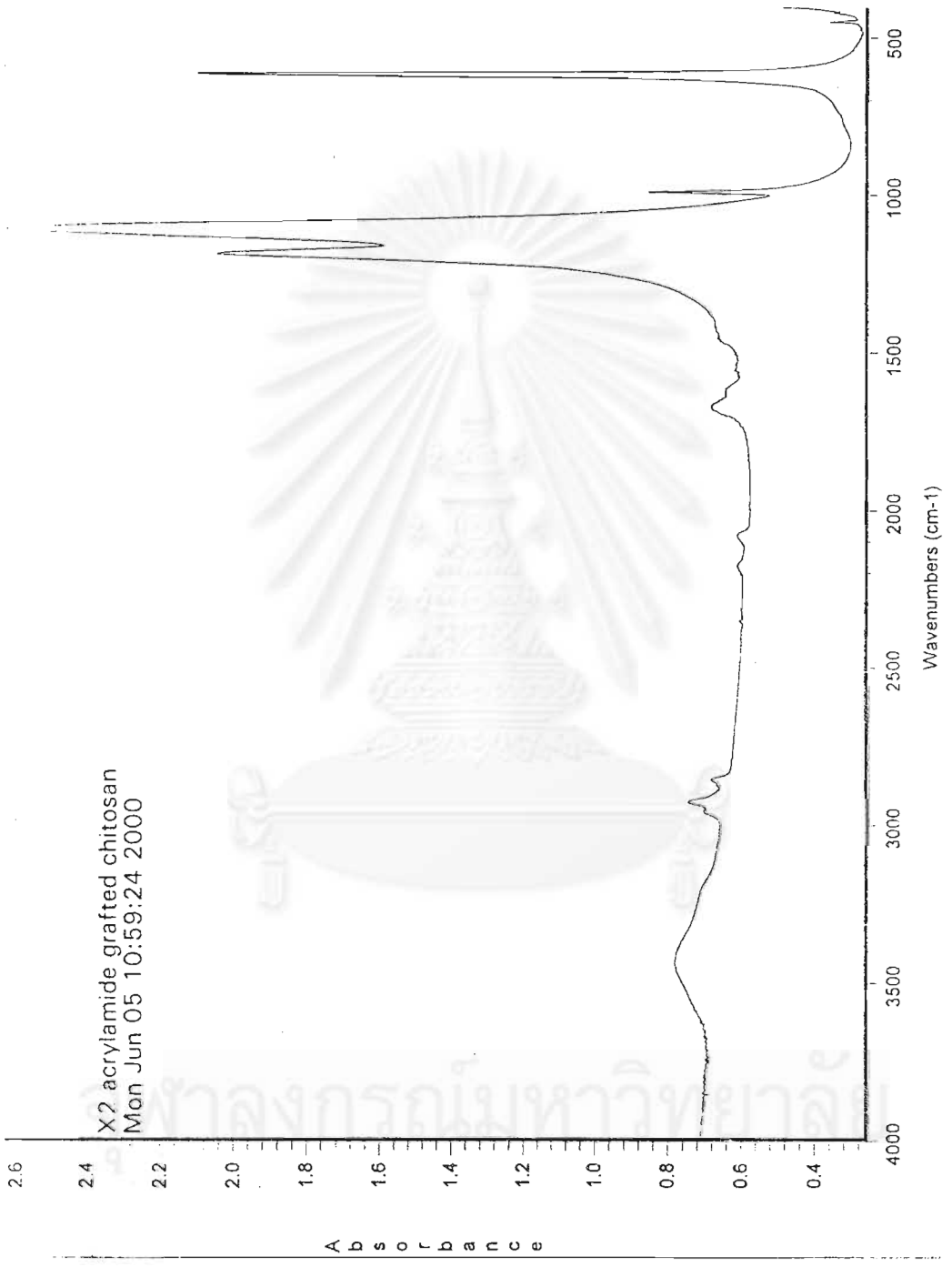


Figure A.7 1.68 g graft yield, acrylamide grafted chitosan

Hofmann degradation: NaOCl/NaOH 6.5/1 mole ratio

2-3 °C, 120 minutes

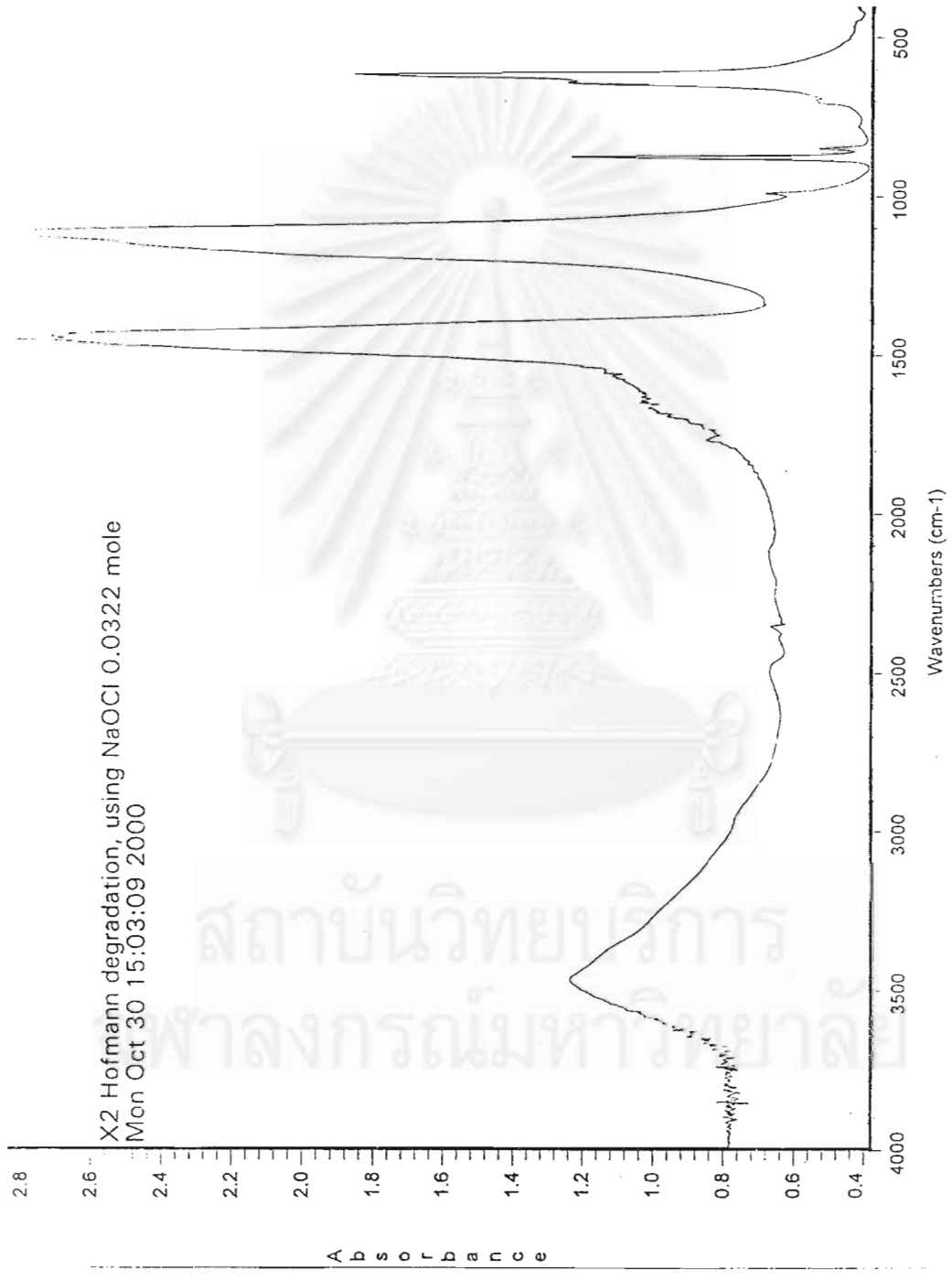


Figure A.8 1.68 g graft yield, acrylamide grafted chitosan

Hofmann degradation: NaOCl/NaOH 13.5/1 mole ratio

2-3 °C, 120 minutes

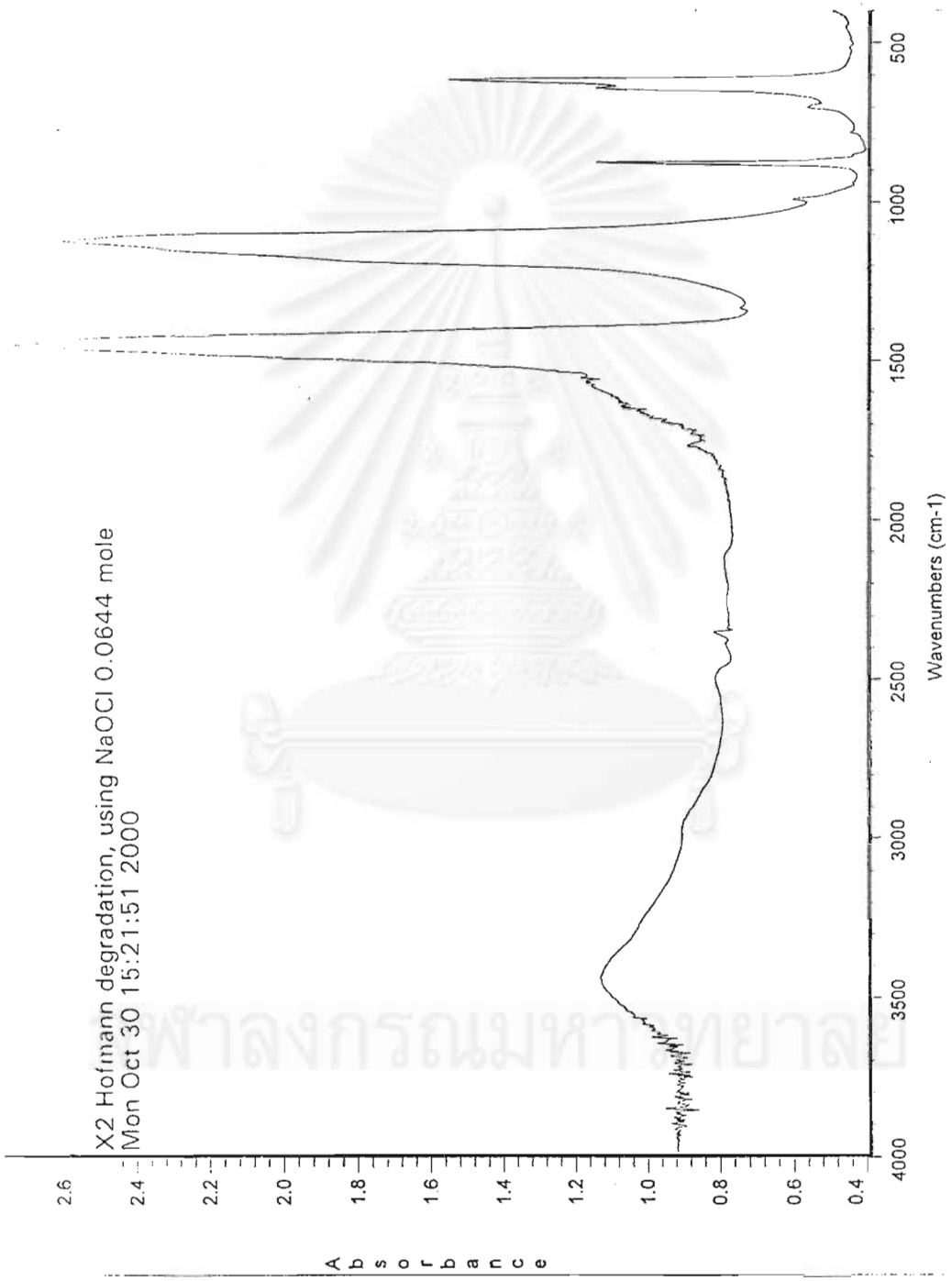


Figure A.9 1.68 g graft yield, acrylamide grafted chitosan

Hofmann degradation: NaOCl/NaOH 19.5/1 mole ratio

2-3 °C, 120 minutes

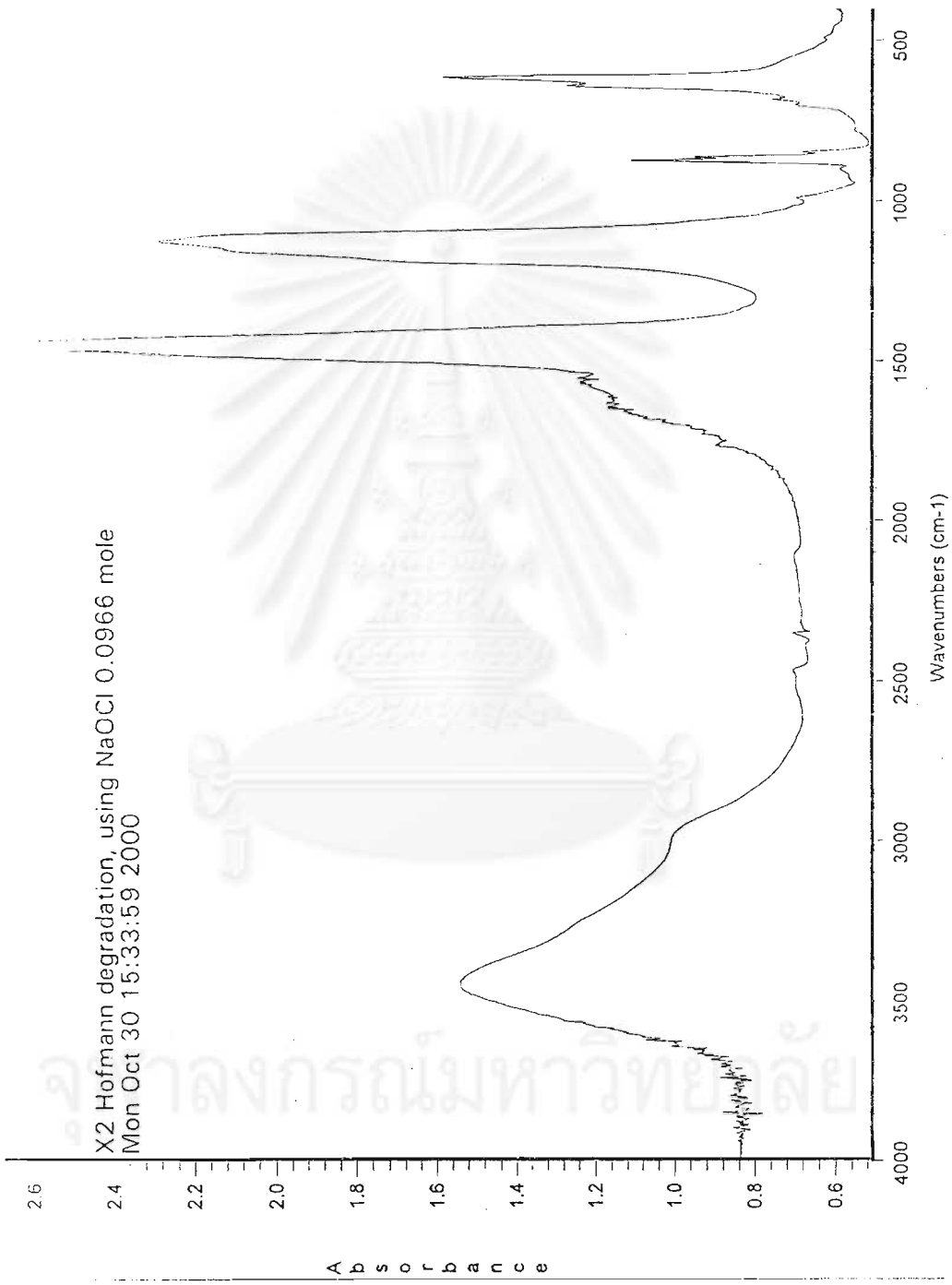


Figure A.10 1.38 g graft yield, acrylamide grafted chitosan

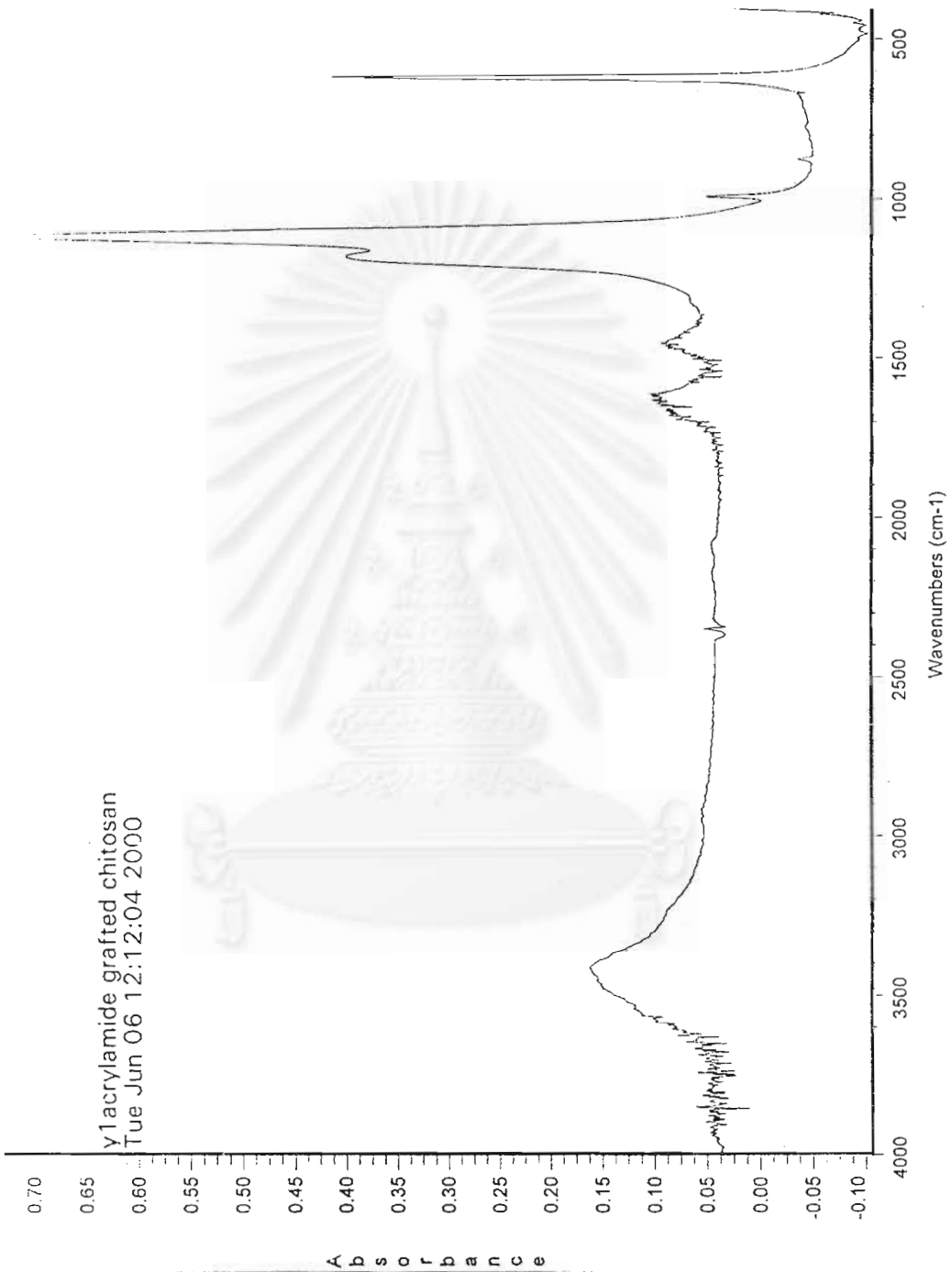


Figure A.11 1.38 g graft yield, acrylamide grafted chitosan
Hofmann degradation: NaOCl/NaOH 6.5/1 mole ratio
2-3 °C, 120 minutes

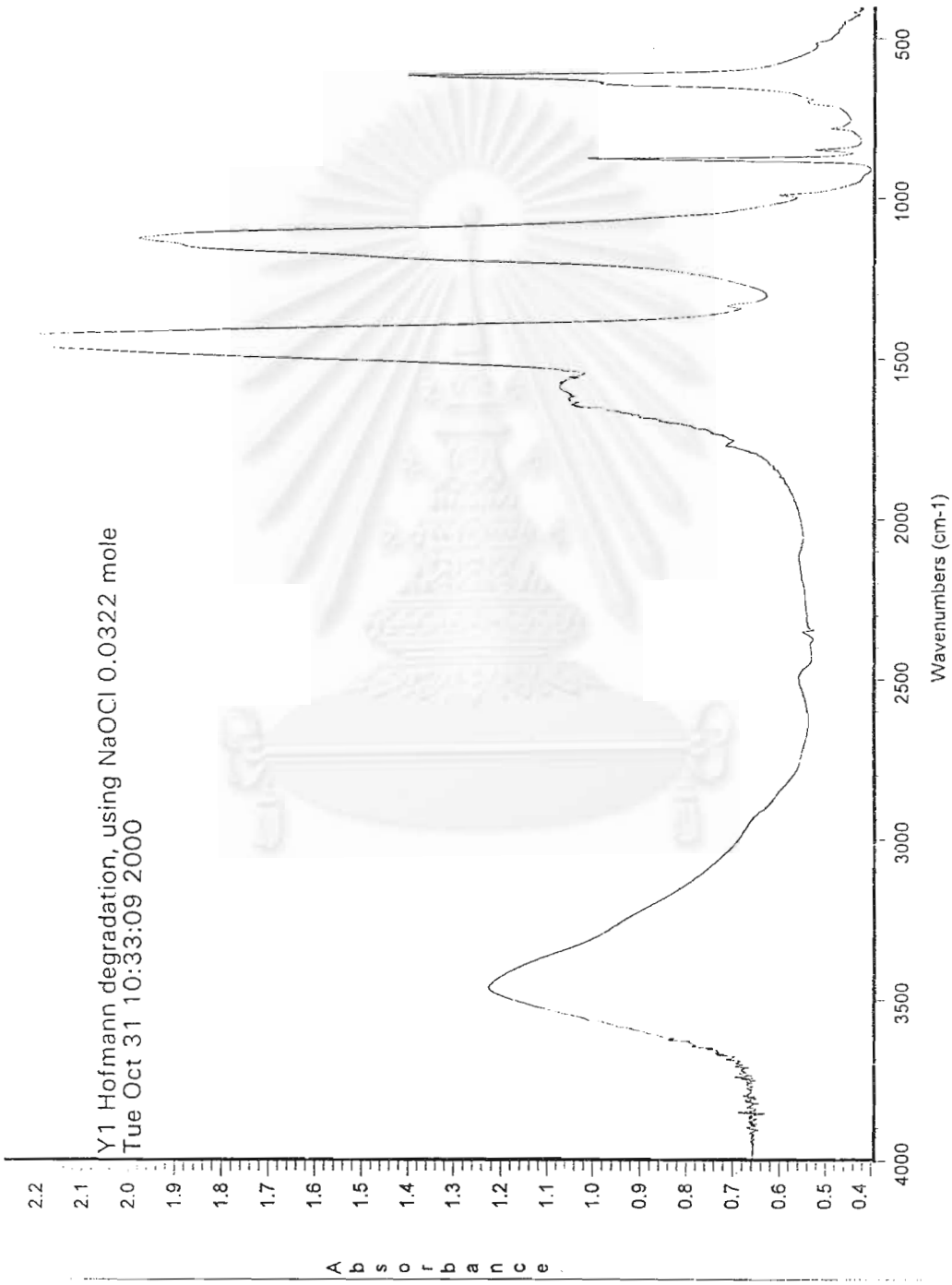


Figure A.12 1.38 g graft yield, acrylamide grafted chitosan
Hofmann degradation: NaOCl/NaOH 13/1 mole ratio
2-3 °C, 120 minutes

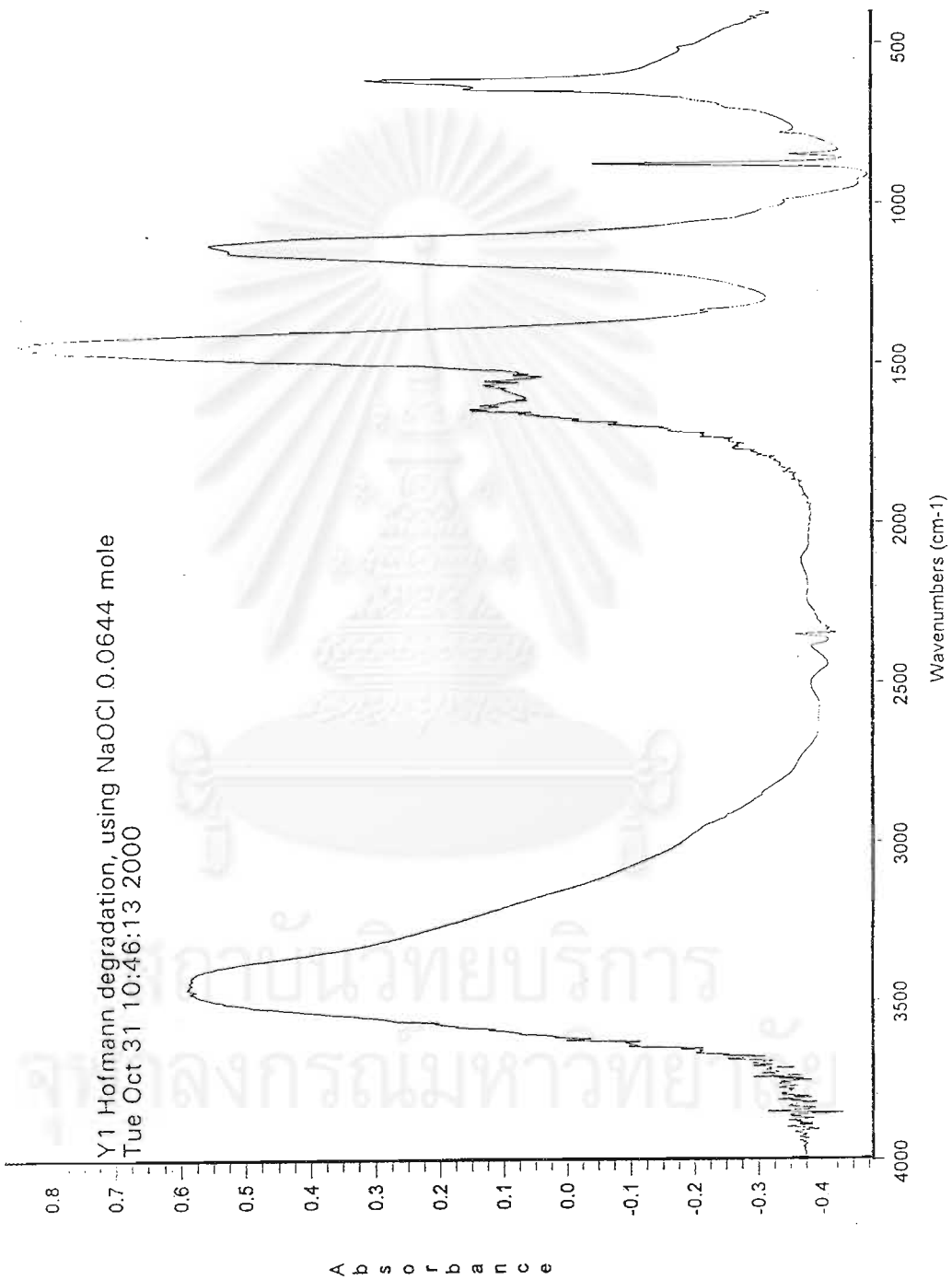
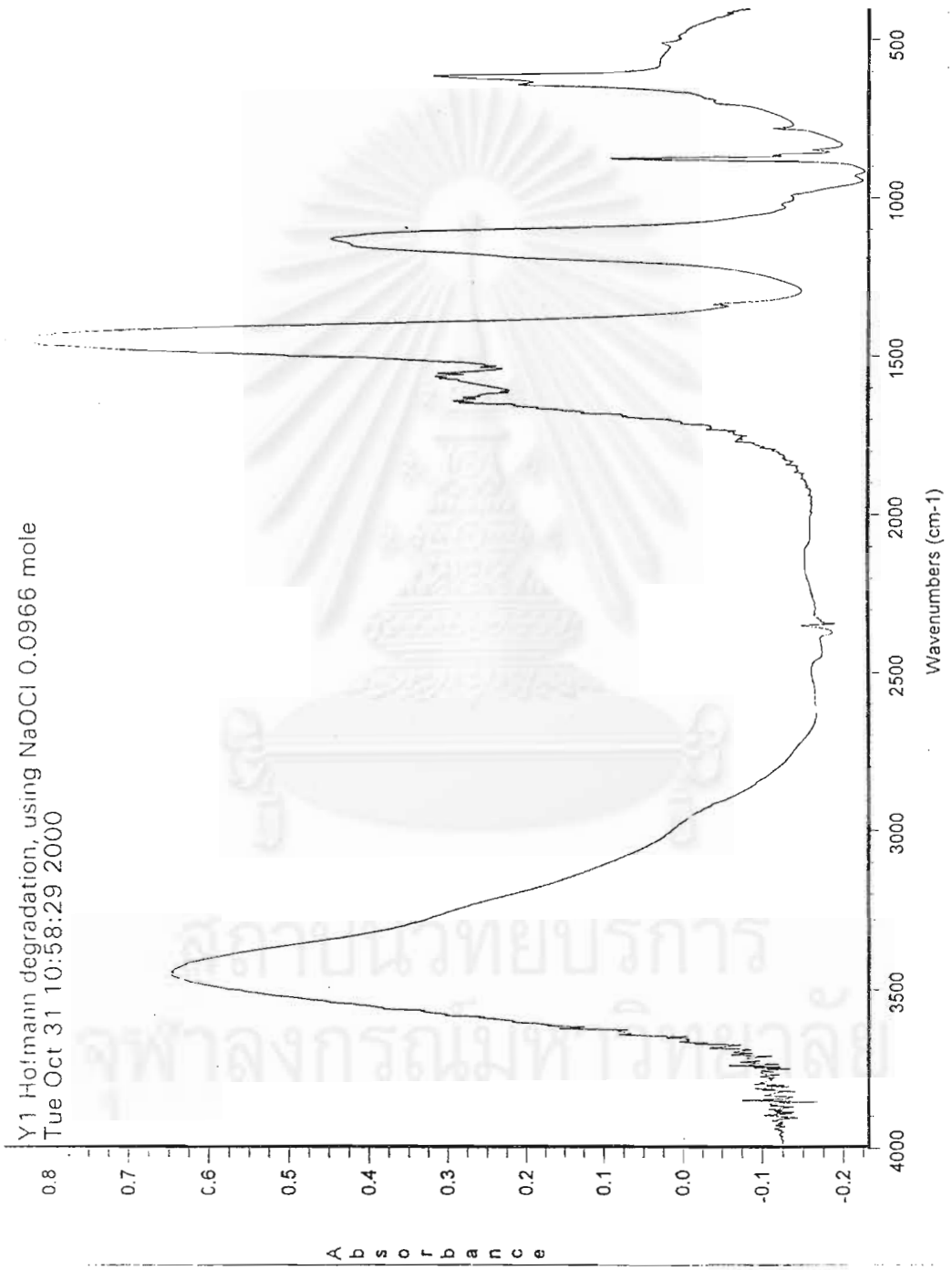


Figure A.13 1.38 g graft yield, acrylamide grafted chitosan
Hofmann degradation: NaOCl/NaOH 19.5/1 mole ratio
2-3 °C, 120 minutes



Appendix B

PotentiometricTitration

Effect of NaOCl on conversions

Figure B.1, 1.68 g graft yield acrylamide grafted chitosan,
1 hour, 2-3 °C

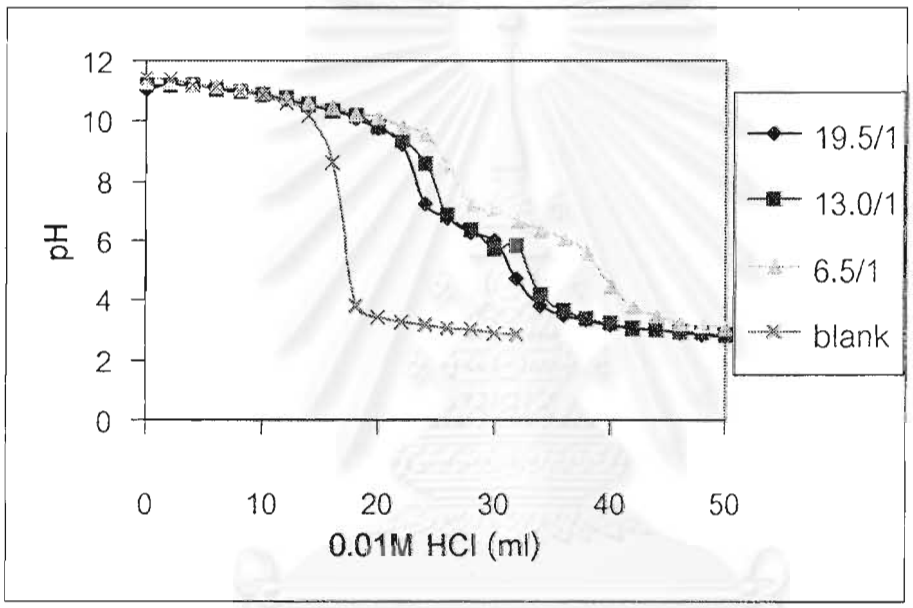


Figure B.2, 1.38 g graft yield acrylamide grafted chitosan 1 hour, 2-3 °C

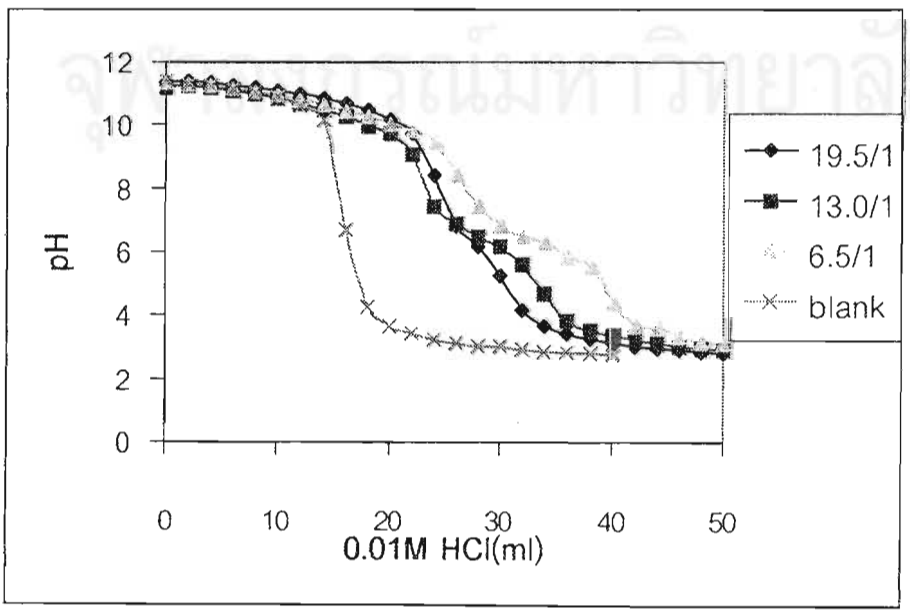
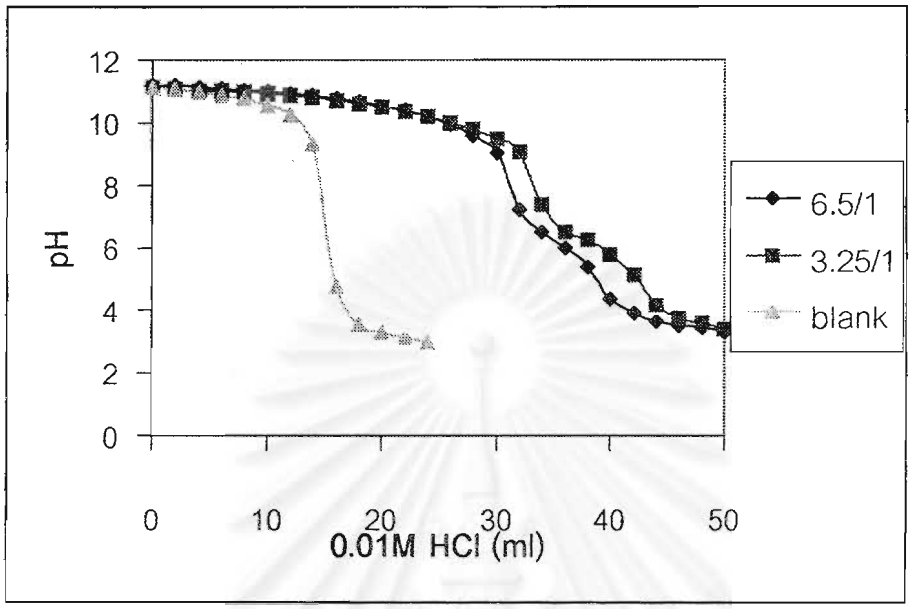


Figure B.3, acrylamide grafted chitosan, 15 minutes, 2-3 °C



Appendix C

Gel permeation chromatography

Figure C.1 1.20 g graft yield, acrylamide grafted chitosan

Using NaOCl 0.0322 mole(NaOCl/NaOH 6.5/1 mole ratio)

Polymer Laboratories
PL LogiCal GPC Software

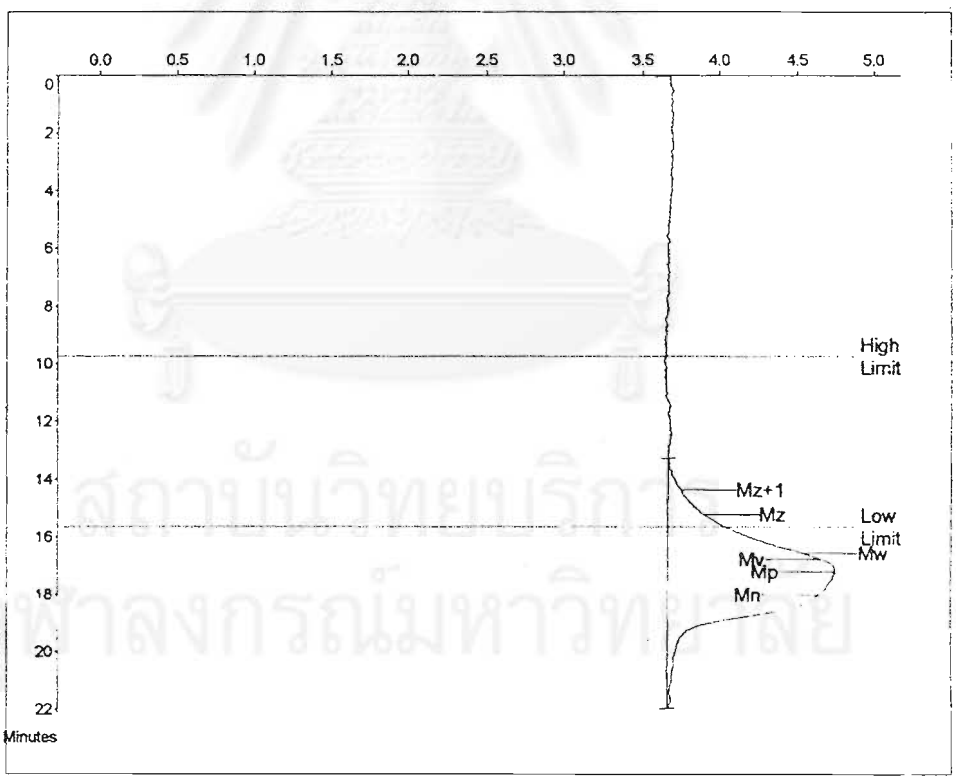
10:59 Thu Nov 30 2000

Unknown w12	T1116.005	Acquired :	15:03 Thu Nov 16 2000
		Operator	Temsiri Wangtaveesab
Concentration :		Detector :	RI
Injection Volume :		Temperature :	30 C
Solvent :	deionised water	Flow Rate :	0.600
Column Set :	Ultralinear hydrogel	Standards :	Polysaccharide

Method 12
Comments :

Calibration Using :	Narrow Standards			Curve Used :	1st Order Polynomial
Calibration Limits :	9.75	to 15.65	Mins	Last Calibrated :	Wed Jan 19 09:24:10 2000
Flow Rate Marker :		found at : Not Found		in Standards at :	0.00 Mins

Broad Peak Start : 13.27 End : 21.95 Mins



Molecular Weight Averages

Mp =	1287	Mz =	8647
Mn =	606	Mz+1 =	19264
Mw =	2401	Mv =	1955
Polydispersity =	3.960	Peak Area =	39242

Figure C.2 1.68 g graft yield, acrylamide grafted chitosan

Using NaOCl 0.0322 mole(NaOCl/NaOH 6.5/1 mole ratio)

Polymer Laboratories
PL LogiCal GPC Software

10:55 Thu Nov 30 2000

Unknown T1116.002
x22

Acquired : 13:03 Thu Nov 16 2000
Operator Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent : deionised water
Column Set : Ultralinear hydrogel

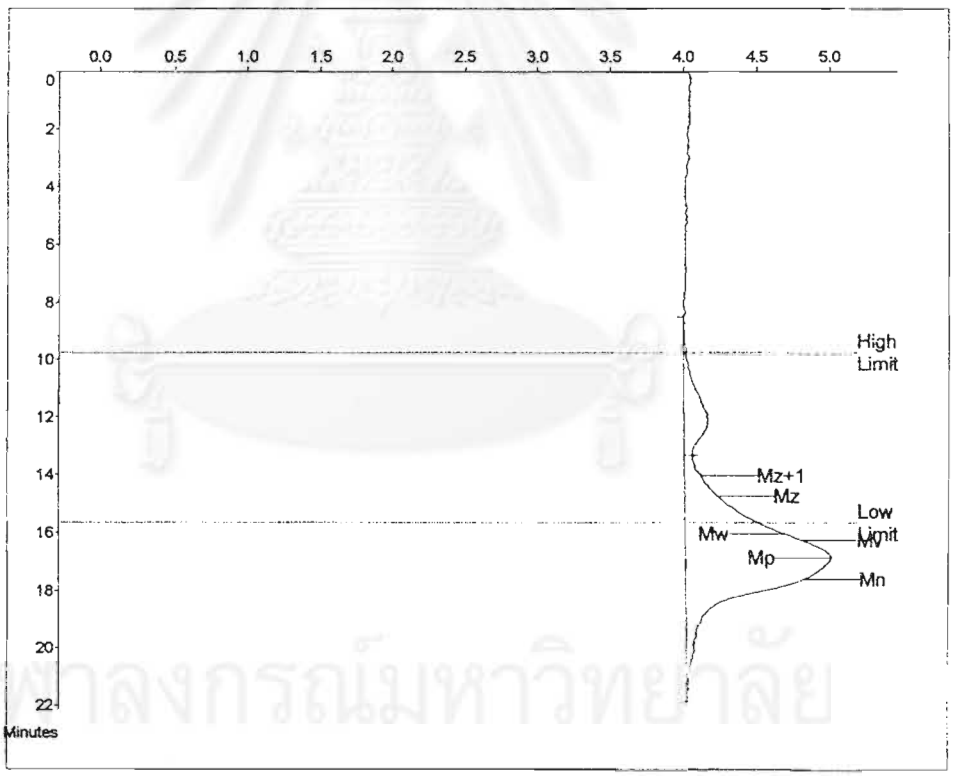
Detector : RI
Temperature : 30 C
Flow Rate : 0.600
Standards : Polysaccharide

Method 12
Comments :

Calibration Using : Narrow Standards
Calibration Limits : 9.75 to 15.65 Mins
Flow Rate Marker : found at : Not Found

Curve Used : 1st Order Polynomial
Last Calibrated : Wed Jan 19 09:24:10 2000
in Standards at : 0.00 Mins

Broad Peak Start : 13.32 End : 21.72 Mins



Molecular Weight Averages

Mp =	1824	Mz =	13572
Mn =	895	Mz+1 =	26342
Mw =	3988	Mv =	3242
Polydispersity =	4.456	Peak Area =	33194

Figure C.3 1.68 g graft yield, acrylamide grafted chitosan

Using NaOCl 0.0644 mole(NaOCl/NaOH 13/1 mole ratio)

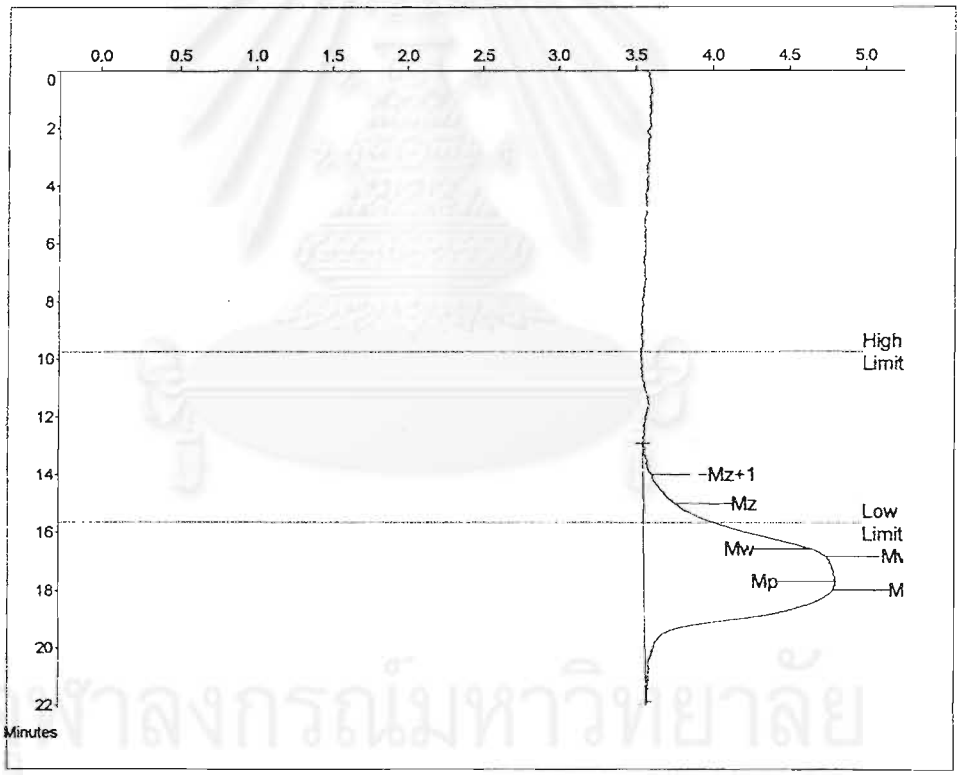
Polymer Laboratories
PL LogiCal GPC Software ***11:00 Thu Nov 30 2000***

Unknown	T1116.006	Acquired :	15:35 Thu Nov 16 2000
x24		Operator	Temsiri Wangtaveesab
Concentration :		Detector :	RI
Injection Volume :		Temperature :	30 C
Solvent :	deionised water	Flow Rate :	0.600
Column Set :	Ultralinear hydrogel	Standards :	Polysaccharide

Method 12
Comments :

Calibration Using :	Narrow Standards			Curve Used :	1st Order Polynomial
Calibration Limits :	9.75	to 15.65	Mins	Last Calibrated :	Wed Jan 19 09:24:10 2000
Flow Rate Marker :		found at : Not Found		in Standards at :	0.00 Mins

Broad Peak Start : 12.92 End : 21.87 Mins



Molecular Weight Averages

Mp =	839	Mz =	10601
Mn =	632	Mz+1 =	28055
Mw =	2408	Mv =	1921
Polydispersity =	3.808	Peak Area =	49323

Figure C.4 1.68 g graft yield, acrylamide grafted chitosan

Using NaOCl 0.0966 mole(NaOCl/NaOH 19.5/1 mole ratio)

Polymer Laboratories
PL LogiCal GPC Software

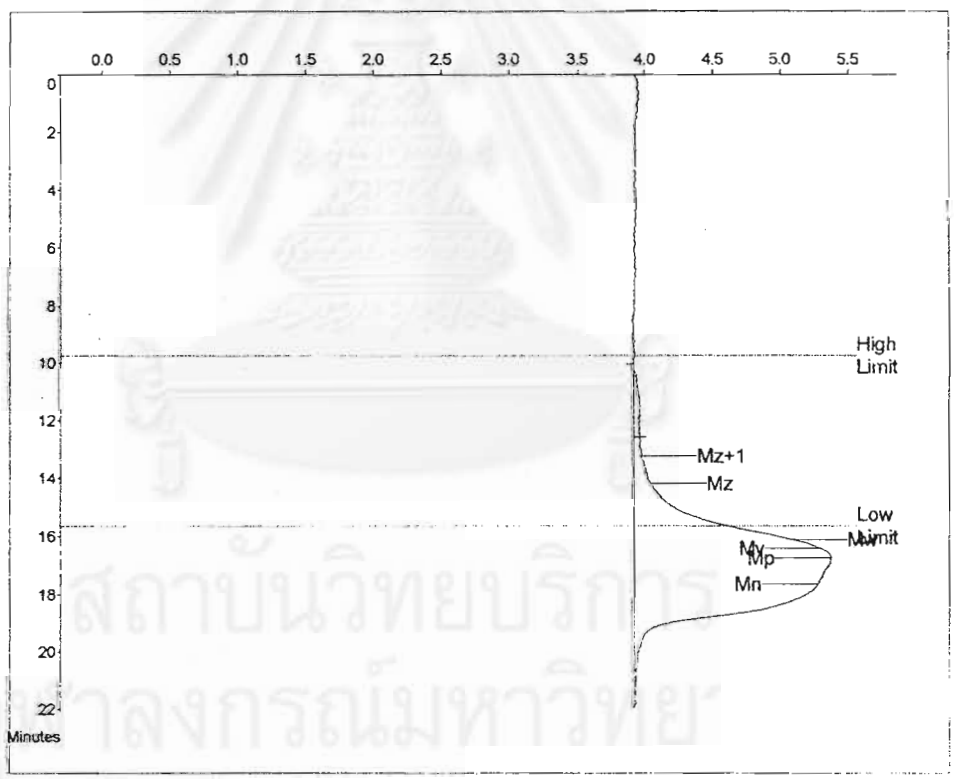
10:56 Thu Nov 30 2000

Unknown	T1116.003	Acquired :	13:41 Thu Nov 16 2000
x26		Operator	Temsiri Wangtaveesab
Concentration :		Detector :	RI
Injection Volume :		Temperature :	30 C
Solvent :	deionised water	Flow Rate :	0.600
Column Set :	Ultralinear hydrogel	Standards :	Polysaccharide

Method 12
Comments :

Calibration Using :	Narrow Standards			Curve Used :	1st Order Polynomial
Calibration Limits :	9.75	to 15.65	Mins	Last Calibrated :	Wed Jan 19 09:24:10 2000
Flow Rate Marker .		found at : Not Found		in Standards at :	0.00 Mins

Broad Peak Start : 12.58 End : 21.82 Mins



Molecular Weight Averages

Mp =	2006	Mz =	22900
Mn =	848	Mz+1 =	56898
Mw =	3669	Mv =	2773
Polydispersity =	4.326	Peak Area =	56134

Figure C.5.1.38 g graft yield, acrylamide grafted chitosan

Using NaOCl 0.0322 mole(NaOCl/NaOH 6.5/1 mole ratio)

Polymer Laboratories
PL LogiCal GPC Software

10:57 Thu Nov 30 2000

Unknown T1116.004
y12

Acquired : 14:29 Thu Nov 16 2000
Operator Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent : deionised water
Column Set : Ultralinear hydrogel

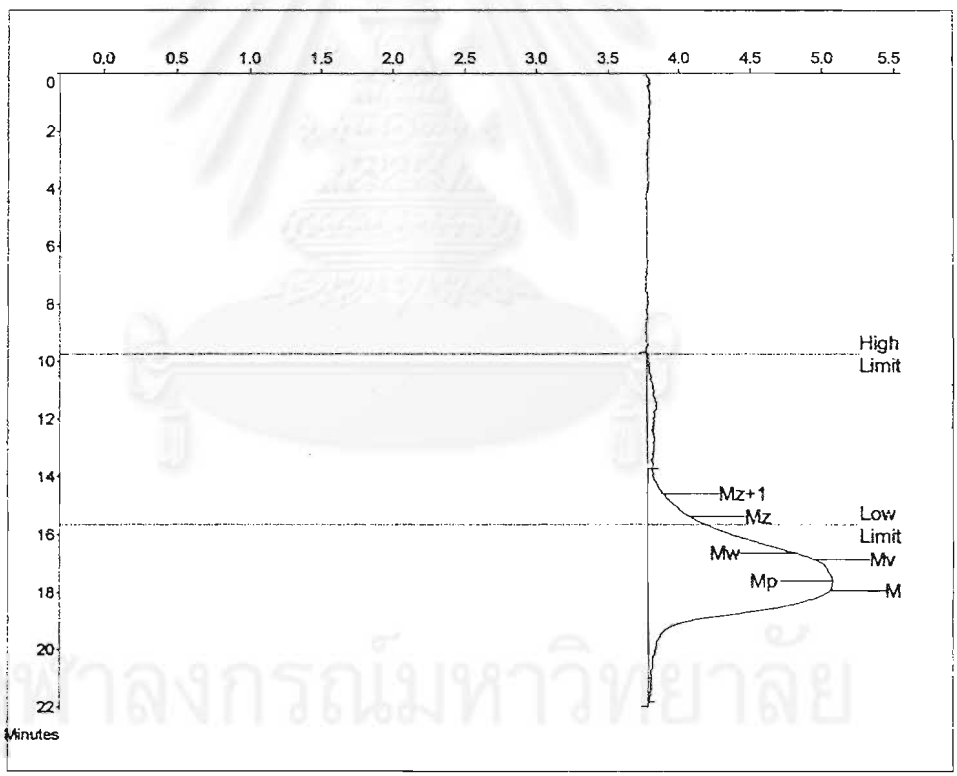
Detector : RI
Temperature : 30 C
Flow Rate : 0.600
Standards : Polysaccharide

Method 12
Comments :

Calibration Using : Narrow Standards
Calibration Limits : 9.75 to 15.65 Mins
Flow Rate Marker : found at : Not Found

Curve Used : 1st Order Polynomial
Last Calibrated : Wed Jan 19 09:24:10 2000
in Standards at : 0.00 Mins

Broad Peak Start : 13.72 End : 21.82 Mins



Molecular Weight Averages

Mp =	894	Mz =	7437
Mn =	653	Mz+1 =	15849
Mw =	2225	Mv =	1837
Polydispersity =	3.403	Peak Area =	45198

Figure C.5 virgin chitosan

Polymer Laboratories
PL LogiCal GPC Software

10:41 Fri Jan 19 2001

Unknown I0117.004
chitosan

Acquired : 13:49 Wed Jan 17 2001
Operator Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent : AcOH : NaOAc (1:1)
Column Set : Ultralinear hydrogel

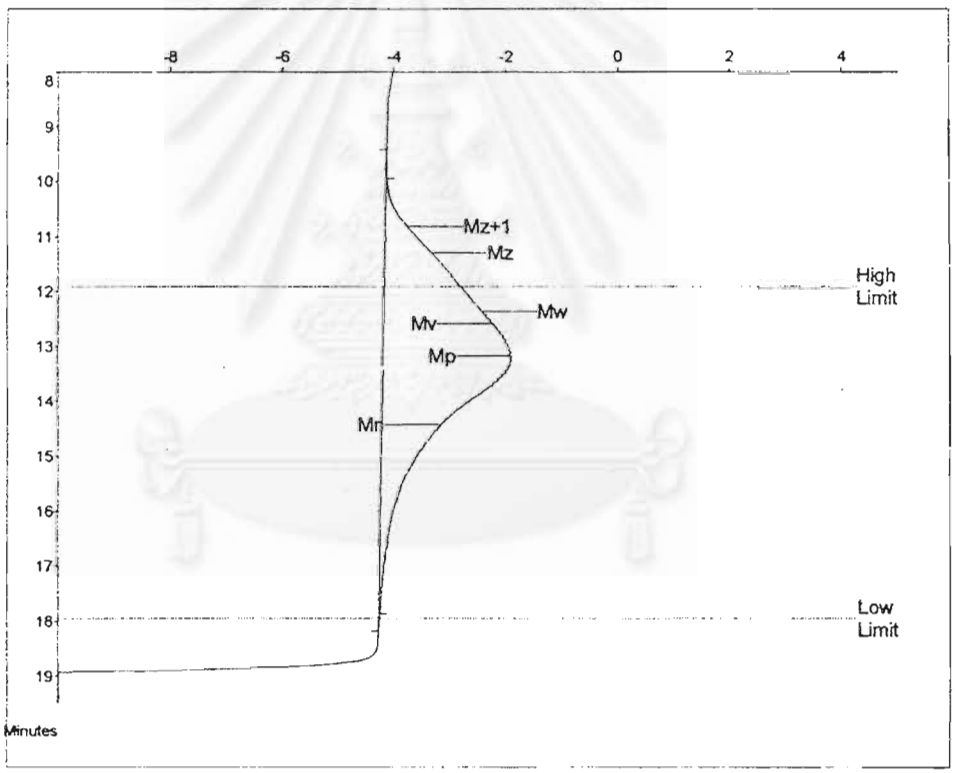
Detector : RI
Temperature : 30 C
Flow Rate : 0.600
Standards : Pullulan

Method 18
Comments : 1M NaOAc + 1M AcOH (Buffer)

Calibration Using : Narrow Standards
Calibration Limits : 11.92 to 17.95 Mins
Flow Rate Marker : found at : Not Found

Curve Used : 1st Order Polynomial
Last Calibrated : Fri Jul 07 09:13:02 2000
in Standards at : 19.55 Mins

Broad Peak Start : 9.95 End : 17.87 Mins



Molecular Weight Averages

Mp =	299393	Mz =	3068678
Mn =	64191	Mz+1 =	5688609
Mw =	819039	Mv =	629255
Polydispersity =	12.759	Peak Area =	82495

Figure C.6 virgin chitosan using NaOCl 0.00322 mole

Polymer Laboratories
PL LogiCal GPC Software

10:43 Fri Jan 19 2001

Unknown I0117.009
NaOCl 2 ml

Acquired : 16:17 Wed Jan 17 2001
Operator Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent : AcOH : NaOAc (1:1)
Column Set : Ultralinear hydrogel

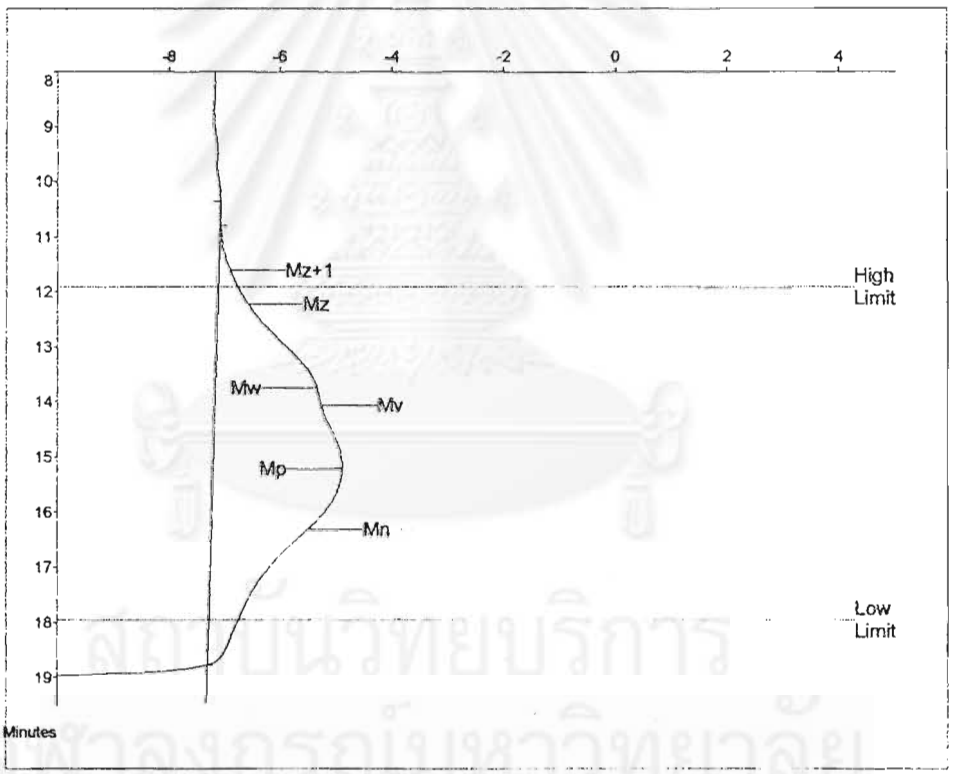
Detector : RI
Temperature : 30 C
Flow Rate : 0.600
Standards : Pullulan

Method 18
Comments : 1M NaOAc + 1M AcOH (Buffer)

Calibration Using : Narrow Standards
Calibration Limits : 11.92 to 17.95 Mins
Flow Rate Marker : found at : Not Found

Curve Used : 1st Order Polynomial
Last Calibrated : Fri Jul 07 09:13:02 2000
in Standards at : 19.55 Mins

Broad Peak Start : 10.80 End : 18.78 Mins



Molecular Weight Averages

Mp =	24066	Mz =	977613
Mn =	6119	Mz+1 =	2106922
Mw =	147903	Mv =	99120
Polydispersity =	24.169	Peak Area =	116683

Figure C.7 virgin chitosan using NaOCl 0.00644 mole

Polymer Laboratories
PL LogiCal GPC Software

10:44 Fri Jan 19 2001

Unknown I0118.009
reNaOCl 4ml

Acquired : 14:28 Thu Jan 18 2001
Operator Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent : AcOH : NaOAc (1:1)
Column Set : Ultralinear hydrogel

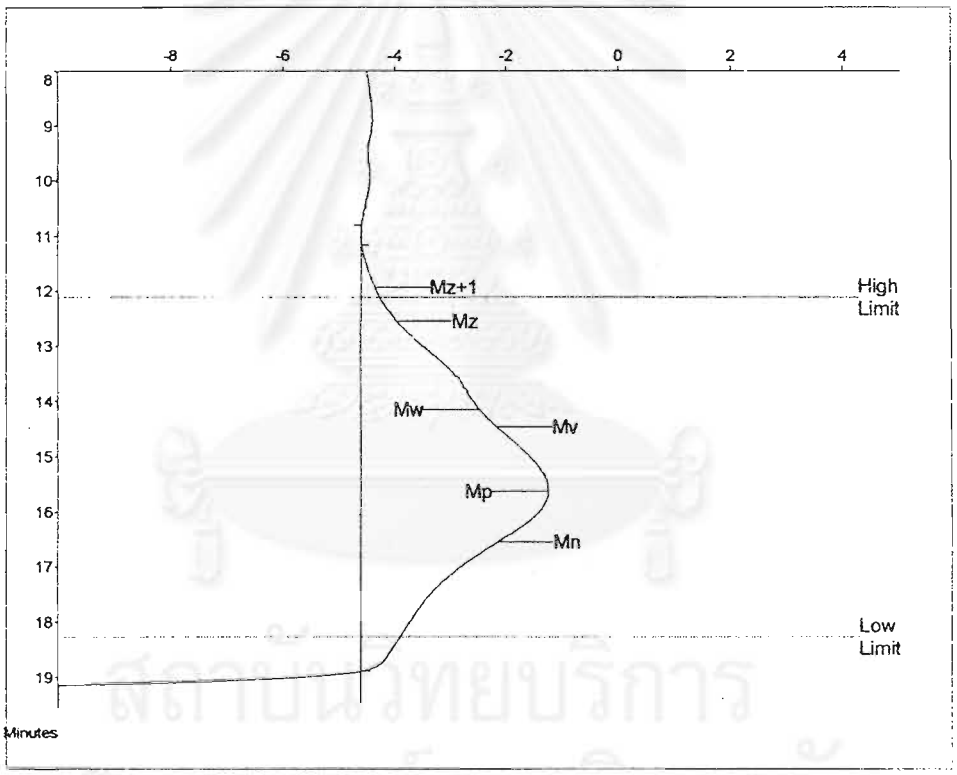
Detector : RI
Temperature : 30 C
Flow Rate : 0.600
Standards : Pullulan

Method 18
Comments : 1M NaOAc + 1M AcOH (Buffer)

Calibration Using : Narrow Standards
Calibration Limits : 12.12 to 18.27 Mins
Flow Rate Marker : found at : 19.90

Curve Used : 1st Order Polynomial
Last Calibrated : Fri Jul 07 09:13:02 2000
In Standards at : 19.55 Mins

Broad Peak Start : 11.17 End : 18.88 Mins



Molecular Weight Averages

Mp =	20189	Mz =	856112
Mn =	6613	Mz+1 =	1811737
Mw =	124089	Mv =	82812
Polydispersity =	18.764	Peak Area =	153169

Figure C.8 virgin chitosan using NaOCl 0.00966 mole

Polymer Laboratories
PL LogiCal GPC Software

10:47 Fri Jan 19 2001

Unknown I0118.007
NaOCl 6ml

Acquired : 13:31 Thu Jan 18 2001
Operator Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent : AcOH : NaOAc (1:1)
Column Set : Ultralinear hydrogel

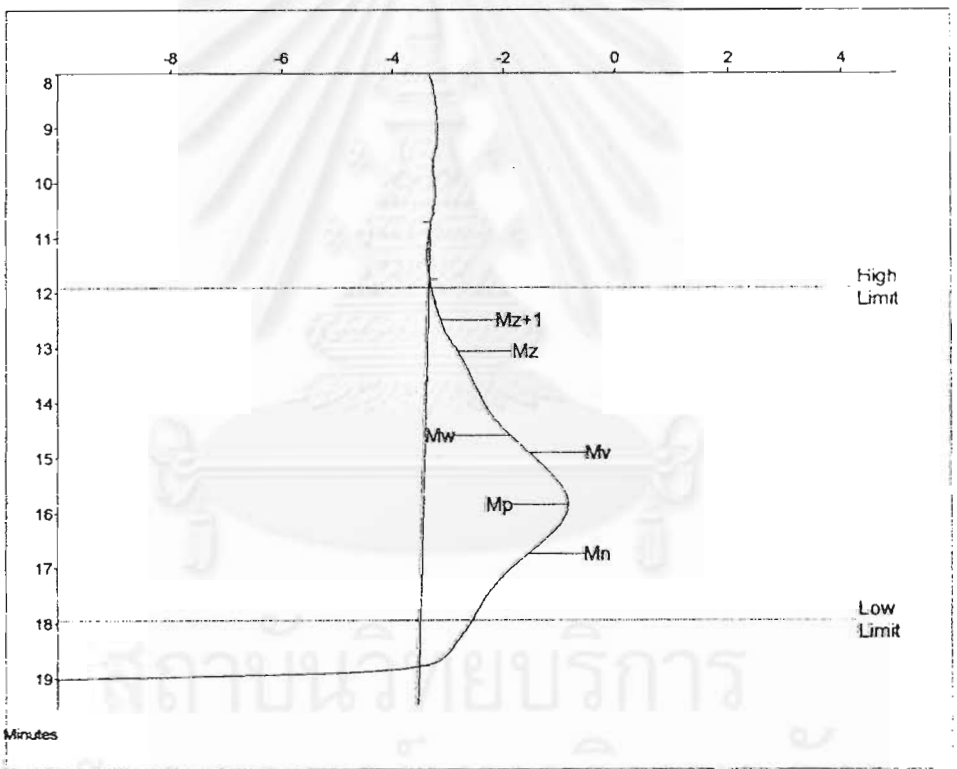
Detector : RI
Temperature : 30 C
Flow Rate : 0.600
Standards : Pullulan

Method 18
Comments : 1M NaOAc + 1M AcOH (Buffer)

Calibration Using : Narrow Standards
Calibration Limits : 11.92 to 17.95 Mins
Flow Rate Marker : found at : Not Found

Curve Used : 1st Order Polynomial
Last Calibrated : Fri Jul 07 09:13:02 2000
in Standards at : 19.55 Mins

Broad Peak Start : 11.75 End : 18.78 Mins



Molecular Weight Averages

Mp =	10975	Mz =	347337
Mn =	3565	Mz+1 =	697255
Mw =	51727	Mv =	34741
Polydispersity =	14.509	Peak Area =	107031

Appendix D

Calculation of dye removal

Nylosan green N-GL; CI. Acid green 25, λmax 639 nm

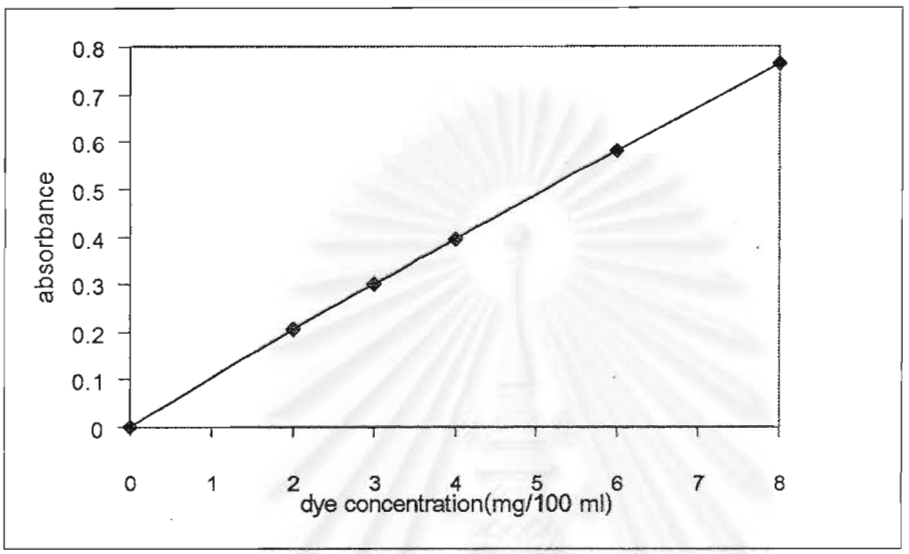


Figure D.1, standard curve of absorbance relate to dye concentration

Table D.1, Dye concentration relate with absorbance

Absorbance, λmax 639 nm	Dye concentration (mg/100 ml)
0.764	8
0.581	6
0.395	4
0.299	3
0.207	2
0.00	0

Linear equation

$Y=aX + B$

Y=0.0929X + 0.02144; X=(Y – 0.02144)/0.0929

Table D.2, 1.20 g of graft yield

NaOCl	Abs(Y)	X=(Y –0.02144) / 0.0929	Conc.sol.(C) =8X/6	Dye removal = Start (8mg) - C
0.0322	0.254	2.5025	2.7366	4.67 mg
0.0644	0.397	4.0413	5.388	2.61 mg
0.0966	0.407	4.1489	5.53	2.46 mg

Table D.3, 1.68 g of graft yield

NaOCl	Abs(Y)	X=(Y –0.02144) / 0.0929	Conc.sol.(C) =8X/6	Dye removal = Start (8mg) - C
0.0322	0.238	2.333	3.10	4.89 mg
0.0644	0.346	3.4925	4.656	3.343 mg
0.0966	0.460	4.719	6.29	1.7 mg

Table D.4, virgin chitosan

Sample	Abs(Y)	X=(Y –0.02144) / 0.0929	Conc.sol.(C) =8X/6	Dye removal = Start (8mg) - C
Chitosan (Mw 800,000)	0.02	0.0	0.0	8.0 mg
Chitosan (Mw 50,000)	0.44	4.5	6.0	2.0 mg

Sample	Abs(Y)	X=(Y –0.02144) / 0.0929	Conc.sol.(C) =8X/6	Dye removal = Start (8mg) - C
Acrylamide grafted chitosan	0.54	5.58	7.44	0.56 mg

Appendix E

Determination of Nickel removal

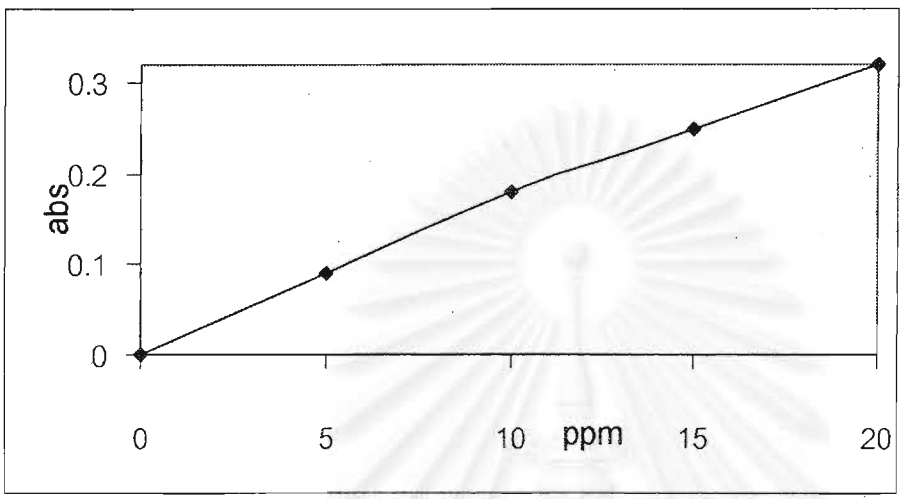


Table E.1, standard curve of Nickel concentration

BIOGRAPHY

Mr. Thorsak Kittikorn was born in April 15, 1974 in Haadyai, Songkla. He received his Bachelor degree in Science majoring in Polymer Science from the Faculty of Science, Prince of Songkla University in the academic year 1995. He worked in HDPE, TPI, Rayong since year 1996-1997. He began his Master degree study in June 1998 and complete the programme in April 2001