สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV
CHITOSAN-PEG: AN APPROACH FOR A SELF SUPRAMOLECULAR DERIVATIVE

Abstract

A self supramolecular structured chitosan is designed. The polyethyleneglycol chain is successfully conjugated onto chitosan chain via carbonyl diimidazole coupling agent. The amino group of chitosan is protected by benzaldehyde through all preparation steps. The compound obtained is characterized by FT-IR, TGA, and XRD. The inclusion property is qualitatively and quantitatively analyzed by UV spectrophotometer, using potassium picrate salt as a model ion.

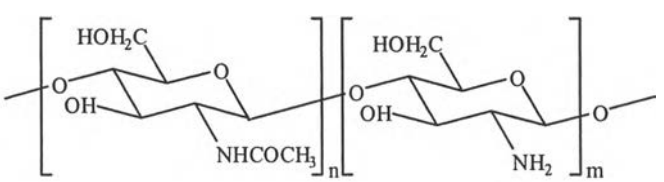
Keywords: Low molecular weight chitosan/ Polyethyleneglycol monomethylether/ Self supramolecule/ Inclusion property

Introduction

Chitin, β-(1-4)-linked 2-deoxy-2-acetamido-D-glucose, is the second most natural occuring abundant polysaccharide obtained from the shells of crustaceans, the cuticles of insects, the cell-walls of fungi and yeasts. In nature, chitin appears as a copolymer chain with chitosan unit, β-(1-4)-linked 2-deoxy-2-amino-D-glucose. For half a century, chitin-chitosan is known for its bioactivity[1], biocompatibility[2], biodegradability[3] and nontoxicity[4] and has received much development to be applied in the fields of biomedicine, pharmacology, and biotechnology owing to the abundancy and cost performance.

Chitin-chitosan has the reactive groups which are amino, primary and secondary hydroxyl group (Scheme I).

Scheme I



(chitin unit) (chitosan unit)

Basically, the hydroxyl and amino group of chitin-chitosan act as crosslinkable site. Moreover, the nitrogen atom provides lone pair electrons for metal complexation. However, since the chemical structure of chitin-chitosan is controlled by the inter and intramolecular hydrogen bonding, the solubility of chitin-chitosan is limited. This brings the problems of reaction yield and reactivity. The way to overcome the shortcoming is to modify the chemical structure of chitin-chitosan for water-soluble chitin-chitosan derivatives such as CM-chitin and CM-chitosan[5], or organic-soluble derivatives for instance N-phthaloyl chitosan[6]. Recently, molecular weight reduction is accepted as a pathway to improve the solubility as well as reactivity.

At present, various chemically modified chitin-chitosan derivatives are proposed in order to achieve a series of value-added products to obtain the property that never be achieved in natural chitin-chitosan. One of the potential value-added

products is the introduction of molecular recognition mechanism on chitin-chitosan chain in order to improve specific properties at molecular level. For example, chitosan flakes showed Cr^{3+} removal ability from wastewater and the adsorption capacity increased with a decrease in flake size[7]. The metal ion interaction and selectivity can be improved significantly after introducing macrocyclic molecules such as crown ethers, calixarenes, and cyclodextrins. Yang *et al.*[8] demonstrated the adsorption selectivity of crosslinked chitosan azacrown ethers, which synthesized by the reaction of crosslinked chitosan with epoxy-activated-3-hydroxyl-1,5-diazacycloheptane or 3-hydroxyl-1,5-diazacyclooctane. The results showed that the adsorption selectivity of products for Cu^{2+} and Hg^{2+} which was higher than chitosan and crosslinked chitosan in aqueous system containing Hg^{2+}, Cu^{2+}, and Cd^{2+}. Tanida[9] proposed a convenient method for introducing a β-cyclodextrin residue into high molecular weight chitosan by using amination as a key reaction. The compounds showed the product possesses an inclusion ability with *p*-nitrophenolate and it would be useful in cosmetic or pharmaceutical industries. Considering supramolecular specific properties, it can be concluded that most of the approaches for chitosan are from the functionalization of chitosan with macrocyclic unit. In other words, the ionic interaction ability is achieved mainly from the conjugated macrocyclic itself. Here, we proposed a supramolecular structured chitosan generated from a linear ethylene glycol chain where the pseudocyclic cavity for ionic interaction is expected. Yamagishi[10] reported that the ethylene glycol chain attached onto phenolic resin performs host-guest property to entrap metal ion via the pseudocyclic crown ether-like structure of ethylene glycol chain.

Thus, the present work stands on the viewpoint to functionalize chitosan with simple functional group as polyethylene glycol, which can induce molecular recognition onto chitosan chain. The present work will focus on the inclusion phenomena to clarify supramolecular structure of chitosan-PEG.

Experimental

Chemicals. Low molecular weight chitosan ($\overline{M}_v$ = 85 000) with 95% deacetylation was provided by Seafresh Chitosan (Lab) Company Limited, Bangkok, Thailand. *N'-N* carbonyldiimidazole (CDI) was obtained from TCI, Japan. Benzaldehyde, acetic acid, acetone, chloroform, ethanol, and methanol were purchased from Carlo Erba Reagenti, Italy. Sodium hydroxide and hydrochloric acid were the products from Lab-Scan, Ireland. Triethylamine was obtained from Unilab, Australia. Sodium acetate was purchased from Univar, Australia. Polyethyleneglycol 1100 monomethylether was supplied from Fluka Chemika, Switzerland. Poly(ethylene glycol) methylethers; M_w 350, 550, 2000, and 5000 were purchased from Aldrich Chemical Company, Inc., USA. Potassium hydroxide, and picric acid were the products of Ajax chemicals (Australia). All chemicals were analytical grade and used without further purification.

Instruments and Equipment. Qualitative and quantitative Fourier transform infrared spectra were obtained from a Bruker Equinox 55/S with 32 Scans at a resolution of 2 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D*, of 1 x 10^9 cm $Hz^{½}$ w^{-1}. Powder X-ray diffraction (XRD) patterns were recorded over a 2θ range of 2-60° by a RIGAKU RINT 2000 using CuKα as an X-ray source and operating at 40 kV and 30 mA with Ni filter. A Dupont thermogravimetric analyzer applied using a N_2 flow rate of 20 mL/min with a heating rate of 20 °C/min from 30 to 600°C. Intrinsic viscosity [η] of low molecular weight chitosan was measured with a calibrated viscometer Cannon-Ubbelohde (No. 2, A149) in 0.2 M CH_3COOH/0.1 M CH_3COONa aqueous solution at 30 ± 0.05°C. Molecular weight was calculated using the Mark-Houwink equation with K = 1.64 x 10^{-30} x DD^{14} and a = (-1.02 x 10^{-2} x DD) + 1.82 as proposed by Wang *et al.* Potassium picrate concentration in aqueous phase was determined by a Perkin Elmer Lambda-10 UV-VIS spectrophotometer.

***N*-benzylidene chitosan (CTB).** The procedures were done according to Yang *et al.* Low molecular weight chitosan, **1** (1.00 g), was dissolved in 2% v/v acetic acid and diluted with methanol. Benzaldehyde (4.35 mL, 7 moles equivalent

to pyranose rings) was added and stirred at room temperature for 15 h. till the viscous solution obtained. The viscous solution was washed thoroughly with methanol and dried *in vacuo* at 60°C to give CTB, **2** (Scheme II).

***N*-benzylidene chitosan-carbonyldiimidazole (CTB-CDI).** The low molecular weight chitosan precursor was carried out according to the method proposed by Yoksan *et al.* Compound **2** (1.00 g) was dispersed in chloroform (20 mL) and heated to 60°C *in vacuo* for 30 min. CDI (1.97 g, **3** moles equivalent to pyranose rings) was added and reacted at 60°C for 6 h. The crude product was collected and washed thoroughly with acetone and dried *in vacuo* at 60°C to give CTB-CDI, **3** (Scheme II).

***N*-benzylidene chitosan-polyethyleneglycol monomethylether (CTB-PEG).** The reaction of polyethyleneglycol monomethylether (PEG) with the chitosan precursor was achieved as follows. Compound **3** (1.00 g) was dispersed in chloroform (20 mL) at 60°C *in vacuo* for 30 min. Triethylamine (2.04 mL, 5 moles equivalent to pyranose rings) and Polyethyleneglycol monomethylether (2 moles equivalent to pyranose rings) were added and the reaction was carried out at 60°C for 8 h. The temperature was reduced to room temperature and the reaction was continued for overnight under nitrogen. The crude product was collected and washed thoroughly with methanol for several times, follow by drying *in vacuo* at 60°C to yield CTB-PEG, **4** (Scheme II).

Chitosan-polyethyleneglycol monomethylether (chitosan-PEG). Compound **4** (1.00 g) was reacted with diluted ethanolic hydrochloride solution (0.5 N, 20 mL) at room temperature for 2 h. NaOH (1.0 N) was added until the pH was adjusted to 7. The crude product obtained was collected and washed thoroughly with distilled water followed by methanol and dried *in vacuo* at 60°C to yield chitosan-PEG, **5** (Scheme II).

Ion extraction property of chitosan and chitosan-PEG. Each of low molecular weight chitosan and chitosan-PEG (20 mg) was dispersed in distilled water (20 mL). Potassium picrate aqueous solution was prepared at 7×10^{-5} M. The picrate solution was mixed with the low molecular weight chitosan and chitosan-PEG, respectively. The solutions were filtered and the precipitation was collected.

The change in concentration of potassium picrates was determined by a UV-VIS spectrophotometry at λ 354 nm.

Results and discussion

The molecular weight of chitosan starting material with the degree of deacetylation 95% was $8.5x10^4$ which is less than most common chitosan material, thus, we termed it as a low molecular weight chitosan. The low molecular weight chitosan shows the weight loss at 56°C and 322°C, which is similar to that of chitosan with $\overline{M}_v$ about $8.0x10^5$ or $8.0x10^6$ as illustrated in Figure 2. The broad peak refers to the loss of moisture and/or water content for 4.9% while the sharp peaks at 322°C implies the degradation of the low molecular weight chitosan after the loss of intramolecular hydrogen bonding and glycoside linkage. This implied that the thermal stability is maintained.

Protection of Amino Group on Low Molecular Weight Chitosan: *N*-benzylidene chitosan (CTB)

In this work, the modification of low molecular weight chitosan at only hydroxyl groups as considered. To achieve this goal, the protection of amino groups with benzaldehyde[12] was carried out in the first step.

FT-IR spectrum (Figure 1(b)) clarifies that the product after the reaction with benzaldehyde shows the characteristic peaks of aromatic ring at 756, 692, and 1581 cm^{-1}. The peak at 1644 cm^{-1} corresponding to the C=N group was also observed.

Thermogravimetry analysis was used to evaluate thermal stability of the derivatives obtained. The *N*-benzylidene chitosan, **2** (Scheme II) shows the degradation temperature at 318°C (Figure 2(b)), implying the protecting group of benzaldehyde leads to the small decrease of thermal stability. This might be due to the decrease of inter and intramolecular hydrogen bonding after the introduction of the bulky benzylidene group.

The observation of the change in XRD pattern is a practical way to evaluate the chemical reaction of chitosan indirectly. Generally, chitosan shows the two significant peaks at 13° 2θ and 20° 2θ referring to d-spacing of the chain between the sheet and in the sheet, respectively.

After benzylidene functionalization the compound obtained shows the peaks at 13° significantly shifted to 5°, whereas the peak at 20° 2θ slightly shifted to 18°.

This implies that by introducing benzyl group the packing structure between the sheet was widely open. In other words, the intermolecular hydrogen bond between chitosan chain was destroyed. However, the intramolecular chain distance of chitosan chain was slightly affected as evidenced from the small change of 20° 2θ to 18° 2θ.

Low Molecular Weight Chitosan Precursor: *N*-benzylidene chitosan-carbonyldiimidazole (CTB-CDI)

In the present work, CDI was chosen as a coupling agent due to its high reactivity with alcohols, carboxylic acids, and amines, which are important functional groups in carbohydrate polymers.[14,15] Here, most of the amino groups at C-2 were protected and the reaction is expected to occur mainly at OH groups (C-3 and C-6).

As shown in Figure 1(c), the product obtained gives ester peak at 1767 cm^{-1} and carbamate peak at 1706 cm^{-1}. This confirms the successful reaction of **2** with CDI to give **3**.

Thermal property study reveals the changes of packing structure from **2** to **3** (Figure 2(c)). The new weight loss peak at 168°C may refer to the loss of ester group while the peak at 319°C could be the cleavage of the intermolecular hydrogen bonding and glycosidic linkage.

The XRD pattern was observed to confirm the compound **3**. Figure 3(c) shows that CTB-CDI gives the peaks at 5° 2θ and 18° 2θ, which are at the same positions as those of CTB, however the peaks are broader. This implies that the unit cell of CTB-CDI is maintained as CTB, whereas the crystallite size is smaller. The increase in amorphous like structure reflects the introduction of CDI onto the chitosan unit.

Conjugation of Low Molecular Weight Chitosan Precursor with Polyethyleneglycol: *N*-benzylidene chitosan-polyethyleneglycol monomethylether (CTB-PEG)

In order to obtain a chitosan chain with pseudocyclic ether, polyethyleneglycol monomethylether (PEG) was applied. The reaction of polyethyleneglycol monomethylether with **3** was expected to proceed at reactive

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ester group. After 8 h, the reactive ester imidazole and carbamate groups are consumed as evidenced by the disappearances of the peak at 1767 and 1706 cm^{-1}, respectively (Figure 1(d)). The peak at 1756 cm^{-1} referred to the carbonate group confirms the structure of **4** (Scheme II).

The TGA diagram of **4** (Figure 2(d)) shows the weight loss at 302°C referring to the thermal degradation. This indicates the more amorphous like structure of **4** as compared to the low molecular weight chitosan starting material. In this case, the bulky groups of the polyethyleneglycol monomethylether may interfere the molecular packing leading to the decrease of thermal stability.

The XRD pattern of **4** shows similar result as **3** (Figure 3(d)). This reflects that the introduction of bulky group onto the low molecular weight chitosan chain destroys the crystallite size, as evidenced from the broad peak at 18° 2θ.

Removal of Protecting Group: Chitosan-polyethyleneglycol monomethylether (Chitosan-PEG)

In order to maintain the unique aminosaccharide of chitosan, in the final step, the deprotection of benzylidene group was carried out to obtain **5**.

The FT-IR spectrum of **5** (Figure 1(e)) shows the disappearance of peaks belong to aromatic ring at 756, 692, and 1581 cm^{-1}. The disappearance of the peak at 1644 cm^{-1} implies the completion of benzylidene removal. The peak at 1750 cm^{-1} belonging to the carbonate group is identified suggesting that the condition of benzylidene removal was not affected to the ester bonds with polyethyleneglycol monomethylether group.

The TGA diagram of **5** (Figure 2(e)) shows the degradation temperature at 262°C. This implies that the amino deprotection leads to the decrease of thermal stability, which might be due to the decrease of inter and intramolecular hydrogen bonding.

Structural analysis by XRD (Figure 3(e)) gives the very broad single peak at 25° 2θ. The disappearance of the peak at 5° 2θ implies that the deprotection of CTB-PEG leads to the collapse in packing structure of chitosan chain. In other words, **5** loses the chain packing framework of chitosan. The broad peak at 25° 2θ also

implies the small d-spacing of unit cell which reflects the local packing of chitosan unit having PEG chain.

Ion Extraction ability

Figure 4 demonstrates the ion interaction ability of the low molecular weight chitosan and chitosan-PEG, which are different in repeating unit (m) of PEG chain. Among all samples, the low molecular weight chitosan shows the lowest ion extraction ability comparing to chitosan-PEG. This might be due to the potassium ion has high interaction with the ether group of PEG chain more than the amino group of chitosan. The chitosan-PEG with m = 6, and 10 shows the highest ion extraction percentage (100%). While that of the ones with m = 23, 43 and 111 were 71.17, 69.87, and 60.32%, respectively. Here, it is speculated that the short ethylene glycol chain (m = 6, and 10) may form a proper cavity size of pseudocyclic ether for potassium ion more than the long ethylene glycol chain (m = 23, 43 and 111). It might also relate to the reaction of mPEG with chitosan which the chain may effectively be grafted on the chitosan. In other words, the long chain ethylene glycol may have the difficulty in conjugation onto chitosan chain owing to the chain length and the steric effect. As a result, the function of ionic interaction for chitosan-PEG with m = 6, and 10 is significant. At present, we are studying on the degree of substitution by means of quantitative FT-IR, and elemental analysis.

Conclusions

A self supramolecular structured chitosan can be prepared by introducing polyethyleneglycol monomethylether onto low molecular weight chitosan chain. The products obtained were confirmed the structure by FT-IR, TGA, and XRD. This self supramolecular structured chitosan (chitosan-PEG) shows very high swelling ability in water. The inclusion property of the chitosan-PEG for potassium ion is better than the low molecular weight chitosan chain. The increasing in the repeating unit of PEG chain shows the decreasing in the ion extraction percentage.

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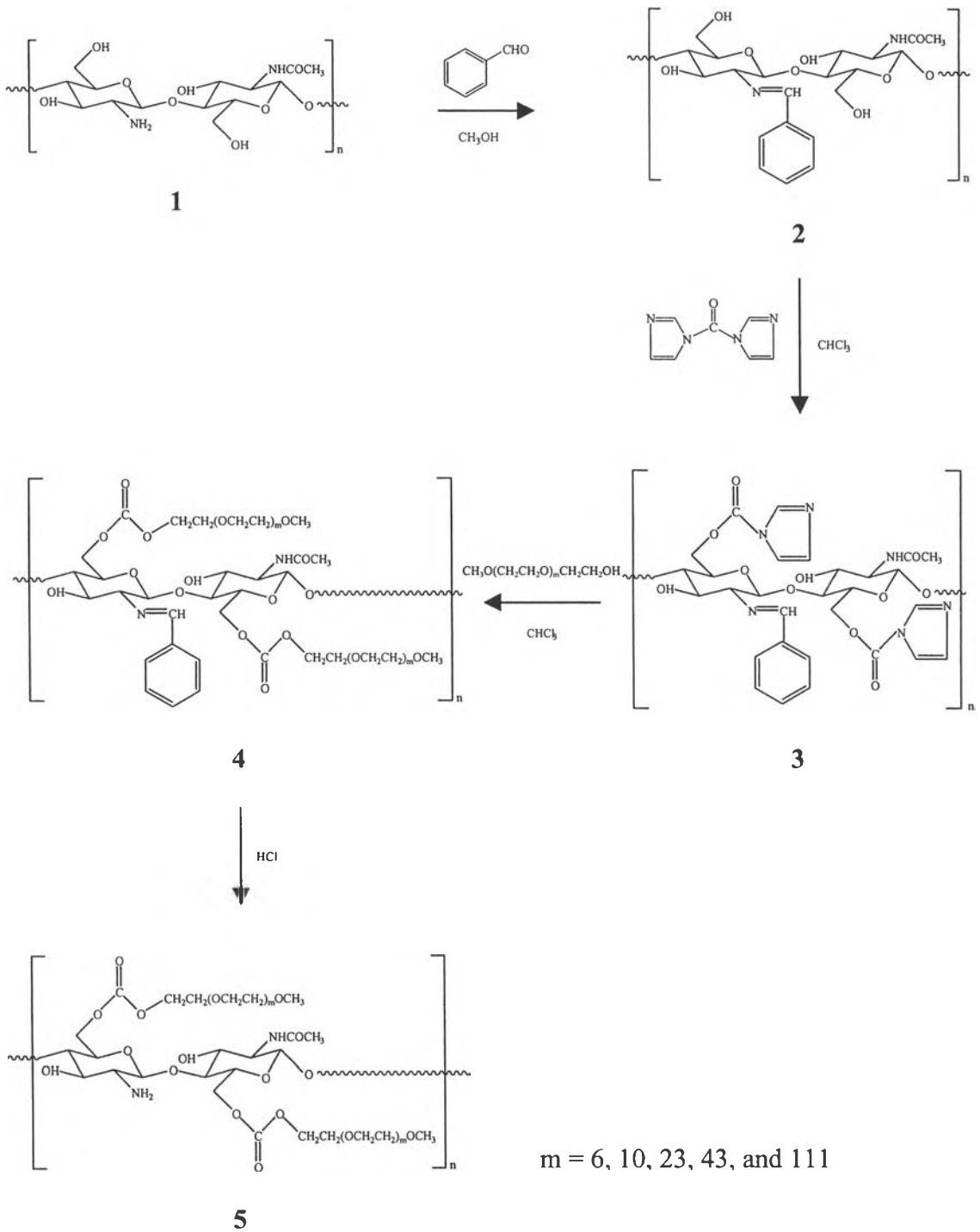

1 **2** **3** **4** **5**

Scheme II (Sasiprapha et al.)

Figure Captions

Figure 1 FT-IR spectra of: (a) low molecular weight chitosan, (b) *N*-benzylidene chitosan (CTB), (c) *N*-benzylidene chitosan-carbonyldiimidazole (CTB-CDI), (d) *N*-benzylidene chitosan-polyethyleneglycol monomethylether (CTB-PEG), (e) chitosan-polyethyleneglycol monomethylether (chitosan-PEG)

Figure 2 TGA diagram of: (a) low molecular weight chitosan, (b) *N*-benzylidene chitosan (CTB), (c) *N*-benzylidene chitosan-carbonyldiimidazole (CTB-CDI), (d) *N*-benzylidene chitosan-polyethyleneglycol monomethylether (CTB-PEG), (e) chitosan-polyethyleneglycol monomethylether (chitosan-PEG)

Figure 3 X-ray diffractograms of: (a) low molecular weight chitosan, (b) *N*-benzylidene chitosan (CTB), (c) *N*-benzylidene chitosan-carbonyldiimidazole (CTB-CDI), (d) *N*-benzylidene chitosan-polyethyleneglycol monomethylether (CTB-PEG), (e) chitosan-polyethyleneglycol monomethylether (chitosan-PEG)

Figure 4 Extraction percentage of potassium picrate at the concentration of 7 x 10^{-5} M by: (a) low molecular weight chitosan, (b) chitosan-PEG with m = 6, (c) chitosan-PEG with m = 10, (d) chitosan-PEG with m = 23, (e) chitosan-PEG with m = 43, (f) chitosan-PEG with m = 111

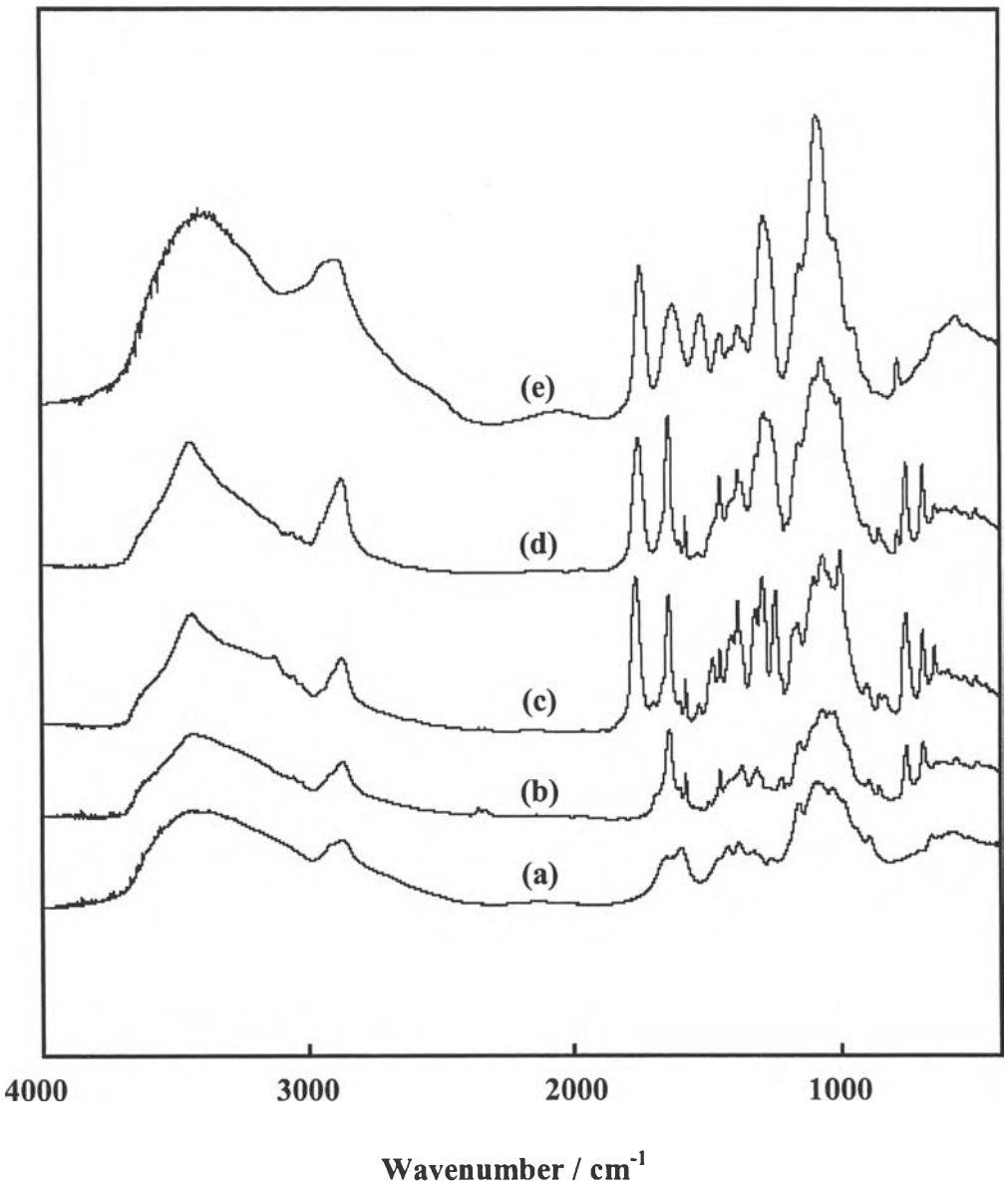

Figure 1 (Sasiprapha et al.)

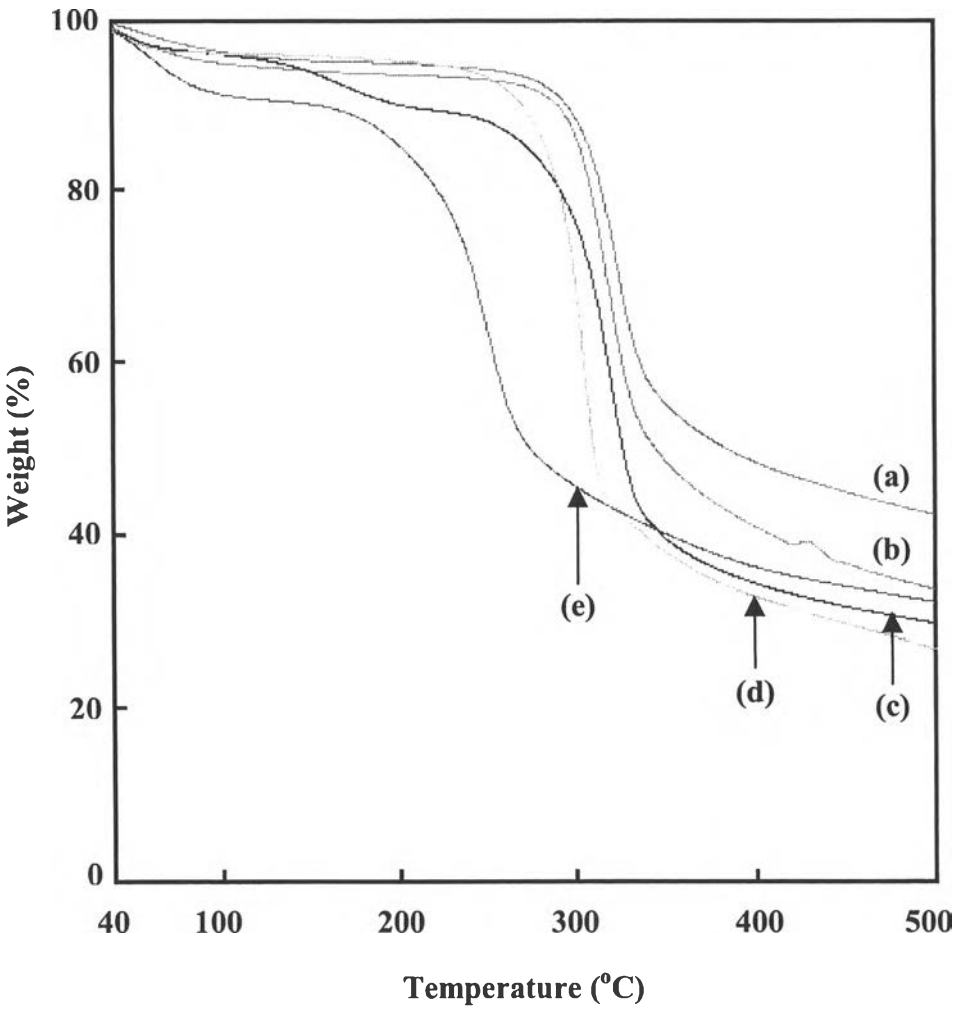

Figure 2 (Sasiprapha et al.)

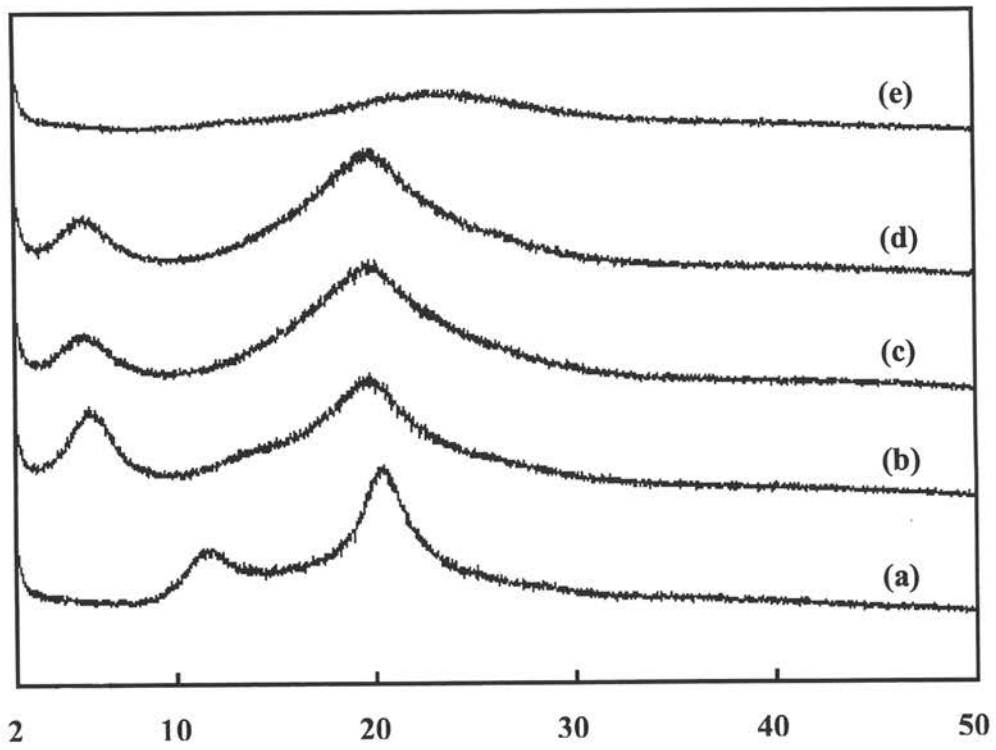

Figure 3 (Sasiprapha et al.)

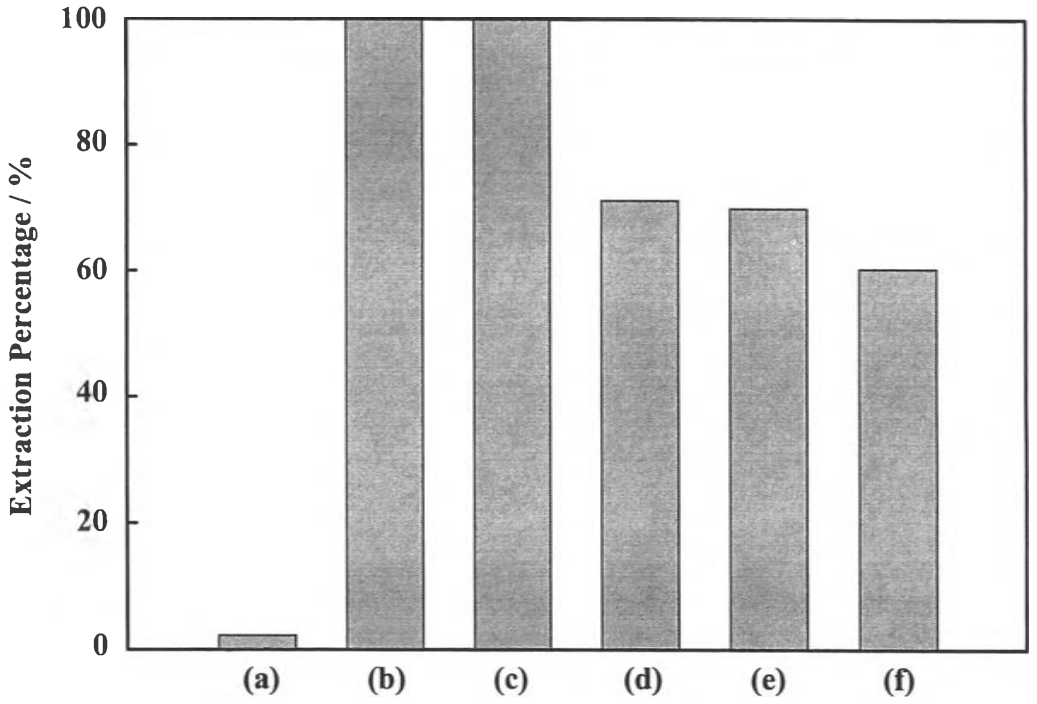

Figure 4 (Sasiprapha et al.)