นาโนสเฟียร์จากอนุพันธ์ไคโทซานที่ดูดกลืนยูวี

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NANOSPHERES FROM UV-ABSORBING CHITOSAN DERIVATIVES

Miss Pimsiree Deemak

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By	Miss Pimsiree Deemak
Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Associate Professor Supason Wanichwaecharungruang, Ph.D.
Thesis Co-Advisor	Assistant Professor Rath Pichayangkura, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partail Fulfillment of the Requirements for the Master's Degree

……Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITEE

.....Chairman
(Associate Professor Supawan Tantayanon, Ph.D.)

.....Thesis Advisor
(Associate Professor Supason Wanichwaecharungruang, Ph.D.)

….....Thesis Co-Advisor
(Assistant Professor Rath Pichayangkura, Ph.D.)

……..Examiner
(Associate Professor Nuanphan Chantarasiri, Ph.D.)

…….External examiner
(Nantanit Wanichacheva, Ph.D.)

พิมพ์สิรี ดีมาก : นาโนสเฟียร์จากอนุพันธ์ไคโทซานที่ดูดกลืนยูวี
(NANOSPHERES FROM UV-ABSORBING CHITOSAN DERIVATIVES)
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ในงานวิจัยนี้อนุพันธ์ไคโทซานที่มีสมบัติดูดกลืนรังสียูวี 4 ชนิดคือ 1) อนุพันธ์ดูดกลืนรังสียูวีบีซินนาโมอิลไคโทซาน 2) อนุพันธ์ดูดกลืนรังสียูวีเอ 2,4,5-ไตรเมทอกซีซินนาโมอิลไคโทซาน 3) อนุพันธ์ดูดกลืนรังสียูวีเอ 4-ไฮรอกซี-3-เมททอกซีซินนามอลลีดีนซัคซีนิลไคโทซาน 4) อนุพันธ์ดูดกลืนรังสียูวีบี 2,4-ไดไฮดรอกซีเบนซาลลีดีนซัคซีนิลไคโทซานถูกสังเคราะห์ขึ้น การเตรียมอนุภาคนาโนจากอนุพันธ์ไคโทซานทั้งสี่โดยวิธีโซลเวนท์ดิสเพลสเมนต์ พบว่าขนาดของอนุภาคแปรผันตามเปอร์เซนต์การแทนที่ของหมู่ซิลนาโมอิลบนสายโซ่ของไคโทซาน 2,4,5-ไตรเมทอกซีซินนาโมอิลไคโทซานมีความเสถียรต่อแสงมากกว่าซินนาโมอิลไคโทซาน 2,4-ไดไฮดรอกซีเบนซาลลีดีนซัคซีนิลไคโทซานมีความเสถียรมากกว่า 4-ไฮรอกซี-3-เมททอกซีซินนามอลลีดีนซัคซีนิลไคโทซาน 2,4-ไดไฮดรอกซีเบนซาลลีดีนซัคซีนิลไคโทซาน ให้สมบัติในการดูดกลืนรังสียูวีบีที่ใกล้เคียงกับ 2-เอทิลเฮกซิล-พารา-เมทอกซีซินนาเมทซึ่งเป็นสารกรองรังสียูวีบีที่นิยมใช้กันทั่วไป

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PIMSIREE DEEMAK: NANOSPHERES FROM UV-ABSORBING CHITOSAN DERIVATIVES. THESIS ADVISOR: ASSOC. PROF. SUPASON WANICHWEACHARUNGRUANG, Ph.D. THESIS CO-ADVISOR: ASST. PROF. RATH PICHYANGKURA, Ph.D., 85 pp.

In this work, four groups of UV-absorbing chitosan derivatives were synthesized. They include 1) UVB filter cinnamoylchitosan (CC) 2) UVA filter 2,4,5-trimethoxycinnamoylchitosan (245CC) 3) UVA filter 4-hydroxy-3-methoxycina malidene succinylchitosan (43CSC) and 4) UVB filter 2,4-dimethoxybenzalidenesuc cinylchitosan (24BSC). Fabrication of nanoparticles from the obtained derivatives was carried out by solvent displacement method. It was found that the size of the particles varied with degree of cinnamoyl substitution. 2,4,5-Trimethoxycinnamoyl chitosan and 2,4-dimethoxybenza lidenesuccinylchitosan are more photostable than cinnamoylchitosan and 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan, respectively. In addition, 2,4-dimethoxybenzalidenesuccinylchitosan possess similar UVB filtering efficiency to the popularly used UVB filter 2-ethylhexyl-4-methoxycinnamate.

Field of Study..Petrochemistry and Polymer Science..Student's Signature…….
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List of Abbreviations

δ	chemical shift
J	coupling constant
°C	degree Celsius
DS	degree of substitution
d	doublet
dd	doublet of doublet
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
EHMC	2-Ethylhexyl-p-methoxycinnamate
g	gram (s)
Hz	hertz
h	hour
IR	Infrared
J	joule
mL	milliliter
mV	millivolt
mW	milliwatt
min.	minute
ε	molar absorptivity
M.W	molecular weight
nm	nanometer (nm)
NMR	nuclear magnetic resonance
ppm	parts per million
%	Percent
cm^{-1}	per centimeter (s)
SEM	Scanning electron microscope
s	singlet
TEM	Transmission electron microscope
t	triplet
UV	ultraviolet

cm^{-1}	unit of wavenumber (IR)
λ	wavelength

CHAPTER I

Introduction

1.1 Chitosan

Chitosan, alpha-(1-4)-amino-2-deoxy-beta-D-glucan, has structural characteristics similar to glycosaminoglycans. This polycationic biopolymer is generally obtained by alkaline deacetylation of chitin (Figure 1.1), which is the main component of the exoskeleton of crustaceans (table 1.1), such as shrimps [1]. The chitosan is insoluble in water, but it dissolves in aqueous solutions of organic acids such as acetic, formic, citric, and inorganic acids such as diluted hydrochloric acid, resulting in viscous solutions [2]. It is well known that some of the structural characteristics such as degree of acetylation (DA) and molecular weight, controlled the solubility in chitosan. Chitosan has interesting biopharmaceutical properties (table 1.2) such as pH sensitivity [3], biocompatibility [4] and low toxicity [5]. Moreover, chitosan is metabolized by certain human enzymes, especially lysozyme, and is considered as biodegradable [6]. Due to these favorable properties, the interest in chitosan and its derivatives as excipients in drug delivery has been increased in recent years [7, 8]. The interest in chitosan arises mainly from the fact that this natural polysaccharide allows the production of biocompatible and biodegradable drug delivery systems. It is extremely important that chitosan be aqueous soluble and positively charged [9]. These properties enable it to interact with negatively charged polymers, macromolecules and poly anions on contact in an aqueous environment.

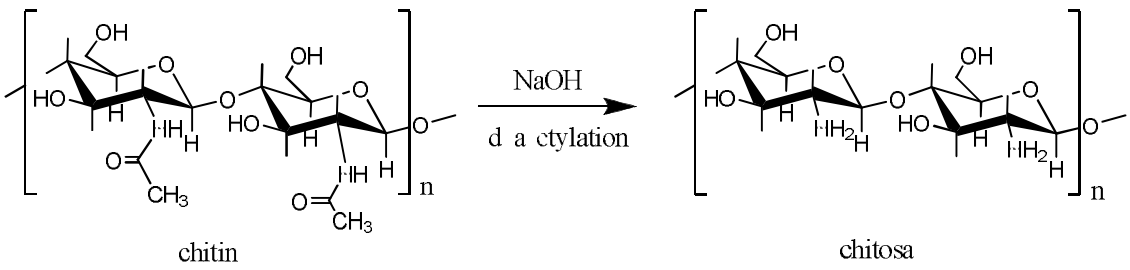


Figure 1.1 Deacetylation of chitin

Sea animals	Insects	Microorganisms
Annelida	Scorpions	Green algae
Mollusca	Spiders	Yeast (β-type)
Coelenterata	Brachiopods	Fungi (cell walls)
Crustaceans	Ants	Mycelia Penicillium
Lobster	Cockroaches	Brown algae
Crab	Beetles	Spores
Shrimp		Chytridiaceae
Prawn		Ascomydes
Krill		Blastocladiaceae

Table 1.1 Sources of chitin and chitosan [6]

Potential Biomedical application	Principal characteristics
Surgical sutures	Biocompatible
Dental implants	Biodegradable
Artificial skin	Renewable
Rebuilding of bone	Film forming
Corneal contact lenses	Hydrating agent
Time release drugs for animals and humans	Nontoxic, biological, tolerance
Encapsulating material	Hydrolyzed by lyzosyme Wound healing properties Efficient against bacteria, viruses, fungi

Table 1.2 Principal properties of chitosan in relation to its use in biomedical applications [6]

1.2 Chemical modification of chitosan

Recently, there has been a growing interest in the chemical modification of chitosan in order to improve its solubility and widen its applications [10-12]. Derivatization by introducing small functional groups to the chitosan structure, such as alkyl or carboxymethyl groups [13, 14] can drastically increase the solubility of chitosan at neutral and alkaline pH values without affecting its cationic character. Among the various methods of modification, graft copolymerization has been the most used. Grafting of chitosan allows the formation of functional derivatives by covalent binding of a molecule, the graft onto the chitosan backbone. Chitosan has two types of reactive groups that can be grafted. First, the free amine groups on deacetylated units and second, the hydroxyl groups on the C3 and C6 carbons on acetylated or deacetylated units. Recently researchers have shown that after primary derivation followed by graft modification chitosan would possess much improved water solubility, antibacterial and antioxidant property [15, 16]. Although the grafting of chitosan modifies its properties, it is possible to maintain some interesting characteristics such as mucoadhesivity [17], biocompatibility [4] and biodegradability [18].

O- and *N*-carboxymethylchitosan

Carboxymethylchitosan (CM-chitosan) is the most fully explored derivatives of chitosan; it is an amphoteric polymer, whose solubility depends on pH. Under controlled reaction condition (with sodium monochloracetate in the presence of NaOH), one gets *O*- and *N*-carboxymethylation. The yield of substituents on the three position was determined by NMR [19]. This reaction extends the range of pH (pH>7) in which chitosan is water-soluble, but a phase separation due the balance between positive and negative charge on the polymer was observed at pH<6.5.

Most interesting is the preparation of *N*-carboxymethylchitosan by reaction with glyoxylic acid in the presence of a reducing agent [20] (Figure 1.2). The distribution of monosubstituted (-NH-CH_2COOH) and disubstituted (-N (-CH_2COH)$_2$) groups was established by ^{1}H and ^{13}C NMR. Disubstitution is easily obtained, giving an interesting derivative for ion complexation. A specific oxidation of the C-6 position hydroxyl group was relized using the TEMPO reactant on chitin to produce a

chitin-base hyaluronic acid analog [21]. This derivative is water soluble in a wide range of pH, but only if it is prepared from a fully acetylated chitin.

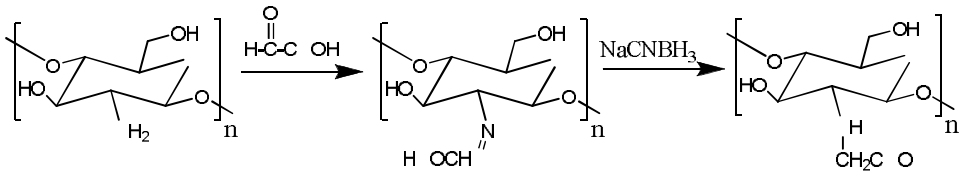
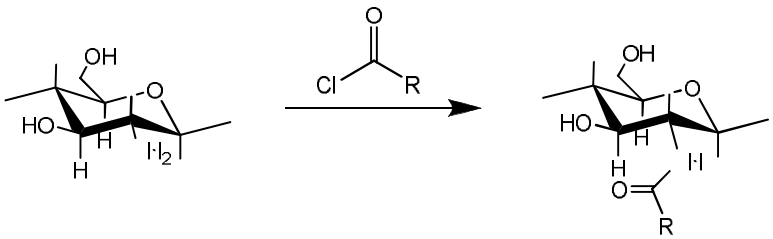


Figure 1.2 *N*-carboxymethylation of chitosan

N-Alkyl and Acylated Chitosan

The introduction of an alkyl or acyl chain offers several possibilities in molecular design of chitosan. The utility of modifying chitosan with hydrophobic branches for controlling solubility properties has also been demonstrated [22, 23]. The introduction of an alkyl chain onto a water soluble modified chitosan (*N*-methylene phosphonic chitosan) offer the presence of both hydrophobic and hydrophilic branches in its structure. A methodology for the preparation of *N*-lauryl-*N*-methylene phosphonic chitosan has been developed by Ramos et al. [23] on the water soluble *N*-methylene phosphonic chitosan. The presence of alkyl groups in *N*-lauryl-*N*-methylene phosphonic chitosan seems to weaken the hydrogen bond and provides good solubility in organic solvents. As a result of the amphiphilic properties, like surface activity typical for surfactants, this derivative opens new perspectives in pharmaceutical and cosmetic field.



Chitosan N- cylated chitosan

R = $-(CH_2)_4CH_3$ – Caproyl chitosan

R = $-(CH_2)_6CH_3$ – Octanoyl chitosan

R = $-(CH_2)_{12}CH_3$ – Myristoyl chitosan

R = $-(CH_2)_{14}CH_3$ – Palmitoyl chitosan

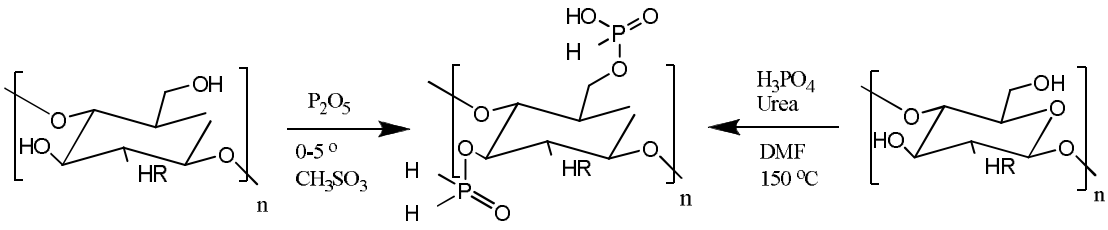
Figure 1.3 Chitosan derivatization with fatty acyl chlorides

N-acylation of chitosan with various fatty acid (C6–C16) chlorides increased its hydrophobic character and made important changes in its structural features. Tien et al. [24] described the *N*-acylation of chitosan with fatty acyl chlorides to introduce hydrophobicity for use as matrix for drug delivery (Figure 1.3). It was expected that such derivatization would reduce hydration of the matrix and play a role in network stabilization by hydrophobic interactions. In this study, unmodified chitosan exhibited a low degree of order and a weak tablet crushing strength.

Chitosan acylated with a short chain length (C6) possessed similar properties, but exhibited significant swelling. Acylation with longer side chains (C8–C16) resulted in a higher degree of order and crushing strength but lower swelling. The best mechanical characteristics and drug release properties were found for palmitoyl chitosan (substitution degree 40–50%) tablets with 20% acetaminophen as a tracer. The high stability of these nanolithic tablets appears to be due to hydrophobic interactions between side chains. Drug dissolution kinetics showed longer release times for higher degrees of functionalization, i.e., 30 h (for 47% substitution) and 90 h (for 69% substitution), suggesting palmitoyl chitosan excipients as interesting candidates for oral and subdermal pharmaceutical applications. It was also reported that the chitosan derivatives with both hydrophobic groups (long acyl groups) and hydrophilic groups (sulphate groups) could form micelles and solubilize hydrophobic compounds [25]. Moreover, it has been reported that polymer-micelle is better than other carriers for use as passive targeting carrier of anticancer drugs. *N*-lauryl carboxymethyl chitosan with both hydrophobic and hydrophilic groups was studied by Miwa et al. [26] in connection with delivery of taxol to cancerous tissues. Zhang et al. [27] studied the entrapment efficiency of amphiphilic *N*-alkyl-*O*-sulfated chitosan using taxol as a model drug. The drug was dissolved into the polymeric micelle by physical entrapment. The result showed that the taxol concentration in the *N*-alkyl-O-sulfated chitosan micelle solution was found to be 2.01 mg/mL, which was much higher than that in water indicating that *N*-alkyl-O-sulfated chitosan may be used as a potential drug delivery carrier

Phosphorylated chitin and chitosan

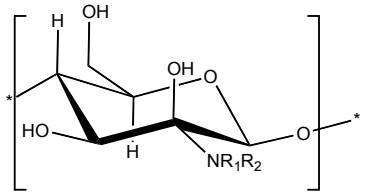
The reaction of chitin with phosphorous pentoxide was found to give water-soluble phosphorylated chitin of high degree of substitution (DS), giving a strategy to overcome this major drawback of chitin and its derivatives. Phosphorylated chitin (P-chitin) and chitosan (P-chitosan) were prepared by heating chitin or chitosan with orthophosphoric acid and urea in DMF (Figure 1.4). P-chitin and P-chitosan were also prepared by the reaction of chitin or chitosan with phosphorous pentoxide in methane sulphonic acid [28]. The phosphorylation reactions of chitin and chitosan in phosphorous pentoxide–methane sulphonic acid were found to be very efficient. However, in this case it was found that only the P-chitosan with low DS was water soluble. The incorporation of methylene phosphonic groups into chitosan allowed solubility in water under neutral conditions [23]. A water-soluble *N*-methylene phosphornic chitosan (NMPC) was also synthesized using chitosan, phosphorous acid and formaldehyde [23].



R = H or $COCH_3$

Figure 1.4 Synthesis of phosphorylated chitin and chitosan

$[\text{-A-/-B-/-C-/-D-/-E-/}\ldots]_n$



	R_1	R_2
A	-H	$-CO-CH_3$
B	-H	-H
C	-H	$-CH_2-PO_3H_2$
D	$-CH_2-PO_3H_2$	$-CH_2-PO_3H_2$
E	-H	$-(CH_2)_{11}-CH_3$

Figure 1.5 Chemical structure of *N*-lauryl-*N*-methylene phosphonic chitosan (LMPC)

A simple methodology for the preparation of a new chitosan derivative surfactant, *N*-lauryl-*N*-methylene phosphonic chitosan (LMPC), has been developed [23] (Figure 1.5). LMPC incorporated *N*-methylene phosphonic groups as hydrophilic moieties and lauryl groups as the hydrophobic ones. *N*-phosphonomethylation of chitosan reaction was studied and optimized using different reaction conditions [29]. The reaction was conducted with a large excess of both phosphorous acid and formaldehyde at 70 ˚C. The obtained white solid was found to be soluble in neutral and acidic aqueous solutions.

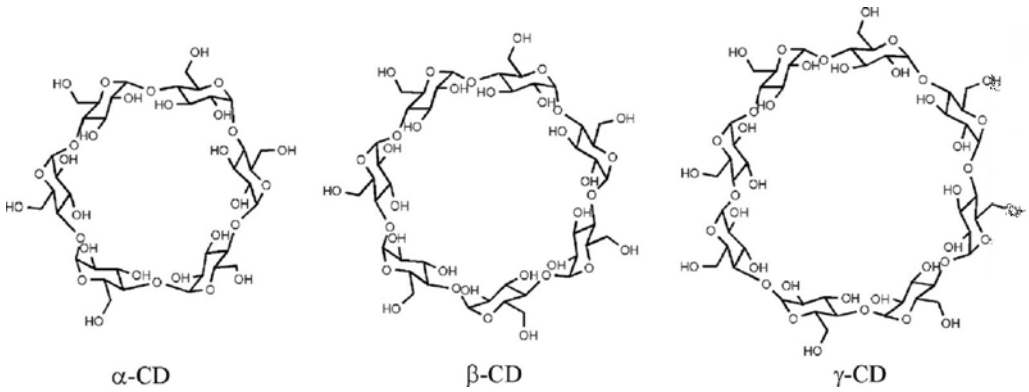


Figure 1.6 Chemical structure of Cyclodextrin [30]

Chitosan-graft-Cyclodextrin

Cyclodextrins (CDs) (Figure 1.6) have gained prominence in recent years because its hydrophobic cavity is capable of binding aromatic and other small organic molecules, and therefore provides ideal binding sites. CD linked chitosan is interesting for the viewpoint of pharmaceutics, including drug delivery, cosmetics, and analytical chemistry. Although functionalization at the 6-position of OH in CD is relatively easy, the secondary 2 or 3 position has been shown to be the more important site of cyclodextrin in binding studies. Tojima et al. [30] prepared a-CD linked chitosan using 2-formylmethyl-α-CD beads by reductive *N*-alkylation [28-30] (Figure 1.7). Their inclusion ability was examined by the use of *p*-nitrophenol and 3-methyl-4-nitrophenol as model compounds. Although these two guest molecules have closely resembling structures, the methyl group of the later strongly inhibited the formation of an inclusion complex due to steric hindrance of the methyl group. The potent inclusion ability was observed on a-CD-g-chitosan beads toward p-nitrophenol while 3-methyl-4-nitrophenol was not adsorbed on the beads. Controlled release study suggested that *p*-nitrophenol entrapped with a-CD-g-chitosan beads was released slowly into the buffer and that equilibrium was reached after 15 h. In contrast to these result, chitosan beads, which have little ability to form inclusion complexes, released almost all of the p-nitrophenol within several hours. These experiments suggested that a-CD-g-chitosan may serve as an adsorbent for controlled release of drugs.

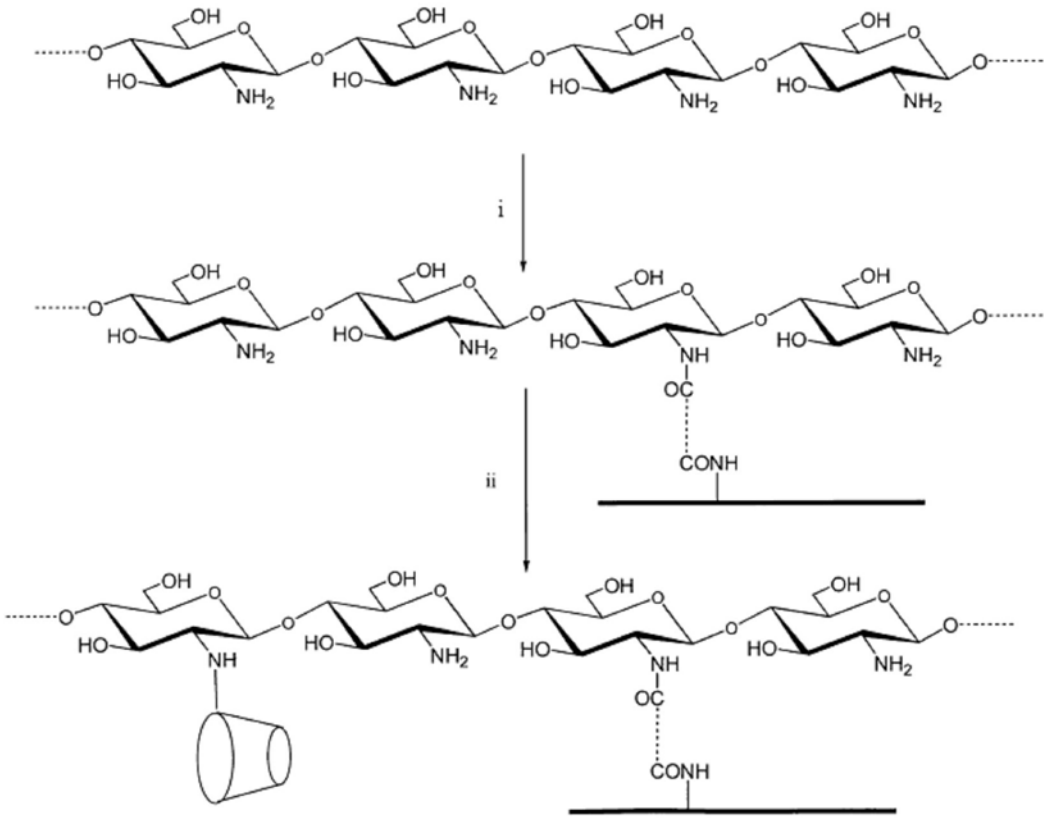


Figure 1.7 Praparation of CD-linked chitosan beads. Reagents and conditions: (1) hexamethylene diisocyanate, DMF, r.t.,overnight; (ii) formylmethyl-α-CD, $NaBH_3CN_3$, 0.2 M acetate buffer at pH 4.4, r.t., 4 d [30]

Schiff base formation

Condensation of the free amino group of chitosan with aldehydes or ketones affords Schiff base (Figure 1.8), and the reaction proceeds smoothly in a mixed solvent of an aqueous acetic acid and methanol. Though the reaction is homogenous in the initial stage, gel formation is frequently observed because of the poor solubility of the resulted Schiff base [31]. A derivative of decanal showed glass transition because of the long side chains [32]. The Schiff base formation can therefore be used for the protection of the amino functionality of chitosan during the modification of the remaining hydroxyl groups [32, 33].

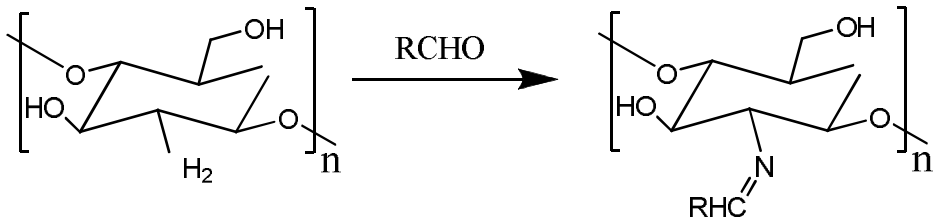


Figure 1.8 Schiff base product from chitosan and aldehyde

O- and N-pthaloylation of chitosan

Phathaloylation of the water-soluble chitosan (50% deacethylated chitin) with phthalic anhydride afforded an *N*-substituted derivative soluble in dimethyl sulfoxide (DMSO) [34]. When fully deacetylated chitosan could be phthaloylated and the product showed higher solubility; it was readily soluble in aprotic solar organic solvents such as pyridine, DMSO, dimethylacetamide (DMAc) and *N, N*-dimethylformamide (DMF) [32]. The reaction typically carried out with three equivalents of phthalic anhydride in DMF at 120-130 °C. The reaction was found to involve partial *O*-phthaloylation in addition to the expected *N*-phthaloylation, and the degree of substitution was up to 1.5. The *O*-phthaloyl groups could be replaced by the triphenylmethyl group or removed by transesterification leading to *N*-phthaloylated chitosan with a degree of substitution of 1.0. *O*-Phthaloylation was completely suppressed by conducting the reaction in DMF containing 5% water to give regiospecifically substituted 2-*N*-phthaloyl-chitosan in one-step (Figure 1.9) [32]

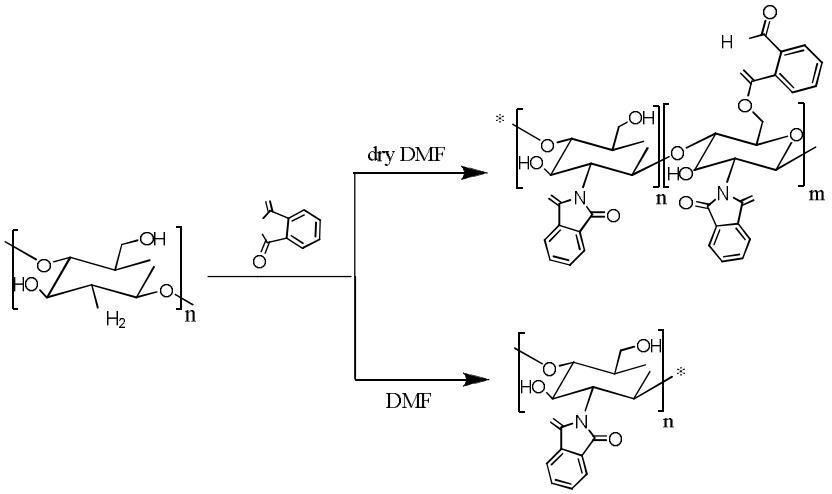


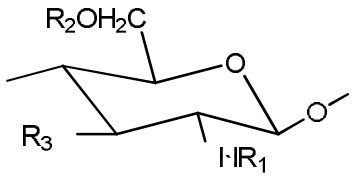
Figure 1.9 Phthaloylation of chitosan in dry DMF and DMF

1.3 Chitosan-based nanoparticle as drug carrier

Chitosan has been reported as material suitable for the use as drug carriers in pharmaceutical, medical and biotechnological areas. The self-assembly of chemically modified chitosan into nanoparticles has been investigated for the delivery of macromolecule.

In 1998, Kawahara *et al.* [26] synthesized novel chitosan derivatives with both hydrophobic and hydrophilic groups to solubilize taxol. Several chitosan derivatives with lauryl groups attached to the amino groups to provide hydrophobic moieties and carboxymethyl groups attacted to the hydroxyl groups to provide hydrophilic moieties (*N*-lauryl-carboxymethyl-chitosan (LCC)) (Figure 1.10) were synthesized. They found that LCC micelles formed nanoparticles less than 100 nm in size. It may be useful as passive targeting carriers of hydrophobic cancer drugs for solid tumors.

$[\text{-A-/-B-/-C-/-D-/-E-/-F-/-G-/-H-/...}]_n$



	R1	R2	R3
A	$-(CH_2)_{11}CH_3$	$-CH_2COOH$	$-CH_2COOH$
B	-H	$-CH_2COOH$	$-CH_2COOH$
C	$-(CH_2)_{11}CH_3$	-H	$-CH_2COOH$
D	$-(CH_2)_{11}CH_3$	$-CH_2COOH$	-H
E	$-(CH_2)_{11}CH_3$	-H	-H
F	-H	$-CH_2COOH$	-H
G	-H	-H	$-CH_2COOH$
H	-H	-H	-H

Figure 1.10 Chemical structure of *N*-lauryl-carboxymethyl-chitosan (LCC).

In 2004, Yokson *et al*. [35] grafted a hydrophilic poly (ethylene glycol) methyl ether terminated carboxylic acid (mPEG-COOH) (Figure 1.11), onto the hydroxyl group of *N*-phthaloylchitosan. The grafted product formed self aggregates into nanoparticles with size 80-100 nm (Figure 1.12). The product formed a milky dispersion in water and a series of solvents.

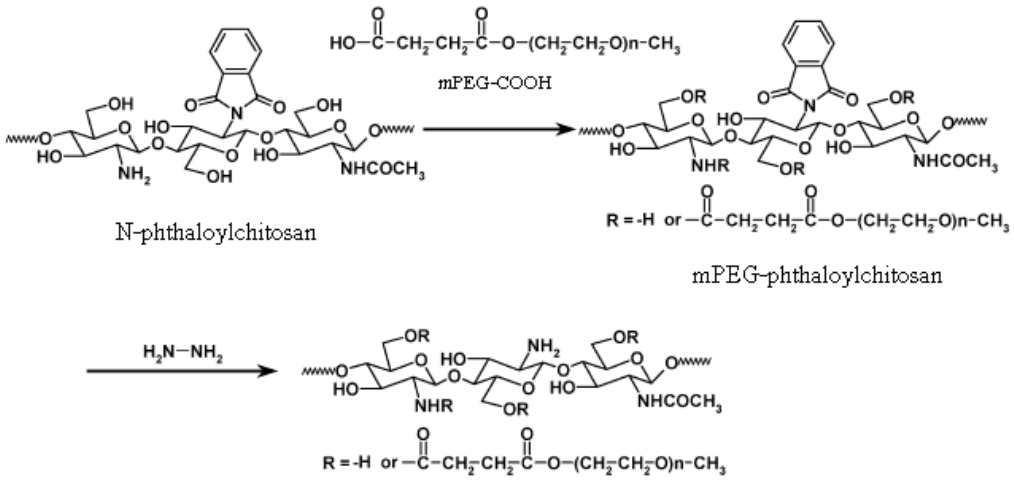


Figure 1.11 Synthesis pathway of *N*-Phthaloylchitosan-grafted poly(ethyleneglycol) methylether (mPEG).

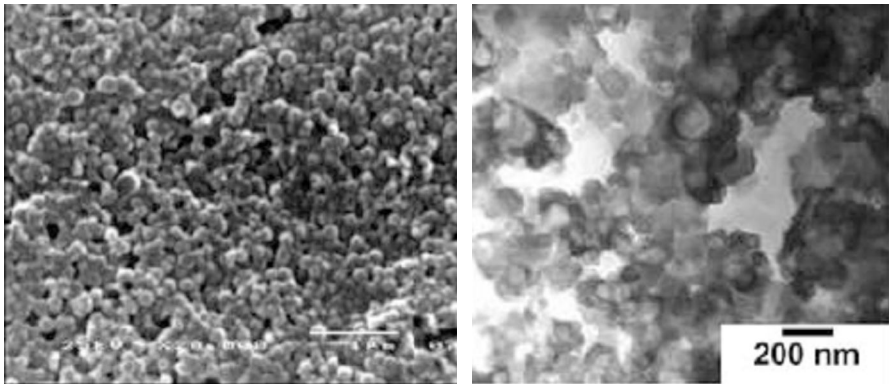


Figure 1.12 SEM and TEM photograph of *N*-phthaloylchitosan-grafted poly (ethyleneglycol) methyl ether (mPEG).

In 2005, Wu *et al*. [36] grafted DL-lactide onto water soluble chitosan, Forming a novel amphiphilic chitosan–polylactide (Figure 1.13) graft copolymers

which could form into polymeric micelles. The micelles possessed unimodal size distribution (Figure 1.14). Since chitosan–polylactide polymeric micelles possessed a hydrophobic core, they could be used as a promising delivery carrier for the entrapment and controlled release of hydrophobic drugs.

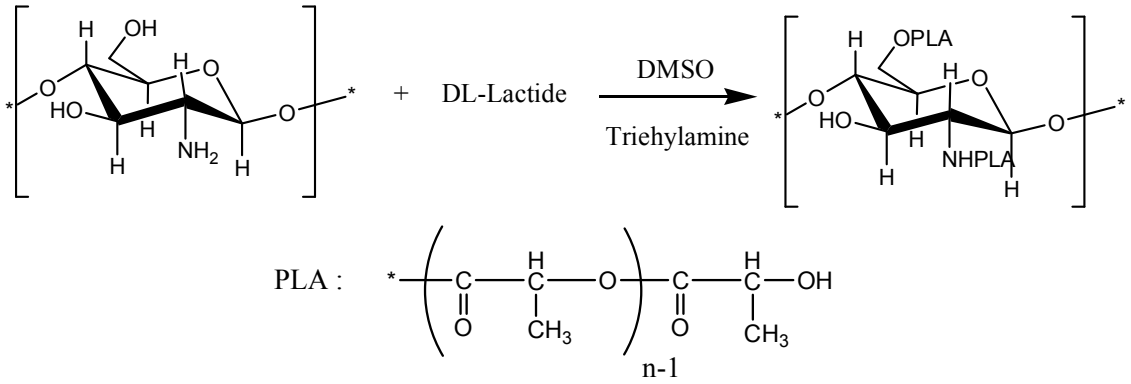


Figure 1.13 Graft copolymerization of DL-lactide onto chitosan.

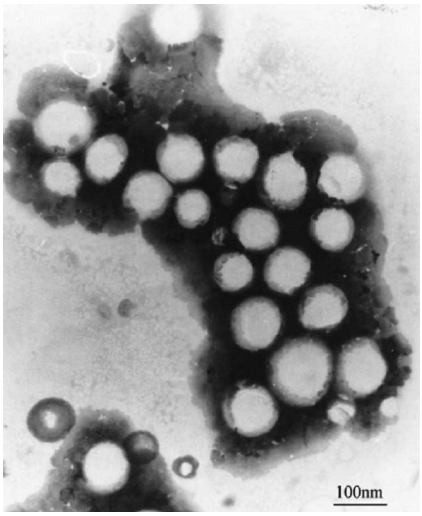


Figure 1.14 TEM photograph of chitosan polylactide copolymer micelle.

In 2005, Chae *et al*. [37] modified chitosan oligosaccharides (COSs) with hydrophobic moiety of deoxycholic acid (DOCA) (Figure 1.15). Due to their amphiphilic character, the hydrophobized DOCA-conjugated COSs (COSDs) also formed core-shell type nanoparticles in aqueous milieu. Compared to unmodified COSs, the COSDs nanoparticles showed elevated potential as gene carriers.

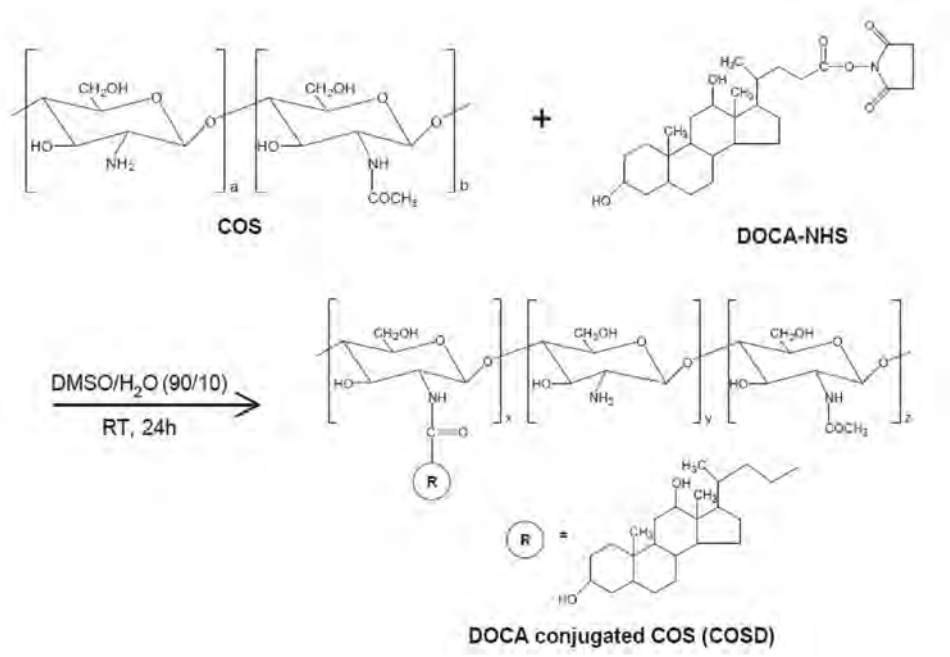


Figure 1.15 Synthetic schemes for deoxycholic acid conjugation on chitosan oligosaccharides**.**

In 2006, Amidi *et al*. [38] prepared *N*-trimethyl chitosan nanoparticles (TMC) under mild conditions using tripolyphosphate (TPP) as crosslinker. The product was for application as investigated as a vehicle for nasal delivery of proteins. The nanoparticles had an average size of about 350 nm (Figure 1.16). The TMC nanoparticles possessed an excellent loading capacity for proteins (ovalbumin), and a positive surface charge, suitable to attach to nasal mucosa.

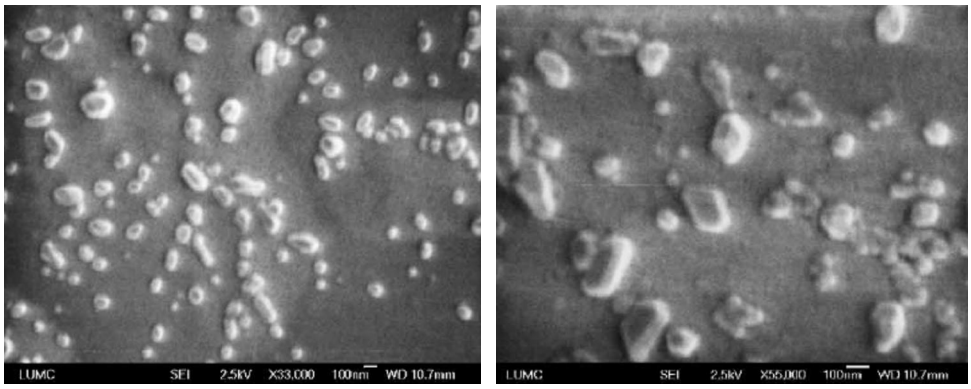


Figure 1.16 SEM photographs of TMC nanoparticles a) non-loaded particles b) ovalbumin loaded particles.

In 2006, Zhu *et al*. [39] synthesized a novel biocompatible chitosan derivative, *N*-succinylchitosan (NSCS) with well-designed structure. NSCS could self-assembly into regular nanosphere morphology (Figure 1.17) in distilled water. The mechanism of self-assembly of NSCS in distilled water are believed to be the intermolecular H-bonding and hydrophobic interaction among the hydrophobic moieties such as $–CH_2CH_2–$, acetyl groups and glucosidic rings in NSCS. Within the nanospheres, there are the hydrophobic domains formed. NSCS possessed non-toxic and cell-compatible properties. All these results suggest that NSCS nanosphere has great potential to be used as a novel drug matrix in the controlled released delivery.

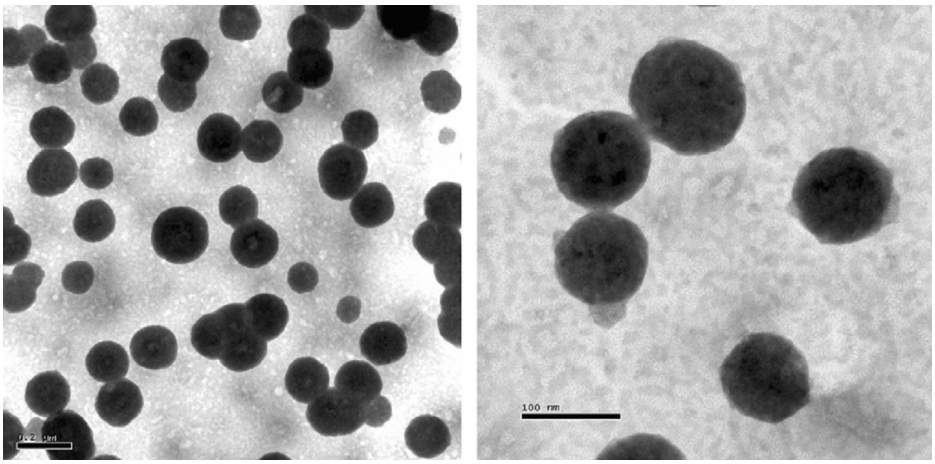


Figure 1.17 TEM micrograph of *N*-succinylchitosan nanosphere (0.5 mg/ml NSCS colloidal dispersion).

In 2008, Anumansirikul *et al*. [40] synthesized m-PEG-phthaloylchitosan derivative (PPLC) and m-PEG-phthaloylchitosan derivative grafted with a UVB absorptive chromophore, the 4-methoxycinnamoyl group (PCPLC), at two different degrees of substitution (PCPLC1 and PCPLC2) (Figure 1.18). 2-ethylhexyl-4-methoxycinnamate (EHMC) was encapsulated into PPLC, PCPLC1 and PCPLC2 nanoparticles. The morphology of product gave spherical nanoparticles (Figure 1.19) The results indicated that the grafted UVB absorptive chromophore acted as a UV-filtering barrier, protecting the encapsulated EHMC from the UVB radiation.

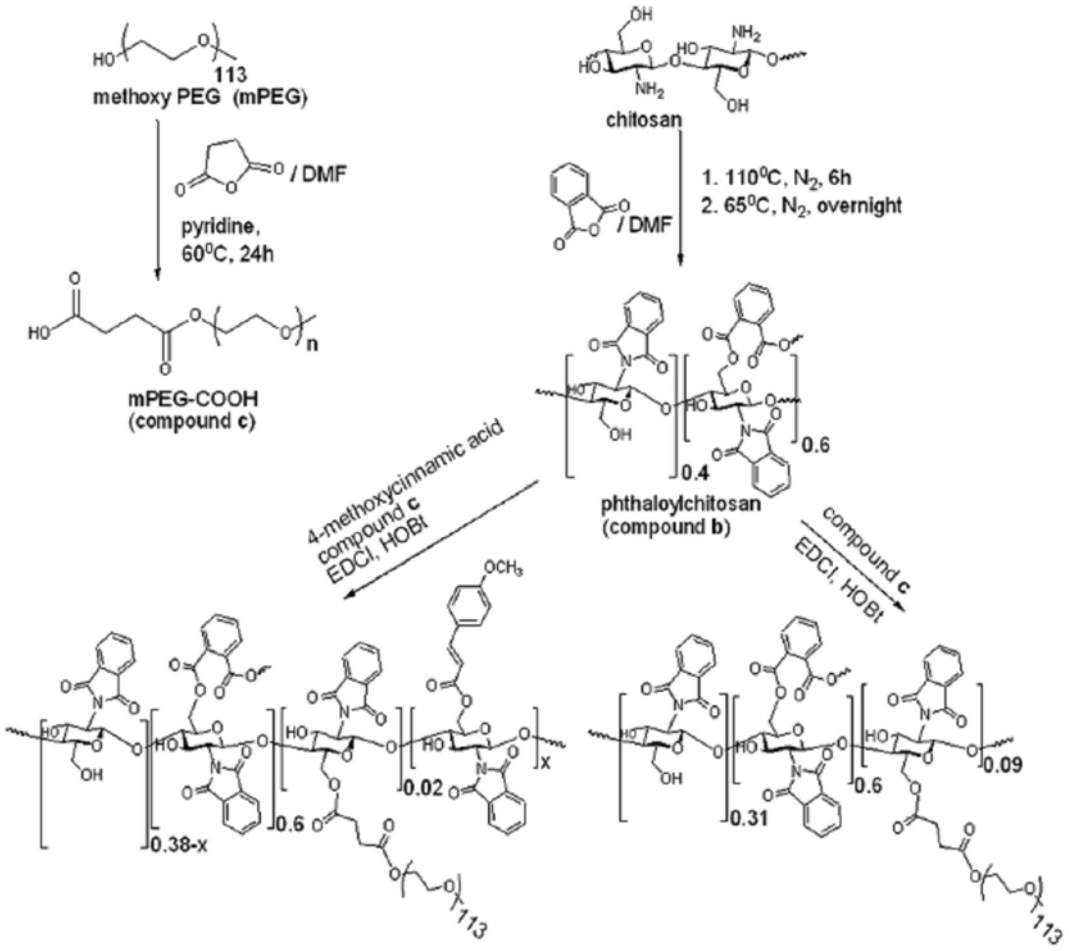


Figure 1.18 Synthetic pathway of PPLC, PCPLC1 and PCPLC2.

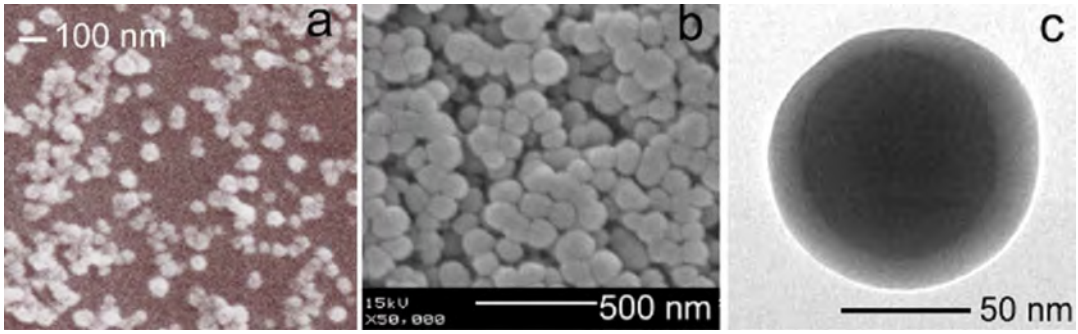


Figure 1.19 SEM micrographs of a) PCPLC2 particles b) EHMC-encapsulated PCPLC2 particles and c) TEM micrograph of EHMC-encapsulated PCPLC2 particles.

However, in Anumasirikul work (Figure 1.18), the synthesis process was multistep and thus, is quite difficult to prepare commercially. Therefore, the present work focuses on the preparation of UV-absorbing chitosan derivatives nanoparticles using a more simple process.

1.4 Research goals

The objectives of this research is to synthesized both UVA- and UVB-absorptive chitosan derivatives nanospheres. Two simple synthetic strategies are employed, amide formation and imine formation. The works include:

1. Amide derivatives
   - Synthesis of 2,4,5-trimethoxycinnamic acid.
   - Synthesis of 2,4,5-trimethoxycinnamoylchloride.
   - Synthesis of cinnamoylchloride and 2,4,5-trimethoxycinnamoylchloride onto chitosan at various degree of substitution.
2. Schiff base form of chitosan
   - Synthesis of succinylchitosan.
   - Synthesis of Schiff base product from 4-hydroxy-3-methoxycinnamaldehyde and succinylchitosan at various degree of substitution.
   - Synthesis of Schiff base product from 2,4-dimethoxybenzaldehyde and succinylchitosan at various degree of substitution.

CHAPTER II

EXPERIMENTAL

2.1 Materials and Chemicals

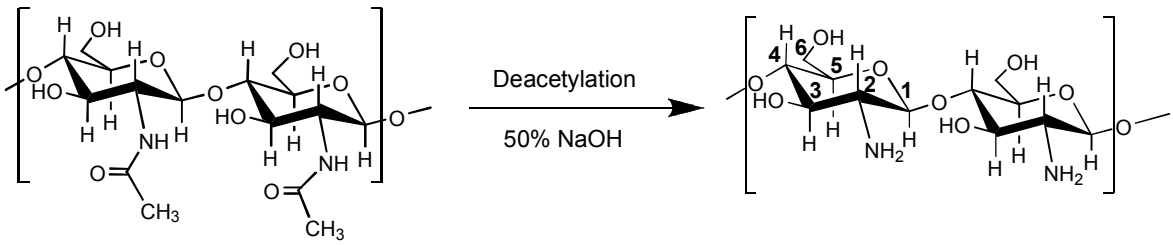
Chitosan (MW 100,000 Daltons, 85% deacetylated) was purchased from seafresh industry Public Co., Ltd. (Bangkok, Thailand). Chitin was purchased from Ta Ming Enterprises Co., Ltd (Samutsakon, Thailand). Dialysis membranes with a molecular weight cutoff of 12000-14000 Daltons (size 76 mm. x 49 mm) were purchased from Aldrich Chemical Company (Steinheim, Germany). Dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) were purchased from Labscan (Dublin, Ireland). Thionyl chloride was double distilled before use. Cinnamic acid (CA) was purchased from Fluka Chemical Company (Buchs, Switzerland). 4-Methoxycinnamicacid (4-MCA), 2, 4-dimethoxybenzaldehyde and 2, 4, 5- trimethoxybenzal dehyde were purchased from Across Organics (Geel, Belgium). Pyridine and benzene was purchased from J.T. Baker Inc. (New Jersey, USA). 2-Ethylhexyl-p-methoxycinnamate (EHMC, Eucolex 2292) was obtained from Merck Co., Ltd. (Darmstadt, Germany). 2,4-dimethoxycinnamaldehyde, trifluoroacetic acid (TFA) and malonic acid were purchased from Fluka Chemical Company (Missouri, USA).

2.2 Instruments and Equipments

The UV spectra were obtained with the aid of UV/Vis spectrophotometer (Agilent Technologies, California, USA) using 1 cm pathlenght quartz cell. Centrifugation was performed on a high speed centrifuge (Allegra 64R centrifuge, Beckman Coulter, Inc., Fullerton, California, USA). Transmission Electron Microscopy (TEM) and Scanning Electron Microscope (SEM) photographs were acquired through the JEM-2100 (JEOL, Tokyo, Japan) and JSM-6400 (JEOL, Tokyo, Japan), respectively. Particle size distribution and zeta potential was obtained with Zetasizer nano series instrument (Zs, Malvern Instrument, Worcestershire, UK). ^{1}H Nuclear magnetic resonance (NMR) spectra were obtained in deuterated dimethylsulfoxide (DMSO-d_6) with tetrametyhlsilane (TMS) as an internal reference using ACF 200 spectrometer which operated at 400.00 MHz (Varian Company,

California, USA). FT-IR spectrometer attached with continuous infrared microscope (Nicolet 6700, USA) was used to obtain FT-IR of the samples. For UV radiation, broad band UVB (280-320 nm) was generated by the FSX24T12/BL/HO lamp (National Biological Corporation, Twinsburg, Ohio, USA). UV Irradiance was measured using an UVA-400C and an UVB-500C power meter (National Biological Corporation, Twinsburg, Ohio, USA). The glass transition and crystallite melting temperatures of derivertizes chitosans were measured by differential scanning calorimeter (DSC) at scan rate of 10 °C/min under nitrogen from 20-300 °C.

2.3 Preparing of chitosan



Chitin **Chitosan**

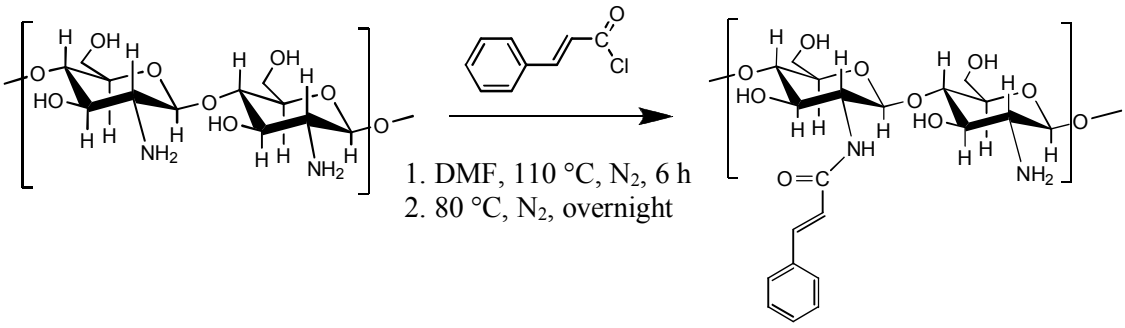
Scheme 2.1

The chitosan used in this experiment was prepared from chitin (squid pen). The demineralization step was accomplished by soaking 100 g of chitin in 1 litters of 1 M hydrochloric acid (HCl) for 24 h. The deproteinization step was accomplished by soaking the protein containing squid pen, from the demineralization step, in 1 liters of 1 M sodium hydroxide (NaOH) for 24 h. The resulted chitin product was then deacetylated in 50% (w/w) sodium hydroxide for 72 h. The chitosan product was washed in distilled water until the pH was neutralized and dried at 50 °C. After that, the pale yellow product was dissolved in 1% acetic acid solution (4 liters) and 200 mL of 2 M NaOH were added. The homogenous solution was mixed with enzyme chitinase and was left overnight at 50 °C. The clear yellow solution was reprecipitated in NaOH solution and washed in distilled water until the pH was neutralized. Final, the obtained product was lyophilized.

Chitosan: 57 % yields of pale yellow solid. T_g: 46.33 °C. T_m: 203.08 °C. Degree of *N*-deacethylation: 85%. MW by GPC: 33,652 daltons. FTIR (cm^{-1}): 3,000-3500 (OH), 3,353 and 3279 (N-H stretching of 1°amine), 2,874 (C-H stretching), 1,640 (C=O, acethylate amino group of chitin) and 1,589 (N-H bending), 1,376 (C-O stretching of $–CH_2$-OH). ^{1}H-NMR (D_2O with 0.05% CD_3OOD,δ, ppm): 2.0 (s, 3H, NCOC**H**$_3$), 2.9 (s, 1H, H-2 of glucosamine), 3.3-4.0 (m, 5H, H-2 proton of acetylglucosamine and H3-H6 of glucosamine ring), 4.7 (br, H-1 of glucosamine ring).

2.4 Synthesis of Amide derivatives

Preparing of cinnamoylchitosan



1 **2**

Scheme 2.2

Two grams (0.0125 mole) of chitosan [**1**] were added into 40 ml of DMF under N_2 atmosphere with stirring. Then, the mixture was heated at 110 °C for 6 h. After that, the reaction temperature as reduced to 80 °C, before adding cinnamoylchloride (0.00125 mole, 0.3 g) and pyridine (2-3 drops). The mixture was left for 12 h. Next, the solution was dialyzed against methanol for the removal of residual cinnamoyl derivatives (4 x 250 mL methanol) and subsequently dialyzed against water (6 x 1000 mL water) to obtain an aqueous dispersion of cinnamoylchitosan [**2**]. Finally, the solution was freeze dried.

Table 2.1 Condition used during the synthesis of cinnamoylchitosan (CC)

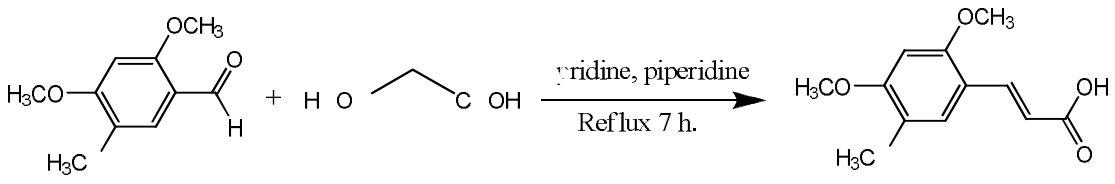
MW of chitosan	Compound	Amounts of chitosan	Amounts of cinnamoylchloride
100,000	CC1	2.0 g, 0.0125 mole	0.3 g, 1.25×10^{-3} mole
30,000	CC2	2.0 g, 0.0125 mole	0.3 g, 1.25×10^{-3} mole
	CC3	2.0 g, 0.0125 mole	1.2 g, 5.00×10^{-3} mole

Cinnamoylchitosan (CC1): 34% yields of pale yellow solid. T_g: 37.97 °C. T_m: 187.63 °C DS of cinnamoyl group: 0.051. FTIR (cm^{-1}): 3,279 (OH), 2,867 (C-H stretching), 1,645 (C=O stretching of amide) and 1,542, 1,406, 888 (C=C of aromatic ring). ^{1}H-NMR (D_2O with 0.05% CD_3OOD, δ, ppm): 2.0 (s, 3H, NCOC**H**$_3$), 3.3-4.0 (m, 5H, H2 proton of acetylglucosamine and H3-H6 of pyranose ring), 4.7 (br, H1 of pyranose ring), 6.4 (d, *J*=16 Hz, 1H, Ar-CH=C**H**-COOR) and 7.0-7.6 (m, 6H, Ar-**H** and Ar-**H**C=CH-COOR). UV-vis (DMSO) λ_{max}: 289 nm.

Cinnamoylchitosan (CC2): 45% yields of pale yellow solid. T_g: 37.53 °C. T_m: 209.27 °C DS of cinnamoyl group: 0.155. FTIR (cm^{-1}): 3,296 (OH), 2,853 (C-H stretching), 1,640 (C=O stretching of amide) and 1,516, 1,401, 894 (C=C of aromatic ring). ^{1}H-NMR (D_2O with 0.05% CD_3OOD, δ, ppm): 2.0 (s, 3H, NCOC**H**$_3$), 3.3-4.0 (m, 5H, H2 proton of acetylglucosamine and H3-H6 of pyranose ring), 4.7 (br, H1 of pyranose ring), 6.4 (d, *J*=16 Hz, 1H, Ar-CH=C**H**-COOR) and 7.0-7.6 (m, 6H, Ar-**H** and Ar-**H**C=CH-COOR). UV-vis (DMSO) λ_{max}: 289 nm.

Cinnamoylchitosan (CC3): 49% yields of pale yellow solid. T_g: 37.06 °C. T_m: 186.71 °C DS of cinnamoyl group: 0.321. FTIR (cm^{-1}): 3,284 (OH), 2,852 (C-H stretching), 1,643 (C=O stretching of amide) and 1,536, 1,404, 882 (C=C of aromatic ring). ^{1}H-NMR (D_2O with 0.05% CD_3OOD, δ, ppm): 2.0 (s, 3H, NCOC**H**$_3$), 3.3-4.0 (m, 5H, H2 proton of acetylglucosamine and H3-H6 of pyranose ring), 4.7 (br, H1 of pyranose ring), 6.4 (d, *J*=16 Hz, 1H, Ar-CH=C**H**-COOR) and 7.0-7.6 (m, 6H, Ar-**H** and Ar-**H**C=CH-COOR). UV-vis (DMSO) λ_{max}: 289 nm.

Preparing of 2, 4, 5-trimethoxycinnamic acid



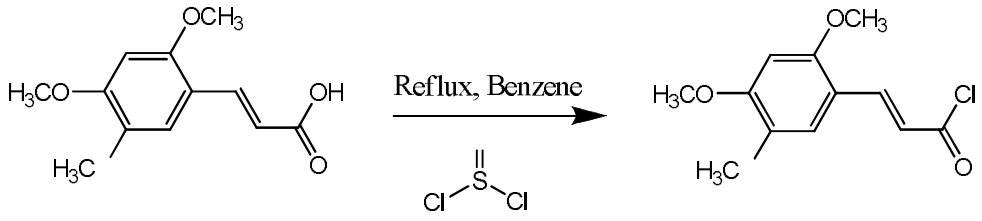
3 **4** **5**

Scheme 2.3

2,4,5-Trimethoxybenzaldehyde [**3**] (0.981 g, 0.005 mole) was dissolved in 43.80 mL of pyridine and malonic acid [**4**] (0.52 g, 0.005 mole) was added. Then, piperidine (2.50 mL, 25.25 mmole) was added to the homogenous solution. The solution was heated at 80 °C for 6-8 h. The solution was precipitated in 10% aqueous HCl and washed thoroughly with ethanol to obtain 2,4,5-trimethoxycinnamic acid [**5**] (Scheme 2.3).

2,4,5-Trimethoxycinnamic acid: 89% yields of yellow solid. ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.8 and 3.9 (s, 9H, OC**H**$_3$), 6.4 (d, 1H, CH=C**H**COOH), 8.1 (d, 1H, Ar-C**H**=CH), 6.4 and 7.3 (s, 1H, Ar-H).

Preparing of 2,4,5-trimethoxycinnamoylchloride



5 **6**

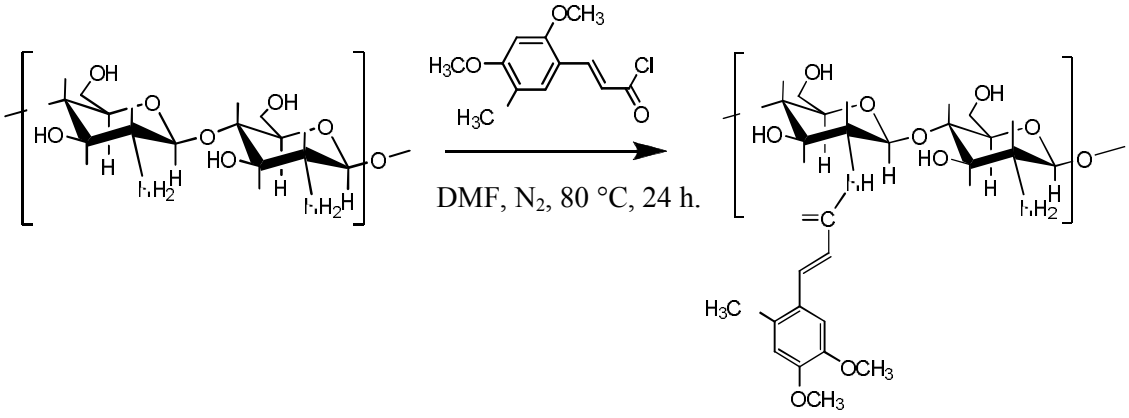
Scheme 2.4

2,4,5-Trimethoxycinnamoylchloride [**6**] was prepared by reacting 2,4,5-trimethoxyacid [**5**] with thionyl chloride (2.5-flod) (Scheme 2.4) in a presence of pyridine (2 drops), in 40 ml dry benzene. The mixture was refluxed overnight. Then,

the solvent and the excess thionyl chloride were removed by evaporation under reduced pressure. Finally, benzene addition (5 ml) and evaporation were performed to remove the last traces of thionyl chloride.

2,4,5-trimethoxycinnamoylchloride: 81% yields of yellow solid. ^{1}H-NMR of ethyl-2,4,5-trimethoxycinnamate ($CDCl_3$, δ, ppm): 1.2 (tr, 2H, -C**H**$_2$CH$_3$), 4.2 (qu, 3H, -CH$_2$C**H**$_3$ of ethylacetate), 3.8 (s, 9H of -OCH$_3$), 6.3 (d, 1H, CH=C**H**COO-), 7.9 (d, 1H, Ar-C**H**=CH), 6.4 and 6.9 (s, 1H, Ar-**H**).

Preparing of 2,4,5-trimethoxycinnamoylchitosan



1 **7**

Scheme 2.5

Chitosan [**1**] 2.0 g. (0.0125 mole) and 40 mL of distilled DMF were then added to the flask and the mixture was heated for 6 h at 110 °C. The reaction mixture was cooled to room temperature. After that, the reaction temperature was reduced to 80 °C, and 2,4,5-trimethoxy cinnamoylchloride [**7**] (0.00125 mole, 0.3025 g) (Scheme 2.5) and pyridine (2-3 drops) were added. Next, the solution was dialyzed against methanol (4 x 250 mL mathanol) for removal of residual cinnamoyl derivatives and followed with dialysis against water (6 x 1000 mL) to obtained an aqueous dispersion of 2,4,5-trimethoxycinnamoylchitosan nanoparticles. Final, dried particles were obtained by freeze dry technique.

Table 2.2 Condition used during the synthesis of 2,4,5-trimethoxycinnamoylchitosan (245CC)

MW of chitosan	Compound	Amounts of chitosan	Amounts of 2,4,5-trimethoxycinnamoylchloride
100,000	245CC1	2.0 g, 0.0125 mole	0.3025 g, 1.25×10^{-3} mole
30,000	245CC2	2.0 g, 0.0125 mole	0.3025 g, 1.25×10^{-3} mole
	245CC3	2.0 g, 0.0125 mole	1.2100 g, 5.00×10^{-3} mole

2,4,5-Trimethoxycinnamoylchitosan (245CC1): 47% yields of pale yellow solid T_g: 37.15 °C. T_m: 271.61 °C. DS of 2,4,5-trimethoxycinnamoyl group: 0.043. FTIR (cm^{-1}): 3,279 (OH), 2,860 (C-H stretching), 1,645 (C=O of amide) and 1,529, 1,401, 894 (C=C of aromatic ring). ^{1}H-NMR (DMSO-d_6 with 0.05% TFA, δ, ppm): 3.3-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring and 9H of OCH_3), 6.4 (d, *J*=8 Hz, 1H, Ar-CH=C**H**-COOR), 7.8 (d, *J*=8 Hz, 1H, Ar-C**H**=CH-COOR and 7.0-7.4 (m, Ar-**H**). UV-vis (DMSO) λ_{max}: 348 nm.

2,4,5-Trimethoxycinnamoylchitosan (245CC2): 53% yields of pale yellow solid. T_g: 37.37 °C. DS of 2,4,5-trimethoxycinnamoyl group: 0.150. FTIR (cm^{-1}): 3,277 (OH), 2,844 (C-H stretching), 1,642 (C=O of amide) and 1,536, 1,396, 889 (C=C of aromatic ring). ^{1}H-NMR (DMSO-d_6 with 0.05% TFA, δ, ppm): 3.3-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring and 9H of OCH_3), 7.0 (d, *J*=8 Hz, 1H, Ar-CH=C**H**-COOR), 7.5 (d, *J*=8 Hz, 1H, Ar-C**H**=CH-COOR and 7.2-7.4 (m, Ar-**H**). UV-vis (DMSO) λ_{max}: 348 nm.

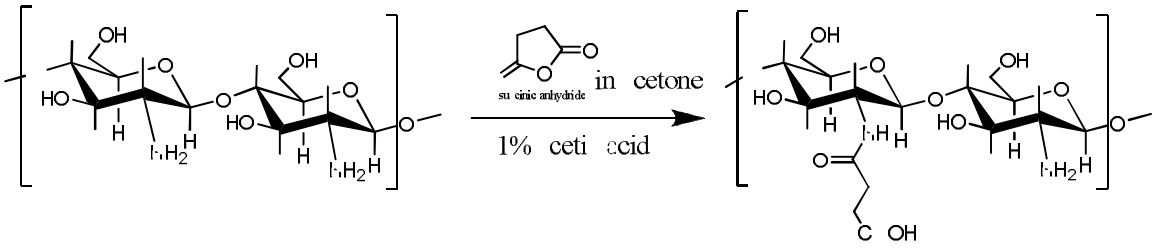
2,4,5-Trimethoxycinnamoylchitosan (245CC3): 49% yields of pale yellow solid. T_g: 39.27 °C. T_m: 263.99 °C. DS of 2,4,5-trimethoxycinnamoyl group: 0.285. FTIR (cm^{-1}): 3,282 (OH), 2,857 (C-H stretching), 1,642 (C=O of amide) and 1,534, 1,401, 892 (C=C of aromatic ring). ^{1}H-NMR (DMSO-d_6 with 0.05% TFA, δ, ppm): 3.3-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring and 9H of OCH_3), 6.4 (d, *J*=8 Hz, 1H, Ar-CH=C**H**-COOR), 7.8 (d, *J*=8 Hz, 1H, Ar-C**H**=CH-COOR and 7.0-7.4 (m, Ar-**H**). UV-vis (DMSO) λ_{max}: 348 nm.

2.5 Nanoparticle formation from amide derivatives of chitosan

Preparation of the nanoparticles from CC1-CC3 and 245CC1-245CC3 were carried out by a solvent displacement method. Sixty milligrams of the polymer were dissolved in 10 mL, DMF. The solution was dialyzed against against methanol for the removal of residual cinnamoyl derivatives (4 x 250 mL methanol) and subsequently dialyzed against water (6 x 1000 mL water) to obtain an aqueous dispersion of the polymer. The obtained colloidal suspension was subjected to zeta potential analysis using the dynamic light scattering technique (DLS) and particle phomorlogy evaluation using scanning electron microscopy and transmission electron microscopy. The pale yellow solid were obtained by freeze dry technique.

2.6 Synthesis of imine derivatives

Preparing of succinylchitosan



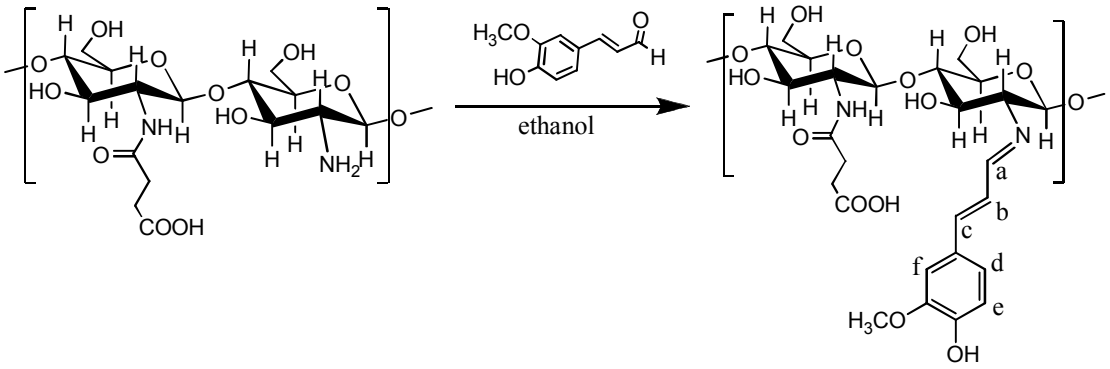
8

Scheme 2.6

One grams of chitosan (6.25 mmole) was dissolved in 1 wt % acetic acid solution (100 mL). Succinic anhydride (0.0625 g, 0.625 mmole) was dissolved in acetone (10 mL) and the solution was slowly dropped into the flask (30 min) at room temperature. Then, the reaction was stirred for 12 h. The reaction mixture was cooled to room temperature and precipitated by 5% w/v NaOH solution. The pH of the mixture was adjusted to 7. The precipitated was collected by filtration and dispersed in 50 mL of H_2O. The pH of the dispersion was adjusted to 10-12 with 5% w/v NaOH solution. The solution was dialyzed against water (6×1000 mL) and lyophilized.

Succinylchitosan (SC): 71 % yields of pale yellow solid. DS of succinyl group: 0.09. FTIR (cm^{-1}): 3,294 (O-H streching), 2,877 (C-H stretching), 1,649 (C=O of amide) and 1,583 (N-H bending), 1,420 (O-H bending of carboxylic), 1,369 (C-N stretching), 1,028 (C-O stretching). ^{1}H-NMR (D_2O with 0.05% CD_3OOD,δ, ppm): 2.9 (s, 1H, H2 of glucosamine), 2.48 (br, 2H, -NHCOC**H$_2$**CH_2COOH), 2.50 (s, 2H, -NHCOCH_2C**H$_2$**COOH), 3.4-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring).

Preparing of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan



5

Scheme 2.7

4-Hydroxy-3-methoxycinnamaldehyde (0.53 g, 3.00×10^{-3} mole) were dissolved in 5 mL of ethanol. This solution was poured into the aqueous dispersion of succinylchitosan (1.0 g, 0.006 mole of succinylchitosan in 50 mL of water) and stirred at room temperature for 12 h. Finally, dry particle was obtained by freeze drying.

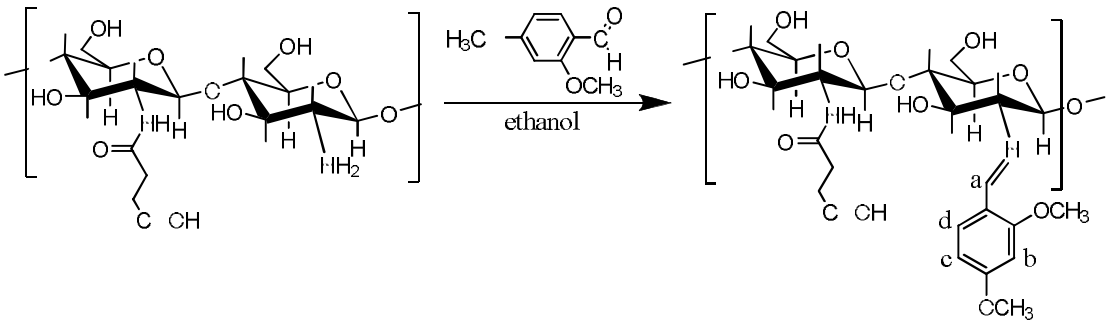
Table 2.3 Condition used during the synthesis of 4-Hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC)

MW of chitosan	Compound	Amounts of succinylchitosan	Amounts of aldehyde derivative group
30,000	43CSC1	1.0 g, 0.006 mole	0.27 g, 1.50×10^{-3} mole
	43CSC2	1.0 g, 0.006 mole	0.53 g, 3.00×10^{-3} mole

4-Hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC1): 69% yields of orange solid. DS of cinnamaldehyde group: 0.196. FTIR (cm^{-1}): 3,323 (OH), 2,884 (C-H stretching), 1,670 (C=N streching) and 1,604, 1,504 (C=C streching), 1,421 (O-H bending of carboxylic), 1,026 (C-O stretching), 1,604, 1,264 and 825 (C=C of aromatic ring), ^{1}H-NMR (D_2O with 0.05% CD_3OOD, δ, ppm): 2.9 (s, 1H, H2 of glucosamine), 3.4-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring), 6.5 (dd, *J*=16 Hz, *J*=8 Hz, 1H, H_b proton of Ar-CH=C**H**), 6.8 (d, *J*=8 Hz, 1H, H_e proton of aromatic ring), 7.0 (d, *J*=8 Hz, 1H, H_d proton of aromatic ring), 7.2 (s, 1H, H_f proton of aromatic ring), 7.5 (d, *J*=16 Hz, 1H, H_c proton of Ar-C**H**=CH), 9.2 (d, *J*=8 Hz, 1H, H_a proton of C**H**=N). UV-vis (DMSO) λ_{max}: 332 nm.

4-Hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC2): 71% yields of orange solid. DS of cinnamaldehyde group: 0.810. FTIR (cm^{-1}): 3,323 (OH), 2,904 (C-H stretching), 1,674 (C=N streching) and 1,604, 1,504 (C=C streching), 1,421 (O-H bending of carboxylic), 1,026 (C-O stretching), 1,604, 1,264 and 828 (C=C of aromatic ring). ^{1}H-NMR (D_2O with 0.05% CD_3OOD, δ, ppm): 2.9 (s, 1H, H2 of glucosamine), 3.4-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring), 6.6 (dd, *J*=16 Hz, *J*=8 Hz, 1H, H_b proton of Ar-CH=C**H**), 6.8 (d, *J*=8 Hz, 1H, H_e proton of aromatic ring), 7.0 (d, *J*=8 Hz, 1H, H_d proton of aromatic ring), 7.2 (s, 1H, H_f proton of aromatic ring), 7.5 (d, *J*=16 Hz, 1H, H_c proton of Ar-C**H**=CH), 9.3 (d, *J*=8 Hz, 1H, H_a proton of C**H**=N). UV-vis (DMSO) λ_{max}: 332 nm.

Preparing of 2,4-dimethoxybenzalidenesuccinylchitosan



6

Scheme 2.8

2,4-Dimethoxybenzaldehyde (0.500 g, 3.00×10^{-3} mole) were dissolved in 5 mL of ethanol. This solution was poured into the aqueous dispersion of succinylchitosan (Scheme 2.6) (1.0 g, 0.006 mole of succinylchitosan in 50 mL of water) and stirred at room temperature for 12 h. Finally, dry particle was obtained by freeze drying.

Table 2.4 Condition used during the synthesis of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC)

MW of chitosan	Compound	Amounts of succinylchitosan	Amounts of aldehyde derivative group
30,000	24BSC1	1.0 g, 0.006 mole	0.250 g, 1.50×10^{-3} mole
	24BSC2	1.0 g, 0.006 mole	0.500 g, 3.00×10^{-3} mole

2,4-Dimethoxybenzalidenesuccinylchitosan (24BSC1): 73% yields of pale yellow solid. DS of benzaldehyde group: 0.180. FTIR (cm^{-1}): 3,374 (OH), 2,877 (C-H stretching), 1,631 (C=N streching), 1,594, 1,516 (C=C stretching) 1,377 (O-H bending of carboxylic), 1,033 (C-O stretching), 1,286 and 808 (C=C of aromatic ring). ^{1}H-NMR (D_2O with 0.05% CD_3OOD, δ, ppm): 2.9 (s, 1H, H2 of glucosamine),

3.4-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring), 6.4 (s, 1H, H_b proton of Ar-**H**), 6.5 (dd, *J*=10 Hz, 1H, H_c proton of Ar-**H**), 7.6 (d, *J*=10 Hz, 1H, H_d proton of Ar-**H**), 9.8 (s, 1H, H_a proton of C**H**=N). UV-vis (DMSO) λ_{max}: 314 nm.

2,4-Dimethoxybenzalidenesuccinylchitosan (24BSC2): 71% yields of pale yellow solid. DS of benzaldehyde group: 0.710. FTIR (cm^{-1}): 3,362 (OH), 2,882 (C-H stretching), 1,631 (C=N streching), 1,594, 1,516 (C=C stretching), 1,376 (O-H bending of carboxylic), 1,031 (C-O stretching), 1,286 and 807 (C=C of aromatic ring). ^{1}H-NMR (D_2O with 0.05% CD_3OOD, δ, ppm): 2.9 (s, 1H, H2 of glucosamine), 3.4-4.0 (m, 5H, H2 proton of acetylglucosamine, H3-H6 of pyranose ring), 6.4 (s, 1H, H_b proton of Ar-**H**), 6.5 (dd, *J*=10 Hz, 1H, H_c proton of Ar-**H**), 7.5 (d, *J*=10 Hz, 1H, H_d proton of Ar-**H**), 9.7 (s, 1H, H_a proton of C**H**=N). UV-vis (DMSO) λ_{max}: 314 nm.

2.7 Photostability test

In these studies, samples were prepared as suspension in water at concentration of 20 ppm. The suspension as then irradiated with Ultraviolet radiation (UVA and UVB) (Ohio, USA) at the UVA intensity of 8.8 mW/cm^2 and UVB intensity of 0.6 mW/cm^2) for 0, 60, 90, 120, 180 minutes and 0, 30, 60, 90, 120, 180 minutes, respectively which corresponded to the UV exposure of 2,4,5-trimethoxy cinnamoylchitosan (245CC2) and 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC2). Next, the irradiated samples were subjected to UV-Visible spectroscopic analysis by scanning wavelength between 200-500 nm.

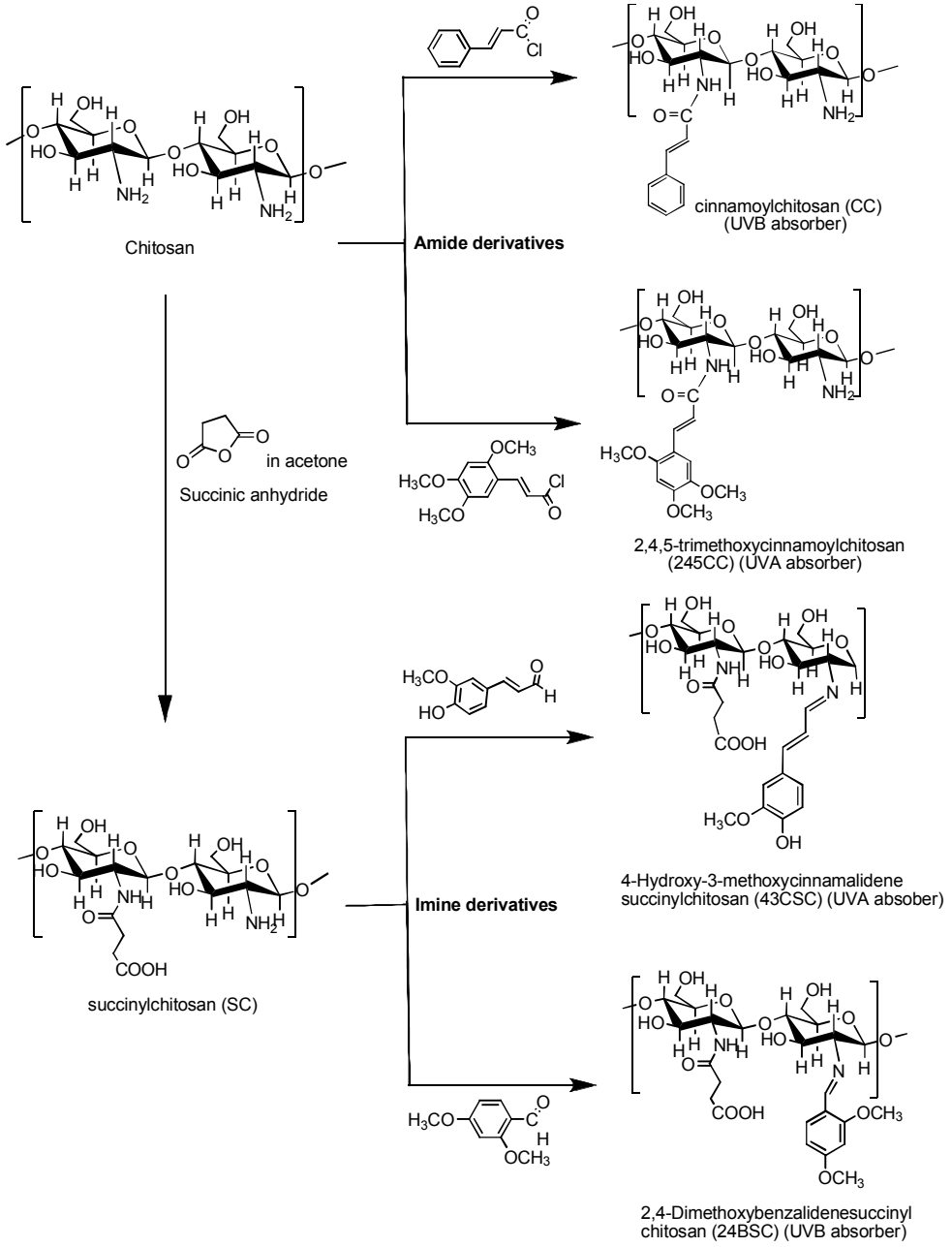
2.8 UV filtering efficiency

UV absorption efficiency of 2,4-dimethoxybenzalidenesuccinylchitosan was compared with that of 2-ethylhexyl-4-methoxycinnamate (EHMC). Cream base formulation containing 5% of the UV-filter was prepared by homogenizing the UV filter into the cream base. Then, each formulation was diluted to the final concentration of 20 ppm and the UV absorption spectrum was acquired.

CHAPTER III

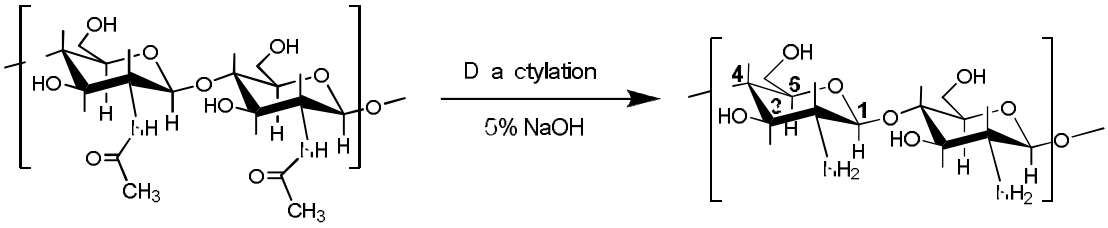
RESULTS AND DISCUSSION

This research presents two synthetic pathways for the preparation of UV absorptive chitosan derivatives. Scheme 3.1 summarizes all the derivatives prepared in this work.



Scheme 3.1

3.1 Deacetylation of chitin

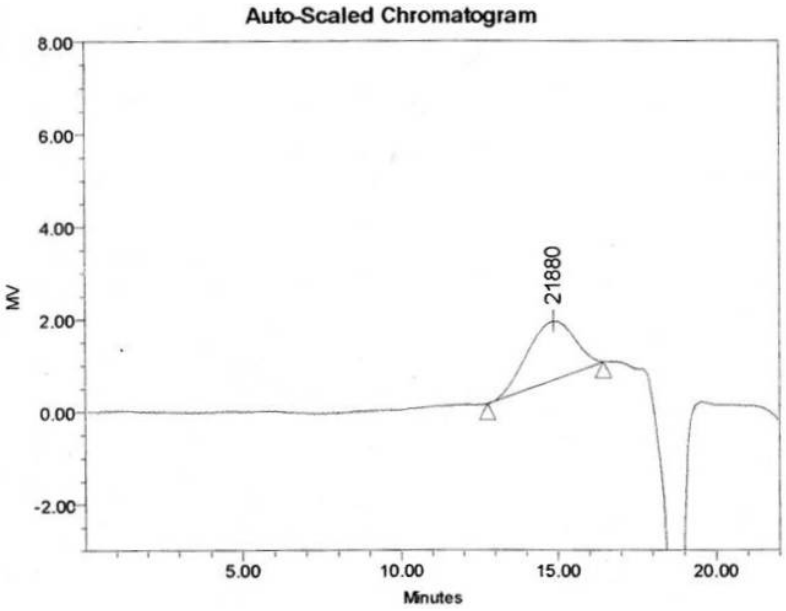


Chitin **Chitosan**

Scheme 3.2

When the product of 2.3 was lyophilized, pale yellow solid (57% yield) was observed. FT-IR spectra of chitosan indicated that deacetylation occurred at acetylate amino functionality of the chitin chain. In other words, the FT-IR showed absorption peaks at 3,353 and 3,279 cm^{-1} (N-H streching, 1° amine and overlap with O-H streching), 1,589 cm^{-1} (N-H bending) from amine group (Figure 3.1). ^{1}H-NMR showed a singlet at 2.0 ppm (acetyl of the N-acetyl glucosamine) and peak at 2.9 ppm was assigned to the protons of glucosamine (H-2). The chemical shift at 3.3–4.0 ppm was from the proton of acetylglucosamine (H-2) and glucosamine ring (H-3, H-4, H-5, H-6). A small singlet at 4.7 ppm (H-1) corresponds to the glucosamine ring (Figure 3.2). The degree of deacetylation was estimated from integration peak (^{1}H-NMR) of the N-acetyl signal (s, 2.0 ppm, NCOC**H**$_3$) and H-2 of glucosamine ring (s, 2.9 ppm). From NMR and FT-IR information, it was concluded that the deacetylated chitin product was chitosan with the DS of 0.85. The result of gel permeation chromatograp found that this chitosan possess molecular weight ~30,000 daltons.

Table 3.1 Result from gel permeation chromatography (GPC) of native chitosan



product	M_n	M_w	Polydisperse index
chitosan	18,932	33,652	1.777

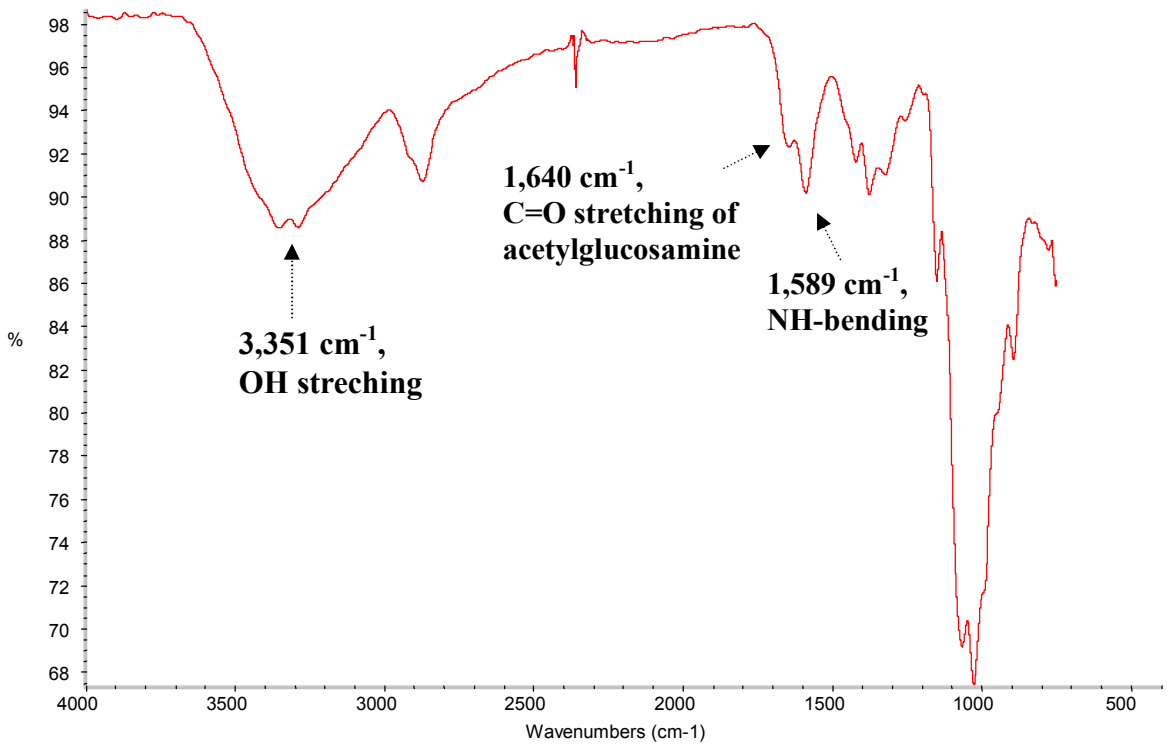


Figure 3.1 FT-IR spectrum of chitosan (DD=85%)

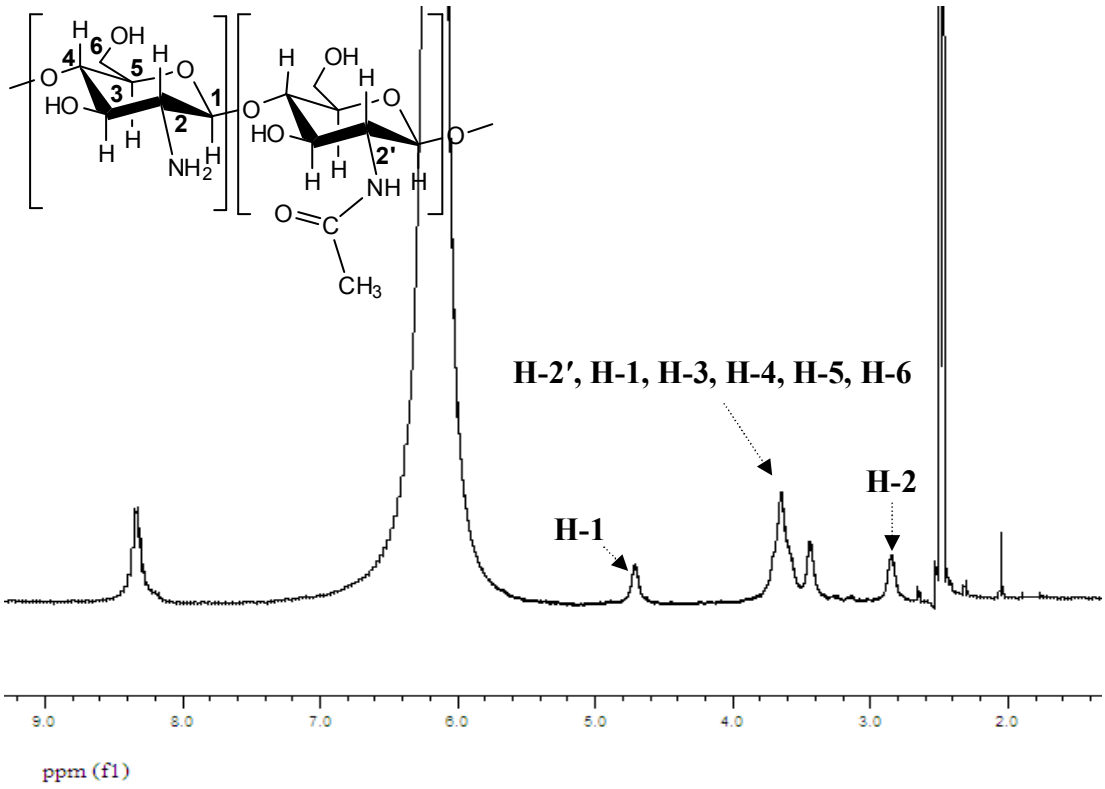
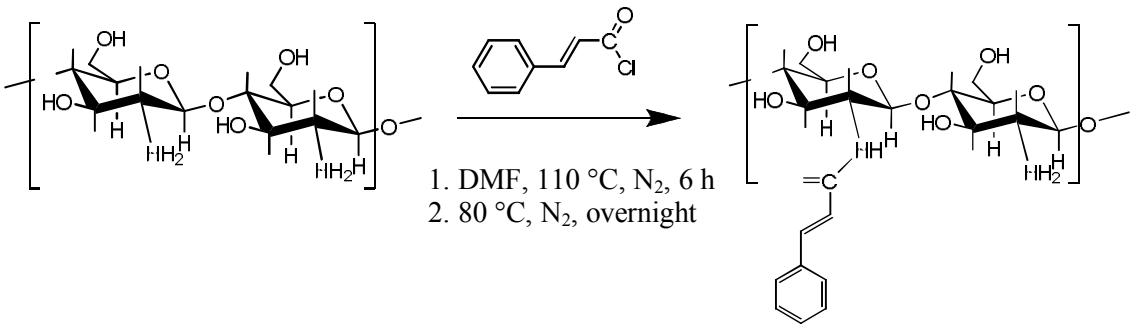


Figure 3.2 ^{1}H-NMR (DMSO-d_6 and 0.05%TFA) spectrum of chitosan (DD=85%)

3.2 Synthesis of Amide derivatives

Preparation of cinnamoylchitosan (CC)



Scheme 3.3

After cinnamoylchloride was reacted with chitosan for a total of 12 h (Scheme 3.3) the mixture was filtered to separate the remaining undissolved chitosan. The remaining undissolved chitosan in the reaction mixture probably was chitosan with

very low degree of deacethylation. When the cinnamoylchitosan (CC) (Scheme 3.3) was dialyzed against water, aqueous dispersed pale yellow particles were observed. After drying, pale yellow solid product could be obtained.

Formation of amide bond between cinnamoyl and chitosan was confirmed by FT-IR (Figure 3.3) and ^{1}H-NMR (Figure 3.4) spectrum. FT-IR spectrum of cinnamoyl chitosan shows amide bond at 1,645 cm^{-1} (C=O stretching) and 1,542 cm^{-1} (NH-bending). The substitution degree of cinnamoyl moieties in each product was estimated using ^{1}H-NMR information; signals~ 6.4 ppm (1H, Ar-HC=C**H**-COOR) of cinnamoyl groups) against 4.7 (H-1 from glucosamine ring). Grafting of cinnamoyl chloride onto chitosan, gave cinnamoylchitosan (CC1-CC3) of various degrees of substitution. In case of the starting chitosan with MW 30,000, the degrees of substitution of cinnamoyl groups were 0.155 (CC2) and 0.321 (CC3). When increasing MW of chitosan, cinnamoylchitosan possess degree of substitution of 0.051 (CC1). Efficiency of the condensation reactive was much lower for higher MW. chitosan.

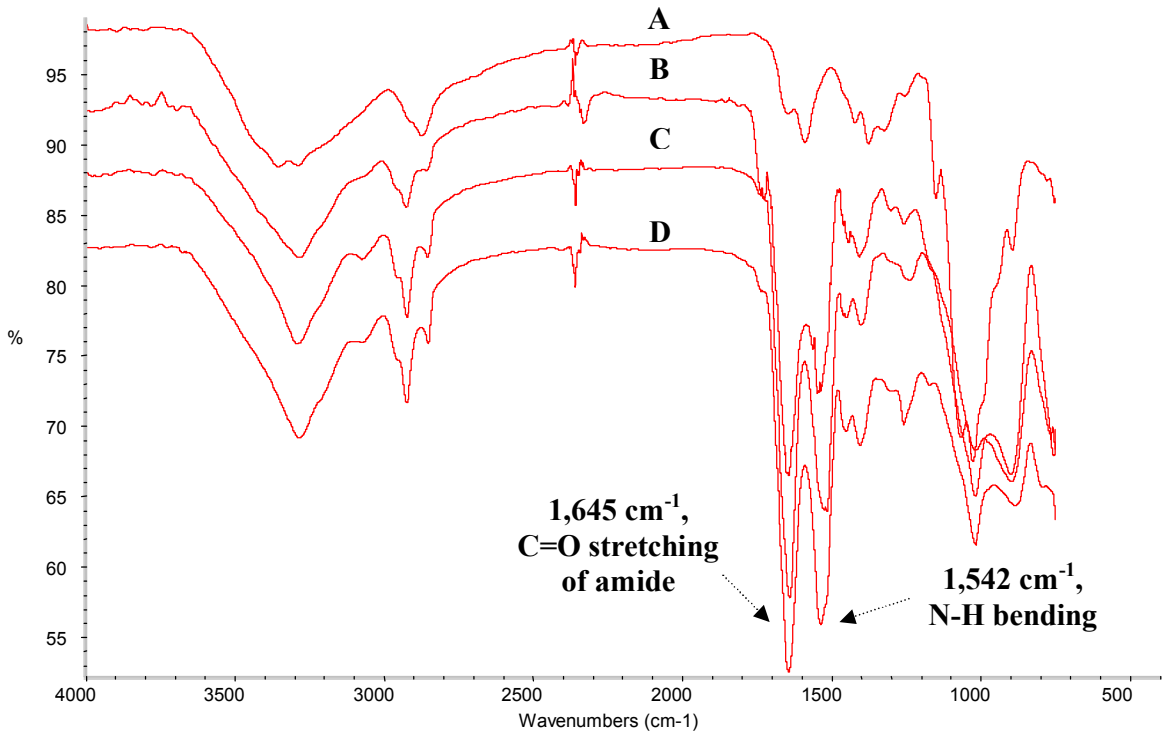


Figure 3.3 FT-IR spectra of A) Chitosan B) CC1 (DS = 0.051) C) CC2 (DS = 0.155) and D) CC3 (DS = 0.321)

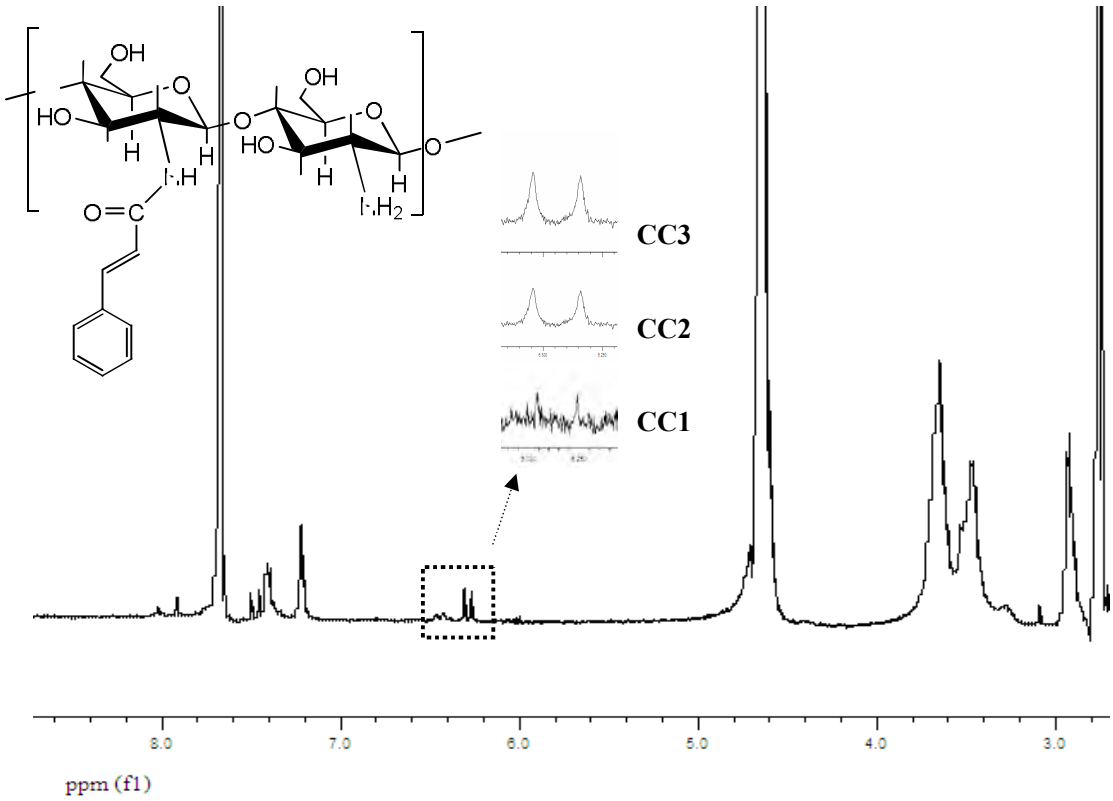
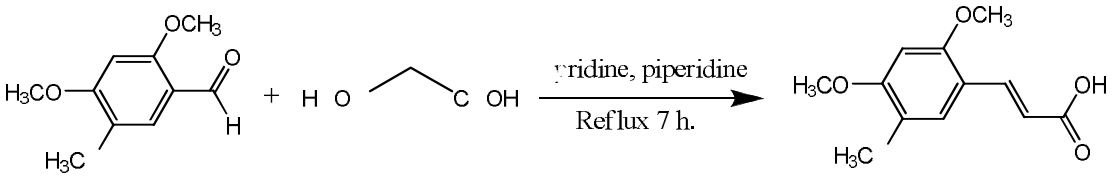


Figure 3.4 ^{1}H-NMR spectra of A) CC1 (DS = 0.051) B) CC2 (DS = 0.155) and C) CC3 (DS= 0.321)

Preparation of 2,4,5-trimethoxycinnamic acid



Scheme 3.4

2,4,5-Trimethoxycinnamic acid was synthesized using Knoevenagel-Doebner condensation between benzaldehyde and malonic acid (Scheme 3.4)**.** Yellow solid product of 89 % yeild could be obtained. ^{1}H-NMR (Figure 3.5) of the product confirmed the structure of 2,4,5-trimethoxy cinnamic acid. Peaks at 3.8 and 3.9 ppm were assigned to the protons of methoxy group (O-C**H**$_3$). The proton signal of ethylene (Ar-C**H**$_1$=C**H**$_2$COOH) were assigned at 6.4 (H-1) and 8.1(H-2) ppm, respectively. Protons on aromatic ring were at position 6.6 (H-3) and 7.0 (H-4) ppm.

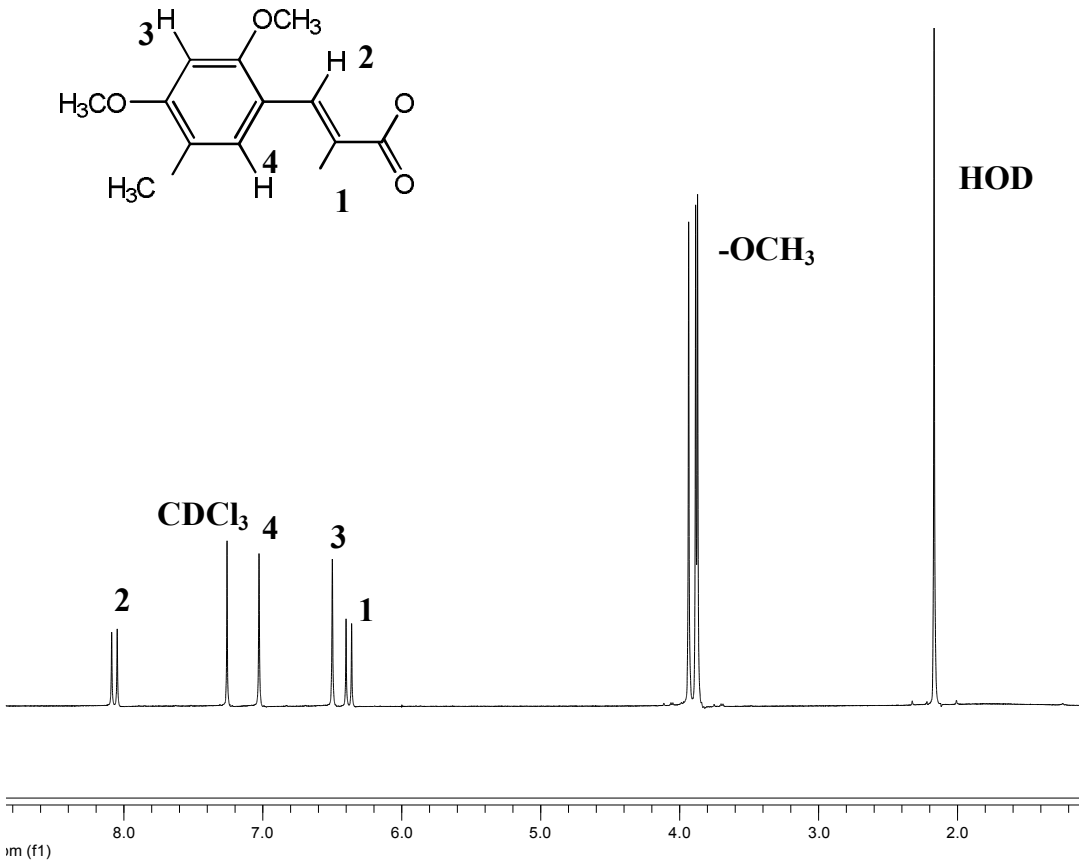
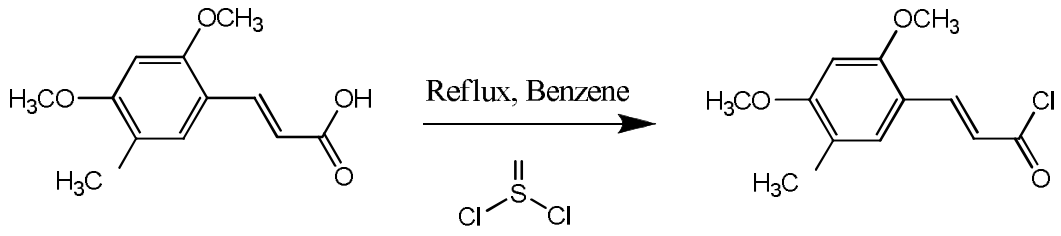


Figure 3.5 ^{1}H-NMR ($CDCl_3$) spectrum of 2, 4, 5-trimethoxycinnamic acid

Preparation of 2, 4, 5-trimethoxycinnamoylchloride



Scheme 3.5

First, the 2,4,5-trimethoxycinnamoylchloride was prepared by reacting 2,4,5-trimethoxycinnamic acid (Scheme 3.5) with excess thionyl chloride in dry benzene. After excess thionyl chloride were removed by vacuum distillation. The obtained product was yellow solid (81 % yield). To confirm the product, ethanol was reacted with 2,4,5-trimethoxycinnamoylchloride to obtain 2,4,5-trimethoxycinnamoylethyl

acetate. Which was characterized by ^{1}H-NMR: 1.2 and 4.2 ppm (2H, -C**H**$_2$CH$_3$ and 3H, -CH$_2$C**H**$_3$ of ethylacetate) and 3.8 ppm (9H from OCH$_3$ of 2, 4, 5-trimethoxy cinnamoyl). The proton signals of ethylene (C**H**$_1$=C**H**$_2$) were assigned at 6.3 (H-1) and 7.9 (H-2) ppm respectively. Protons on aromatic ring gave resonance peaks at position 6.4 (H-3) and 6.9 (H-4) ppm (Figure 3.6).

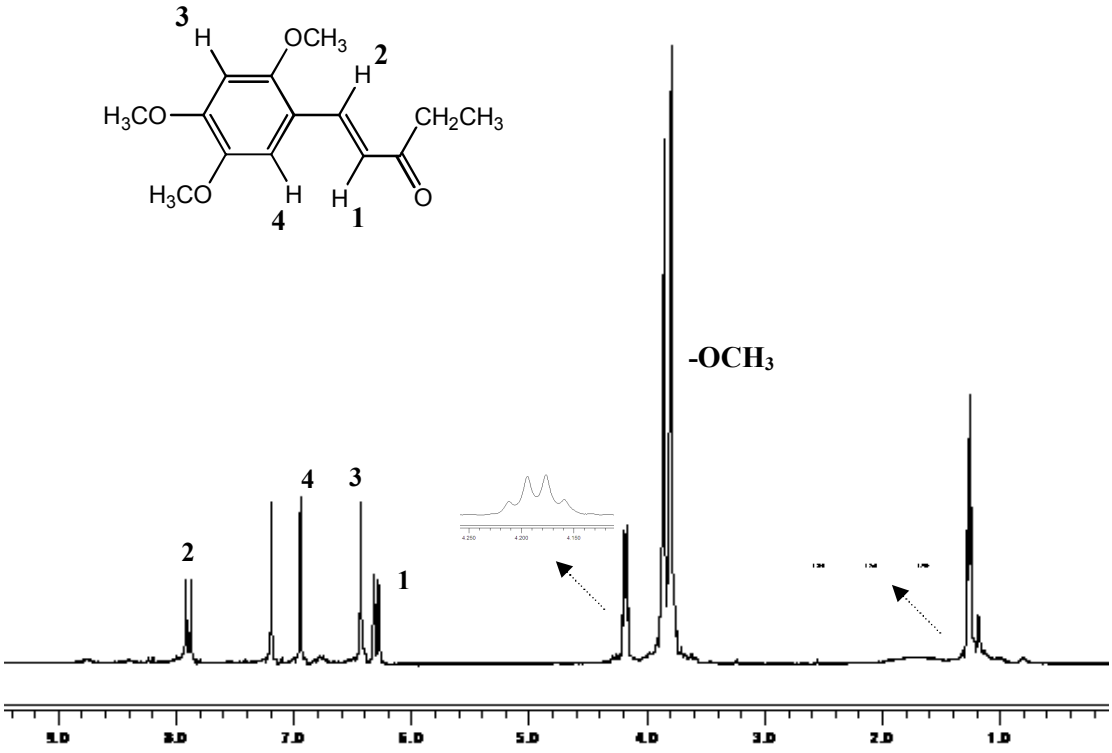
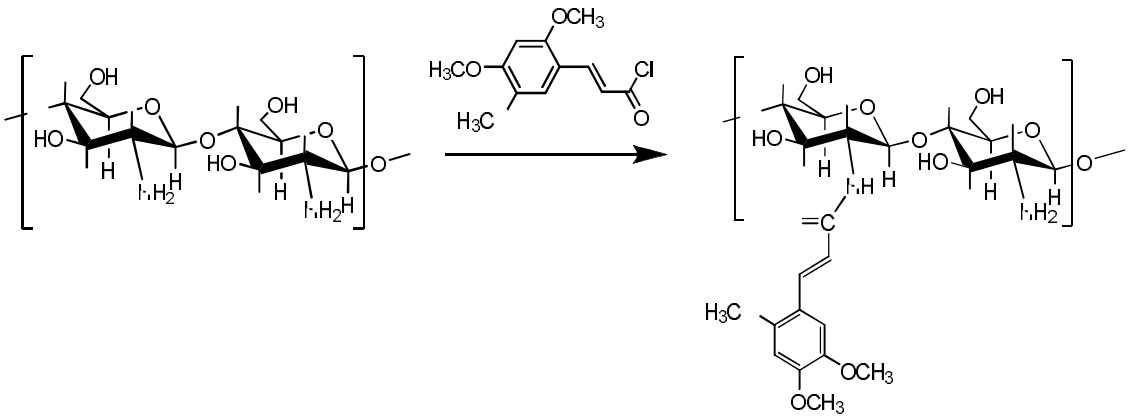


Figure 3.6 ^{1}H-NMR (CDCl$_3$) spectrum of 2, 4, 5-trimethoxycinnamoylethylacetate

Preparation of 2, 4, 5-trimethoxycinnamoylchitosan (245CC)



Scheme 3.6

After 2, 4, 5-trimethoxycinnamoylchloride was reached with chitosan for a total of 12 h the mixture was filtered to separate the remaining undissolved chitosan. When the 2, 4, 5-trimethoxycinnamoylchitosan (Scheme 3.6) was dialyzed against water, aqueous dispersed yellow particles were observed. After drying, yellow solid product could be obtained.

Formation of amide bond between 2,4,5-trimethoxycinnamoyl moieties and chitosan was confirmed by FT-IR (Figure 3.7) and ^{1}H-NMR (Figure 3.8). FT-IR spectrum of 2,4,5-trimethoxycinnamoylchitosan shows amide bond at ~1,645 cm^{-1} (C=O stretching) and 1,529 cm^{-1} (N-H bending). The substitution degree of 2,4,5-trimethoxycinnamoyl moieties in each product was estimated using ^{1}H-NMR information; signals ~ 6.4 ppm (1H, Ar-HC=C**H**-COOR) of 2,4,5-trimethoxycinna moyl groups) against 3.3-4.0 (H2-H6 from chitosan back bone and 9H of 3×OCH_3) (Figure 3.8). Grafting of 2,4,5-trimethoxycinnamoyl moieties onto chitosan, gave 2,4,5-trimethoxy cinnamoylchitosan (245CC1-245CC3) of various degree of substitution. The degree of substitution of 2,4,5-trimethoxy groups induced onto chitosan were 0.150 (245CC2) and 0.285 (245CC3), respectively. When increasing MW of starting chitosan, 2,4,5-trimethoxycinnamoylchitosan possess degree of substitution at 0.043.

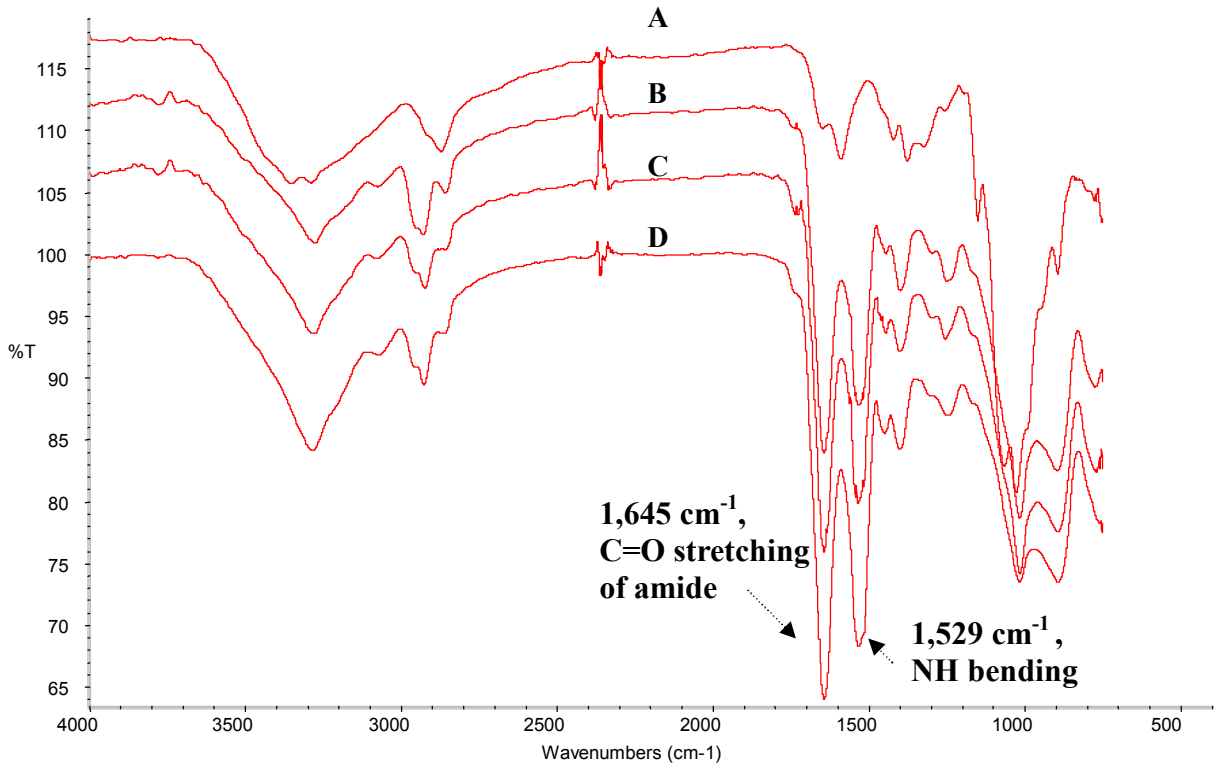


Figure 3.7 FT-IR spectra of A) Chitosan B) 245CC1 (DS = 0.043) C) 245CC2 (DS = 0.150) and D) 245CC3 (DS= 0.285)

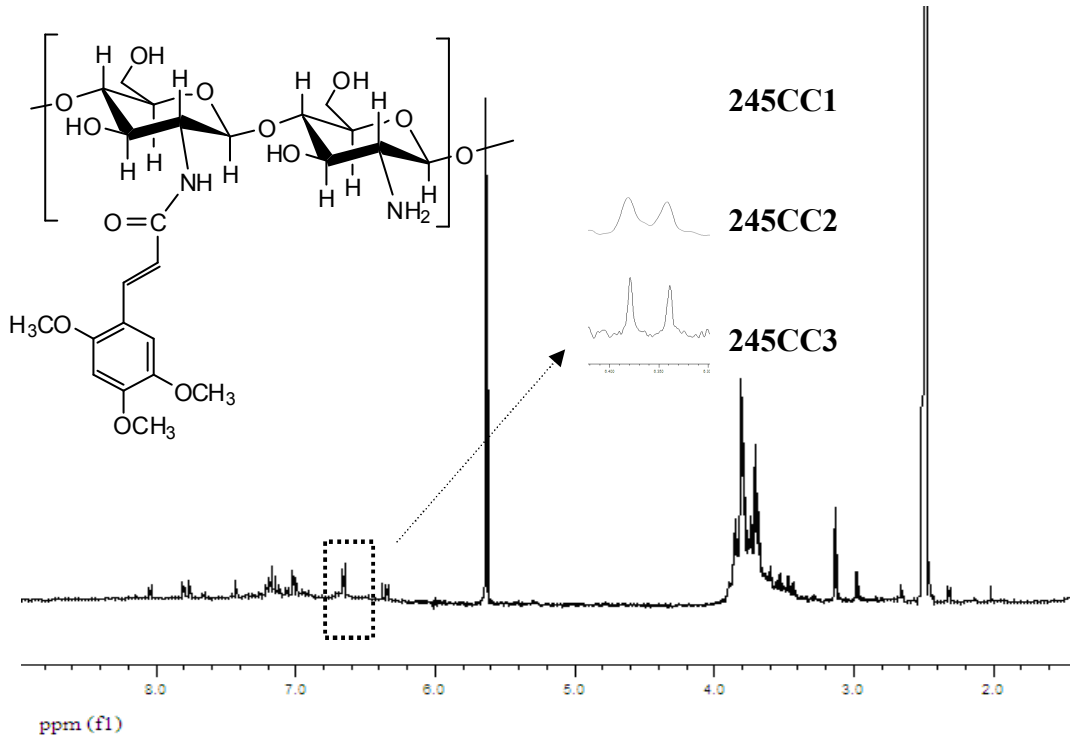
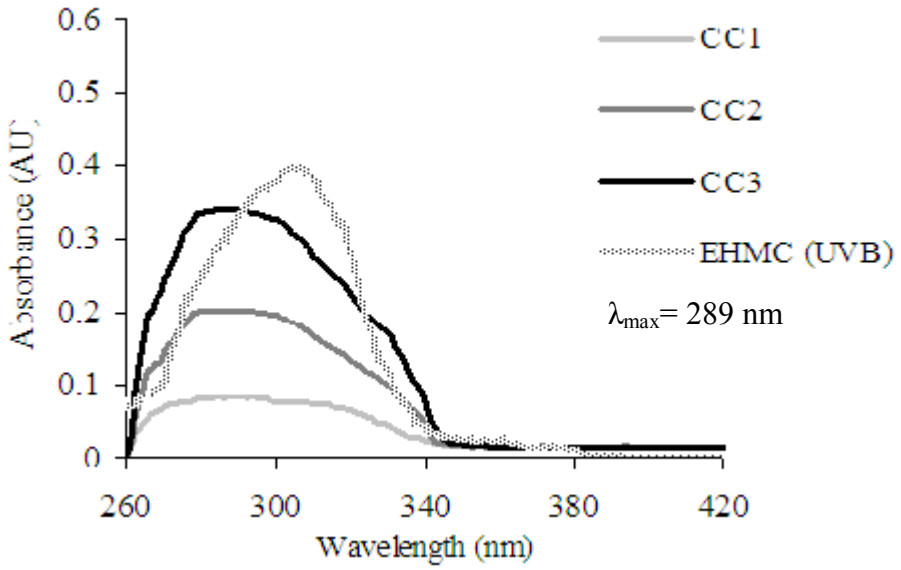


Figure 3.8 ^{1}H-NMR spectrum of 2, 4, 5-trimethoxycinnamoylchitosan (245CC) A) 245CC1 (DS = 0.043) B) 245CC2 (DS = 0.150) C) 245CC3 (DS= 0.285)

The three CC and the three 245CC products gave two new absorption bands (Figure 3.9), λ_{max} of 289 nm (UVB) and 348 nm (UVA) in DMSO. Appearance of the UVB and UVA absorption characteristics of the grafted chromorphores product correlated well with the increased degree of substitution of the chromophores on the chains. One interesting feature of all the obtained derivatives is that their UV-absorption profile is all broaden comparing to the commercial sunscreen (Figure 3.9). This, in facts, is very good to be use as UV filter because all the light in those region will be absorbed.

The thermal properties (Table 3.2) of chitosan of M.W. 30,000 and 120,000 Daltons showed glass transition temperature (T_g) at 46 and 46 °C, respectively. The CC1, CC2, CC3, 245CC1, 245CC2 and 245CC3 polymer possess glass transition temperature of 37-39 °C. This indicates that the thermal properties of chitosan changed upon the grafting of cinnamoyl and 2,4,5-trimethoxycinnamoyl groups onto its backbone. The decrease in the T_g of all the products implied that the presence of cinnamoyl moiety in the chitosan structure, lessen the inter-molecular H-bonding.

A)



B)

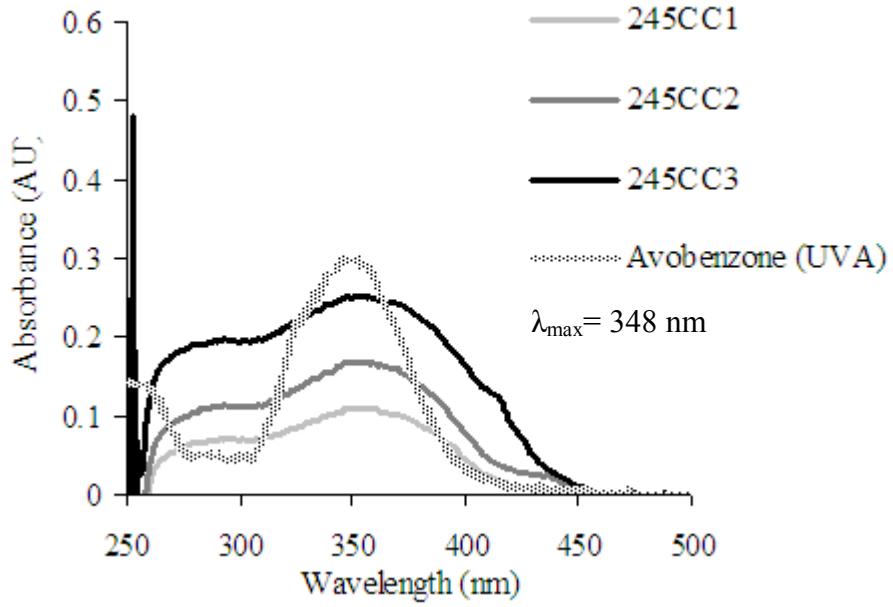
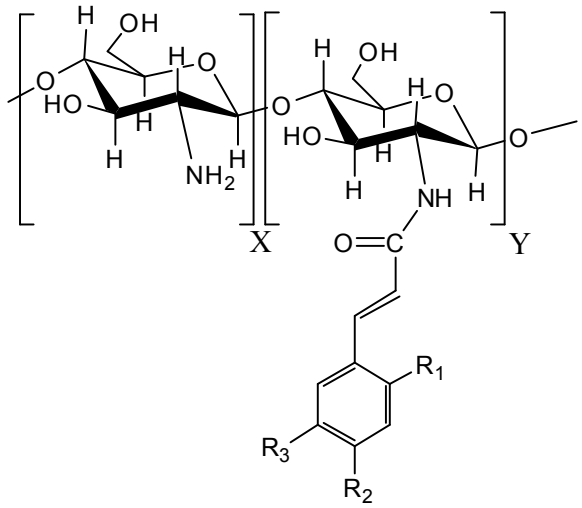


Figure 3.9 UV absorption properties of A) cinnamoylchitosan (CC) and 2-ethylhexyl-p-methoxycinnamate (UVB absorber) B) 2,4,5-trimethoxycinnamoylchitosan (245CC) and avobenzone (UVA absorber).

Table 3.2 Chemical structure, degree of substitution, UV absorption properties and thermal properties of cinnamoylchitosan (CC1-CC3) and 2,4,5-trimethoxycinnamoyl chitosan (245CC1-245CC3).



Product	R_1	R_2	R_3	X	Y(DS)	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$ per the monomeric units	T_g (°C)	T_d (°C)
Chitosan1	-	-	-	-	-	-	-	46	203
Chitosan2	-	-	-	-	-	-	-	46	219
CC1	H	H	H	0.949	0.051	289	1,128	37	187
CC2				0.845	0.155		2,778	37	209
CC3				0.679	0.321		5,557	37	186
245CC1	OCH_3	OCH_3	OCH_3	0.957	0.043	348	2,057	37	271
245CC2				0.850	0.150		3,192	37	-
245CC3				0.715	0.285		4,826	39	263

Chitosan1: chitosan with molecular weight 30,000 daltons

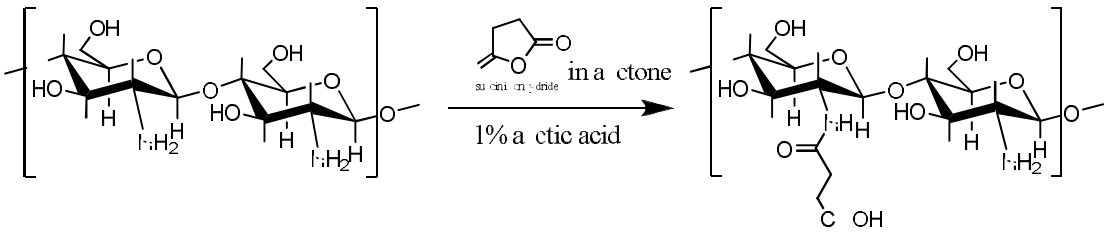
Chitosan2: chitosan with molecular weight 100,000 daltons

T_g : Glass transition temperature

T_d : Decomposition temperature

3.3 Synthesis of imine derivatives

Preparation of succinylchitosan (SC)



Scheme 3.7

After succinic anhydride was reacted with chitosan (MW 30,000) for a total of 12 h and the mixture was precipitated. The obtained product was pale yellow solid (71 % yield). Structural change of chitosan was confirmed by FT-IR spectra (Figure 3.10). The increase of amide peaks (1,649 cm^{-1} and 1,583 cm^{-1}) indicated increase of the amidation. ^{1}H-NMR (D_2O with 0.05% CD_3COOD) of succinylchitosan also confirmed successful amidiation (the presence of peak at 4.8 ppm (Figure 3.11) from protons of the ethyl group on succinyl chain).

The degree of substitution of succinyl groups onto chitosan chain was estimated from the ^{1}H-NMR to be 0.09 (see Figure 3.12). In short, integration at 4.8 ppm represented 4H from succinyl moiety were determined in relative to the integration from 2.9 ppm which represented 1H from the glucosamine ring (H-2). From these results, it was concluded that the succinyl group was successfully introduced onto chitosan. The product can be dispersed in water.

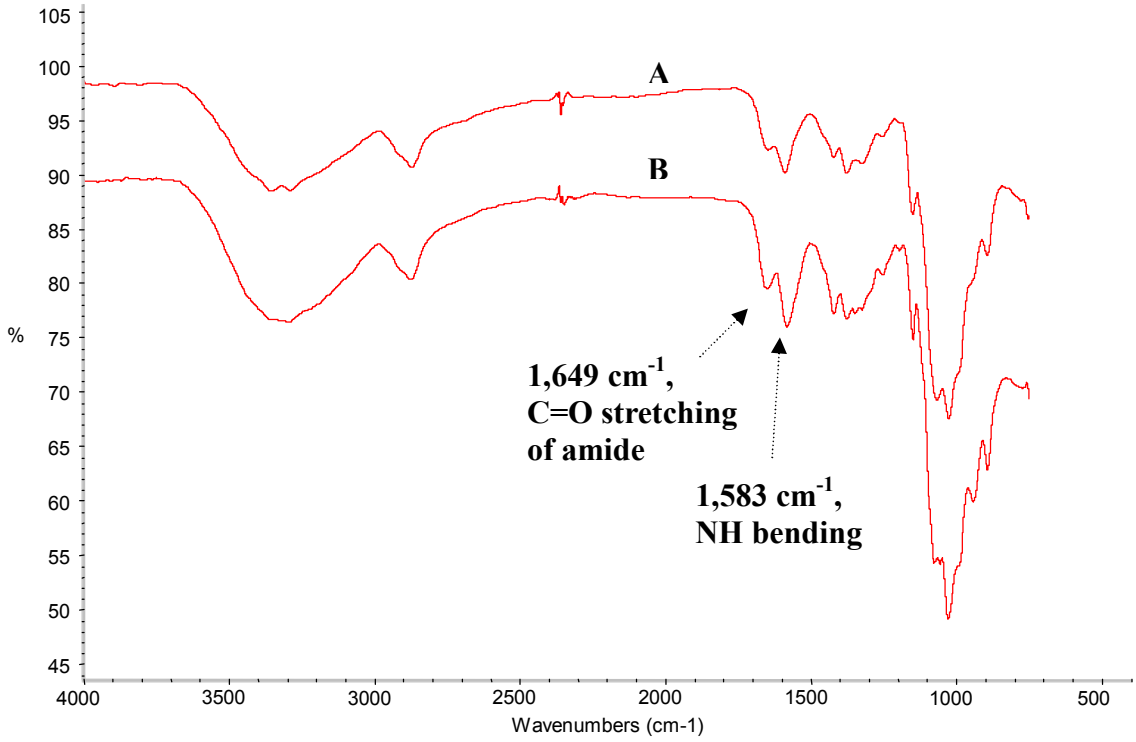


Figure 3.10 FTIR spectrum of a) chitosan b) succinylchitosan (SC) (DS=0.09)

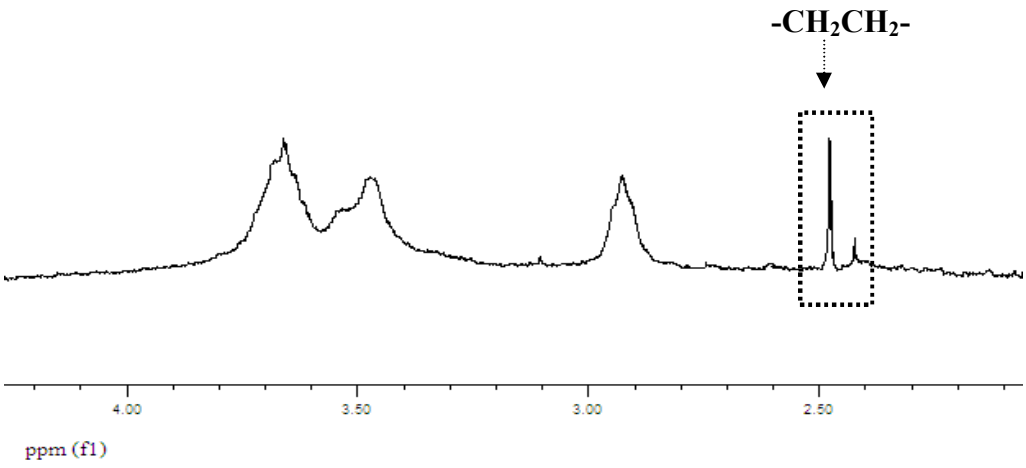


Figure 3.11 ^{1}H-NMR spectrum in D_2O with 0.05% CD_3COOD of succinylchitosan (SC) (DS=0.09)

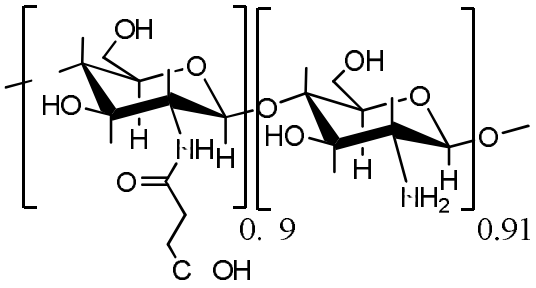
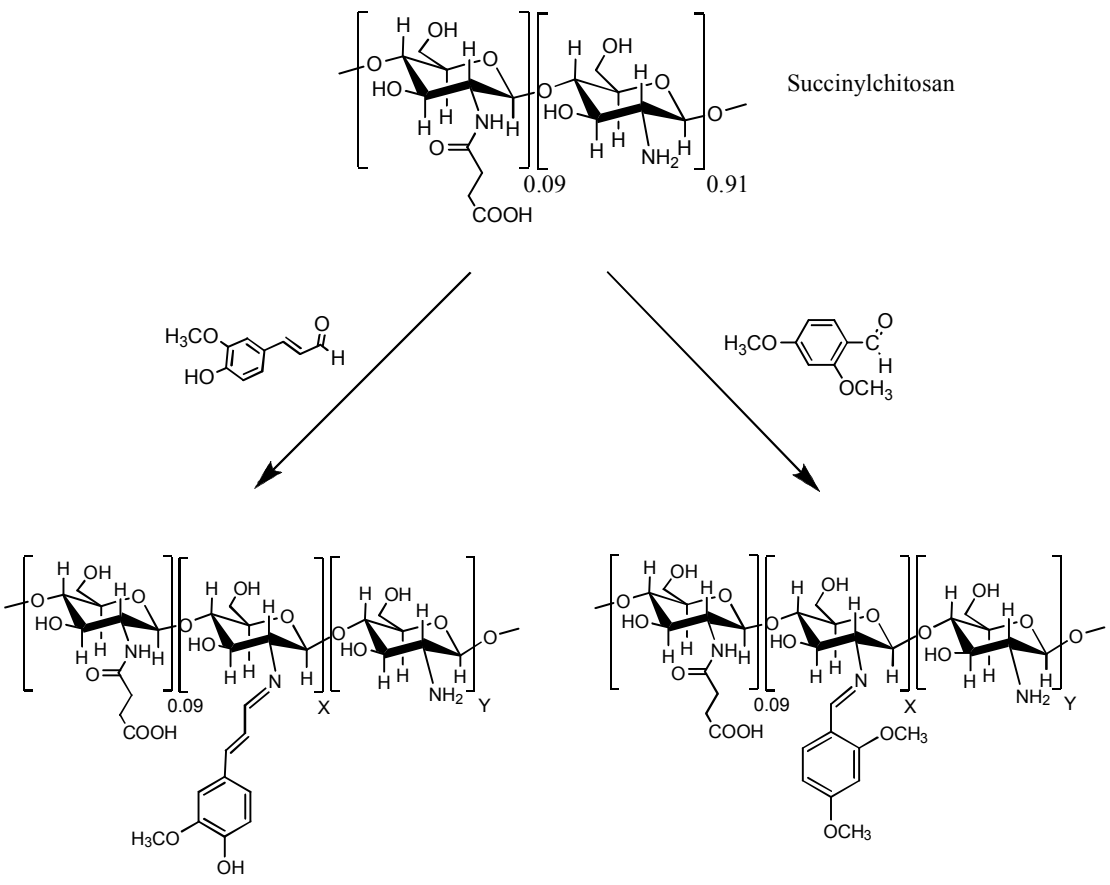
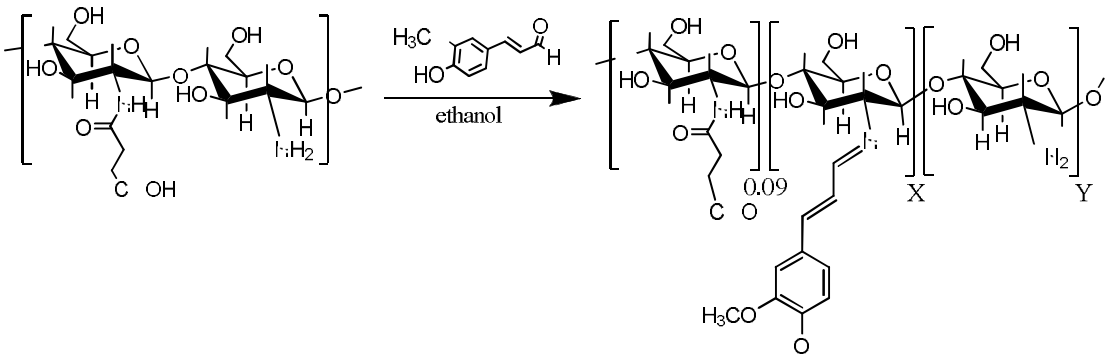


Figure 3.12 Chemical structure of succinylchitosan (DS=0.09)



Scheme 3.8

Preparation of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC)



Scheme 3.9

In the imine derivatives preparation (Scheme 3.8), chitosan acts as the primary amine reacting with aromatic aldehyde to produce the corresponding UV-absorptive polymeric derivative. The obtained product was orange solid (69 % yield). The IR showed absorption peaks at 1,670 cm^{-1} and 1,604 cm^{-1} of C=N stretching and C=C stretching, respectively (Figure 3.13). ^{1}H-NMR (D_2O with 0.05% CD_3COOD) of 43CSC also confirmed successful imine formation. The presence of peak at 6.8 ppm from protons of Ar-C**H**=CH (Figure 3.14). The substitution degree of 4-hydroxy-3-methoxycinnamaldehyde group in each product was estimated using ^{1}H-NMR information; signal ~6.8 ppm (1H, Ar-**H** of the 4-hydroxy-3-methoxycinnamal dehyed moieties) and 2.9 ppm (1H, H-2 of chitosan back bone) (Figure 3.14). The degree of substitution of 4-hydroxy-3-methoxycinnamaldehyde groups induced onto succinylchitosan were 0.196 and 0.810, respectively.

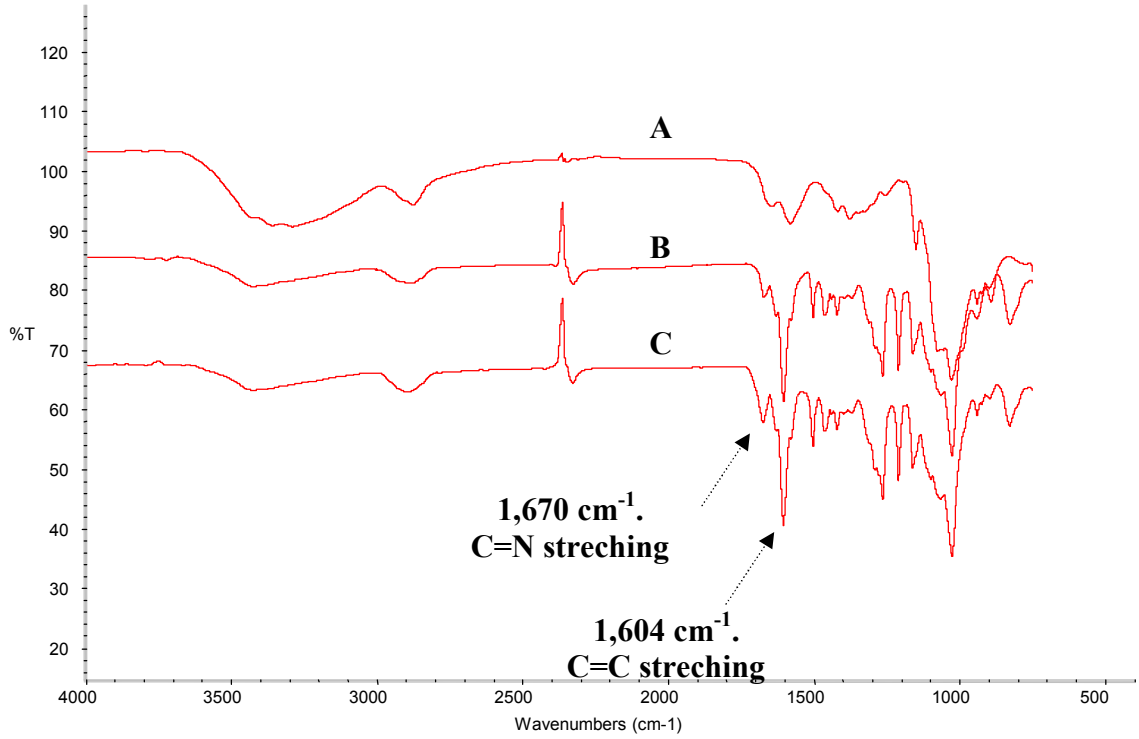


Figure 3.13 FT-IR spectrum of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC) A) succinylchitosan (DS=0.09) B) 43CSC1 (DS=0.196) C) 43CSC2 (DS=0.810)

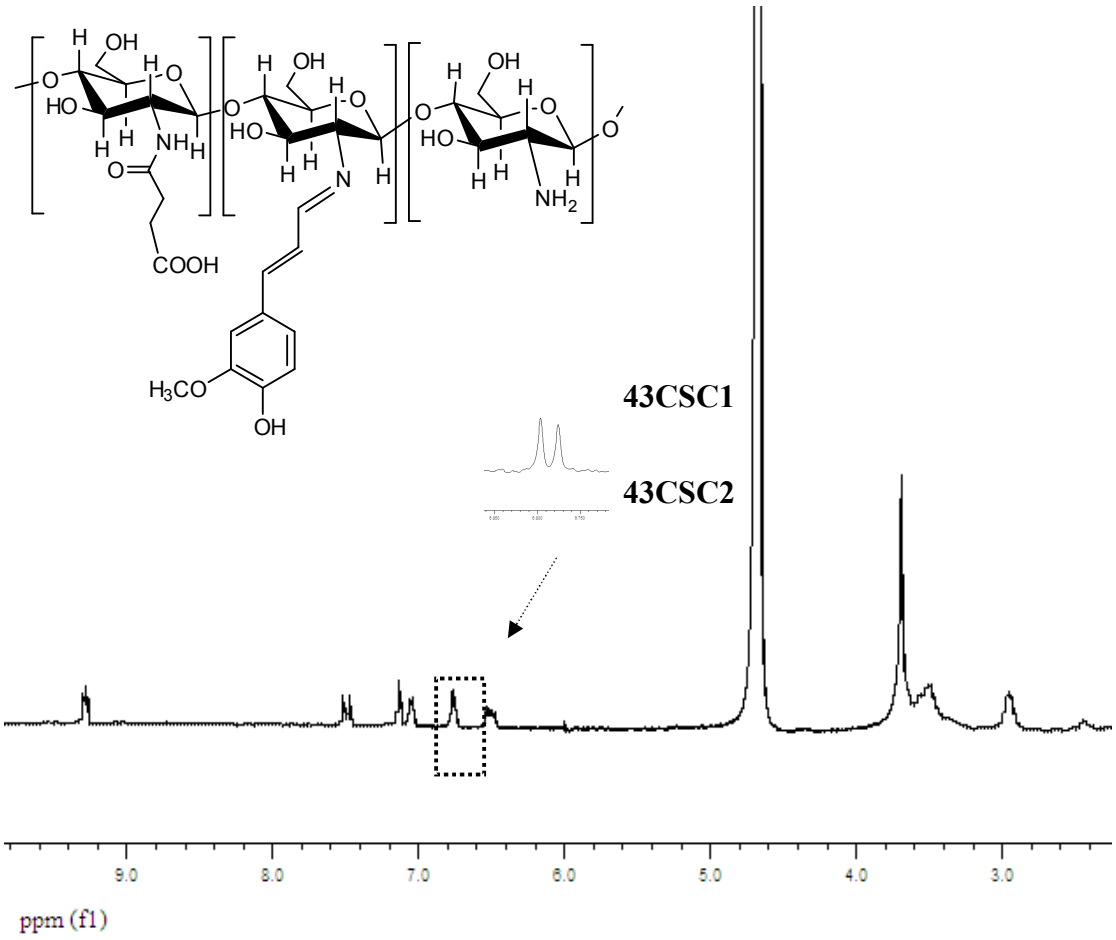
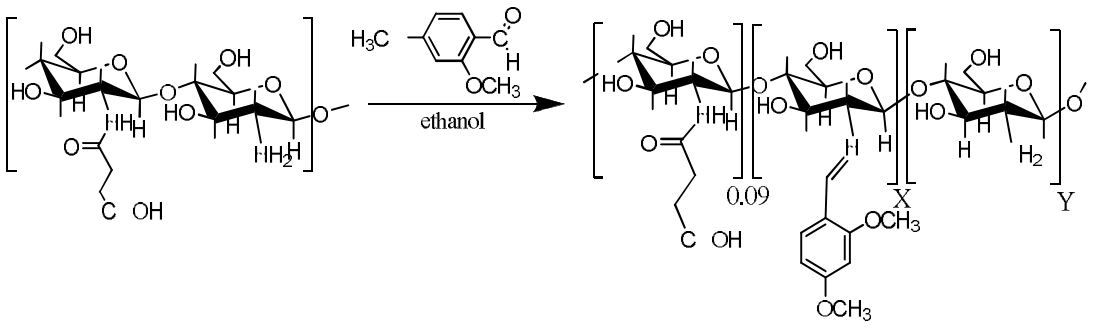


Figure 3.14 ^{1}H-NMR spectrum of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC) A) 43CSC1 (DS=0.196) B) 43CSC2 (DS=0.810)

Preparation of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC)



Scheme 3.10

After chitosan reacted with 2,4-dimehtoxybezaldehyde for a total 12 h the suspension was dried to obtain a pale yellow solid (73% yield). FT-IR spectra (Figure

3.15) of product indicated that primary amine reacting with aromatic aldehyde. In other words, the IR showed absorption peak at 1,631 cm^{-1} and 1,594 cm^{-1} of C=N stretching and C=C stretching, respectively. ^{1}H-NMR (D_2O with 0.05% CD_3COOD) of 24BSC also confirmed successful imine formation. The presence of peak at 6.8 ppm from protons of Ar-C**H**=CH-) (Figure 3.16). The substitution degree of 2,4-dimethoxy benzalmaldehyde group in each product was estimated using ^{1}H-NMR information; signal ~7.5 ppm (1H, Ar-**H** of the 2,4-dihydroxybenzaldehyed moieties) and 2.9 ppm (1H, H-2 of chitosan back bone) (Figure 3.16). The degree of substitution of 2,4-dimethoxybenzalmaldehyde groups induced onto succinylchitosan were 0.180 and 0.710, respectively.

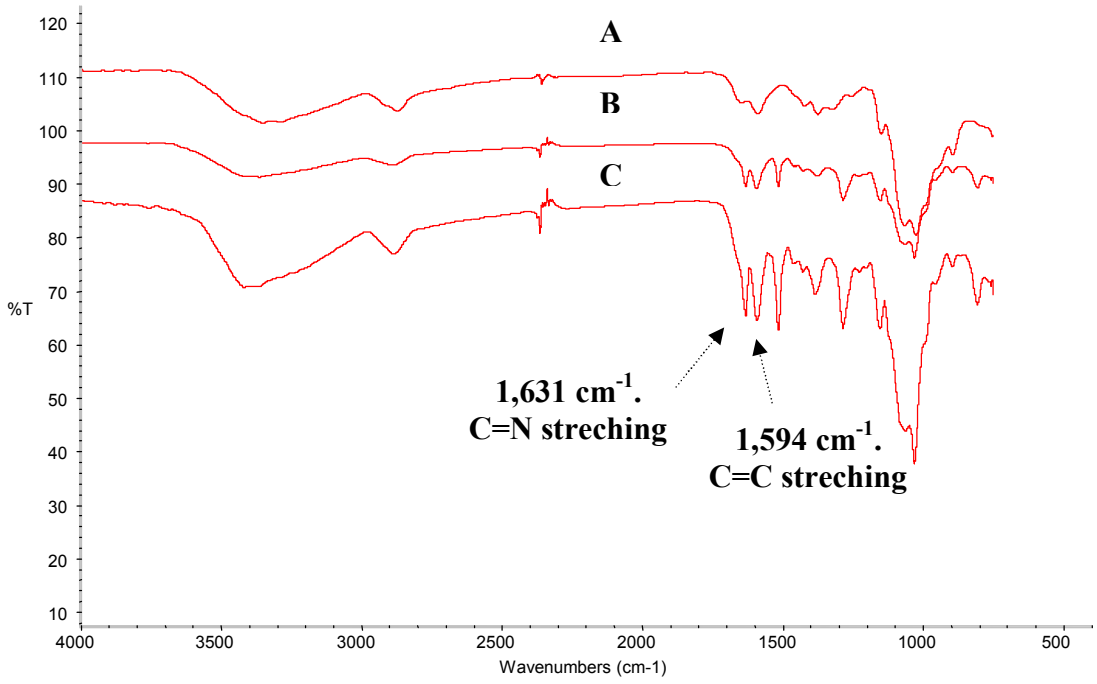


Figure 3.15 FT-IR spectrum of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) a) succinylchitosan (SC) b) 24BSC1 (DS=0.180) c) 24BSC2 (DS=0.710)

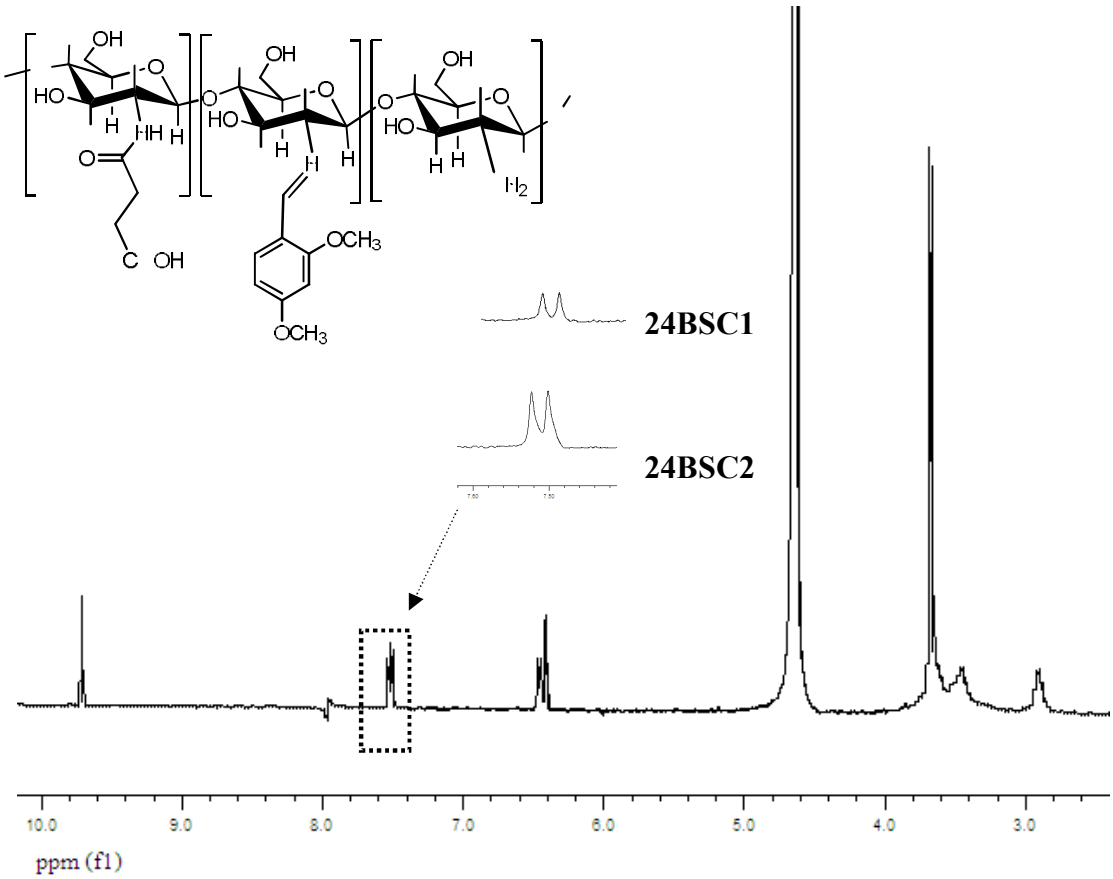


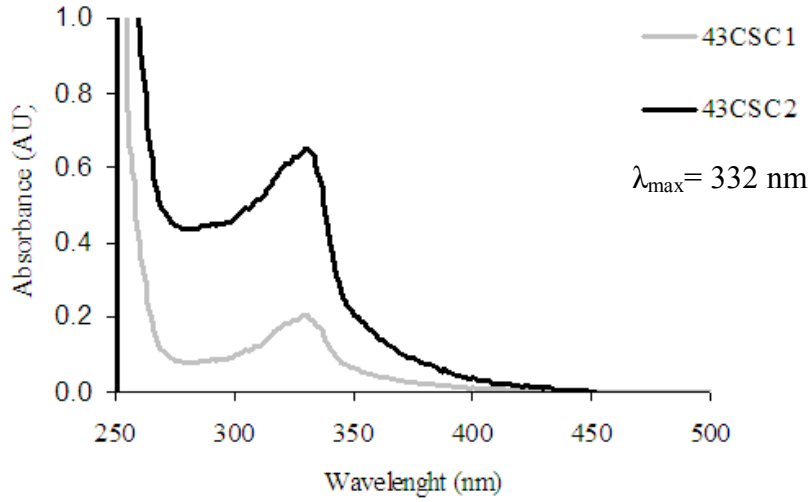
Figure 3.16 ^{1}H-NMR spectrum of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) A) 24BSC1 (DS= 0.180) B) 24BSC2 (DS= 0.710)

The two 43CSC and the two 24BSC products gave two new absorption bands, λ_{max} of 332 nm and 314 nm in DMSO, respectively (Figure 3.17). Appearance of the UVA and UVB absorption characteristics of the grafted chromophore product correlated well with the increased degree of substitution of those groups on the chains. Absorption profile of 24BSC products was all broadened, indicating very good absorption of the UVB radiation from 290-320 nm.

The thermogram of succinylchitosan (SC) showed the glass transition temperature (T_g) at 33 °C, with no melting characteristic (Table 3.3). The decrease of T_g in the grafted product indicated that crystallinity in chitosan decreased upon succinyl group grafting due to some disruption of intramolecular H-bonding. The 43CSC1, 43CSC2, 24BSC1 and 24BSC2 gave similar glass transition temperature

(~33-36 °C) and showed increase decomposition temperature (T_d) when comparing to the starting polymer (Table 3.3 and 3.4).

A)



B)

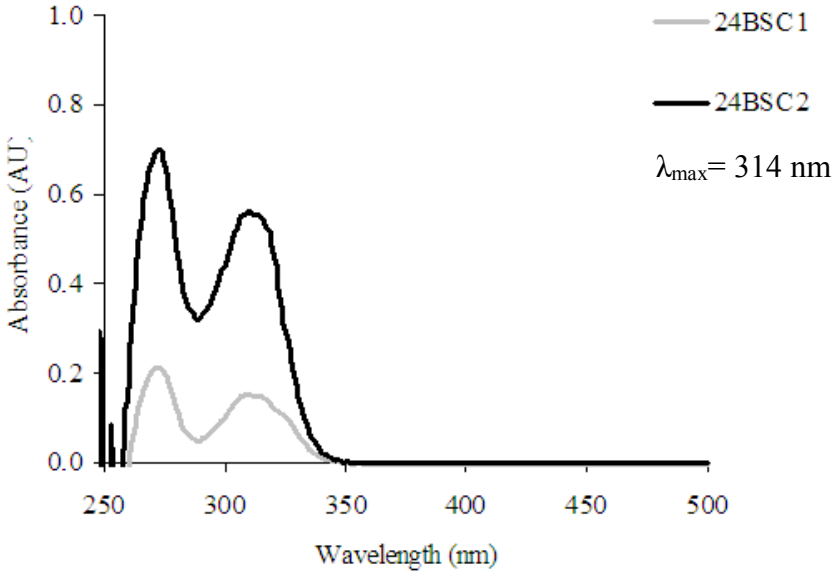
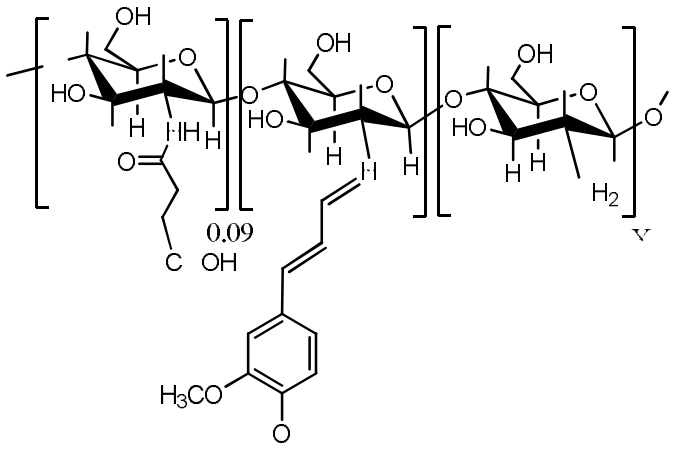


Figure 3.17 UV absorption properties of A) 4-hydroxy-3-methoxycinnamalidene succinylchitosan (43CSC) B) 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) 20 ppm in DMSO

Table 3.3 Chemical structure, degree of substitution, UV absorption properties and thermal properties of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC1 and 43CSC2)



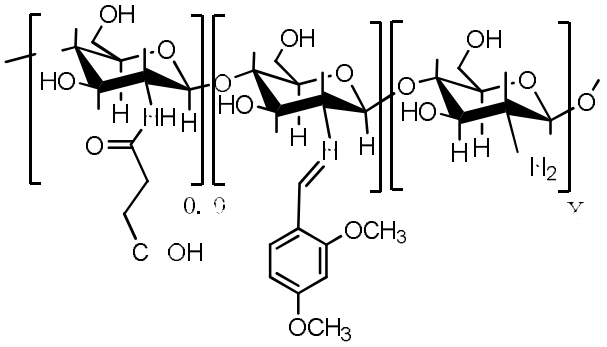
Product	**X(DS)**	**Y**	**λ_{max} (nm)**	**ε ($M^{-1}cm^{-1}$ per the monomeric units**	**T_g (°C)**	**T_d (°C)**
Chitosan1	-	-	-	-	46	203
SC (DS=0.09)	-	-	-	-	33	255
43CSC1	0.196	0.715	332	3,585	34	-
43CSC2	0.810	0.100	332	10,300	33	293

Chitosan1: chitosan with molecular weight 30,000 daltons

T_g : Glass transition temperature

T_d : Decomposition temperature

Table 3.4 Chemical structure, degree of substitution, UV absorption properties and thermal properties of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC1 and 24BSC2)



Product	**X(DS)**	**Y**	**λ_{max} (nm)**	**ε ($M^{-1}cm^{-1}$ per the monomeric units**	**T_g (°C)**	**T_d (°C)**
Chitosan1	-	-	-	-	46	203
SC (DS=0.09)	-	-	-	-	33	255
24BSC1	0.180	0.705	314	4,650	35	-
24BSC2	0.710	0.200	314	8,055	36	292

Chitosan1: chitosan with molecular weight 30,000 daltons

T_g : Glass transition temoerature

T_d : Decomposition temperature

3.4 Preparation of amide derivatives nanoparticles

The obtained polymers (CC1-CC3 and 245CC1-245CC3) (6000 ppm) were induced into nanoparticles by dialysis method using DMF as a solvent and water as an anti-solvent (Figure 3.18). When DMF was displaced by water the hydrophobic groups (cinnnamoyl and 2,4,5-trimethoxycinnamoyl) are probably directed inwards while the hydrophilic domains of the chitosan backbone will probably arrange themselves to give maximum interaction with the hydrophilic water leading to spontaneous particle formation.

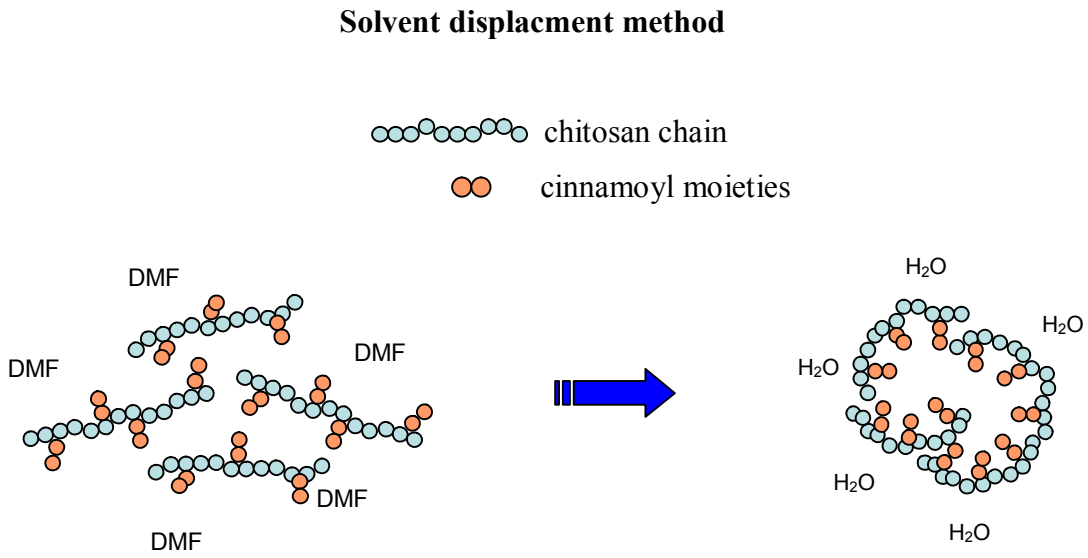


Figure 3.18 Particles formation by solvent displacement technique

The cinnamoylchitosan nanoparticles (CC1-CC3) showed transparent colloidal suspension of the polymeric particles. The suspension of the nanoparticles was subjected to SEM, TEM and dynamic light scattering analyses. The shape, size, and surface characteristics of CC1, CC2 and CC3 nanoparticles are shown in Figure 3.19. All cinnamoylchitosan nanoparticles (CC1, CC2 and CC3) are spherical. The 2,4,5-trimethoxycinnamoylchitosan (245CC1-245CC3) showed yellow colloidal suspension. Shape of 245CC2 particles (degree of substitution of 0.150) and 245CC1 (DS: 0.043) was spherical while that of 245CC2 (DS: 0.285) was rod-like (Figure 3.20). As can be seen in Table 3.4, the size of CC2, CC3, 245CC2 and 245CC3

particles showed increase with increasing degree of cinnamoyl and 2,4,5-trimethoxy cinnamoyl moieties substitution. In addition, CC1 and 245CC1 particles size also increase with M.W. of chitosan used.

Zeta potential is a useful indicator of surface charge property and can be employed as an index to the stability of the nanoparticles. In most circumstances, the higher the absolute value of the zeta potential of the particles, the larger amount of charge on their surface. These might result in stronger repellent interactions among the particles, and hence, higher stability of the suspension. It should be noted here that the values of < -30 or +30 mV were usually used as indicator of particle stability [41]. It was not surprised to see the aggregation of CC particles since in such condition, the zeta potential value of the particles was quite low (-11 - -26 mV) (see Figure 3.19) while the particles of 245CC showed high zeta potential values (-20 - -34 mV. This mean that 245CC colloidal was stable than CC particles.

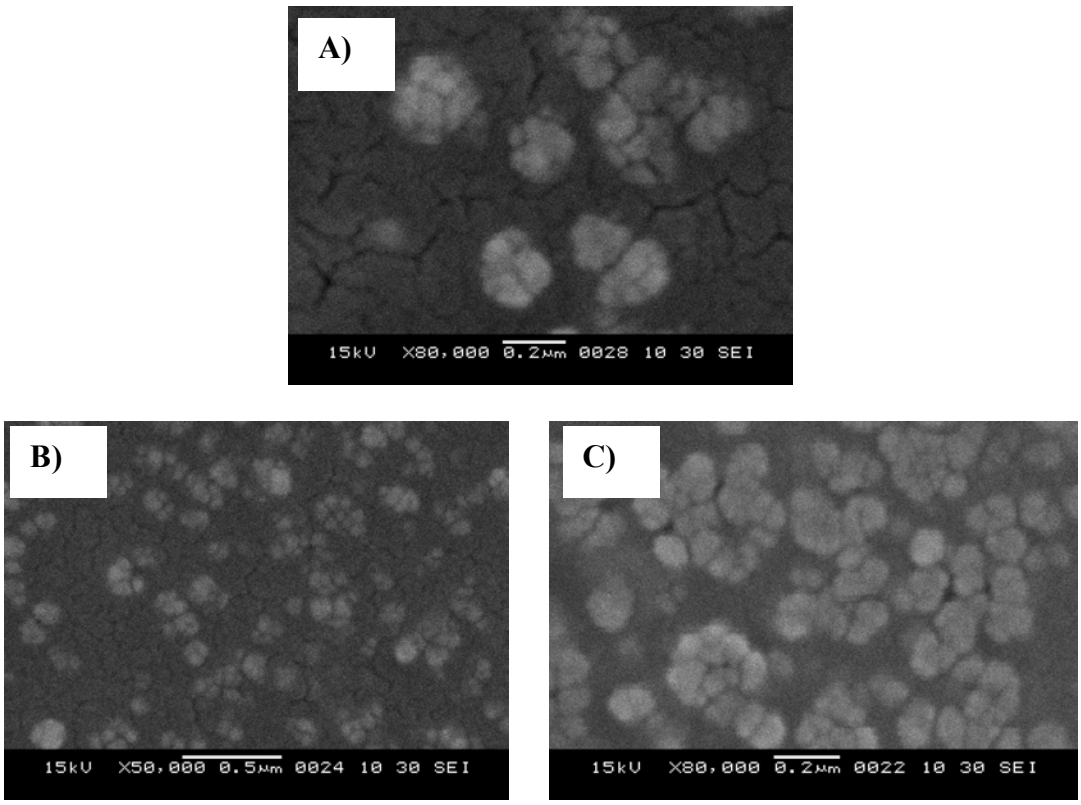


Figure 3.19 SEM photographs at 15 kV of A) CC1 (×80,000) B) CC2 (×50,000) C) CC3 (×80,000)

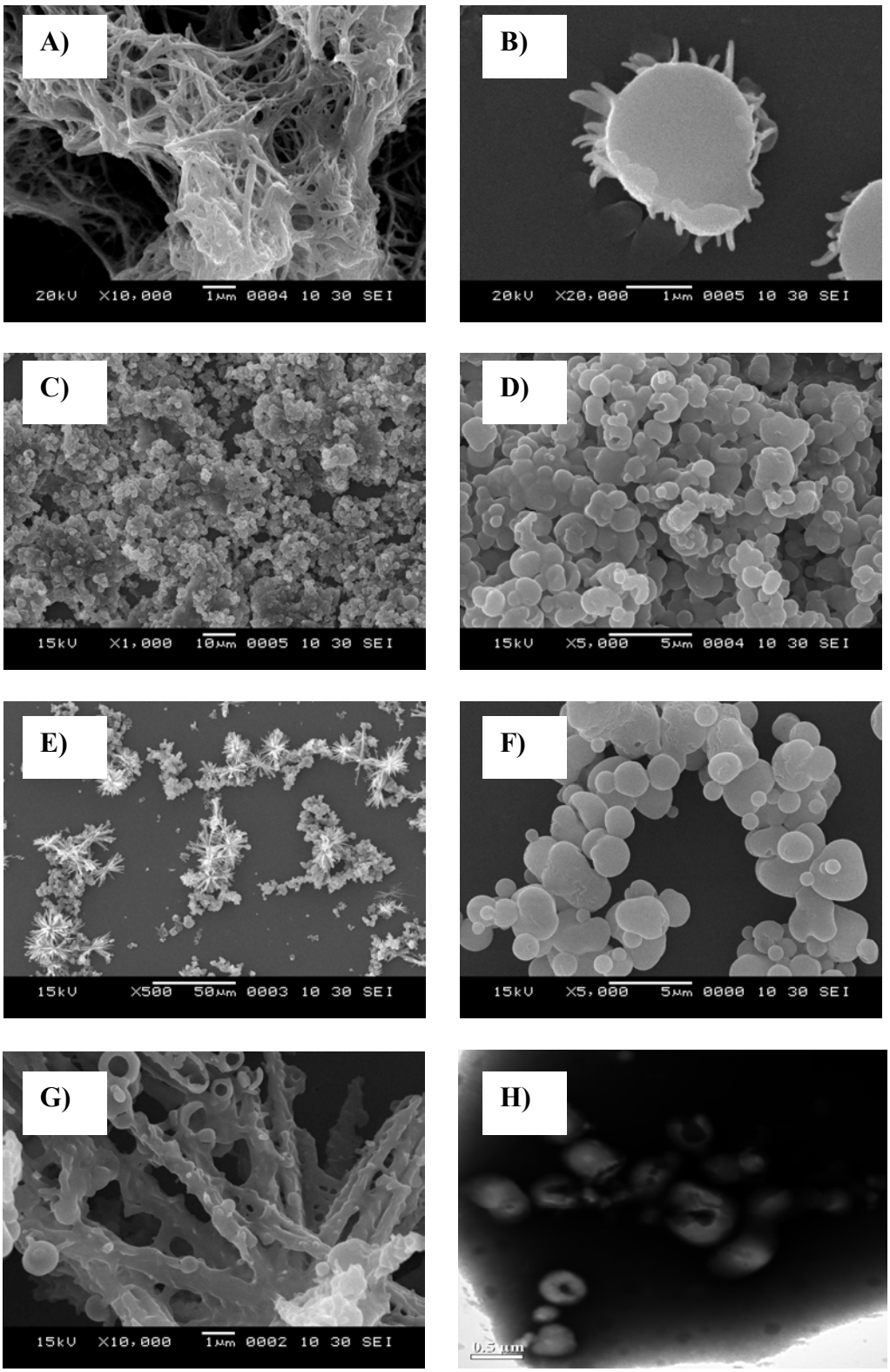
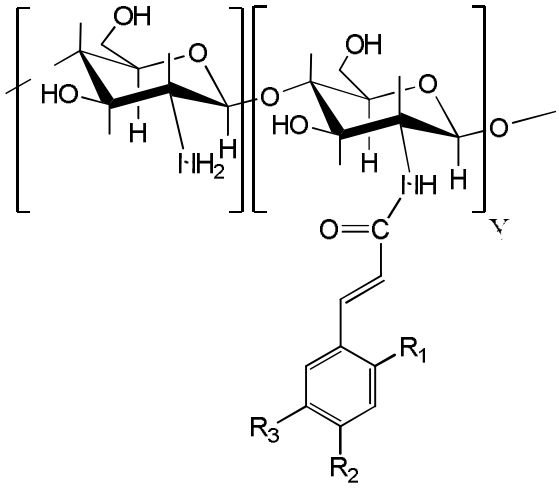


Figure 3.20 SEM photographs at 15 kV of A, B) 245CC1 (×10,000 and 20,000) C, D) 245CC2 (×1,000 and 5,000) E, F, G) 245CC3 (×1,000,× 5,000 and ×10,000) and H) TEM photograph of 245CC3

Table 3.5 Sizes, shapes and zeta potentials of cinnamoylchitosan and 2,4,5-trimethoxycinnamoylchitosan (CC) and 2,4,5-trimethoxycinnamoylchitosan (245CC)



Product	R_1	R_2	R_3	X	Y(DS)	Shape	Average size by SEM (nm)	Zeta potential (mV)
CC1[a]	H	H	H	0.949	0.051	sphere	111.11±8.67	-17.06±2.18
CC2[b]				0.845	0.155		66.67±2.81	-26.80±2.10
CC3[b]				0.679	0.321		107.14±1.33	-11.02±2.09
245CC1[a]	OCH_3	OCH_3	OCH_3	0.957	0.043	sphere, rod	1,621±98.32	-20.91±0.28
245CC2[b]				0.850	0.150	sphere	1,130±66.14	-30.33±0.31
245CC3[b]				0.715	0.285	sphere, rod	1,487±45.20	-34.21±0.11

[a] This products were synthesized from chitosan with MW 120,000

[b] This products were synthesized from chitosan with MW 30,000

3.5 Preparation of imine derivatives nanoparticles

The succinylchitosan nanoparticles (SC) obtained from self assembly in water show spherical shape with 50 nm size as declared by AFM technique. Well-defined sphere existed in the colloidal aqueous solution as shown in Figure 3.22. The result of zeta potential (Table 3.6) indicates a marked negative charge of the succinylchitosan (-18.26 mV).

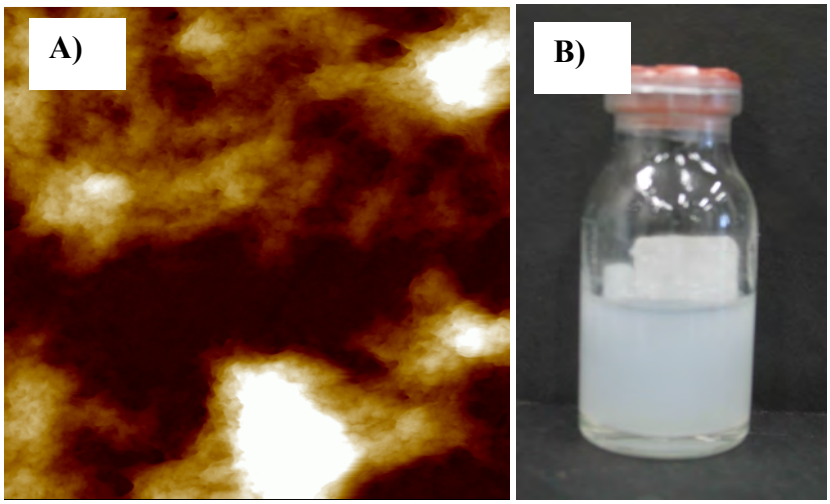


Figure 3.21 A) AFM photograph with 50.54±0.25 nm of succinylchitosan B) suspension of succinylchitosan (DS=0.09) at 1,000 ppm in Milli-Q water.

The 43CSC1-43CSC2 and 24BSC1-24BSC2 nanoparticles were directly prepared by imine formation on the SC nanosphere. The 43CSC and 24BSC nanoparticles showed orange and yellow colloidal suspension of the polymeric particles. The shape, size, and surface characteristics of all products are shown in Figure 3.23. The four products are spherical. As shown in Table 3.6 and 3.7, the size of 43CSC and 24BSC particles showed increase with increasing degree of 4-hydroxy-3-methoxycinnamalidenesuccinyl and 2,4-dimethoxybenzalidenesuccinyl moieties substitution. Both of particles (43CSC and 24BSC) showed positive charge on surface of spheres (Table 3.6 and 3.7). As shown in Table 3.7, the most stable aqueous colloid is 24BSC2, the product with the highest positive zeta potential value (30.3 mV).

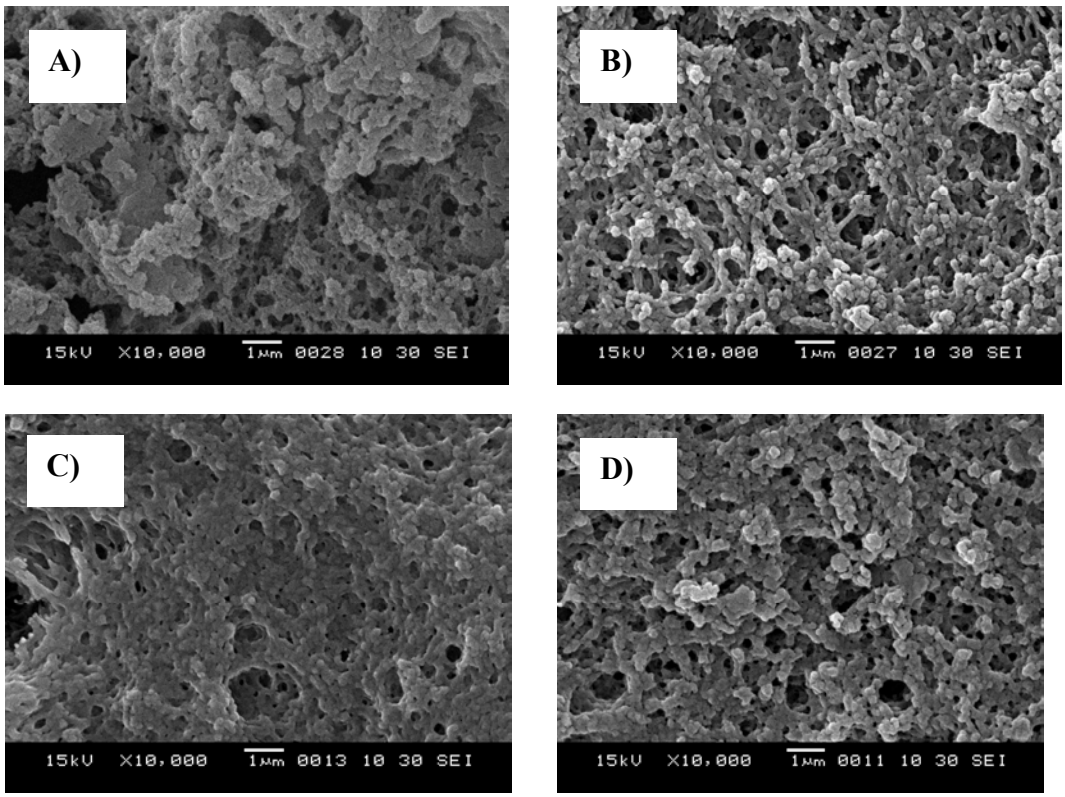
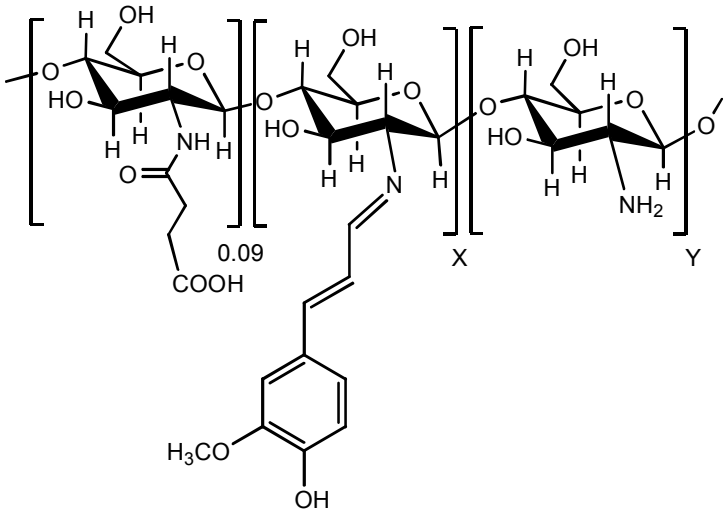


Figure 3.22 SEM photographs of A) 43CC1 (×10,000) B) 43CC2 (×10,000) C) 24BSC1 (×10,000 D) 24BSC2 (×10,000) nanoparticles at 6,000 ppm in Milli-Q water

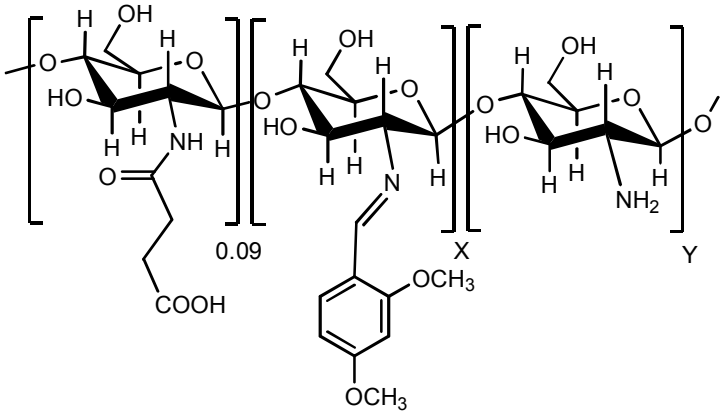
Table 3.6 Chemical structure, degree of substitution, sizes, shape and zeta potential of 4-hydroxy-3-methoxycinnamalidenesuccinyl chitosan (43CSC1 and 43CSC2).



Product	Y	X(DS)	Shape	Average size by SEM (nm)	Zeta potential (mV)
SC[b] (DS=0.09)	-	-	sphere	-	-18.26±1.46
43CSC1[b]	0.715	0.196	sphere	92.85±17.09	8.51±0.23
43CSC2[b]	0.100	0.810	sphere	123.11±20.75	21.38±0.13

[b] This products were synthesized from chitosan with MW 30,000

Table 3.7 Chemical structure, degree of substitution, shape, sizes and zeta potential of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC1 and 24BSC2)



Product	Y	X(DS)	Shape	Average size by SEM (nm)	Zeta potential (mV)
SC[b] (DS=0.09)	-	-	sphere	-	-18.26±1.46
24BSC1[b]	0.730	0.180	sphere	302.36±28.27	11.23±0.15
24BSC2[b]	0.247	0.710	sphere	176.23±27.97	30.3±0.16

[b] This products were synthesized from chitosan with MW 30,000

3.6 Photostability of amide and imine products

Because among amide and imine derivative products 245CC2 and 24BSC2 showed the most stable in water, these products were chosen for photostability test. Photostability of 245CC2 and 24BSC2 particles were monitored in water under simultaneous UVA and UVB exposure, respectively. The decrease in absorption was recorded at the absorption maximum wavelength. As can see Figure 3.28 and Figure 3.29, the absorbance ($A_t/A_{0)}$ decreased slightly. This means that both products show photostable in the UVA and UVB region, respectively.

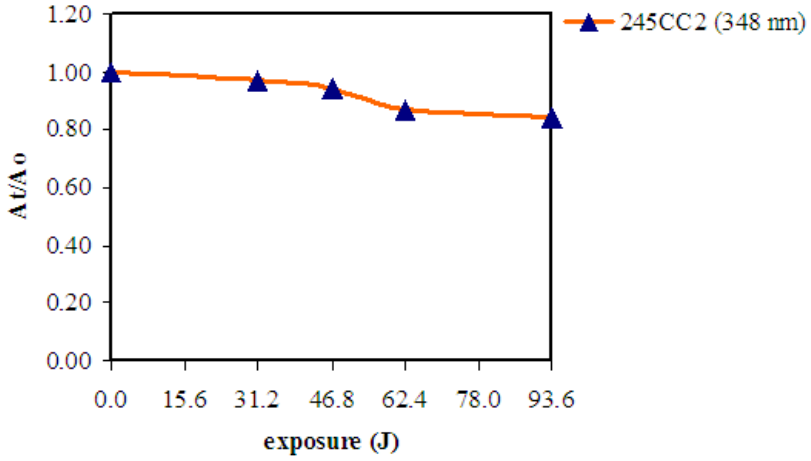


Figure 3.23 Photostability test of 245CC2 particles in water irradiated by 8.8 mW/cm^2 UVA.

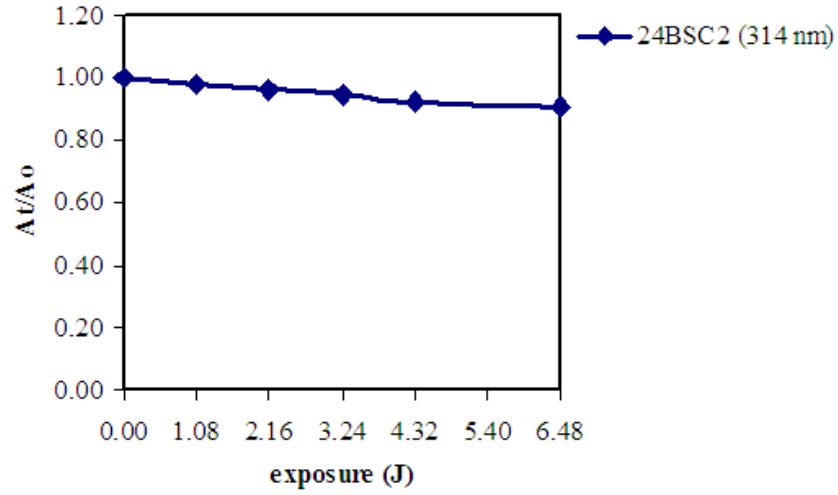


Figure 3.24 Photostability test of 24BSC2 particles in water irradiated by 0.6 mW/cm^2 UVB.

3.8 Comparison of product with standard sunscreen

The two samples, 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) 20 ppm and 2-ethylhexyl-4-methoxycinate (EHMC) 20 ppm (the commercially use UVB filter), were mixed in the cream base. Each cream was then diluted and measured for its UV absorption property. It can be concluded that 24BSC possess similar UV-absorption efficiency to that of EHMC. In addition, the 24BSC can be efficiency used with EHMC, as UV-absorption of the 24BSC and EHMC mixture shows very good absorption in UVB region (Figure 3.30).

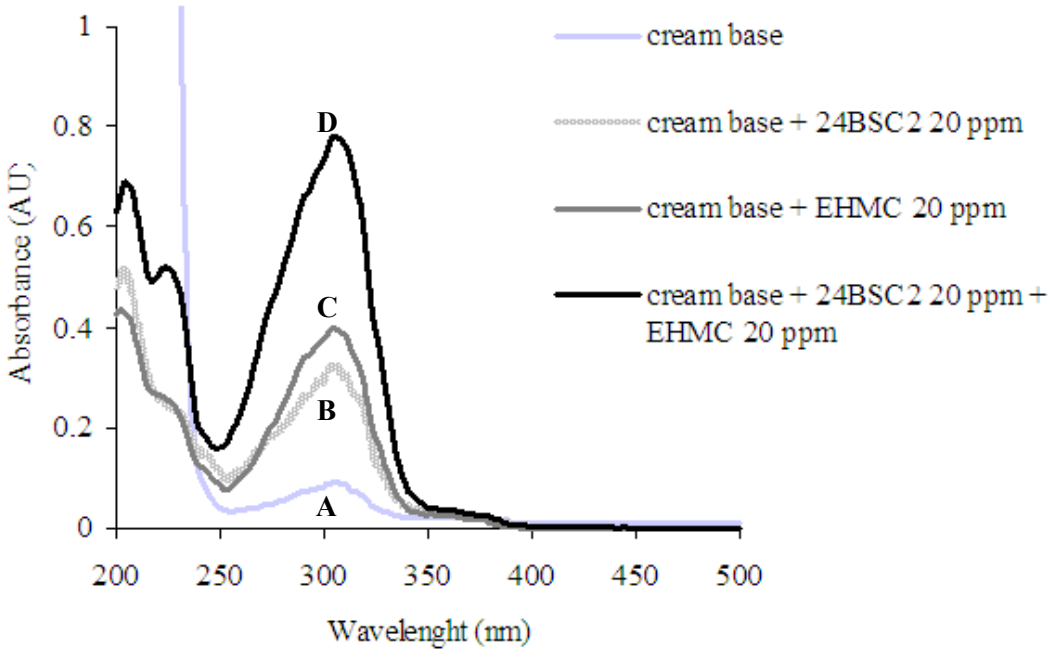
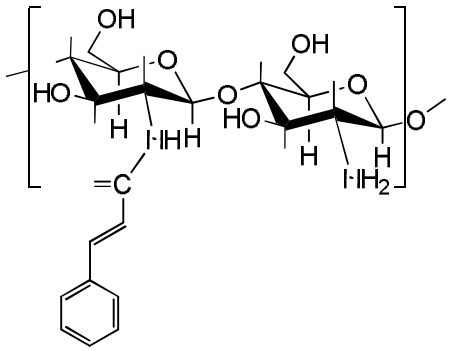


Figure 3.25 UV absorption properties of A) cream base B) cream base + 24BSC2 particles 20 ppm C) cream base + 2-ethylhexyl-4-methoxycinnamate 20 ppm and D) cream base + 24BSC2 particles 20 ppm + 2-ethylhexyl-4-methoxycinnamate 20 ppm.

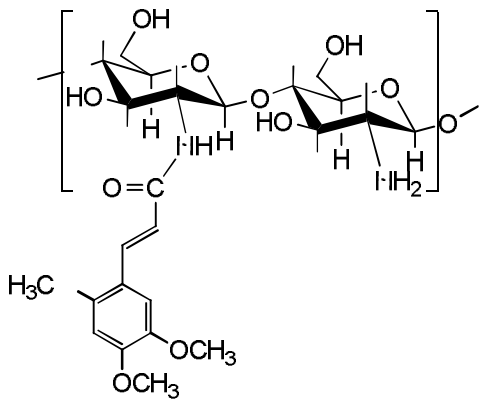
CHAPTER IV

CONCLUSION

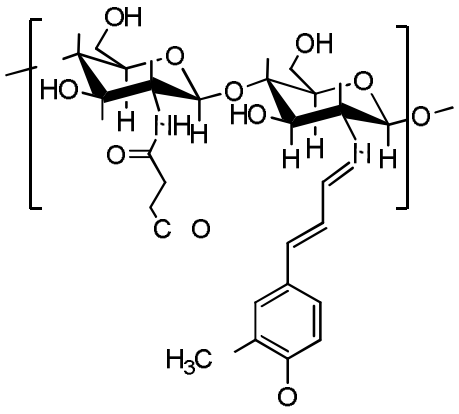
In this research, chitosan derivatives with UV absorption property, cinnamoyl chitosan (CC), 2,4,5-trimethoxycinnamoylchitosan, 4-hydroxyl-3-methoxycinna malidenesuccinylchitosan (43CSC) and 2,4-dimethoxybenzalidenesuccinyl chitosan (24BSC) were prepared. The products were then fabricated into water dispersible nanospheres.



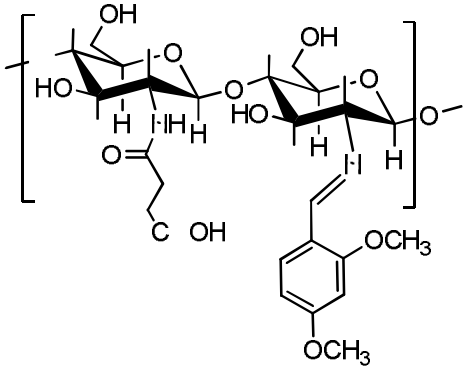
Cinnamoylchitosan (CC)



2,4,5-Trimethoxycinnamoylchitosan (245CC)



4-Hydroxy-3-methoxycinnamalidene-succinylchitosan (43CSC)



2,4-Dimethoxybenzalidenesuccinyl-chitosan (24BSC)

Cinnamoylchitosan (CC) and 2,4,5-trimethoxycinnamoylchitosan were prepared by amide formation between amino groups of chitosan backbone and cinnamoylchloride derivatives. Nanoparticle formation was carried out by self assembly of the obtained polymers using solvent displacement method. Most cinnamoylchitosan nanoparticles were spherical with diameter of 50-70 nm. 2,4,5-Trimethoxycinnamoylchitosan nanoparticles were spherical but with larger diameter of 1,100-1,600 nm.

Succinylchitosan was prepared via the ring-opening reaction of succinic anhydride with chitosan in dilute acetic solution. 4-Hydroxy-3-methoxycinnamalidene succinylchitosan (43CSC) and 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) were then prepared by Schiff base formation between remaining primary amino groups of succinylchito san and aldehyde derivatives. Nanoparticle formation was carried out by self-assembly of the obtained polymer in water. The shape of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan (DS of 0.810) and 2,4-dimethoxybenzalidenesuccinylchitosan (DS of 0.710)

Factors affecting the formation, size and shape of nanoparticles include degree of cinnamoyl substitution and also MW. of chitosan used. 2,4,5-Trimethoxycinna moylchitosan (245CC) nanospheres and 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) nanospheres are good UVA and UVB filters, respectively. Both are photostable and 24BSC is compatible with commercially used UVB filter 2-ethylhexyl-p-methoxycinnamate.

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APPENDICES

APPENDIX A

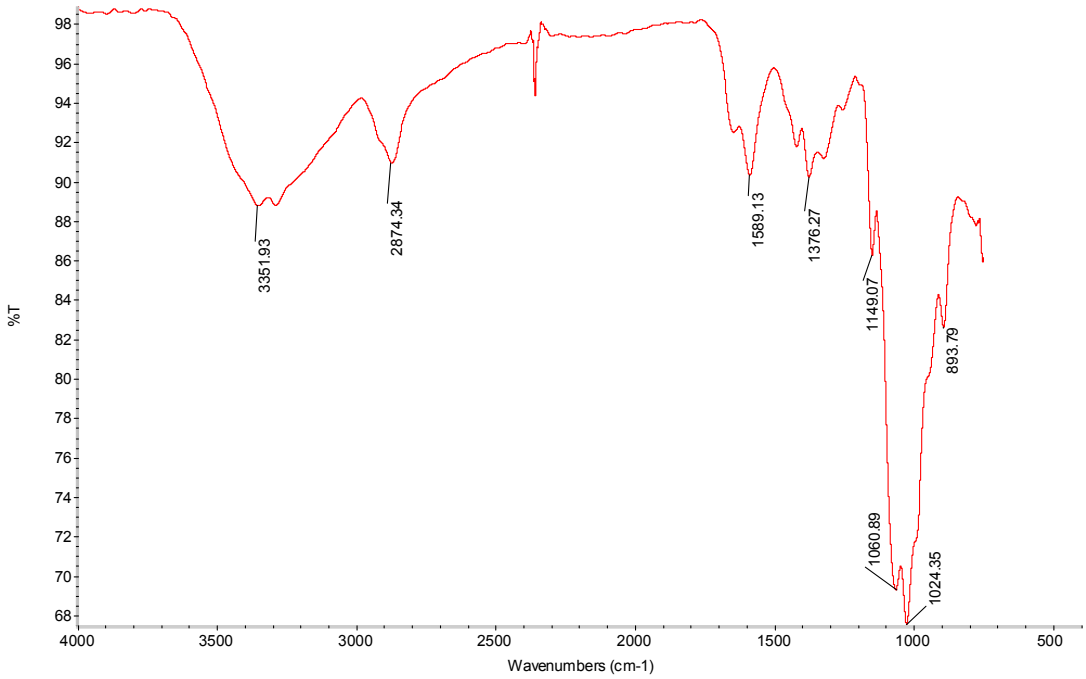


Figure A1 FTIR spectrum of chitosan

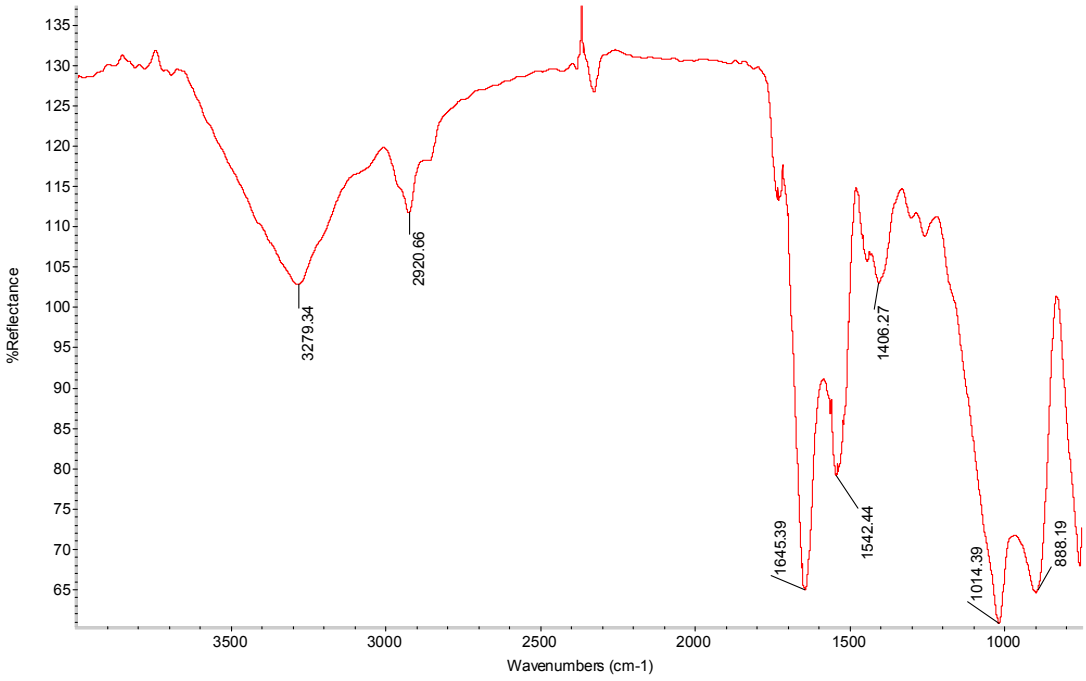


Figure A2 FTIR spectrum of cinnamoylchitosan (CC1) DS= 0.051

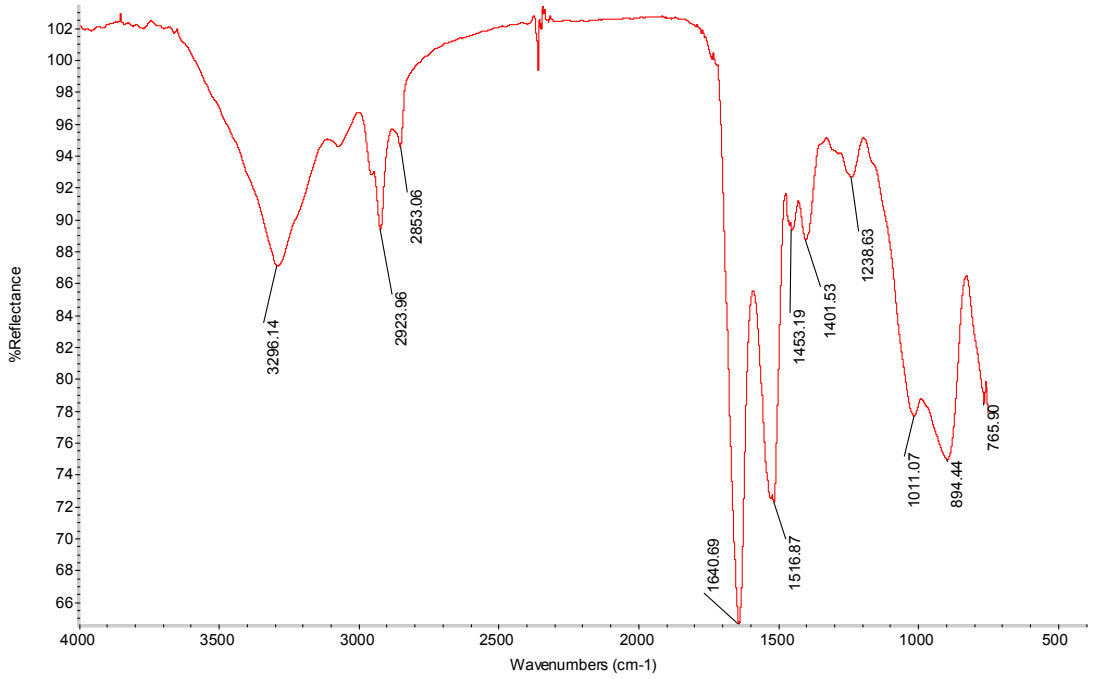


Figure A3 FTIR spectrum of cinnamoylchitosan (CC2) DS= 0.155

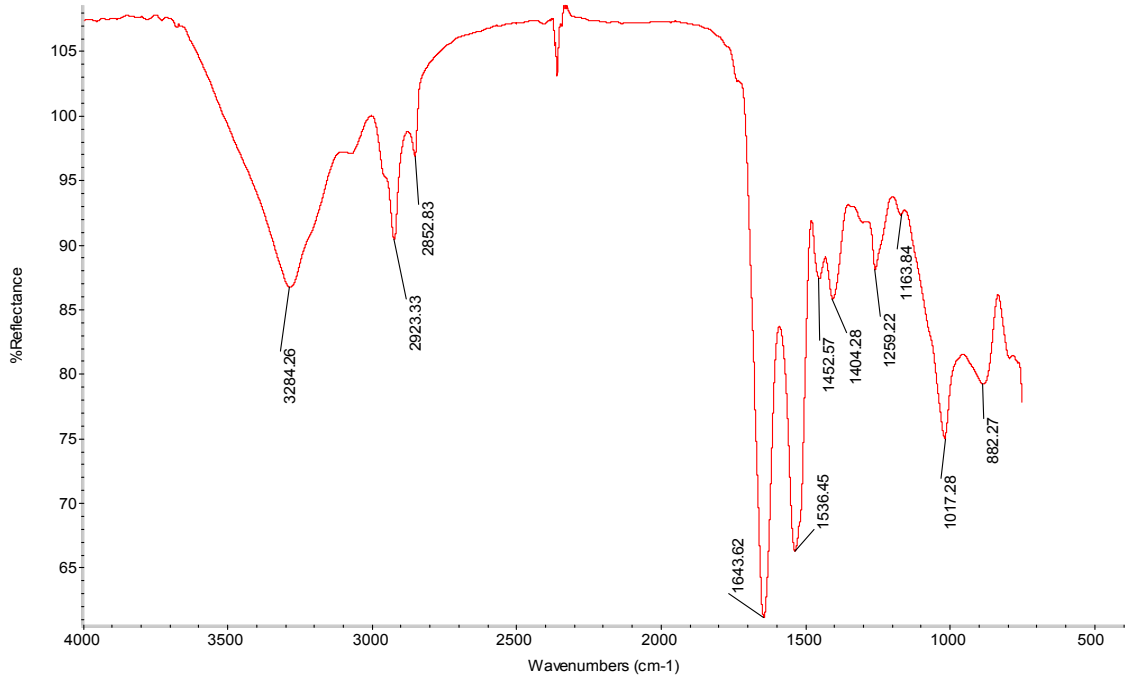


Figure A4 FTIR spectrum of cinnamoylchitosan (CC3) DS= 0.321

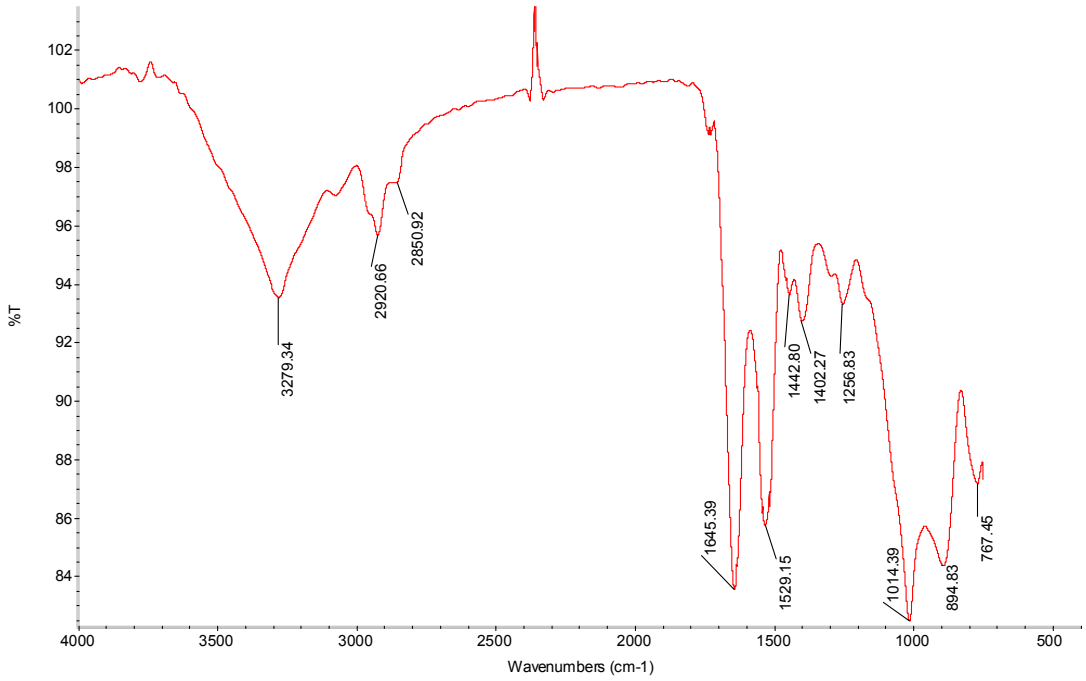


Figure A5 FTIR spectrum of 2,4,5-trimethoxycinnamoylchitosan (245CC1) DS=0.043

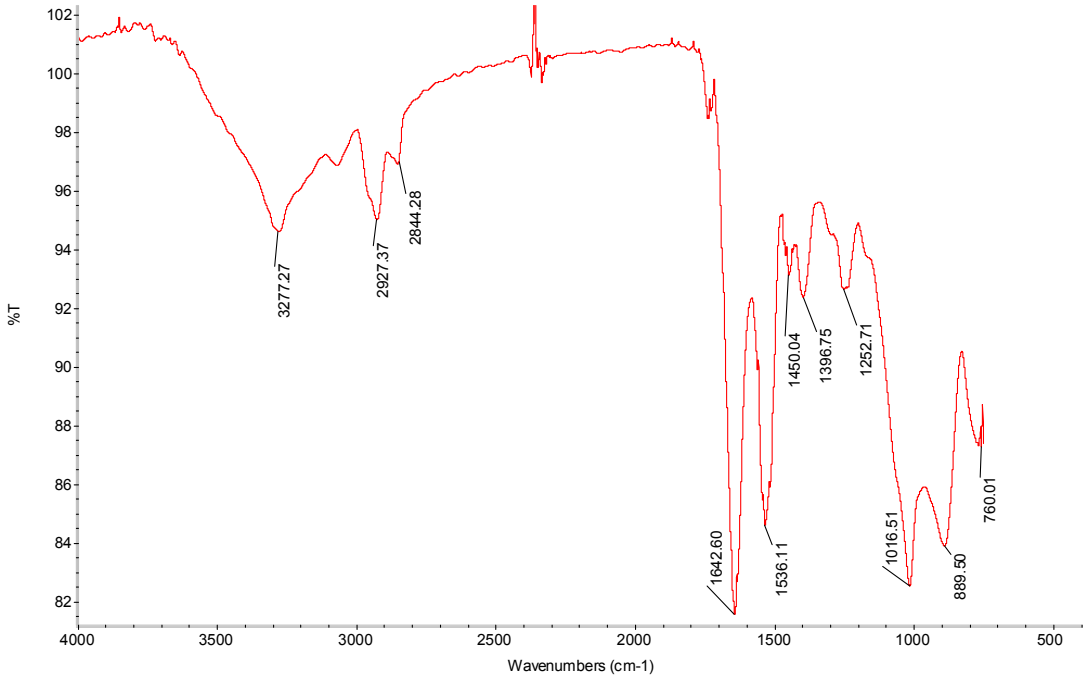


Figure A6 FTIR spectrum of 2,4,5-trimethoxycinnamoylchitosan (245CC2) DS= 0.150

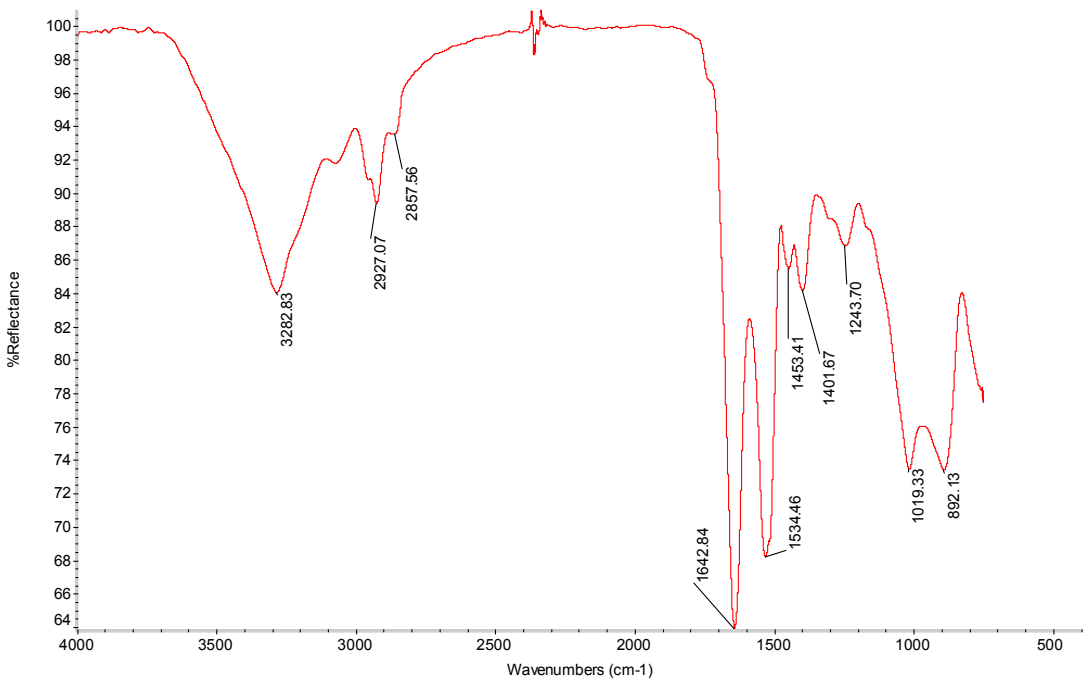


Figure A7 FTIR spectrum of 2,4,5-trimethoxycinnamoylchitosan (245CC3) DS= 0.285

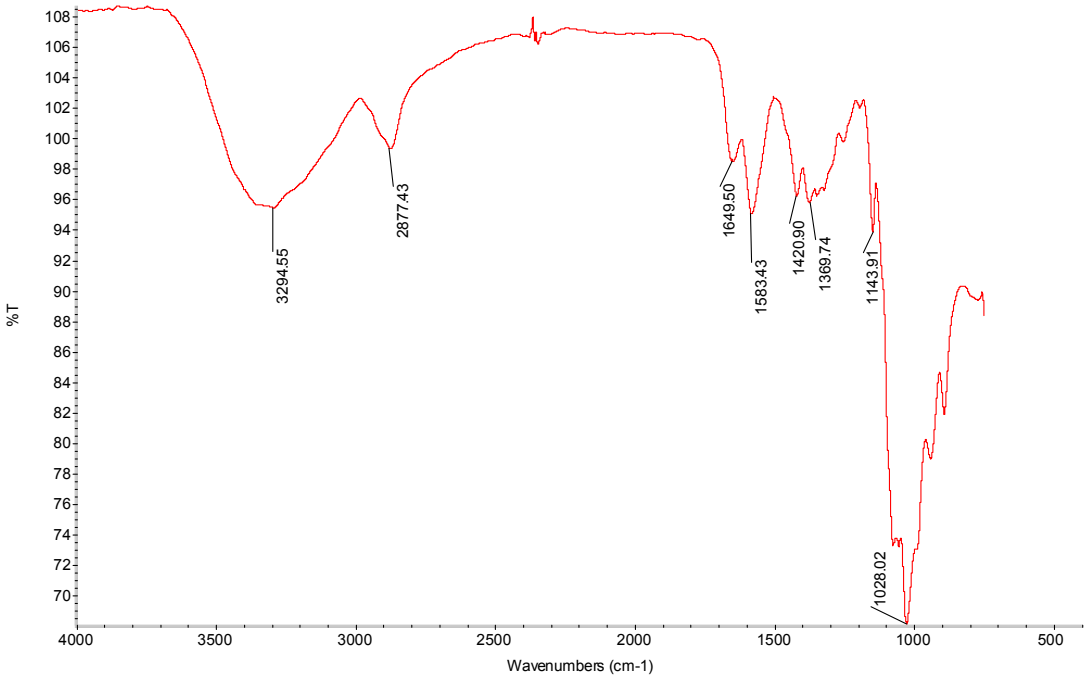


Figure A8 FTIR spectrum of Succinylchitosan (DS=0.09)

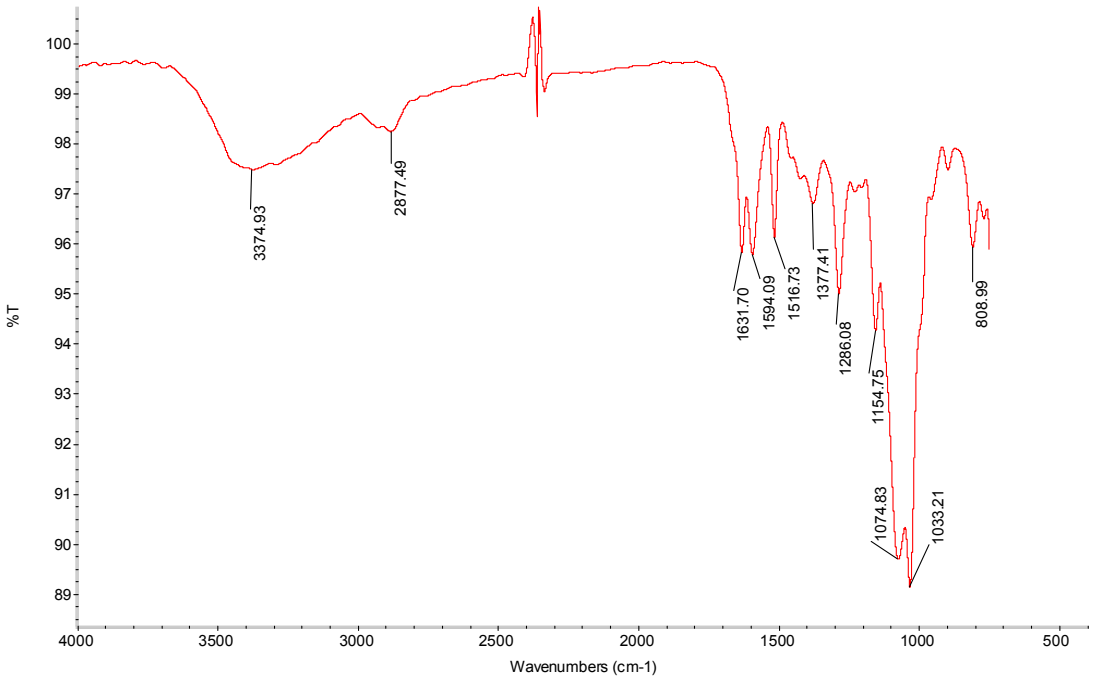


Figure A9 FTIR spectrum of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) DS=0.180

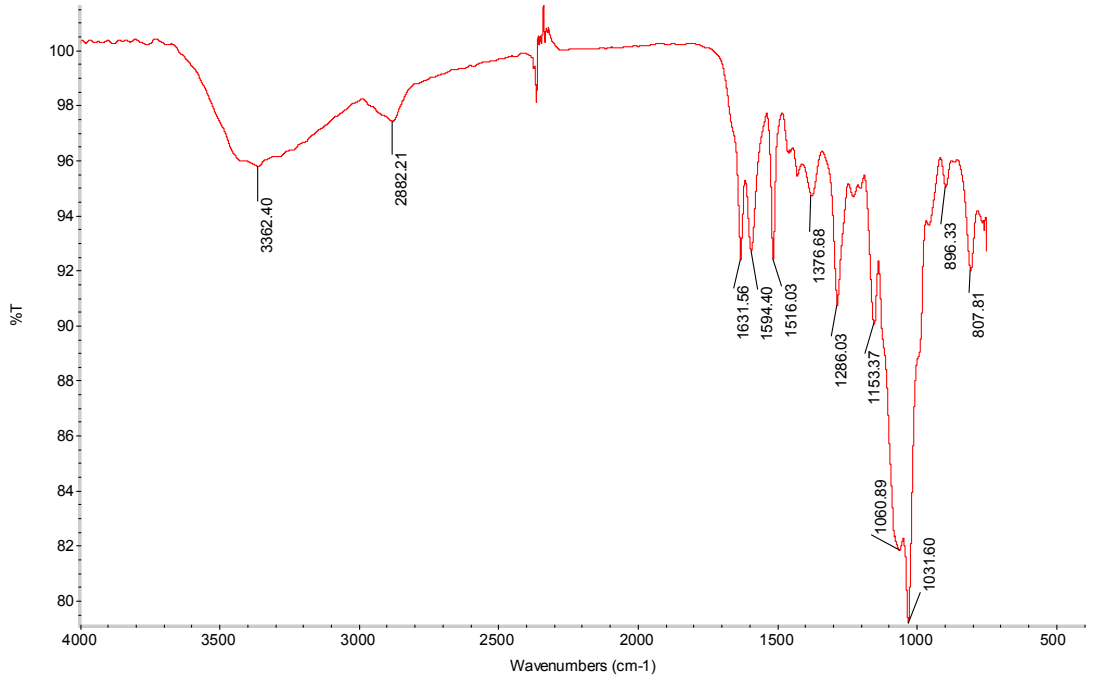


Figure A10 FTIR spectrum of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) DS=0.710

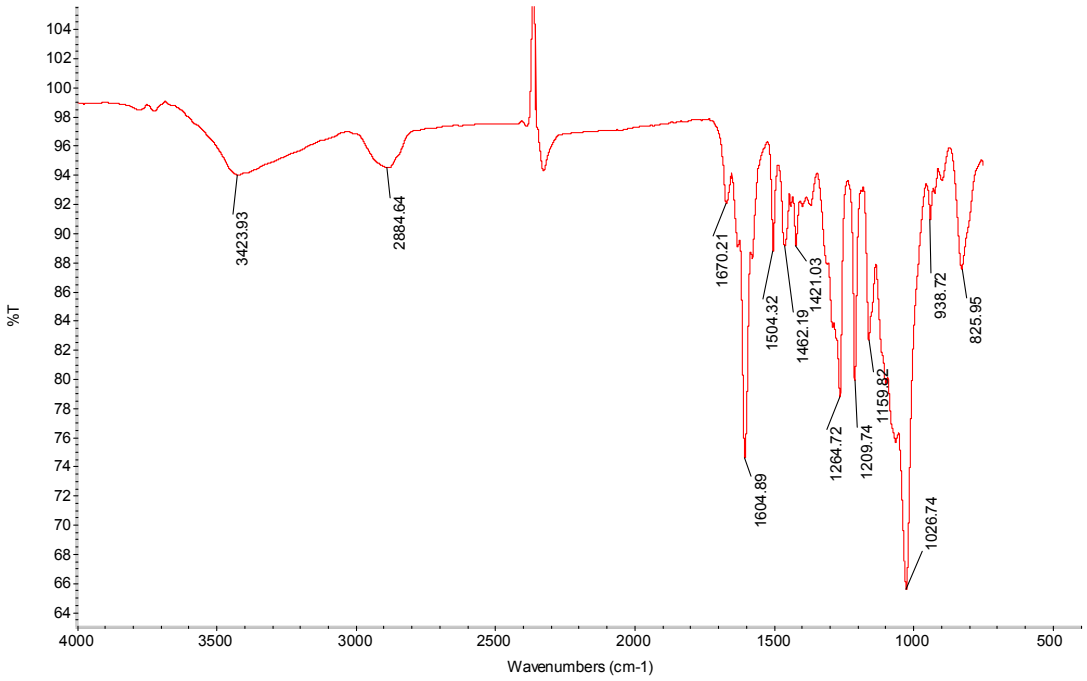


Figure A11 FTIR spectrum of 4-Hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC1) DS=0.196

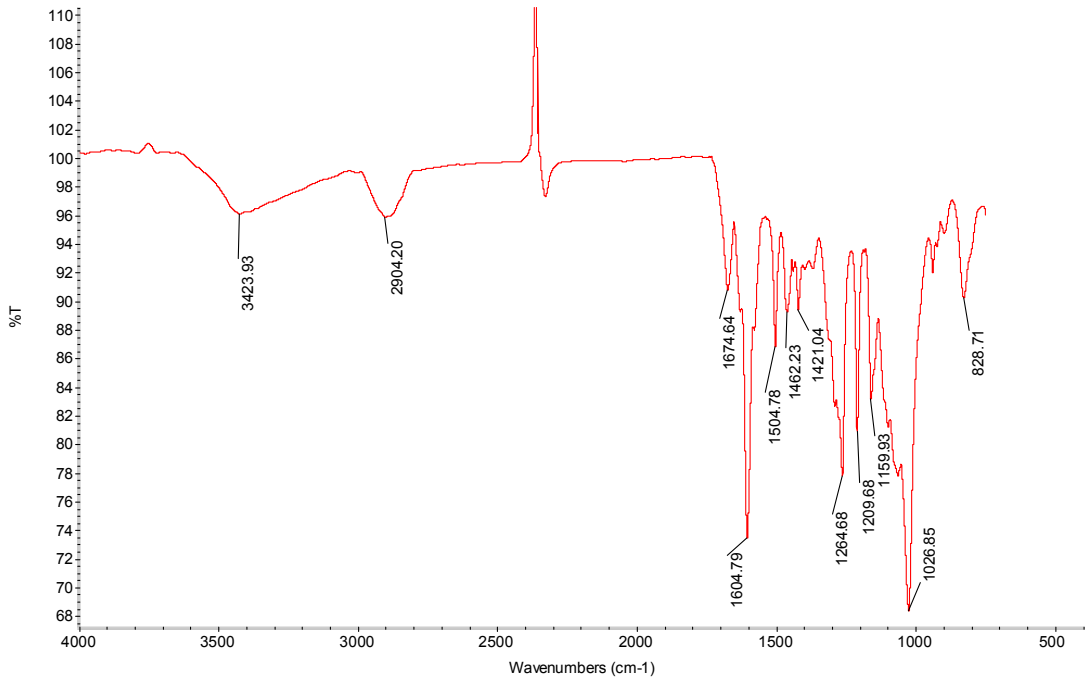


Figure A12 FTIR spectrum of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC2) DS=0.810

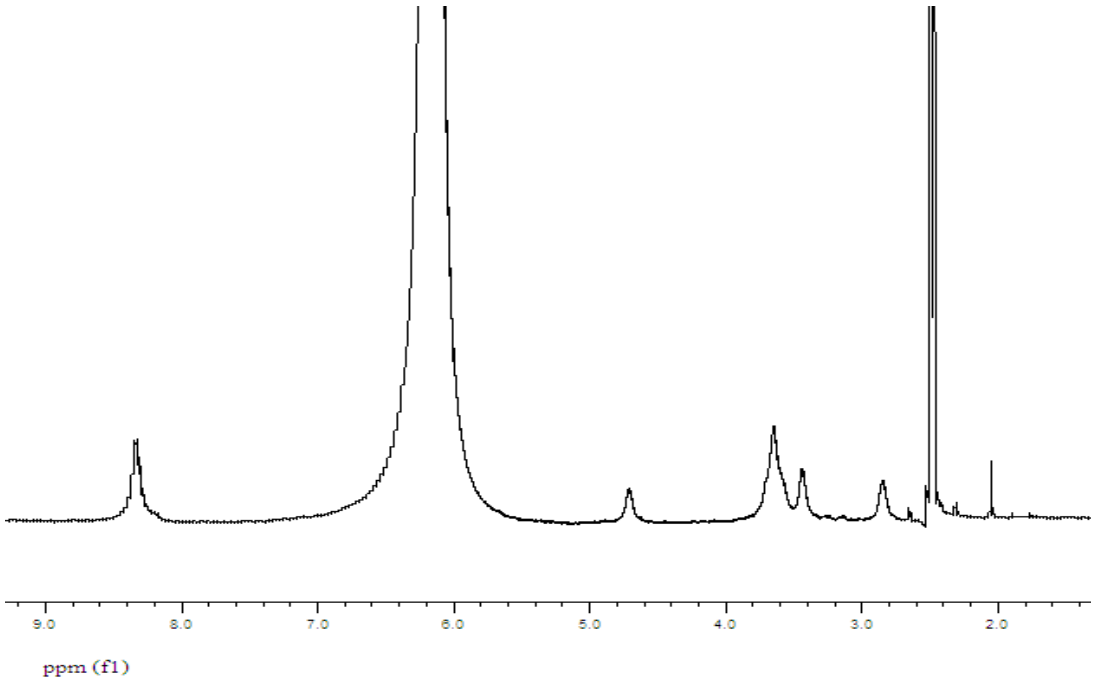


Figure A13 ^{1}H-NMR of native chitosan

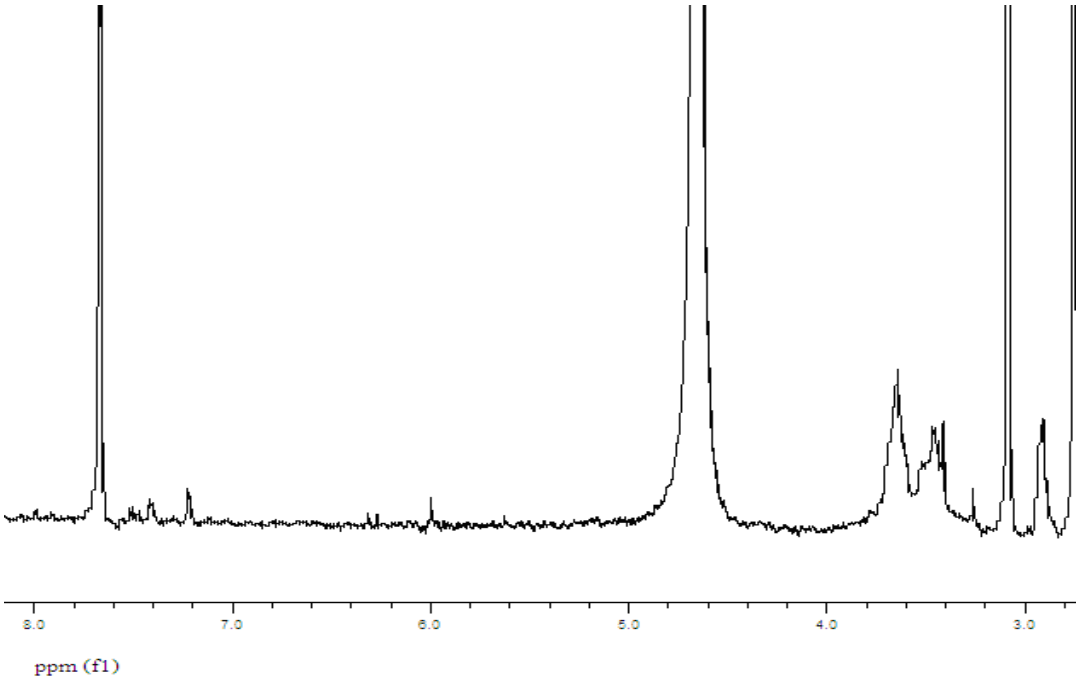


Figure A14 ^{1}H-NMR spectrum of cinnamoylchitosan (CC1) DS=0.051

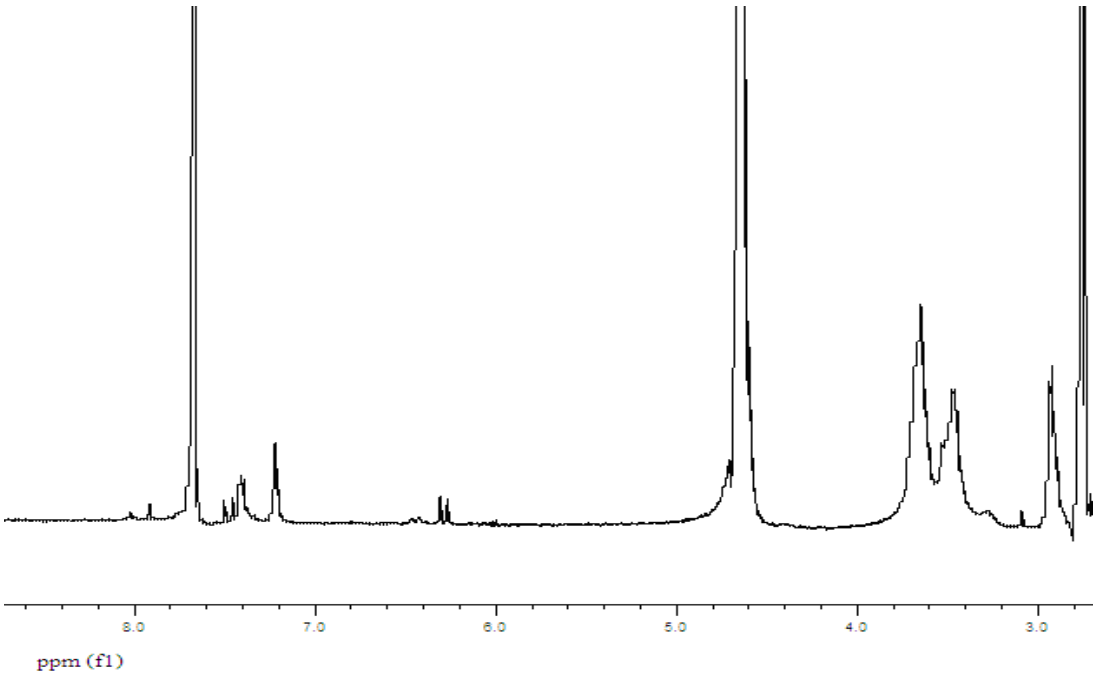


Figure A15 ^{1}H-NMR spectrum of cinnamoylchitosan (CC2) DS=0.155

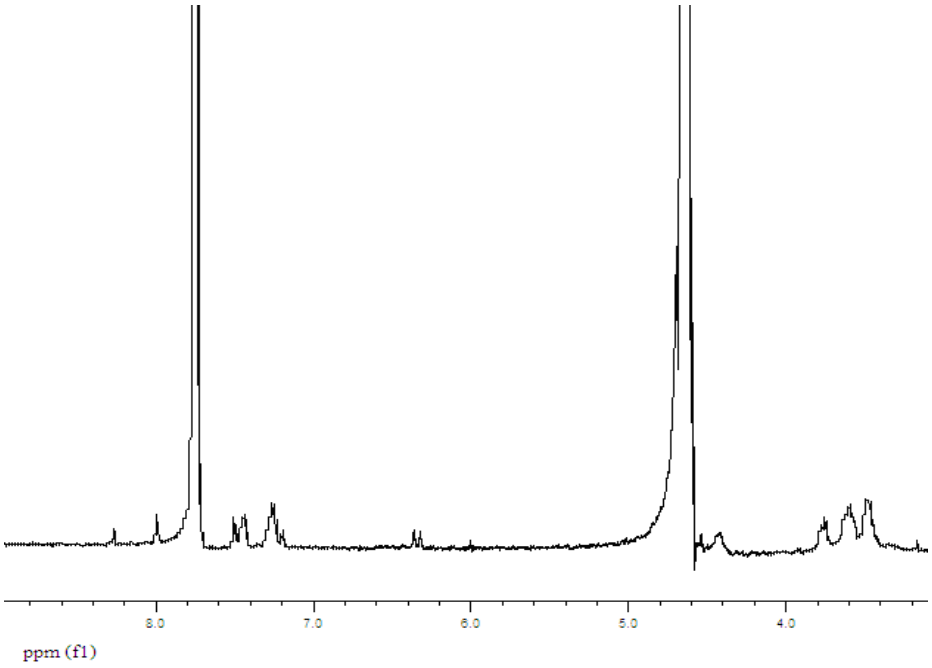


Figure A16 ^{1}H-NMR spectrum of cinnamoylchitosan (CC3) DS=0.321

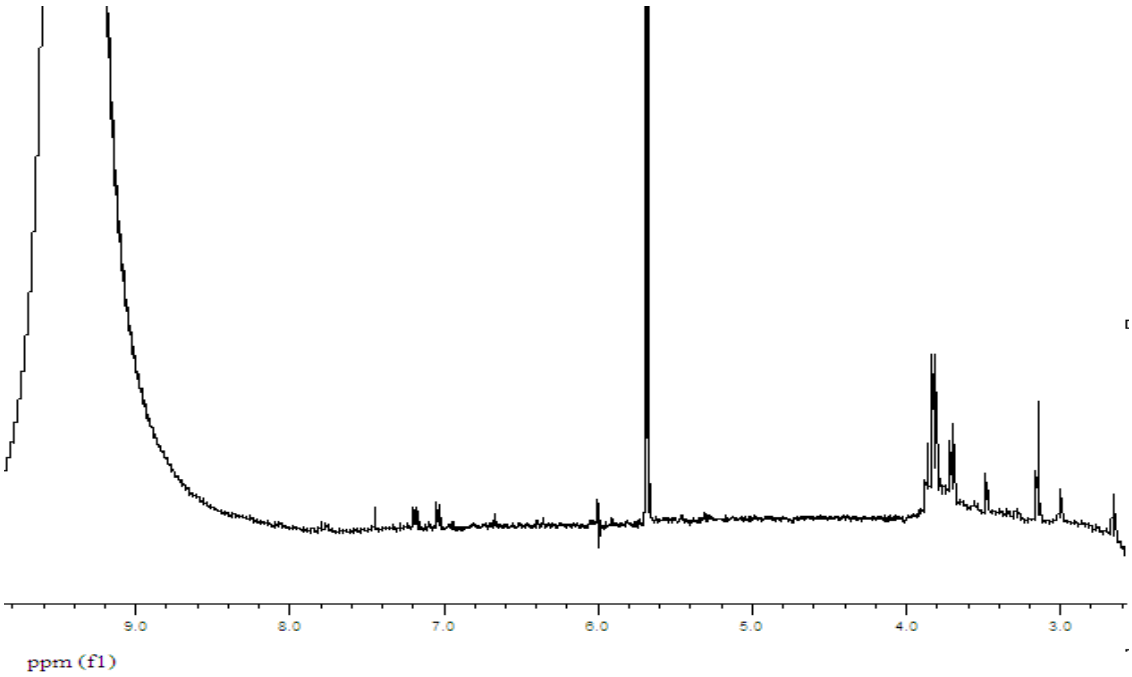


Figure A17 ^{1}H-NMR spectrum in DMSO and 0.05% TFA of 2,4,5-trimethoxycinnamoylchitosan (245CC1) DS=0.043

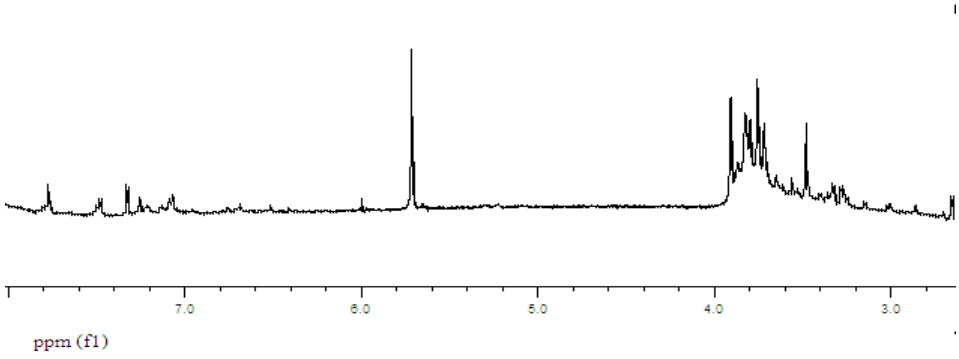


Figure A17 ^{1}H-NMR spectrum in DMSO and 0.05% TFA of 2,4,5-trimethoxycinnamoylchitosan (245CC2) DS=0.150

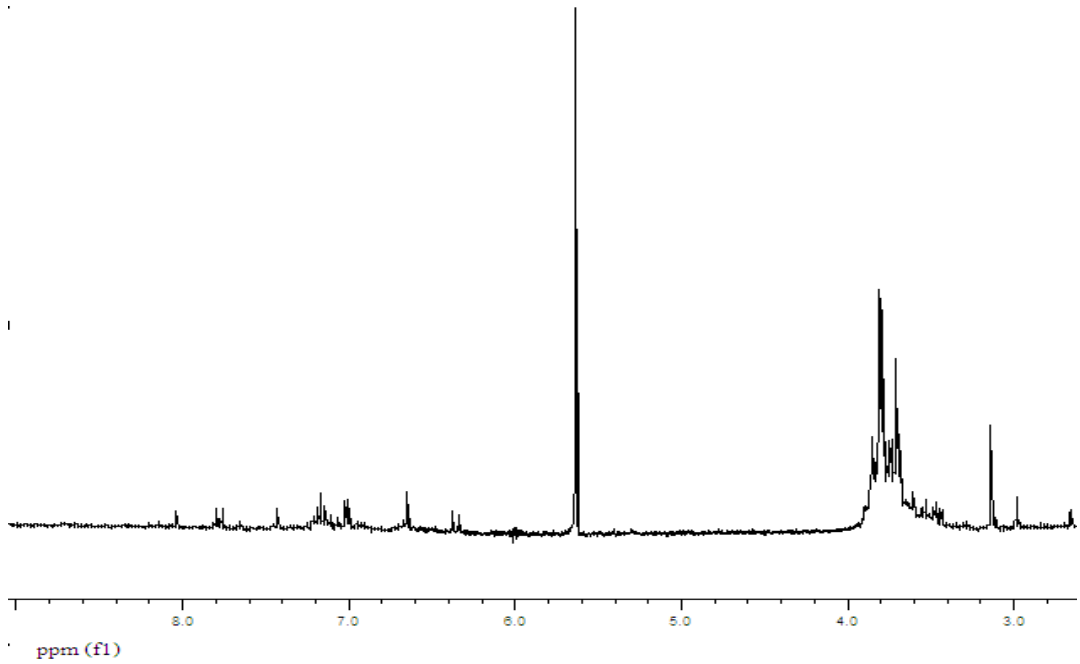


Figure A18 ^{1}H-NMR spectrum in DMSO and 0.05% TFA of 2,4,5-trimethoxycinnamoylchitosan (245CC3) DS=0.285

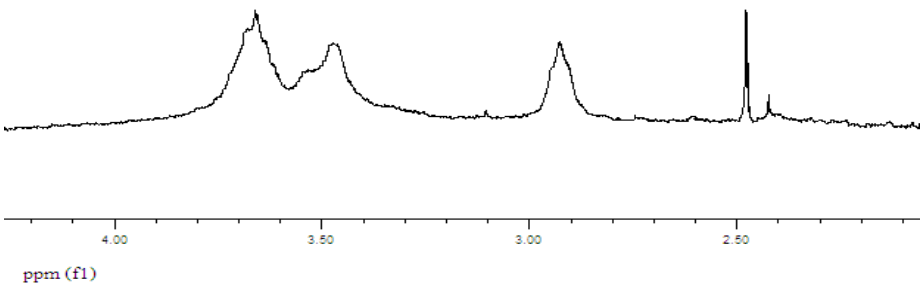


Figure A19 ^{1}H-NMR spectrum in D_2O with 0.05% CD_3OOD of Succinylchitosan (SC) DS=0.09

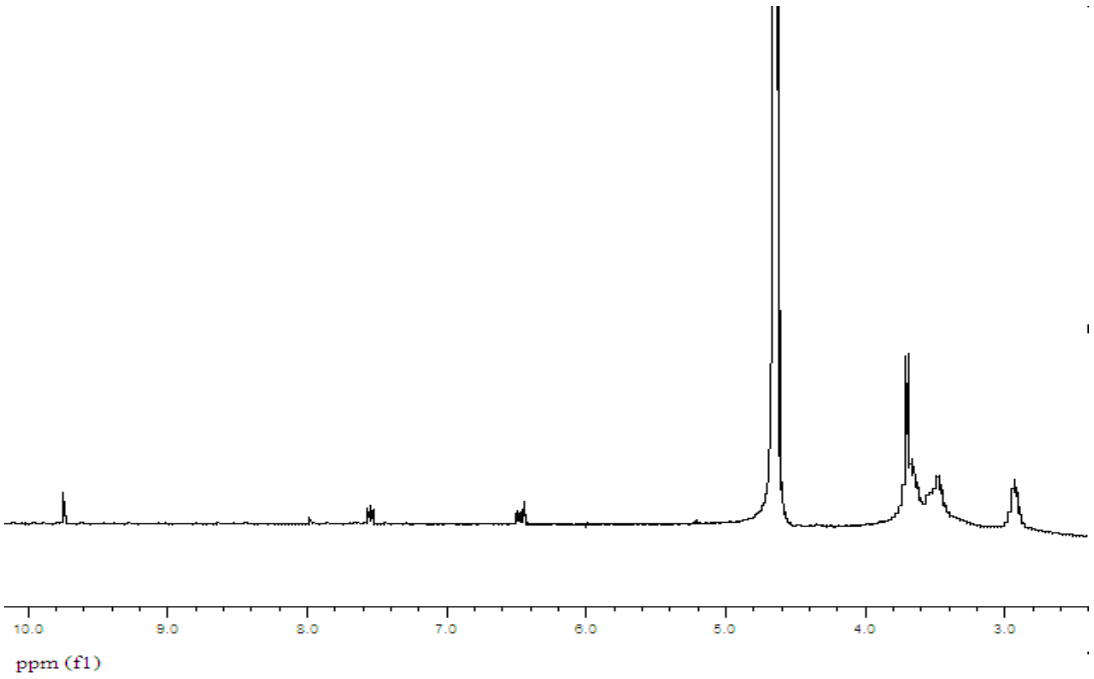


Figure A20 ^{1}H-NMR spectrum in D_2O of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) DS=0.180

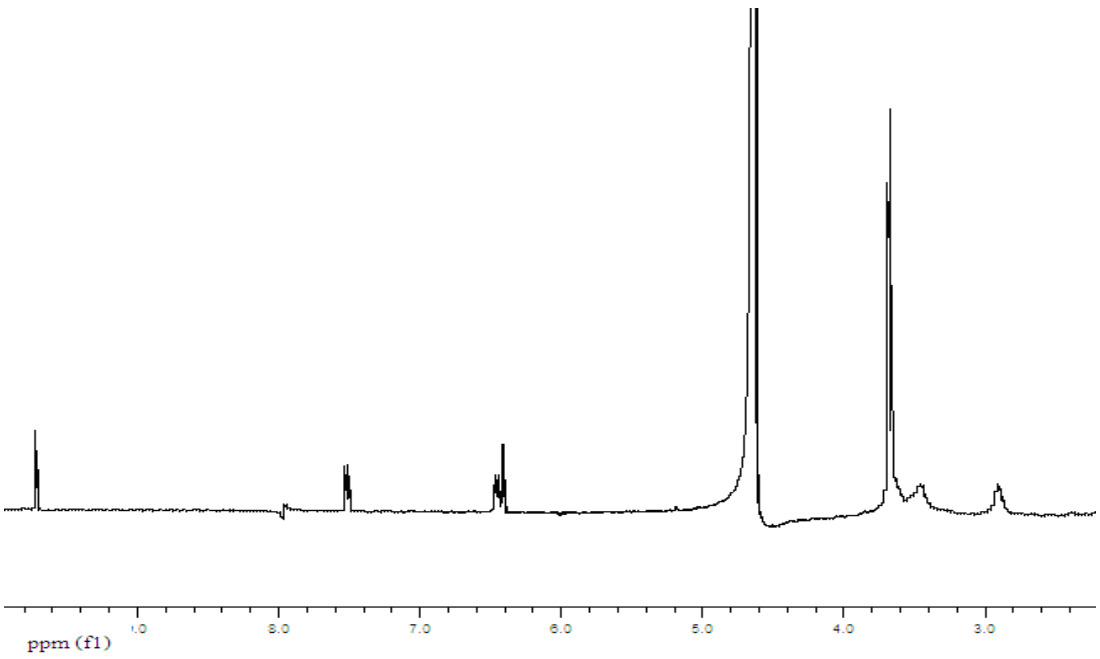


Figure A21 ^{1}H-NMR spectrum in D_2O of 2,4-dimethoxybenzalidenesuccinylchitosan (24BSC) DS=0.710

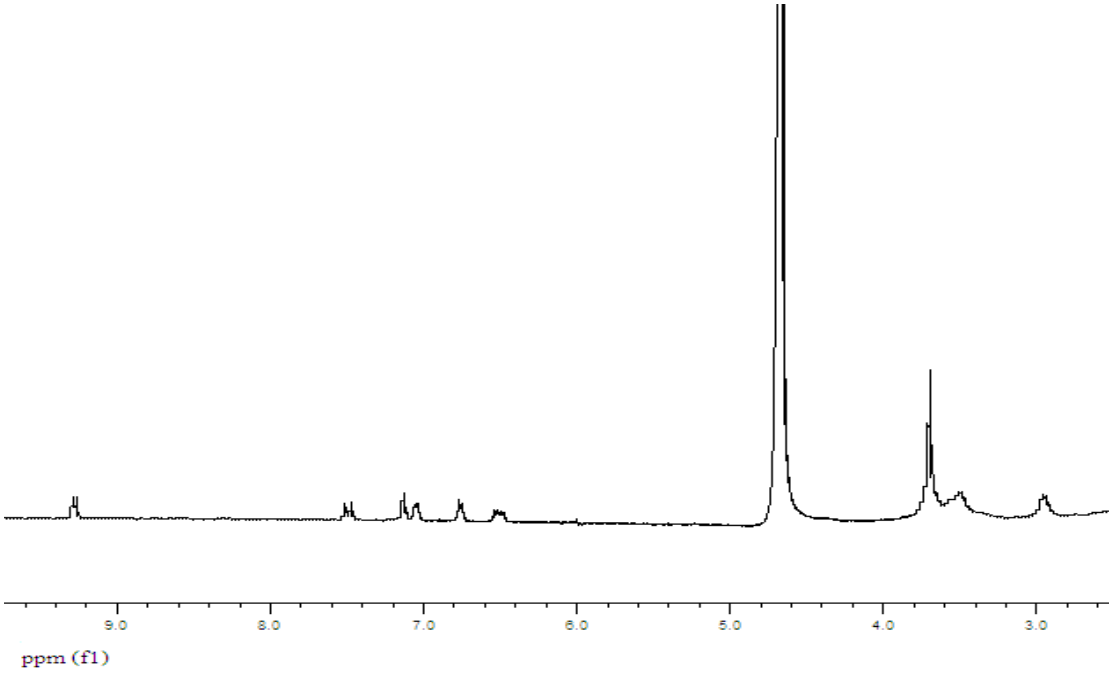


Figure A22 ^{1}H-NMR spectrum in D_2O of 4-Hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC) DS=0.196

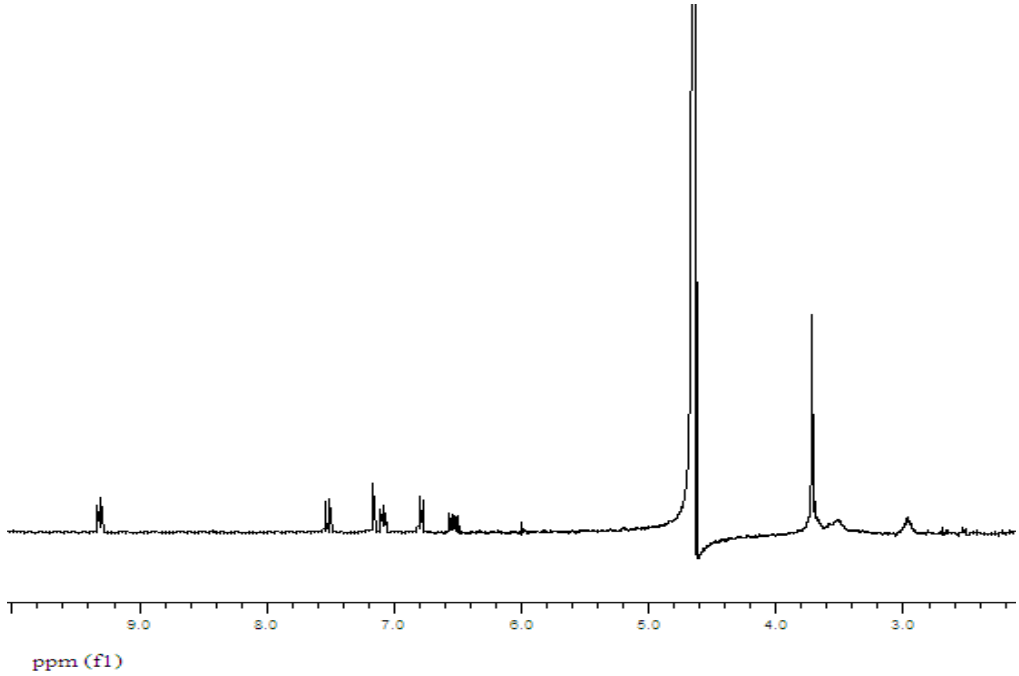
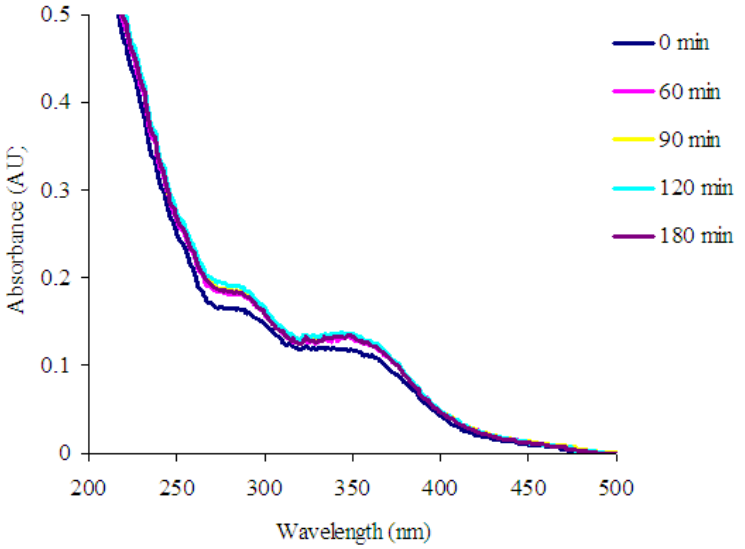


Figure A23 ^{1}H-NMR spectrum in D_2O of 4-hydroxy-3-methoxycinnamalidenesuccinylchitosan (43CSC) DS=0.810

APPENDIX B

A)



B)

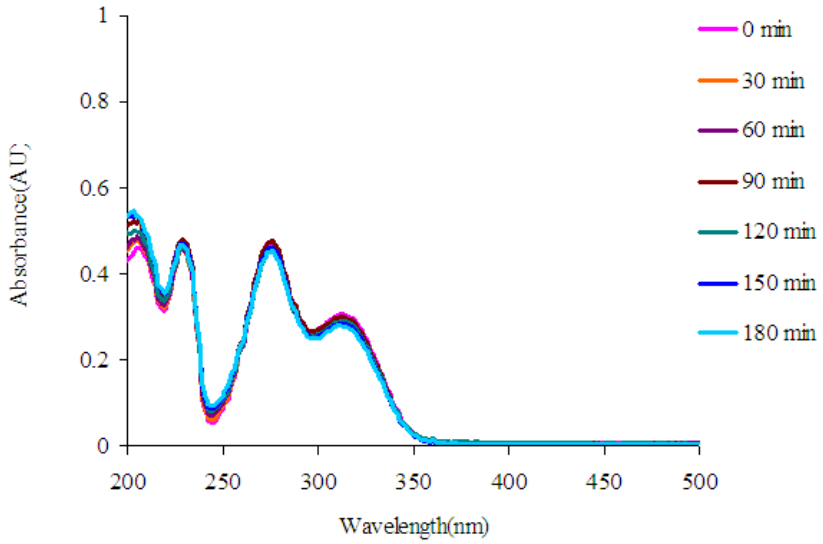


Figure B1 Photostabilities test of A) 2,4,5-trimeyhoxycinnamoylchitosan (245CC) and B) 2,4-dimethoxybenzalidenesuc- cinylchitosan (24BSC), in water irradiated by 8.8 mW/cm^2 UVA and 0.6 mW/cm^2 UVB.

VITA

Ms. Pimsiree Deemak was born on March 5, 1984 in Bankok. She received a Bachelor's Degree of Science in Chemistry from Silpakorn University in 2006. After that, she started her graduate study a Master's degree in the Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University. During she had presented "Nanoparticles of UV-absorbing chitosan derivatives" in the 3rd Asian Conference on Colloid & Interface Science (ACCIS 2009 KOREA) and "Cinnamoylchitosan derivatives: synthesis, characterization and self-assembly" in 6th International Symposium on Advance Material in Asia-Pacific Rim (6th ISAMAP) by poster presentation. The latter got the scholarships from National Center of Excellence, for Petrochemicals, and Advanced Material (NCE-PPAM) and the Graduate School, Chulalongkorn University.

My address is 42, Moo 2, T. Khaonoi, Sichol, Nakornsrithammarat, 80120, Tel. 089-6528163.