ABSTRACT

LIM, SANG-HOON. Synthesis of a Fiber-Reactive Chitosan Derivative and Its Application to Cotton Fabric as an Antimicrobial Finish and a Dyeing-Improving Agent. (Under the direction of Samuel Mack Hudson.)

The purpose of this research has been to develop a textile finish based on chitosan that is a biopolymer. A fiber-reactive chitosan derivative was synthesized from chitosan with a low molecular weight and a high degree of deacetylation. The synthesis was composed of two steps. As a first step, a water-soluble chitosan derivative was prepared by introducing quaternary ammonium salt groups on the amino groups of chitosan. The derivative was further modified by introducing functional groups (acrylamidomethyl) on the primary alcohol groups of the chitosan backbone, which can form covalent bonds with cotton. The fiber-reactive chitosan derivative (NMA-HTCC) itself showed complete bacterial reduction against *Staphylococcus aureus* and *Escherichia coli* at the concentration of 10 ppm. The NMA-HTCC was applied to cotton fabrics by a pad-batch method in the presence of an alkaline catalyst. The 1% NMA-HTCC treated cotton showed 100% bacterial reduction against *S. aureus*. The fabric maintained over 99% of bacterial reduction even after 50 home launderings. The NMA-HTCC cotton was dyed with direct and reactive dyes without addition of salt. The color yield was higher than that of untreated cotton, which required a large amount salt for dyeing. The NMA-HTCC cotton showed better washfastness than untreated cotton, but the lightfastness was inferior to that of untreated cotton. The antimicrobial activity of the NMA-HTCC cotton was considerablely decreased after dyeing due to the blocking of the cationic groups of the NMA-HTCC by dye molecules.

SYNTHESIS OF A FIBER-REACTIVE CHITOSAN DERIVATIVE AND ITS APPLICATION TO COTTON FABRIC AS AN ANTIMICROBIAL FINISH AND A DYEING-IMPROVING AGENT

by

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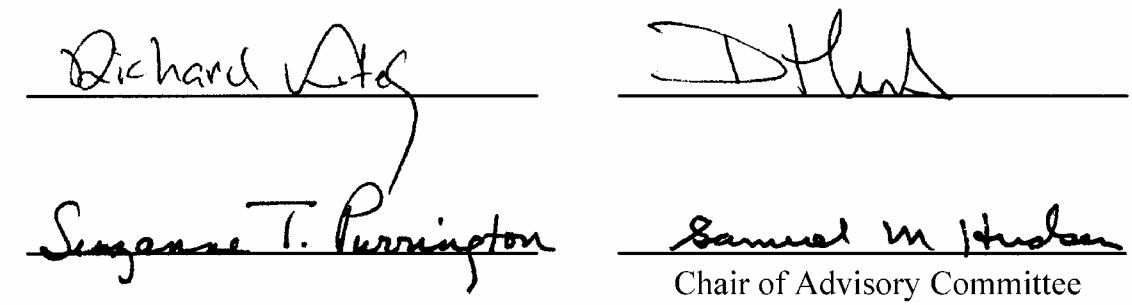

Chair of Advisory Committee

DEDICATION

This dissertation is dedicated to my father, Prof. Yong-Jin Lim, who inspired my interest in Textile Chemistry and to my wife, Ji-Ae, who supported me with ultimate love during the preparation of this dissertation.

BIOGRAPHY

Sang-Hoon Lim, the second son of Young-Jin Lim who is a professor in the Dept. of Dyeing and Finishing at Kyungpook National University and Sook-Ja Chung, was born on December 27, 1971 in Daegu, Korea. He has two brothers, Jung-Hoon who is an ophthalmologist and Kyung-Hoon who is a surgeon. He graduated from Daegu high school in February of 1990 and received a Bachelor of Engineering degree in Dyeing and Finishing from Kyungpook National University, Daegu, Korea in August of 1996. During his undergraduate years, he always has been at the top of his class. After his freshman year, he served his military service in the Korean Army for two years and four months from April of 1991 to August of 1993.

Sang-Hoon came to the United States in June of 1997 to continue his education and started his Master program in Textile Chemistry in College of Textiles at North Carolina State University. He obtained a Master of Science degree in December of 1999 and continued his study to pursue his Doctor of Philosophy degree in Fiber and Polymer Science in the College of Textiles. Throughout his graduate study including Master and Ph.D. programs, Sang-Hoon earned GPA of 4.0/4.0 and passed his final oral examination for the Ph.D. degree on December 16, 2002.

On July 25, 1998, Sang-Hoon married Ji-Ae Chung who is a daughter of Sang-Tack Chung and Soon-Ja Kwon. Sang-Hoon and Ji-Ae are expecting their first baby, whose nickname is Kookie, in early January of 2003.

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Professionally, I would particularly like to thank my advisor, Dr. Samuel Hudson, who provided me with the time, invaluable advice, and countenance to complete this dissertation. It has been a privilege to know and to work with such a dedicated person. I am most appreciative of Dr. Suzanne Purrington, Dr. Richard Kotek, and Dr. David Hinks who served as my committee members. I owe big thanks to Dr. Nam-Sik Yoon in Korea and Dr. Kazuyuki Hattori in Japan for their help and suggestions on my research, and to Dr. David Jenkins for his assistance in my early work. I would like to thank Dr. Brent Smith and Dr. Peter Hauser for providing me dyestuffs and answering my questions. I wish to thank Dr. Keith Beck for teaching me a basic knowledge of the Weather-Ometer and Ms. Amy Hammonds at AATCC for letting me use their Weather-Ometer. A special thank goes to Mr. Jeffrey Krauss for giving me helpful suggestions and letting me have access to the pilot plant.

Personally, I am deeply grateful for my family in Korea including my grandmother, parents, two brothers, and parents-in-law for their constant love,

encouragement, and overall support during the course of my Ph.D. program. I also would like to thank my sisters-in-law and brother-in-law for their concern and encouragement.

Lastly, I would like to acknowledge all the people who were kind enough to ask from time to time, “how’s it going?” and then were gracious enough to listen to what I had to say.

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LIST OF ABBREVIATIONS

AOX	adsorbable organic halogens
BTCA	1,2,3,4-butanetetracarboxylic acid
CA	citric acid
CFU	colony forming unit
CNC	cyanuric chloride
COS	chito-oligosaccharide
DC	degree of carboxyalkylation
DD	degree of deacetylation
DEAE	*N,N*-diethylaminoethyl
DHDMI	4,5-dihydroxy-1,3-dimethyl-2-imidazolidinone
DMDHEU	1,3-dimethylol-4,5-dihydroxyethylene urea
DP	degree of polymerization or durable press
DQ	degree of quaternization
DS	degree of substitution
GTMAC	glycidyltrimethylammonium chloride
HT(A)CC	*N*-[(2-hydroxy-3-trimethylammonium)propyl]chitosan chloride
LAS	linear alkylbenzene sufonate
LMA	lauryl methacrylate
MAA	methacrylic acid
MIC	minimum inhibitory concentration

MW	molecular weight
NCBC	*N*-carboxybutyl chitosan
NCMC	*N*-carboxymethyl chitosan
NCPC	*N*-carboxylpropyl chitosan
NMA	*N*-methylolacrylamide
NOCMC	*N,O*-carboxymethyl chitosan
OCMC	*O*-carboxymethyl chitosan
owb	on weight of bath
owf	on weight of fabric
PCP	pentachlorophenol
PET	polyester
PP	polypropylene
SBC	*N*-sulfobenzoyl chitosan
SC	*N*-sulfonated chitosan
SLS	sodium lauryl sulfate
TEAE	triethylaminoethyl
TMCC	*N,N,N*-trimethyl chitosan chloride
TMCH	*N,N,N*-trimethyl chitosan hydroxide
TMCI	*N,N,N*-trimethyl chitosan iodide
TSP	3-(trimethylsilyl)propionic 2,2,3,3,-d_4 acid sodium salt
% WPU	% wet pick-up
WRA	wrinkle recovery angle

1. INTRODUCTION

Chitosan is the deacetylated derivative of chitin that is the second most abundant polysaccharide found on earth next to cellulose. There is not a sharp boundary in the nomenclature distinguishing chitosan from chitin [1]. When chitin is deacetylated over about 60% it becomes soluble in dilute aqueous acids and is referred to as chitosan. Chitin is the main component in the shells of crustaceans, such as shrimp, crab, and lobster. It is also found in exoskeletons of mollusks and insects, and in the cell walls of some fungi [2,3]. Chitosan is found in some fungi, but its quantity is so limited that it is mainly produced commercially by alkaline deacetylation of chitin [3].

Huge amounts of crab and shrimp shells have been abandoned as wastes by worldwide seafood companies. This has led to considerable scientific and technological interest in chitin and chitosan as an attempt to utilize these renewable wastes. Chitosan has become the preferred commercial form of these materials, as it is more tractable to solution processes than chitin. In the past thirty years, it has been demonstrated by a number of researchers that chitosan has a great potential for a wide range of uses due to its biodegradability, biocompatibility, antimicrobial activity, nontoxicity, and versatile chemical and physical properties. The applications of chitosan include uses in a variety of areas, such as pharmaceutical and medical applications, paper production, textile dyeing and finishing, fiber formation, wastewater treatment, biotechnology, cosmetics, food processing, and agriculture [1,2,4-11].

There is a greater demand for antimicrobial finishes on textile goods as consumers have become aware of the potential advantages of these materials. The textile industry

continues to look for eco-friendly processes that substitute for toxic textile chemicals and reduce dyes in dyehouse wastewater. Chitosan, a natural biopolymer, has many chemical attributes, especially its cationic nature, to make it an interesting candidate for these applications. The use of chitosan as a textiles chemical will be greatly beneficial in the view of the utilization of abandoned seafood wastes. However, the major problems of chitosan are its loss of the antimicrobial activity under alkaline conditions due to its loss of the cationic nature and its poor durability, when applied to textile fabrics, due to its lack of strong bonding with fabrics.

The goal of this work is to develop a textile finish for cotton fabrics based on chitosan, which can overcome the limitations of chitosan. The specific objectives of the work are to 1) synthesize a fiber-reactive chitosan derivative and optimize the reaction conditions, 2) optimize the application conditions of the chitosan derivative to cotton fabric, and 3) evaluate the antimicrobial activity and dyeing property of the treated fabric.

The thesis is organized as follows. The next chapter deals with general properties of chitosan. Chapters 3, 4, and 5 provide extensive literature review on antimicrobial activities of chitosan and its derivatives, and their uses in textile dyeing and finishing, which give background and motivation for this work. Chapters 6, 7, and 8 describe the preparation of a fiber-reactive chitosan derivative (NMA-HTCC) from chitosan. Chapter 9 deals with the optimization of the application conditions of the NMA-HTCC to cotton fabric. The antimicrobial and the dyeing property of the NMA-HTCC treated cotton are discussed in Chapters 10 and 11, respectively. Conclusions and recommendations for future research are provided in Chapter 12.

2. CHEMICAL AND PHYSICAL PROPERTIES OF CHITOSAN

2.1. Chemical and Physical Structure of Chitosan

Chitin and chitosan are naturally occurring *β*-1,4-linked linear polysaccharides similar to cellulose as shown in Figure 1. Chitin has the same backbone as cellulose, but it has an acetamide group on the C-2 position instead of a hydroxy group and its molecular weight, purity, and crystal morphology are dependent on their sources [12].

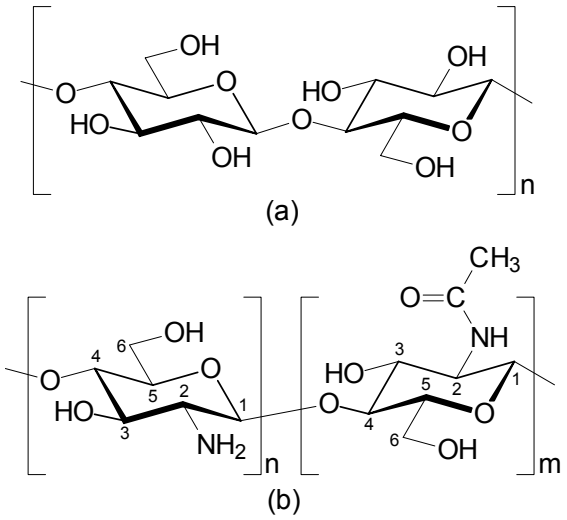

Figure 1. Chemical structures of (a) cellulose and (b) chitin and chitosan (chitin occurs as mostly the "m" or *N*-acetyl form and chitosan occurs as the "n" or amino form).

Chitin is essentially a homopolymer of 2-acetamido-2-deoxy-*β*-D-glucopyranose, although some of the glucopyranose residues exist as 2-amino-2-deoxy-*β*-D-glucopyranose. Chitosan is the *N*-deacetylated derivative of chitin and the majority of its glucopyranose residues are 2-amino-2-deoxy-*β*-D-glucopyranose.

Chitin occurs naturally as one of three crystalline polymorphic forms, known as *α*-, *β*-, and *γ*-chitin. The three forms differ in the packing of the chains within the

crystalline regions [13]. The chains of α-chitin are antiparallel but the β-chitin has a parallel stack structure. The γ-chitin form has not been fully classified but an arrangement of two parallel chains and one antiparallel chain has been suggested. Although both α- and β-chitins possess C=O·····H−N intermolecular hydrogen bonds, the β-chitin does not have the intermolecular hydrogen bonds between $-CH_2OH$ groups, which are present in the α-chitin [14]. This fact makes it easy for the β-chitin to swell in water to produce hydrates unlike the α-chitin, which has a strong three-dimensional hydrogen bond network. The α-chitin is the most abundant and found in crustaceans, insects, and fungi. Because of its abundance, commercial chitosan is prepared exclusively from α-chitin. The occurrence of β-chitin is less common and it is found in squid and marine diatoms.

2.2. Degree of Deacetylation (DD)

The DD is the proportion of glucosamine monomer residues in chitin. It has a striking effect on the solubility and solution properties of chitin. By convention, chitin and chitosan are distinguished by their solubility in dilute aqueous acids such as acetic acid [2]. Chitin does not dissolve in dilute acetic acid. When chitin is deacetylated to a certain degree (~ 60% deacetylation) where it becomes soluble in the acid, it is referred to as chitosan.

A typical deacetylation process of chitin involves the reaction of chitin powder or flake in an aqueous 40-50% sodium hydroxide solution at 100-120°C for several hours to hydrolyze *N*-acetyl linkages [3]. Repetition of the process can give deacetylation values

up to 98% but the complete deacetylation can never be achieved by this heterogeneous deacetylation process without modification. Fully deacetylated (nearly 100%) chitosan can be prepared by the alkaline treatment of a gel form instead of the powder form of chitosan [15].

A number of methods have been employed to measure the DD, such as IR spectroscopy [16-20], UV spectroscopy [21], circular dichroism [22], ^{1}H NMR spectroscopy [23,24], ^{13}C solid-state NMR spectroscopy [25], gel permeation chromatography [21], titration methods [19,25-28], residual salicylaldehyde analysis [19], equilibrium dye adsorption [29], elemental analysis [30], acid degradation followed by HPLC [30], thermal analysis [31], and pyrolysis-mass spectrometry [32].

One of the simplest methods for determining the DD is acid-base titration. In this method, a known amount of chitosan is dissolved in an excess of dilute aqueous acid such as hydrochloric acid and titrated with a standard sodium hydroxide solution. The stoichiometry is determined by pH measurement. Although this method has been used by many researchers, its precision has been questioned by Domard and Rinaudo because of the precipitation of chitosan in the range of neutralization pH and consequently slow and unstable response of the pH reading [33]. They reported that conductivity measurement eliminated this problem. The advantage of conductometric titration against potentiometric titration was confirmed in our laboratory [27]. Figure 2 shows the conductometric titration curve which has two inflection points. The first inflection point corresponds to the neutralization of excess H^{+} ions of HCl by OH^{-} ions of NaOH added. The range between the two inflection points corresponds to the neutralization of the

protonated amino groups of chitosan. After the second inflection point, the conductivity goes up with a higher value of slope, which is due to the excess of NaOH added. The number of moles of NaOH used between the two inflection points equals the number of moles of amino groups in the chitosan sample, which is converted to the DD of chitosan.

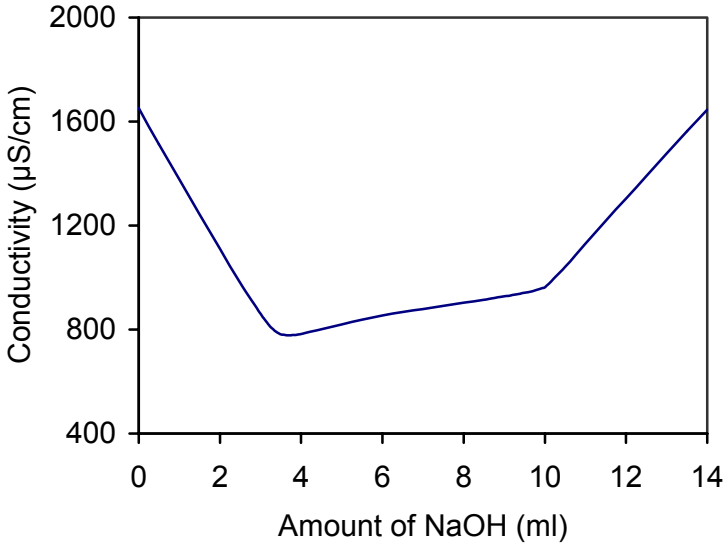

Figure 2. Conductometric titration curve of chitosan.

2.3. Molecular Weight (MW)

MW is a very important parameter for natural and synthetic polymers for their applications. The MW of chitin and chitosan depend on its source and deacetylation conditions (time, temperature, and concentration of NaOH), respectively. Chitosan obtained from deacetylation of crustacean chitin may have a MW over 100,000. Consequently, it is necessary to reduce the MW by chemical methods to much lower MW for easy application as a textile finish. The MW of chitosan can be determined by

several methods, such as light scattering spectrophotometry [33-35], gel permeation chromatography [36-40], and viscometry [40-44].

Although viscometry is not an absolute method for determining the MW of chitosan, it is one of the simplest and most rapid methods. The Mark-Houwink equation is used to calculate viscosity average MW ($\overline{M}_v$) when the intrinsic viscosity [η] and the empirical constants *k* and α are known. The constants vary depending on the solvent systems and temperature employed, and the DD of chitosan.

$$[\eta] = k\overline{M}_v^{\alpha}$$

As a result of the polyelectrolyte nature of chitosan in a dilute aqueous acid, the linear charge density along the chitosan chain increases with the increase in DD, which results in a gradual increase in intrinsic viscosity due to the coil expansion. Wang et al. [43] presented the dependence of *k* and α on the DD of chitosan when chitosan is dissolved in 0.2M CH_3COOH/0.1M CH_3COONa aqueous solution at 30°C.

$$k = 1.64 \times 10^{-30} \times DD^{14}$$

$$\alpha = -1.02 \times 10^{-2} \times DD + 1.82$$

Consequently, knowing the DD of chitosan, the MW can be determined using Wang's equations for *k* and α followed by the Mark-Houwink equation. A number of different values of *k* and α depending on different solvent systems can be found in a recent literature review [40].

2.4. Solubility

The solubility of chitosan is very important for its utilization, such as for chemical modification and film or fiber formation. Neither chitin nor chitosan are soluble in neutral water. Chitin is a semicrystalline polymer with extensive inter- and intra-molecular hydrogen bonds, which make it difficult to dissolve in dilute acids or organic solvents under mild conditions. Although many solvents have been found, a majority of them are not useful due to their toxicity, corrosiveness, or mutagenic properties. Chitosan, a more tractable form than chitin, readily dissolves in dilute mineral or organic acids by protonation of free amino groups at pH below about 6.5. This cationic nature is the basis of a number of applications of chitosan. Acetic and formic acids are most widely used for research and applications of chitosan. A number of solvents for chitin and chitosan can be found in the literature [45]. Generally, the solubility of chitin and chitosan decreases with an increase in MW. Oligomers of chitin and chitosan with a degree of polymerization (DP) of 8 or less are water-soluble regardless of pH [46].

Water-soluble chitin, however, can be prepared by either homogeneous deacetylation of chitin [47] or homogeneous *N*-acetylation of chitosan [48,49]. Water-solubility is obtained only when the DD of chitin is about 0.5. It should be emphasized that the water-soluble chitin is obtained by homogeneous reaction instead of heterogeneous reaction. The former treatment gives a random copolymer of *N*-acetyl-D-glucosamine and D-glucosamine units, whereas the latter one produces a block copolymer of these two units. X-ray diffractometry revealed that the random copolymer was almost amorphous but the block copolymer was highly crystalline although the degree of

deacetylation of the two polymers is the same. Kurita et al. [47,49] concluded that the water-solubility was attributed to the greatly enhanced hydrophilicity resulting from the random distribution of acetyl groups and the destruction of the tight crystalline structure of chitin.

2.5. Chemical Reactivity

Chitosan has three reactive groups, that is, primary (C-6) and secondary (C-3) hydroxyl groups on each repeat unit, and the amino (C-2) group on each deacetylated unit. These reactive groups are readily subject to chemical modification to alter mechanical and physical properties, and solubilities of chitosan. The typical reactions involving the hydroxyl groups are etherification and esterification. Selective *O*-substitution can be achieved by protecting the amino group during the reaction. The presence of a nucleophilic amino group allows selective *N*-substitution, such as *N*-alkylation and *N*-acylation by reacting chitosan with alkyl halides and acid chlorides, respectively. The alternative method for the *N*-alkylation is reductive alkylation, where the amino group is converted to an imine with a variety of aldehydes or ketones, and subsequently reduced to an *N*-alkylated derivative. Chitosan can also be modified by either cross-linking or graft copolymerization. A number of chemically modified chitosan derivatives are listed in the literature [3,50].

3. ANTIMICROBIAL ACTIVITY OF CHITOSAN

The antimicrobial activity of chitosan against various bacteria and fungi is well known and it has been reported by a number of authors [51-75]. This unique property, due to the polycationic nature of chitosan, facilitated its application in a variety of fields, including food science, agriculture, medicine, pharmaceutics, and textiles. In this section the mechanisms of this action and factors affecting antimicrobial activity are described.

3.1. Mechanism

Several different mechanisms for microbial inhibition by chitosan have been proposed, but the exact mechanism is still not known. The most accepted one is the interaction of the positively charged chitosan with the negatively charged residues at the cell surface of many fungi and bacteria, which causes extensive cell surface alterations and alters cell permeability [53,56-58,63,68,69]. This causes the leakage of intracellular substances, such as electrolytes, UV-absorbing material, proteins, amino acids, glucose, and lactate dehydrogenase. As a result, chitosan inhibits the normal metabolism of microorganisms and finally leads to the death of these cells.

For example, Fang et al. [56] reported that the growth of *Aspergillus niger* was inhibited by chitosan. Chitosan at the concentration of 5.0 mg/mL induced considerable leakage of UV-absorbing and proteinaceous materials from *A. niger* at pH 4.8. In contrast, chitosan at pH 7.6 and chitin at pH 4.8 did not induce leakage, which suggests that the antifungal activity of chitosan is related to the polycationic nature of chitosan and is directly affected by the pH value. The leakage of nucleic acid and protein from

Escherichia coli was observed by Hwang et al. [57] in their study on the bactericidal activity of chitosan on *E. coli*. Transmission electron microscopy (TEM) revealed that the outer cell wall of *E. coli* was greatly distorted and frayed, and the cytoplasmic membrane was detached from the inner part of the cell wall after chitosan treatment. Tsai and Su [58] observed the chitosan-induced leakage of glucose and lactate dehydrogenase from *E. coli* cells and suggested that the death of cells resulted from the interaction between chitosan and the *E. coli* cell, that changed the membrane permeability, which resulted in the leakage of intracellular components, such as glucose and lactate dehydrogenase. Young et al. [68,69] suggested that chitosan induces the leakage of electrolytes, protein, and UV-absorbing material from *Glycine max* and *Phaseolus vulgaris* cells. Severe damage to the *G. max* cell membrane by chitosan was indicated by reduced staining with fluorescein diacetate and the leakage of fluorescein from preloaded cells [68].

Another mechanism is that the positively charged chitosan interacts with cellular DNA of some fungi and bacteria, which consequently inhibits the RNA and protein synthesis [60,70]. In this mechanism, chitosan must be hydrolyzed to a lower MW to penetrate into the cell of microorganisms. However, this mechanism is still controversial. Tokura et al. [64] examined the antimicrobial action of chitosan with weight average MW of 2,200 and 9,300 having DD of 0.54 and 0.51, respectively. It was observed that the chitosan of MW 9,300 was stacked on the cell wall and inhibited the growth of *E. coli*. However, the chitosan of MW 2,200, which permeated into the cell wall, accelerated the growth of *E. coli*. They suggested that the antimicrobial action is related to the

suppression of the metabolic activity of the bacteria by blocking nutrient permeation through the cell wall rather than the inhibition of the transcription from DNA.

3.2. Factors Affecting Antimicrobial Activity

The extent of the antimicrobial action of chitosan is influenced by intrinsic and extrinsic factors such as MW, DD, pH, temperature, and so on. It is necessary to understand these factors for the effective application of chitosan as an antimicrobial agent. In fact, the effect may be enhanced by chemical modification, which is discussed in Section 4.

3.2.1. Molecular weight (MW)

Tanigawa et al. [67] reported that D-glucosamine hydrochloride (chitosan monomer) did not show any growth inhibition against several bacteria, whereas chitosan was effective. This suggests that the antimicrobial activity of chitosan is related to not only its cationic nature but also its chain length. Hirano and Nagao [51] examined the relationship between the degree of polymerization (DP) of chitosan and the growth inhibition of several phytopathogens. Three different MW chitosans, high MW (HMW) chitosan (MW 400,000, DD 0.95), low MW (LMW) chitosan (MW and DD: not specified) and chitosan oligomer (DP 2-8), were used. It was observed that the increases in MW of chitosan increased the number of inhibited fungi. The strongest growth inhibition was observed with LMW-chitosan and the weakest was observed with HMW-

chitosan. This was explained by the difficulty of the HMW-chitosan to diffuse into the agar gel containing the test organism due to its high viscosity.

Kendra and Hadwiger [71] examined the antifungal effect of chitosan oligomers on *Fusarium solani* f. sp. *pisi* and *Fusarium solani* f. sp. *phaseoli*. In the assessment of the minimum concentration (μg/mL) at which no fungal growth was detected, the antifungal activity was found to increase as the polymer size increased. Monomer and dimer units did not show any antifungal activity at the concentration of 1000 μg/mL. Heptamer (DP 7) showed maximal antifungal activity and the minimum concentrations were identical to those observed for both native chitosan and the acid-cleaved chitosan.

Shimojoh et al. [72] also suggested that the antimicrobial activity is heavily dependent on the MW of chitosan. Several oral bacteria treated with the same concentration of chitosans with four different MW (DD 0.99) from squid chitin (β-chitin) in 1% lactate buffer (pH 5.8) for 1 min and incubated at 37°C for 24 hrs. It was found that the chitosan with MW 220,000 was most effective and MW 10,000 was the least effective in their bactericidal activities. The antimicrobial activity of chitosan with MW of 70,000 was better than MW 426,000 for some bacteria, but for the others, the effectiveness was reversed. This suggests that the antimicrobial activity of chitosan varies depending on the microorganisms targeted.

Yalpani et al. [73] reported that chitosans (medium and high MW) showed higher antimicrobial activities against *Bacillus circulans* than chito-oligosaccharides (DP 2-30), whereas they were less effective against *E. coli* than chito-oligosaccharides. From the

results of Simojoh [72] and Yalpani [73], one can notice that the relationship between MW of chitosan and the antimicrobial activity can be affected by the test organisms.

Numerous researchers have reported the antibacterial activity of chitosan against *E. coli* [57,60,61,66,67,74]. Hwang et al. [57] concluded that chitosan with MW about 30,000 exhibited the highest bactericidal effect on *E. coli* from their investigation of chitosan MW range of 10,000 - 170,000. Jeon et al. [61] suggested that the MW of chitosan is critical for the inhibition of microorganisms and suggested the required MW be higher than 10,000 for better antimicrobial activity.

To elucidate the relationship between MW of chitosan and its antimicrobial activity against *E. coli*, the existing experimental data are summarized in Table 1.

Table 1. Effect of chitosan MW on its antimicrobial activity against *E. coli*.

References	Effectiveness, MW (DD)
Hwang [57]	29,800 (0.93) > 102,200 (0.93) > 9,800 (0.96) > 174,700 (0.94)
Liu [60]	91,600 (0.86) > 51,100 (0.88) > 8,000 (0.75) > 5,000 (0.73), 274,000 (0.74) > 650,000 (0.85), 1,080,000 (0.85)
Jeon [61]	685,000 (0.89) ≥ 24,000 – 7,000 > 6,000 – 1,500 > ~ 1,000
Ueno [66]	10,500, 9,300 > 8,000, 7,300 > 6,200 > 5,500 > 4,100, 2,200
Tanigawa [67]	80,000 (0.80) > 166,000 (0.91) > 190,000 (0.84) > 2,000 (< 0.80) > 4,000 (< 0.80) > 12,000, 8,000 (< 0.80)
Chang [74]	35,000 > 29,000 > 32,000 > 97,000, 95,000, 68,000 > 293,000, 275,000 > 820,000, 11,000

Although it is difficult to find a clear correlation between MW and antimicrobial activity, generally the antimicrobial activity increases as the MW of chitosan increases. However, the activity decreases over a certain high MW. The discrepancies between data may

result from the different DD and molecular weight distributions (MWD) of chitosan. The evaluation of only the MW dependence of the antimicrobial activity requires a wide MW range of chitosan samples with the same DD and MWD. It is almost impossible to obtain this because chitosan is a natural polymer. From the existing data, it is difficult to determine what the most optimal MW for the maximal antimicrobial activity is. The selection of MW of chitosan could be thought to be more dependent on its application.

3.2.2. Degree of deacetylation (DD)

The antimicrobial activity of chitosan is directly proportional to the DD of chitosan [60,67,72,75]. The increase in DD means the increased number of amino groups on chitosan. As a result, chitosan has an increased number of protonated amino groups in an acidic condition and dissolves in water completely, which leads to an increased chance of interaction between chitosan and negatively charged cell walls of microorganisms.

3.2.3. The pH

The antimicrobial activity of chitosan is strongly affected by pH [53,55,58,60]. Tsai and Su [58] examined the antimicrobial activity of chitosan (DD 0.98) against *E. coli* at different pH values of 5.0, 6.0, 7.0, 8.0, and 9.0 (Figure 3).

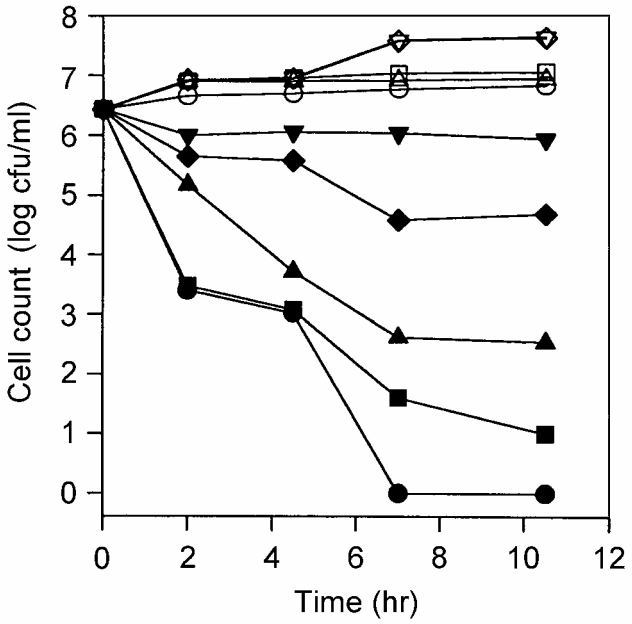

Figure 3. Effect of pH on the antimicrobial activity of chitosan against *E. coli*. *E. coli* cells were inoculated in 5mM phosphate buffer alone (open symbols) or in buffer containing 150ppm chitosan (solid symbols) with various pH values and incubated at 37°C. The surviving cells were counted. (▼ and ▽, pH 9.0; ◆ and ◇, pH 8.0; ▲ and △, pH 7.0; ■ and □, pH 6.0; ● and ○, pH 5.0). (From Ref. 58.)

The greatest activity was observed at pH 5.0. The activity decreased as the pH increased and chitosan had little antibacterial activity at pH 9.0. Other researchers [53,60] reported that chitosan had no antimicrobial activity at pH 7.0 due to the deprotonation of amino groups and poor solubility in water at pH 7.0. This suggests that the antimicrobial activity of chitosan comes from the cationic nature of chitosan.

3.2.4. Temperature

Tsai and Su [58] examined the effect of temperature on the antibacterial activity of chitosan against *E. coli*. The cell suspensions in phosphate buffer (pH 6.0) containing 150 ppm chitosan were incubated at 4, 15, 25, and 37°C for various time intervals and the surviving cells were counted. The antibacterial activity was found to be directly proportional to the temperature. At the temperatures of 25°C and 37°C, the *E. coli* cells were completely killed within 5 hrs and 1 hr, respectively. However, at lower temperatures (4°C and 15°C), the number of *E. coli* declined within the first 5 hrs and then stabilized. The authors concluded that the reduced antimicrobial activity resulted from the decreased rate of interaction between chitosan and cells at a lower temperature.

3.2.5. Cations and polyanions

Young and Kauss [69] reported that chitosan caused the release of Ca^{2+} present on *Glycine max* cell and/or plasma membrane, which destabilized the cell membrane and further induces leakage of intracellular electrolytes. They suggested that the cross-linking of chitosan (polycation) with phospholipid or protein components in the cell membrane affects the membrane permeability, which further causes leakage of intracellular substances, and finally causes the death of cell.

Young et al. [68] observed that chitosan-induced leakage of UV- absorbing material from *G. max* was strongly inhibited by divalent cations in the order of $Ba^{2+} > Ca^{2+} > Sr^{2+} >> Mg^{2+} > Na^{+} > K^{+}$. It was assumed that the cations displaced Ca^{2+} released from the cell surface, formed complexes stabilizing the cell membrane, and consequently

reduced the chitosan-induced leakage. Tsai and Su [58] also reported reduced bactericidal effect of chitosan against *E. coli* by the addition of salts containing alkaline earth metals such as $MgCl_2$, $BaCl_2$, and $CaCl_2$. The order of effectiveness was Ba^{2+} > Ca^{2+} > Mg^{2+}. The authors proposed that the cations form complexes with chitosan and consequently the reduced available amino groups of chitosan led to the reduced bactericidal effect unlike Young's assumption [68].

In addition to the reduced chitosan-induced leakage by cations, the leakage was also reduced by the addition of polyanions such as Na polygalacturonate and Na poly-L-aspartate [68]. The complete prevention of electrolyte leakage was observed when the number of carboxyl groups in the polyanions equaled to that of the amino groups of chitosan. It was attributed to the formation of polycation(chitosan)-polyanion complexes, which was observed by formation of precipitate. However, monomeric galacturonate and aspartate did not show any effect on the leakage and no precipitation of chitosan was observed. The explanation of this provided by the authors was that individual ionic bonds between anionic monomers and polycations could dissociate, but the multiple bonds between polyanion and polycation would not dissociate at the same time.

4. CHITOSAN DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITIES

Although chitosan shows antimicrobial activity against a broad spectrum of microorganisms, its activity is limited to acidic conditions due to its poor solubility above pH 6.5 at which chitosan loses its cationic nature. Water-solubility is an important factor for the application of chitosan as an antimicrobial agent. Therefore, researchers focused on the preparation of chitosan derivatives that can dissolve in water for the entire range of pH.

4.1. Chitosan Salts

The simplest form of chitosan derivative is chitosan salt that is prepared by dissolving chitosan in an appropriate dilute acid. The most common chitosan salt is chitosan acetate (Figure 4) and other available salts are chitosan hydrochloride, lactate, and hydroglutamate. These salts have been studied extensively by researchers and exhibit strong antimicrobial activity against various bacteria and fungi [51-75].

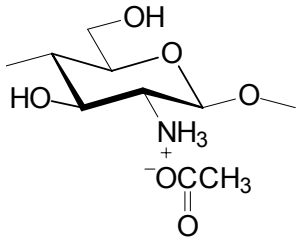

Figure 4. Chitosan acetate.

Nelson [76] prepared chitosan pyrithione, as an antimicrobial agent for personal care products, by dissolving chitosan in a dilute omadine acid (pyrithione) followed by dialysis to remove excess acid. Pyrithione salts, such as zinc and sodium pyrithione are

commercially available and are known to have broad antimicrobial activity. The use of these salts was limited to products for topical application at relatively low concentrations due to their rapid absorption through the skin. The chitosan pyrithione showed an equivalent antimicrobial activity to sodium pyrithione and much better antimicrobial activity than chitosan acetate against several selected bacteria and fungi. The chitosan pyrithione was characterized by a combination of slow release of pyrithione and excellent antimicrobial activity. The inventor suggested its use as an antimicrobial agent in a variety of dermatological items, such as soaps, shampoos, and skin care medicaments.

The drawbacks of chitosan salts are that they precipitate in an alkaline condition and that the antimicrobial activity depends on pH. It was, thus, of interest for researchers to prepare chitosan derivatives that can dissolve in water over the entire pH range.

4.2. Quaternized Chitosan Derivatives

Polymeric quaternary ammonium compounds have received the most attention over the years as biocidal polymers [77]. Introducing quaternary ammonium salts onto the chitosan backbone could be one of the best methods to increase antimicrobial activity as well as the water solubility of chitosan. The simplest form of chitosan derivative having a quaternary ammonium salt is *N,N,N*-trimethyl chitosan chloride (TMCC) (Figure 5).

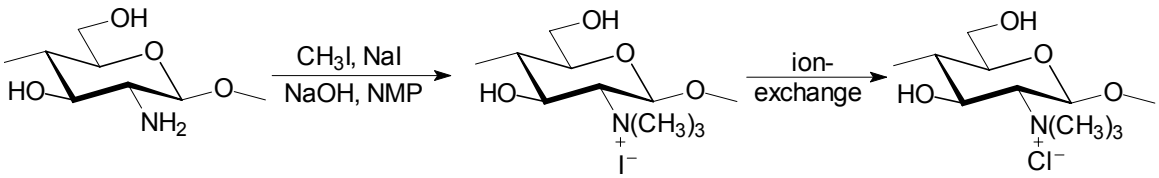

Figure 5. Synthesis of TMCI and TMCC.

A typical method [78] of *N*-trimethylation is dispersion of chitosan in *N*-methyl-2-pyrrolidone (NMP) containing sodium iodide and methyl iodide in the presence of sodium hydroxide as a base. The counter-ion I^- of the reaction product (*N,N,N*-trimethyl chitosan iodide (TMCI)) is exchanged with Cl^- to give TMCC, which is more stable than the iodine form (TMCI). To obtain a high degree of quaternization (DQ), a repeated reaction is necessary [79].

Tanigawa et al. [67] reported that the minimum inhibitory concentrations (MIC) of TMCI (DQ 0.1-0.2) against several bacteria were comparatively lower than those of unmodified chitosan. It was suggested that the inhibition of bacterial growth might be further enhanced by increasing the degree of *N*-trimethylation of chitosan. Jia et al. [80] synthesized TMCIs with DQ 0.89 - 0.91 from three chitosans with different MWs. They reported that all TMCIs showed bactericidal effect on *E. coli* and the TMCI from a high MW chitosan exhibited a higher antibacterial activity. Tsurugai and Hiraide [81] prepared three different *N*-trimethylammonium salts of chitosan, TMCI, TMCC, and *N,N,N*-trimethyl chitosan hydroxide (TMCH). The TMCH was prepared by treating TMCI with aqueous NaOH solution followed by dialysis. TMCI and TMCC were water-soluble while TMCH was insoluble in water. Wide-angle X-ray diffraction patterns revealed that TMCI and TMCC were amorphous but TMCH was crystalline. The thermal decomposition temperatures of TMCI, TMCC, and TMCH were 209.3, 202.0, and 251.3ºC, respectively. All three salts showed antibacterial activities against *E. coli* and *Staphylococcus epidermidis* in the increasing order of TMCH, TMCC, and TMCI.

Lang et al. [82] synthesized *N*-[(2-hydroxy-3-trimethyl ammonium)propyl] chitosan chloride (HTCC) (Figure 6) by reacting chitosan with glycidyltrimethyl ammonium chloride (GTMAC) in water for application in cosmetics. It was also synthesized by Daly and Guerrini [83] by treating chitosan with 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (Quat 188). In the MIC test, HTCC (referred to as CHI-Q188, degree of substitution (DS) 1.00 based on chitosan DD 0.88) showed high antimicrobial activity against *E. coli*, *Pseudomonas aeruginosa*, and uniquely high against *Staphylococcus aureus*. It was suggested that a variety of applications of CHI-Q188 as a biocide could be envisioned.

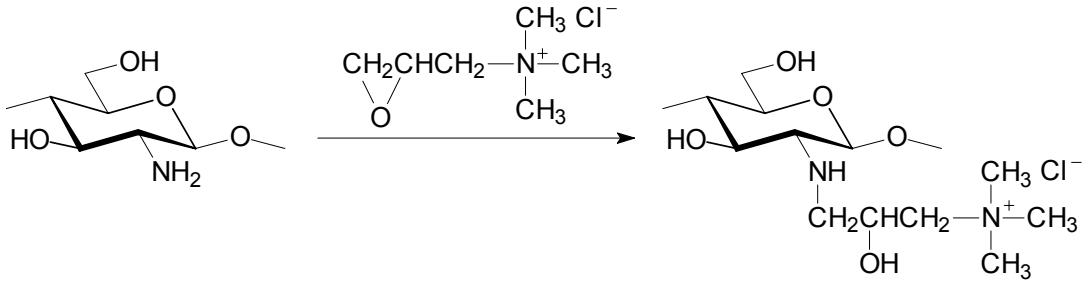

Figure 6. Synthesis of HTCC.

Several different quaternized *N*-alkyl chitosan derivatives were prepared by Kim et al. [84] to evaluate the effect of the chain length of the alkyl substituents on the antimicrobial activity. The *N*-alkyl chitosan derivatives (Figure 7) were prepared by introducing different alkyl groups (butyl, octyl, and dodecyl) onto the amino groups of chitosan via Schiff base intermediates followed by reduction and quaternization of them to produce water-soluble chitosan derivatives. The antimicrobial activity of the chitosan derivatives against *S. aureus* was evaluated by the viable cell counting method in acetate buffer (pH 6.0). The order of antimicrobial activity was as follows: *N,N*-dimethyl-*N*-

dodecyl chitosan > *N,N*-dimethyl-*N*-octyl chitosan > *N,N*-dimethyl-*N*-buthyl chitosan > *N,N,N*-trimethyl chitosan > chitosan. This result suggested that cationic charge as well as alkyl chain length of the substituent strongly affect the antimicrobial activity of the chitosan derivatives. It was assumed that chitosan derivatives with longer alkyl chains have higher hydrophobic affinity to the bacteria cell composed of mainly phospholipids (hydrophobic) and membrane protein, and as a result, show higher antimicrobial activity. Jia et al. [80] reported that the antibacterial activity against *E. coli* of *N,N*-dimethyl-*N*-propyl chitosan, which has a longer alkyl chain, was higher than that of *N,N,N*-trimethyl chitosan. The result was in good agreement with the suggestion of Kim et al. [84].

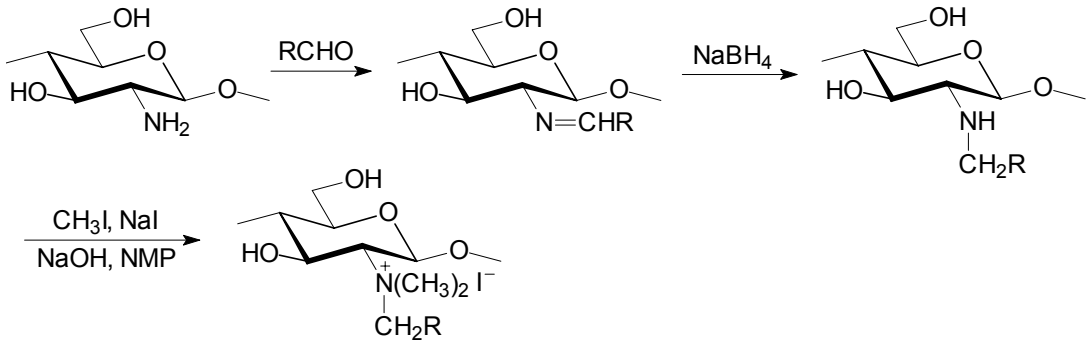

Figure 7. Synthesis of quaternized *N*-alkyl chitosan derivatives.

In another study on chitin by Kim et al. [85], they introduced the diethylaminoethyl (DEAE) group onto the hydroxyl group at the C-6 position of chitin by reacting alkali chitin with *N,N*-diethylaminoethyl chloride hydrochloric acid (DEAE-Cl·HCl). The DEAE-chitin was soluble in water and aqueous acetic acid. DEAE-chitosan and triethylaminoethyl chitin (TEAE-chitin, quaternized chitin) were prepared by treating the DEAE-chitin with 10% NaOH and ethyl iodide, respectively (Figure 8).

In their antibacterial assessment, all three derivatives showed antibacterial activities against *E. coli* and *S. aureus* in the increasing order of DEAE-chitin (DS 0.81 and DD 0.41), DEAE-chitosan (DS 0.67 and DD 0.73), and TEAE-chitin (DQ 0.47 and DD 0.48) with increasing alkyl chains and cationic charge of the substituent. Particularly, the quaternized chitin derivative (TEAE-chitin) at a concentration of 500 ppm completely killed the bacterial cells of *S. aureus* within 2 hrs.

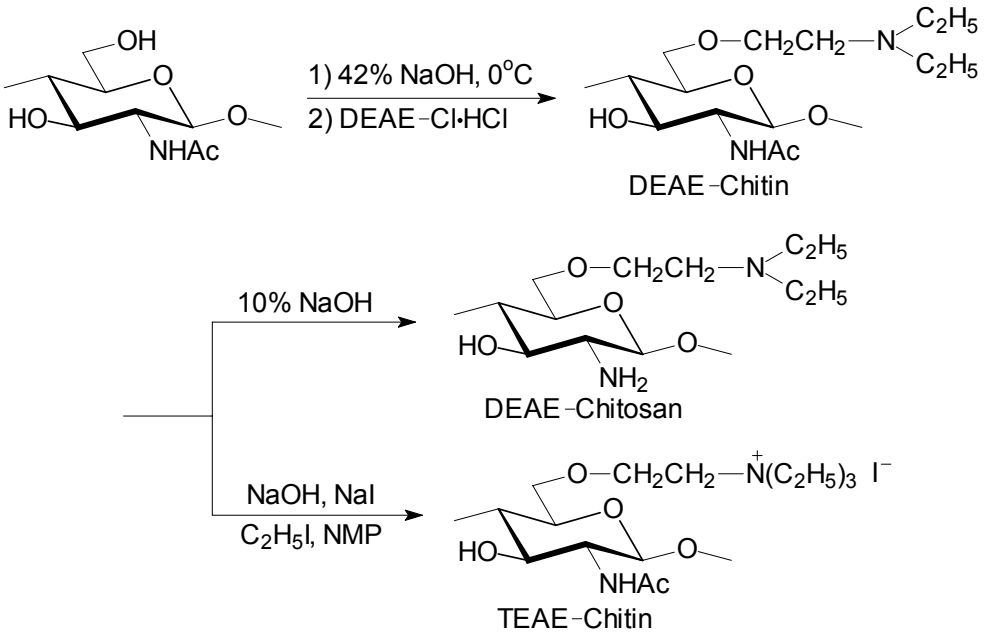

Figure 8. Synthesis of DEAE-chitin, DEAE-chitosan, and TEAE-chitin.

4.3. Carboxyalkylated Chitosan Derivatives

Another method to improve the solubility of chitosan is carboxyalkylation of chitosan. Muzzarelli et al. [86] prepared *N*-carboxymethyl chitosan (NCMC) (Figure 9), as a metal chelating agent, by treating an aqueous suspension of chitosan with glyoxylic acid followed by pH adjustment and reduction with sodium cyanoborohydride. The NCMC was soluble in water at all pH values.

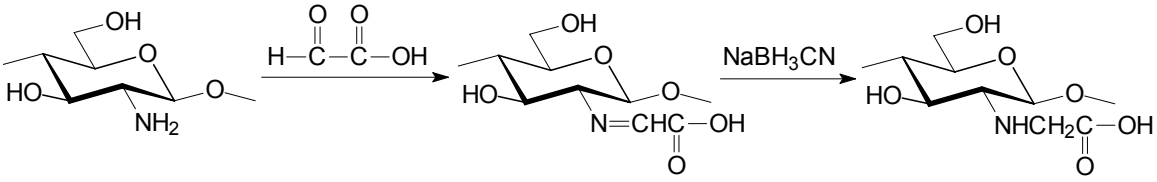

Figure 9. Synthesis of NCMC.

A characteristic feature of carboxyalkylated chitosan is its amphotericity. Delben et al. [87] reported that the NCMC has three different forms depending on pH, such as fully protonated ($-N^{+}H_2-CH_2COOH$), monodissociated ($-NH-CH_2-COOH \leftrightarrow -N^{+}H_2-CH_2COO^{-}$), and fully dissociated ($-NH-CH_2COO^{-}$) forms. According to Figure 10, at pH ~ 5, only the monodissociated form exists and at pH > ~ 9, only the fully dissociated form exists. Between the two pH values, NCMC exists as a mixture of mono and fully dissociated forms. As the pH decreases below ~ 5, NCMC exists as a mixture of monodissociated and fully protonated forms and the amount of the latter increases with decreasing pH.

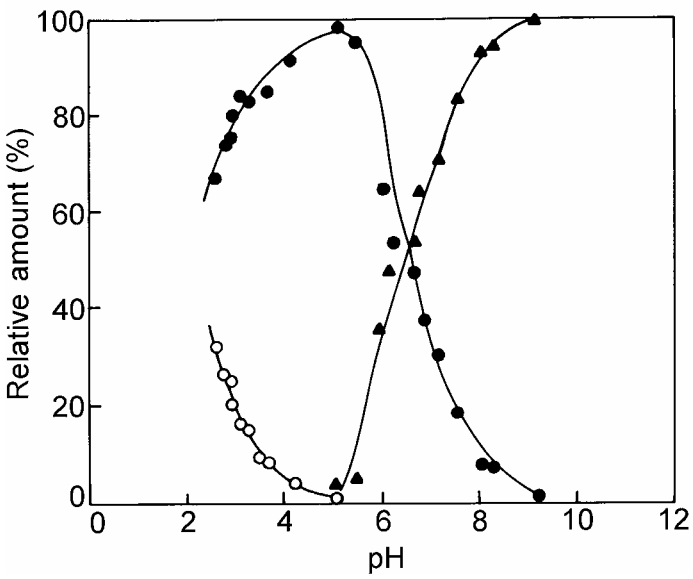

Figure 10. Relative amount (%) of three forms of NCMC in water at 25°C as a function of the pH value (4.8×10^{-3} M NCMC): ○, fully protonated form; ●, monodissociated form; ▲, fully dissociated form. (From Ref. 87.)

Recently, Muzzarelli [88] reported the antifungal activity of NCMC with degree of carboxyalkyation (DC) 0.27 and it was significantly effective in depressing the radial growth of *Saprolegnia parasitica*. Whereas, *N*-dicarboxymethyl chitosan (NDCMC) with mono- (DC 0.30) and di-substituted (DC 0.30) units did not show any antifungal activity against the same fungus. A clear explanation on the different behavior of the two carboxymethyl chitosans was not provided. Cuero et al. [89] observed that aqueous solutions of NCMC suppressed the growth of *Aspergillus flavus* and *A. parasiticus*.

Liu et al. [60] evaluated the antimicrobial activity of *N,O*-carboxymethyl chitosan (NOCMC) and *O*-carboxymethyl chitosan (OCMC) against *E. coli*. The NOCMC (DC 0.48 to 0.98) and OCMC (DC 0.42 and 0.73) were synthesized by reacting chitosan with

monochloroacetic acid at different reaction conditions (Figure 11). For the antibacterial assessment of chitosan and carboxymethyl chitosans, they employed the optical density methods. It was found that the NOCMC did not show any antibacterial activity, whereas OCMC was more effective than chitosan for the inhibition of the growth of *E. coli*. They explained the lack of antibacterial action of NOCMC came from the reduced number of NH_2 groups compared to chitosan due to the substitution of carboxymethyl groups on the amino groups of chitosan. The improved antibacterial activity of OCMC resulted from the facts that the number of amino groups did not change and its −COOH groups might have reacted with the $-NH_2$ groups inter- and intra-molecularly. As a result, the number of $-NH_3^+$ groups of OCMC could be more than that of chitosan in the same condition. This explanation does not seem clear because the number of amino groups on chitosan is higher than that of the OCMC with the same mass and the experimental condition was acidic (the samples were dissolved in 2M acetic acid), where chitosan is protonated.

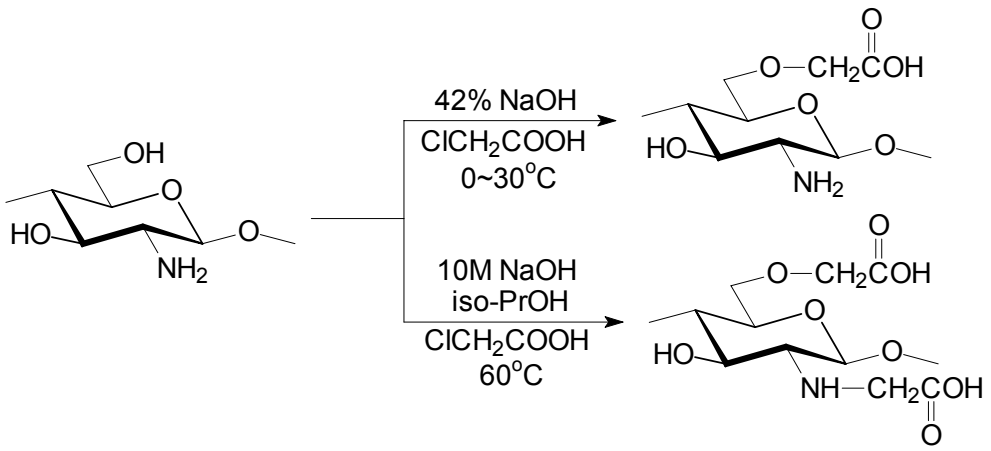

Figure 11. Synthesis of OCMC and NOCMC.

Two different carboxyalkyl chitosan derivatives, *N*-carboxypropyl (DC 0.19) and *N*-carboxybutyl (DC 0.26) chitosan (NCPC and NCBC), were synthesized by reacting chitosan (MW 2,000,000, DD 0.75) with 4-chlorobutyric and 5-chlorovaleric acid in methanol, respectively (Figure 12) [90]. Both derivatives exhibited improved solubility in various solvents and antimicrobial activity against *E. coli* and *S. aureus* as compared with chitosan. Although the derivatives did not dissolve in water due to the low DC, they did swell substantially. The improved solubility was explained by the contribution of decreased crystallinity confirmed by X-ray diffraction and thermogravimetric analysis (decreased thermal decomposition temperatures compared to chitosan). When it comes to the improved antimicrobial activities of the chitosan derivatives, it was mentioned that the amphoteric properties of carboxyalkyl chitosans might lead to their strong interaction with not only zwitterionic but also anionic components of phospholipids in the bacterial cytoplasmic membrane.

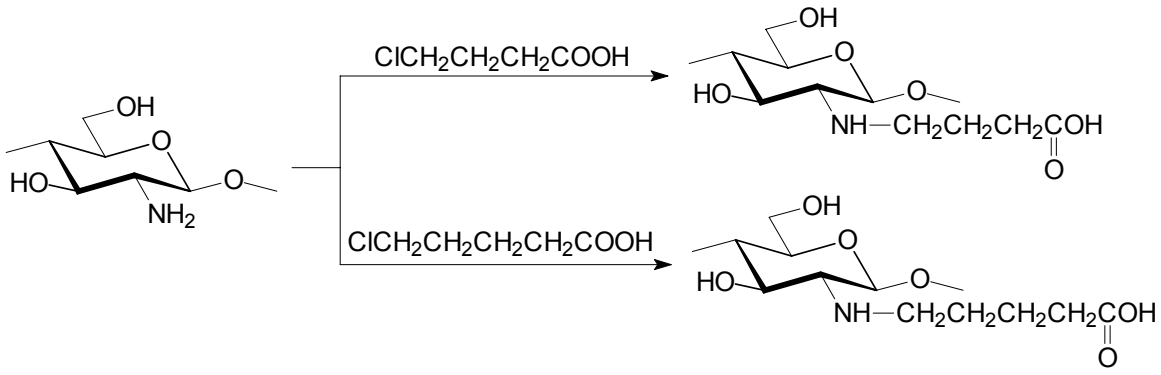

Figure 12. Synthesis of NCPC and NCBC.

Muzzarelli et al. [91] synthesized *N*-carboxybutyl chitosans (NCBC) (DC 0.26 – 0.28) as cosmetic functional ingredients by dissolving chitosan in aqueous levulinic acid followed by reduction using sodium borohydride (Figure 13).

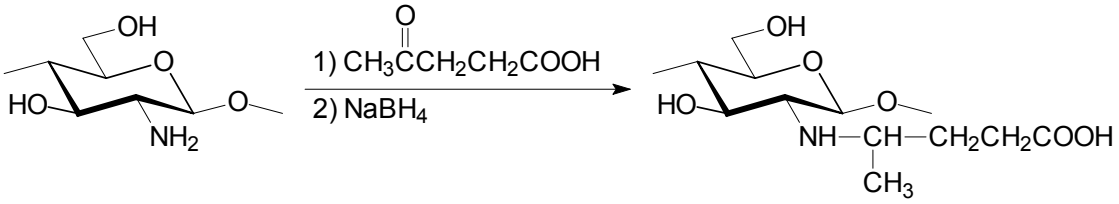

Figure 13. Synthesis of NCBC.

The NCBCs were soluble in water at below pH 8.5, but they were precipitated at pH 8.5 and higher. The freeze-dried product was shown to be completely amorphous from the X-ray spectrum. It was stated that a properly sized chain bearing the carboxyl group imparts solubility to chitosan by reducing inter- and intra-molecular interactions. The authors [92] extensively studied the antimicrobial properties of NCBC (DC ~0.27, from chitosan DD 0.73) against 298 strains of various pathogens. The NCBC was particularly effective against candidae and gram-positive bacteria. It was found that the microbial cells exposed to NCBC underwent remarkable morphological alteration as shown by electron microscopy analysis. Although there was no elucidation of the mechanism of the antimicrobial activity of NCBC, it was mentioned that the polycationic nature of NCBC induced the interaction and formation of polyelectrolyte complexes with acidic polymers produced at the bacterial cell surface. From the antimicrobial actions exerted by NCBC, its high potential application as a wound dressing was suggested.

4.4. Chitosan Derivatives with Sulfonyl Groups

Chen et al. examined the antibacterial effects and physical properties of *N*-sulfonated [93] and *N*-sulfobenzoyl [94] chitosans for food applications (Figure 14).

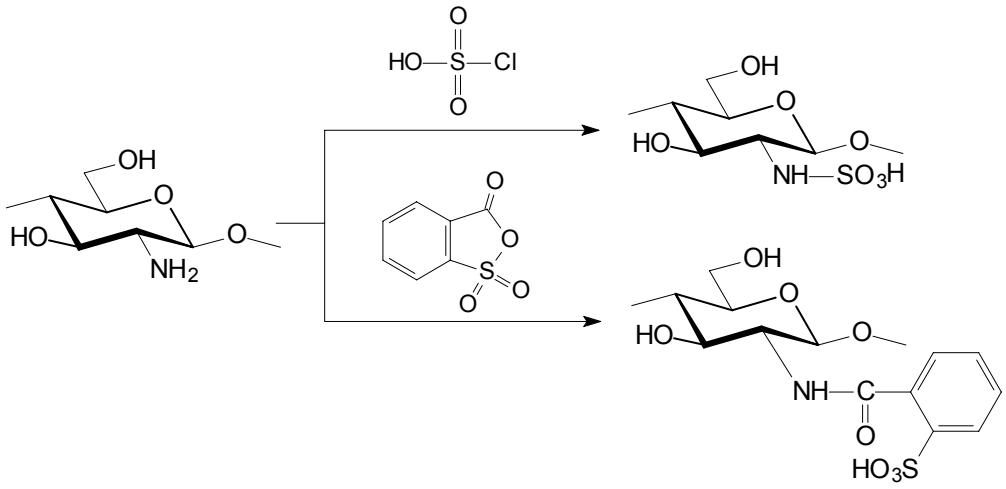

Figure 14. Synthesis of *N*-sulfonated and *N*-sulfobenzoyl chitosans.

N-Sulfonated chitosan (SC) was prepared by reacting regenerated chitosan (DD 0.69) with chlorosulfonic acid in pyridine. SCs with different sulfur contents (0.4, 1.7, 2.5, and 3.4%) were prepared by varying the amount of chlorosulfonic acid. *N*-Sulfobenzoyl chitosans (SBC) with different S-contents (1.7, 2.6, 5.3, and 6.2%) were obtained by reacting chitosan (DD 0.69) with various amounts of 2-sulfobenzoic acid cyclic anhydride in methanol. It was observed that SCs with S-contents of 1.7% and 2.5% were most effective against *Salmonella typhimurium* and *E. coli*, respectively. This suggested that there are different optimal S-contents for different bacteria. The bacteria-dependent optimal S-content is also observed in the study of SBCs. The optimal S-contents found were 5.3%, 1.7%, and 2.6% for *S. aureus*, *E. coli*, and *S. typhimurium*, respectively. In

general, the antibacterial activities and water-solubilities of both SC and SBC were superior to the unmodified chitosan.

The improved antibacterial actions were explained by the better charge interaction between negatively charged residues of bacterial cell walls and chitosan molecules, which resulted from the better solubilities of SC and SBC due to the sulfonyl groups. However, both SC and SBC with a high S-content above certain values showed lower antibacterial activity than that of chitosan. This effect was explained by supposing that the increased negative charges on chitosan caused a repulsive force against negatively charged cell walls of bacteria. The excellent antibacterial activities of SC and SBC against other bacteria were also reported [95].

4.5. Carbohydrate-branched Chitosan Derivatives

Yalpani et al. [73] reported two different water-soluble carbohydrate-branched chitosan derivatives, 1-deoxy-1-glucit-1-yl (DS 0.15) and 1-deoxy-1-lactit-1-yl (DS 0.15) chitosan (Figure 15).

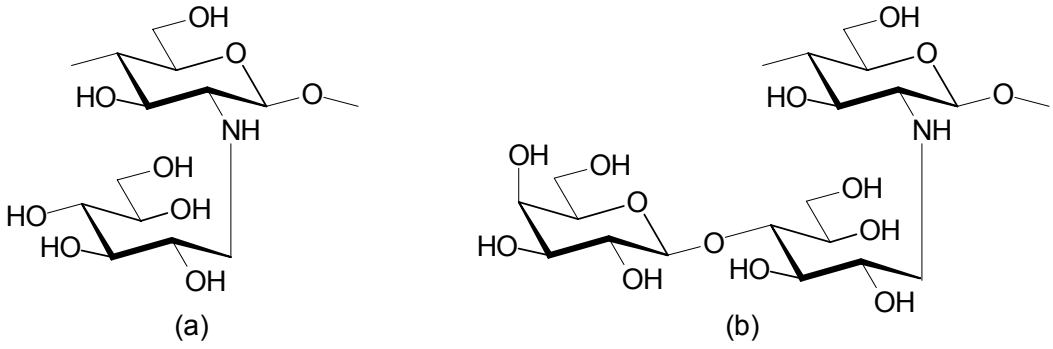

Figure 15. 1-Deoxy-1-glucit-1-yl chitosan (a) and 1-deoxy-1-lactit-1-yl chitosan (b).

These derivatives displayed a significant inhibitory effect against *Bacillus circulans* at concentrations below 400ppm, while chitosan did not show any effect and chito-oligosaccharides (DP 2~30) promoted the growth of *B. circulans* to a small degree at concentrations of 500ppm. The 1-deoxy-1-lactit-1-yl chitosan showed overall higher antimicrobial activity against *B. circulans* than that of 1-deoxy-1-glucit-1-yl chitosan. It was found that both derivatives were not effective against *E. coli*.

Kurita et al. [96] prepared another carbohydrate-branched chitosan having antimicrobial activity. The D-glucosamine branch was introduced onto the hydroxyl group on the C-6 position of chitosan by a series of regioselective reactions through *N*-phthaloyl chitosan, which is a useful chitosan derivative to protect the amino group. The resulting D-glucosamine-branched chitosan (DS 0.45) (Figure 16) was readily soluble in neutral water when the DS was above 0.3 and highly swollen in common organic solvents. This branched chitosan showed higher growth suppression against *Bacillus subtilis*, *S. aureus*, and *Candida albicans* compared to unmodified chitosan. The authors suggested the use of this biodegradable chitosan derivative as a new type of water soluble polymeric antimicrobial agent in the fields of cosmetics, pharmaceutics, biomedical use, food processing, and textiles.

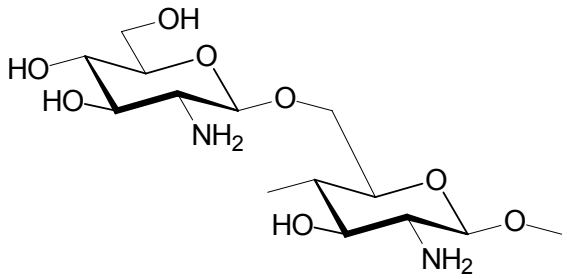

Figure 16. D-Glucosamine-branched chitosan.

4.6. Chitosan-Amino Acid Conjugates

Jeon and Kim [97] synthesized several chitosan derivatives *N*-conjugated with different amino acids by reacting low MW chitosan (MW < 10,000) with Boc-amino acid derivatives followed by deprotection of the Boc group.

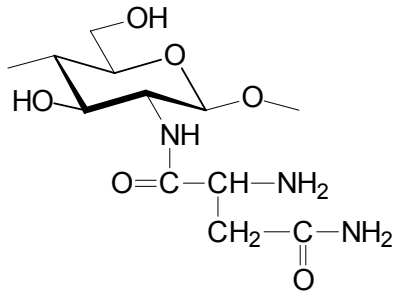

Figure 17. Chitosan-asparagine conjugate.

The chitosan-asparagine conjugate (DS ~ 0.70) (Figure 17) was found to have a much higher bactericidal activity against *E. coli* than unmodified chitosan. However, the other chitosan derivatives *N*-conjugated with different amino acid groups, such as glycine, alanine, aspartic acid, cysteine, and methionine showed much lower antibacterial activity than that of chitosan itself. The higher antibacterial activity of chitosan-asparagine conjugate was explained by the increased number of amino groups (two amino groups per asparagine group) on chitosan molecules, which can be cationized and interact with the negative charges of bacterial cell walls. Whereas, the other chitosan-amino acid conjugates possess fewer amino groups (one amino group per amino acid group) compared to chitosan with the same mass. We believe that there must be another explanation rather than that provided by the authors. The authors suggested that the two amino groups on the asparagine group can be cationized. However, the amino group

attached to carbonyl group is an amide and it cannot be cationized. Therefore, all different amino acid groups conjugated with chitosan have only one amino group, which can be cationized.

4.7. Chitosan-Iodine Complexes

De Rosa et al. [98] prepared complexes of iodine with chitosan or its derivatives by simply exposing chitosan or its derivatives, such as *N*-carboxybutyl, *N*-carboxymethyl, *N,O*-carboxymethyl, and *N,O*-chitosan sulfate, to iodine vapors caused by sublimation of elementary iodine at room temperature or above. This method completely eliminated the use of toxic organic solvents used for formation of chitosan-iodine complex by dispersing chitosan in an iodine solution [99]. They stated that the chitosan-iodine complexes (if the iodine content does not exceed 50% (w/w)) can be dissolved in dilute aqueous acids, such as acetic and glutamic acid, in which the iodine remains stable in the form of a soluble complex with chitosan. It was found that the chitosan-iodine complex both in its dry state and in solution showed a significant bactericidal action against numerous types of microorganisms that commonly infect the skin and wounds. The inventors suggested that the slow release of iodine from the chitosan-iodine complex eliminated the drawbacks of alcoholic iodine solution disinfectant, which include low stability of iodine solution over a period time, the notable aggressiveness of iodine on skin, and the persistent stains on the skin.

4.8. Miscellaneous Chitosan Derivatives

Muzzarelli et al. [88] reported that 5-methylpyrrolidinone chitosan (DS 0.27) and *N*-phosphonomethyl chitosan with monosubstituted (DS 0.24) and disubstituted (DS 0.14) units (Figure 18) exerted effective fungistatic action against *Saprolegnia parasitica*. The authors suggested that the amphotericity of the chitosan derivatives might lead to their effective interaction with some surface components of the *S. parasitica* cell wall, thus leading to increased permeability and consequent cellular leakage and death of the cells.

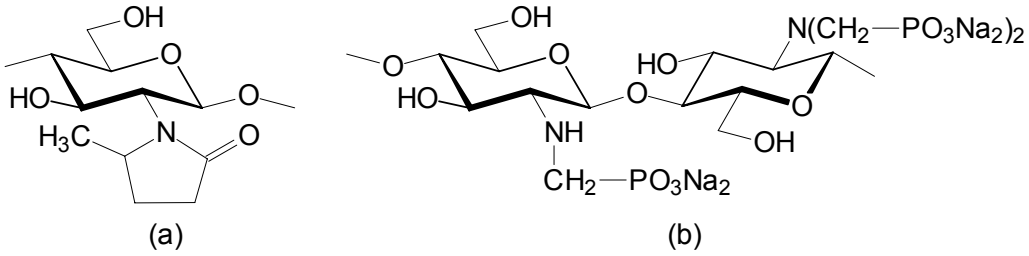

Figure 18. 5-Methylpyrrolidinone chitosan (a) and *N*-phosphonomethyl chitosan (b).

5. CHITOSAN AND ITS DERIVATIVES AS TEXTILE CHEMICALS

The unique properties of chitosan, such as biodegradability, nontoxicity, antimicrobial activity, and polycationic nature, make it suitable for a number of textile applications. The application of chitosan in textiles can be categorized into two main topics; the production of man-made fibers and textile wet processing that includes dyeing and finishing. The extensive investigation of chitin and chitosan as fiber and film formers can be found in the literature [45,100]. Chitosan may be considered a multifunctional finish, and those chemical attributes which contribute to the antimicrobial properties also contribute to other functional uses of chitosan. This chapter deals with the uses of chitosan and its derivatives in textile dyeing and finishing.

5.1. Chitosan in Dyeing of Cotton

Chitosan can easily absorb anionic dyes such as direct, acid, and reactive dyes by electrostatic attraction due to its cationic nature in an acidic condition (Figure 19).

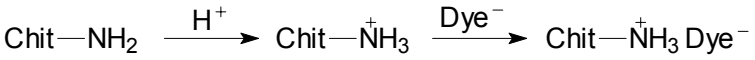

Figure 19. Chitosan-dye interaction.

In the manufacture and dyeing of cotton fabrics, some lots of cotton cause dyeing problems. The cotton does not absorb dye uniformly and creates small lightly colored or white spots. This is the result of the small groups of immature cotton fibers known as neps. The immature cotton results from various reasons, such as disease, insect attack, premature harvesting, and unsuitable weather conditions [101].

Rippon [102] evaluated the application of chitosan to improve the dyeability of immature cotton fibers. The chitosan, which was dissolved in dilute acetic acid, was applied to cotton fabric by three different methods; pad-batch (30 min)-rinse, pad-dry (3min at 150ºC)-rinse, and exhaustion (20 min at 40ºC, liquor ration 28:1)-rinse. The author postulated that the affinity of chitosan to cotton would be by van der Waals' forcess between them because of the similar structures of chitosan and cotton. Another possibility mentioned for the binding chitosan to cellulose was crosslinking by formation of Schiff base between cellulose's reducing end (−CO−H) and the amino group of chitosan. In addition to the two possible bindings suggested by the authors, hydrogen bonding should also play an important role. Although the application of chitosan by a pad-dry method was the most effective in terms of the amount of chitosan on cotton after rinsing, this method caused uneven dyeing on the fabric because of migration of the chitosan during drying. It was reported that a chitosan pretreatment increased the exhaustion of direct dyes and enabled immature fibers to be dyed to the same depth of shade as mature fibers. When the chitosan treated fabrics were aftertreated with a quaternary ammonium compound after dyeing, the fastness properties were comparable to those of untreated cotton.

Metha and Combs [101] also evaluated nep coverage in the dyeing of cotton by the pretreatment of cotton with chitosan. For the pretreatment, they employed an exhaust method (10 min at room temperature and 40 min at 60ºC, liquor ratio 25:1). The chitosan treated fabrics were dyed with direct dyes and the dyed fabrics were further treated with cationic compounds to improve fastness properties. It was found that the addition of

sodium sulfate (Na_2SO_4) during the exhaust process improved the absorption of chitosan on cotton and fully covered the neps. However, in the absence of the salt, the chitosan treated fabric only partially covered the neps when the same amount of chitosan (0.4% owf (on weight of fabric)) was applied. The pretreatment of cotton prior to dyeing resulted in the coverage of neps by direct and reactant fixable direct dyes. However, the pretreatment was not effective for reactive dyes to cover the neps.

Canal et al. [103] evaluated the dye exhaustion of chitosan-treated cotton fabrics compared to untreated ones. The most uniform distribution of chitosan on the fabric was obtained by pad (75% WPU (wet pick-up))-dry (3min at 150ºC) method. The treated fabric was dyed with a direct dye and it was subject to consecutive washes for the evaluation of durability of chitosan on the fabric. It was found that the first washing led to the largest color loss of the dyed fabric and the color loss was stabilized after 15 washes. At this stage, the fabric kept 35% higher K/S (color strength) values than those of untreated fabrics. The authors suggested that the application of chitosan to cotton could reduce the uses of dyes and dyes in wastewaters due to the increased dye exhaustion. The washing and wet rubbing fastness of chitosan-treated fabric was reduced by about one point and dry rubbing fastness was not affected.

Direct and reactive dyes have been widely used for dyeing of cotton fabrics because of their complete color range and ease of application. However, these anionic dyes have a low affinity to the cotton fiber since cotton fibers develop anionic surface charge (zeta potential) in water. The charge repulsion between dyes and cotton can be overcome by adding an electrolyte like sodium chloride or sodium sulfate, which screens

the surface charge of cotton. However, the large amount of salts required in dyeing can cause pollution of rivers and streams [104]. As an attempt to reduce the use of salts, a number of researchers have cationized cotton fibers through chemical modifications with compounds containing cationic groups. The majority of the chemicals used for the cationization of cotton are not safe environmentally. Therefore, the use of chitosan, a polycationic biopolymer is more eco-friendly [105].

Bandyopadhyay et al. [106] applied chitosan as a substitute for synthetic chemicals to cationize cotton fabrics. A chitosan solution in 2% aqueous acetic acid was applied to cotton fabrics by padding (80% WPU) and drying at 110ºC for 2-3 min. The treated fabric was dyed with several reactive dyes. When the fabric was treated with 1% and 2% chitosan solutions, it was possible to decrease the amount of salt required by about 50% in order to produce a comparable shade to that of untreated fabric. The chitosan-treated fabric also showed improvement in fixation of reactive dyes. This result was explained by the increased exhaustion of negatively charged reactive dyes to the cotton, whose negative potential at the fiber surface was suppressed by the cationic chitosan treatment. Consequently, when alkali was added to the dyebath, a substantial quantity of dye was available for the reaction with cotton. It was also suggested that the amino groups of chitosan reacted with the reactive group (vinylsulfone group) in the dye and the fixation was further improved. The chitosan-treated fabric showed comparable color fastness properties to the untreated fabric.

Weltrowski and Masri [107] treated cotton fabrics with chitosan oligomer, which was obtained by acid hydrolysis, to improve dyeability as well as to eliminate the

stiffness of the fabric caused by the treatment of high MW chitosan. A solution of chitosan oligomer was applied to the bleached cotton fabric, which has aldehyde groups formed by oxidation during bleaching. In this step, it was believed that Schiff base was formed by the reaction between the amino group of chitosan and aldehyde group of the cotton. The padded fabric was treated with reducing agents such as sodium borohydride or sodium cyanoborohydride to fix the chitosan on the cotton permanently by stabilizing the Schiff base through reductive alkylation. It was reported that this method of application of chitosan could reduce or eliminate the amount of salt required in the dyeing of cotton with reactive dyes.

Lac is the hardened resin generated by a tiny insect *Kerria lacca* [108]. A lac dye is a natural acid dye obtained as a by-product from the wash-waters of the lac processing. This dye has been used for dyeing protein fibers, such as wool and silk in the past. However, this dye could not be applied to cotton due to its low affinity to cotton. Saxena et al. [108] examined the dyeability of chitosan-treated cotton with lac dye. Cotton fabrics were treated with chitosan solution in 1% acetic acid by a pad (80% WPU)-dry (100ºC for 7 min)-cure (160ºC for 3 min) method and washed with distilled water. The treated fabrics were dyed with lac dye in an acidic condition (pH 4.5). The resulting fabrics had a violet shade but showed poor washfastness. The washfastness was improved by the use of mordants or a crosslinking treatment with 1,3-dimethylol-4,5-dihydroxyethylene urea (DMDHEU).

5.2. Chitosan in Durable Press (DP) Finishing

Cotton fabric has a tendency for wrinkling when it is subject to severe bending. DP finishing, also called easy-care, wrinkle-free, or crease-resistant finishing, is a chemical treatment that imparts dimensional stability against repeated bendings to cotton fabric by the chemical crosslinking between cellulose chains through etherification using *N*-methylol compounds or esterification using polycarboxylic acids.

It is well known that DP-finished cotton shows low affinity for direct and reactive dyes, which are commonly used for cotton. The adverse dyeing property results from the following reasons [109-111]; (a) inability of the crosslinked fibers to swell sufficiently in aqueous dyeing solution, (b) blocking the hydroxyl groups of cellulose by crosslinking, and (c) repulsion between anionic dyes and unreacted free carboxyl groups of polycarboxylic acid type DP finishing agent.

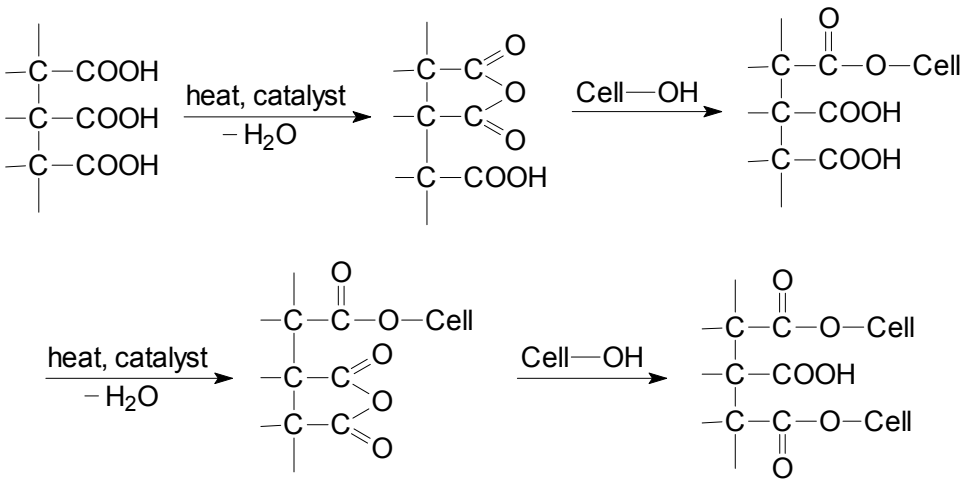

Figure 20. Crosslinking mechanism between cellulose and polycarboxylic acids.

From the mechanism [112] of the crosslinking reactions shown in Figure 20, it can be seen that the carboxyl acid groups are never completely substituted due to the esterification through the polycarboxylic anhydrides. There have been several attempts to improve the dyeability of DP-finished cotton by the addition of reactive nitrogenous additives in the DP finishing formula [109,111,113,114].

Shin and Yoo [115] treated cotton fabrics with a mixture of DMDHEU, catalyst, and chitosan with six different MWs. The treated fabrics (90~100% WPU) were dried at 110ºC for 3 min, cured at 150ºC for 5 min, and washed with water. It was observed that the wrinkle recovery angle (WRA) reduced, as the MW of chitosan increased. When the MW was below 21,000 the values of WRA were higher that that of DMDHEU-treated fabric without chitosan. When it comes to the stiffness and breaking strength retention of treated fabrics, both properties were increased with increasing MW of chitosan. The color strengths (K/S) of DMDHEU/chitosan-treated fabrics dyed with direct and acid dyes were higher than DMDHEU-treated fabric and untreated fabric. The value of K/S was proportional to the MW of chitosan. The dyeability with reactive dyes was not improved due to the loss of the cationic nature of chitosan under the alkaline dyeing condition. However, the dye uptake increased in an acidic condition (pH 5.5-6.0) because of the attraction between the protonated amino groups of chitosan and anionic reactive dye rather than the formation of covalent bonds between dyes and hydroxyl groups of cellulose or chitosan. The washfastness of dyed fabrics were not affected, but the rubbing fastness was slightly reduced compared to that of the untreated fabric.

Another special property reported on DMDHEU/chitosan-treated cotton fabrics is the improved soil release under washing [116]. The highest values of soil removal (%) were observed when the MW of chitosan was in the range of 3,800-14,000, and the values were significantly higher than those of untreated and DMDHEU-treated fabrics. The improvement of soil release was attributed to the increase in hydrophilicity of the fabric and the prevention of deep soiling by oily soils because of the blocking of the microporous structure of cotton fiber by low MW chitosan.

In another study of Shin and Yoo [117] for the evaluation of the effect of chitosan on the dyeability of DP-finished cotton, they used 4,5-dihydroxy-1,3-dimethyl-2-imidazolidinone (DHDMI), which is also called 1,3-dimethyl-4,5-dihydroxyethylene urea (DMeDHEU), as a DP finishing agent. All experiments were carried out with the same methods of their previous work [115]. DHDMI/chitosan-treated fabrics were observed to be more hydrophilic (moisture regains 5.59-5.93%) than DHDMI-treated fabric (moisture regain 4.31%) due to the introduction of hydroxyl and amino groups of chitosan. The dyeing behavior and color-fastness of the DHDMI/chitosan-treated fabrics showed a similar trend with those of the DMDHEU/chitosan-treated cotton fabrics [115].

Polycarboxylic acids have been used as non-formaldehyde DP-finishing agents for cotton fabrics to replace the formaldehyde-based *N*-methylol compounds such as DMDHEU. El-Tahlawy [118] incorporated chitosan in citric acid (CA) DP finishing bath to improve the dyeability of the finished fabrics. An aqueous mixture of CA, sodium hypophosphite, and chitosan was applied to cotton fabrics by a pad (90-100% WPU)-dry (85°C for 5 min)-cure (170°C for 90 sec) method. It was assumed that an

amide bond is formed between chitosan and citric acid, while the remaining two carboxyl groups form an anhydride ring and consequently crosslinked with cellulose through esterification. The author evaluated various performance properties, such as WRA, tensile strength, elongation at break, whiteness index, wettability, and stiffness of the fabrics treated under different finishing conditions. The stiffness of the finished fabrics was reported to be increased with the amount and viscosity of the added chitosan. The values of WRA decreased by increasing the amount and MW of the chitosan. In terms of dyeability of the fabrics with reactive dyes, the color strength (K/S) of CA/chitosan-treated fabric was higher than that of CA-treated fabric without chitosan and the value of K/S increased when the amount of chitosan increased.

Bhattacharyya et al. [119] reported the use of chitosan as a formaldehyde scavenger for DMDHEU-finished cotton fabrics. The reduction of formaldehyde release was attributed to the interaction of formaldehyde with the amino groups of chitosan. It was observed that the use of chitosan as an additive in DMDHEU finishing after dyeing was more effective in reduction of formaldehyde release as compared to the fabric which is chitosan-pretreated, dyed, and then DMDHEU-finished. The authors indicated that this result might be due to the blocking of the amino groups of chitosan by dye molecules on subsequent dyeing in the case of chitosan-pretreated cotton, and as a result, reduced reactions between chitosan and formaldehyde. In another study of theirs [120], cotton fabrics were dyed with direct, Indosol, and reactive dyes and then the dyed fabrics were treated with a mixture of DMDHEU, catalyst, and four different formaldehyde

scavengers, shellac, chitosan, TEA·HCl, and EDTA. It was observed that chitosan was most effective for reducing formaldehyde release from the dyed-finished fabrics.

5.3. Chitosan in Wool Finishing

One of the undesired properties of wool fibers is felting, which is the process of progressive entanglement of fibers under mechanical action in the presence of water. The felting shrinkage results from the interlocking and hooking of contingent fibers due to the scales on the wool fibers [121]. Polymer deposition on wool fibers to coat the scales is one method to prevent laundering-shrinkage of wool fabrics by felting.

Masri et al. [122] reported that the deposition of crosslinked chitosan on woven wool fabric imparted laundering-shrinkage resistance. In a two-step application, chitosan solutions in dilute acids were padded on wool fabrics and dried. The chitosan-deposited fabrics were further treated with crosslinkers like glyoxal or glutaraldehyde, which reacts with chitosan as well as with functional groups on the wool, e.g. lysine, arginine, histidine, or serine residues. In a single-step application, mixtures of chitosan solution and crosslinkers, such as glyoxal, glutaraldehyde, or DMDHEU were padded on the fabrics, dried, and then cured. Both application methods improved resistance to laundering-shrinkage of the fabrics. The authors suggested that the single-step method was simpler, more practical, and resulted in a more even treatment. It was possible to include additives such as a flame retardant to the padding mixture.

It is known that the weathered tip of the wool fiber shows a different dyeing behavior from the relatively undamaged root. Davidson and Xue [123] applied chitosan

(DD ≥ 0.70) on wool fabric to obtain uniform dyeing for both damaged and undamaged portions of the wool fiber. A water-soluble chitosan salt, chitosan lactate, was dissolved in water at different concentrations and padded on wool fabrics (100% WPU), dried at 40°C, and washed with water. The chitosan-treated wool fabrics were dyed with acid and reactive dyes under acidic conditions (pH 4.5-5.7). The rates of dye uptake of the treated fabrics were found to be faster than that of the untreated one due to the additional dye sites on the treated fabrics by the introduction of amino groups of chitosan. The treated fabrics showed higher dye exhaustions than the untreated one by 1.3 to 41.3% depending on the dye. When it comes to the color fastness of the treated wool compared to untreated wool, there was no difference when the dyeing temperature was high (100°C). The authors stated that the even dyeing of the chitosan-treated wool resulted from the formation of a uniform chitosan layer on both damaged and undamaged portions of wool fibers. From a fluorescence microscopy investigation on the cross-sections of dyed fibers it was observed that the dye penetration into the chitosan-treated fiber was more uniform than the untreated one. It was suggested that the improved dyeing behaviors of chitosan-treated wool might reduce dyeing times and wool damage, and save energy.

Jocić et al. [124] reported the role of a nonionic surfactant (octaethyleneglycol mono-n-dodecyl ether) in the application of chitosan to wool. To investigate the interaction of chitosan (MW~70,000) with the surfactant, the viscosity of chitosan solutions containing different surfactant concentrations were measured five days after the preparation of the solutions. It was observed that a sudden viscosity drop occurred in the region close to the critical micelle concentration of the pure surfactant (Figure 21).

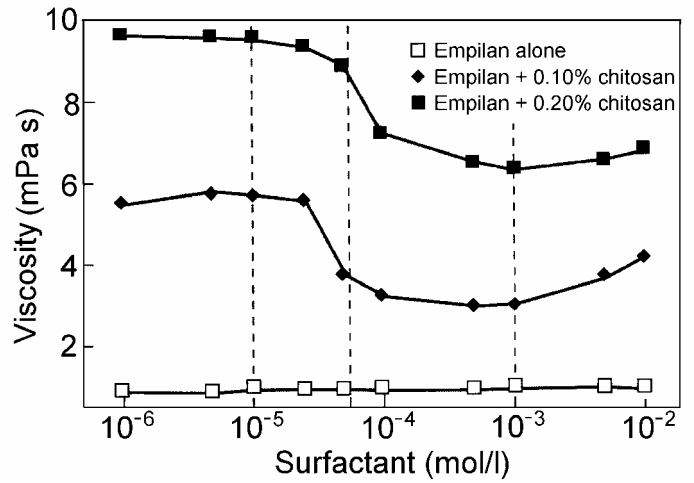

Figure 21. Viscosity of chitosan solutions at 25°C as a function of a nonionic surfactant (Empilan) concentration after 5 days after mixing the solutions. (From Ref. 124.)

The concentration range of the surfactant (Empilan KBS8) was independent of the concentration of chitosan. The wool fabrics treated with chitosan/surfactant mixtures at the surfactant concentrations of 10^{-5} and 10^{-3} mol/L were found to show higher dye uptakes and produced deeper and more vivid colors than untreated wool using the same concentration of a reactive dye. Higher concentration of the surfactant (10^{-3} mol/L) showed a better result than lower concentration (10^{-5} mol/L) at the same concentration of chitosan. Better dyeing results were obtained by using a stored (5 days) chitosan/surfactant solution rather than a freshly prepared one. Also, the chitosan/surfactant-treated wool compared to the wool treated with chitosan alone demonstrated increased dye fixation (wet fastness) after stripping of unfixed dyes by an alkaline aftertreatment. It was concluded that the presence of nonionic surfactant over a

certain critical concentration altered the solution behavior of chitosan and improved the applicability of chitosan on wool.

The chlorine-Hercosett process is one of the most widely used processes to impart shrink-resistance to wool fibers. In this process, the chlorination, neutralization, and resin (polyamide-epichlorohydrin type) application produce substantial amounts of adsorbable organic halogens (AOX) in the processing wastewater. As an alternative pretreatment to chlorination, per-monosulfate, enzymatic, plasma, or corona treatment can be utilized [125].

Julià et al. [126] investigated the use of chitosan for shrink-resistant treatment of wool fabrics, which does not generate AOX. Wool fabrics were pretreated with hydrogen peroxide (H_2O_2) either under alkaline or acidic conditions. The purpose of the pretreatment was to increase the number of cysteic acid groups ($-SO_3H$) by the oxidation of the disulfide bonds on wool fibers and consequently increase the anionic charges on the fiber surface, which could enhance the sorption of chitosan with cationic charge. It was found that both acidic and alkaline H_2O_2 treatments generated similar amounts of cysteic acid groups on the fibers. From the measurement of wetting time of the treated fabrics, the alkaline H_2O_2 treatment imparted a higher hydrophilicity to wool fiber, while the wool treated in acidic conditions showed similar wettability to that of untreated wool. The pretreated fabrics were padded (200% WPU) with chitosan (MW 70,000) solution in dilute acetic acid and dried at room temperature. The laundering-shrinkage measurements revealed that the best shrink-resistance was obtained from the wool fabric pretreated with H_2O_2 under alkaline condition (pH 9.0) at 70°C for 1 hr. However, the

application of chitosan to acidic H_2O_2 pretreated wool did not show any shrinkage reduction and the shrinkage percentage was similar to that of untreated wool. The authors concluded that the shrink-resistance and chitosan sorption were related to the hydrophilicity rather than the cysteic acid content of the wool. They stated that the increased hydrophilicity of the alkaline H_2O_2 pretreated wool resulted from a partial removal of fatty acids on the epicuticle of the wool fiber in alkaline conditions. The microscopic observation of the cross-sectioned fibers stained with C.I. Reactive Red 180 revealed that diffusion of chitosan through the fiber occurred only in the fibers pretreated with H_2O_2 under alkaline conditions. The results of dyeing fabrics with C.I. Reactive Red 116 showed that all chitosan-treated fabrics, regardless of pretreatments, had faster dye exhaustion and higher final exhaustion and fixation than those of untreated and H_2O_2 pretreated fabrics.

In another study of Julià's research group [127], they examined the effect of chitosan MW on the shrink-resistance of H_2O_2 pretreated (at pH 9.0) wool fabrics. For the study, three different chitosans with MWs of 70,000, 150,000, and 750,000 with similar DD were used. The higher the molecular weight was, the greater the shrink-resistance. However, the dyeing behavior was not affected by the MW of chitosan.

As an attempt to promote the sorption of chitosan on wool fibers in shrink-resistance finishing, several different pretreatments of wool, such as anionic surfactant [128], cold plasma [129], and enzyme treatments [130] were evaluated. Julia et al. [128] pretreated wool fabrics with an anionic surfactant (sodium lauryl sulfate (SLS)) at different pH conditions (pH 2.2, 4.0, 6.0, and 11.8). In an acidic condition (pH 2.2), the

highest adsorption of SLS on wool was observed. This was explained by the formation of a double layer of SLS on the wool surface (Figure 22).

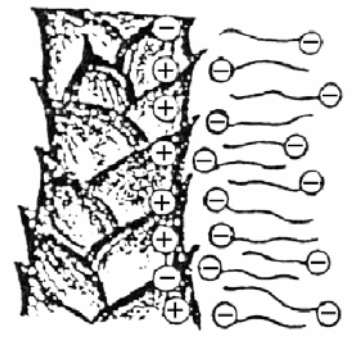

Figure 22. Formation of double layers of SLS on the surface of wool at pH 2.2. (From Ref. 128.)

The protonated amino groups of wool at acidic pH are ionically linked by SLS and the second layer is formed by hydrophobic interaction between the hydrocarbon chains of SLS. Finally, the fiber surface acquires a substantial amount of negative charge. All SLS pretreated fabrics were treated with chitosan (MW 70,000) solution. The lowest laundering-shrinkage was obtained from the fabric pretreated with SLS at pH 2.2.

Erra et al. [129] reported the chitosan treatment on wool fabrics pretreated with cold plasma as a possible ecological wool finishing process. The plasma treatment of wool resulted in a significant reduction in laundering-shrinkage compared to untreated wool, but the plasma treatment alone was not enough as a machine-washable finish. By applying chitosan (MW 70,000) to the plasma-treated wool, the shrink-resistance was further improved. The authors concluded that the plasma treatment increased the hydrophilicity of wool and consequently promoted the spreading and adhesion of chitosan on wool, which provided an additional shrink-resistant effect.

Rybicki et al. [130] suggested enzymatic pretreatment and chitosan deposition on wool fabric as a new ecological method for shrink-proofing of wool fabrics. Wool fabrics were pretreated with PERIZIM LAN (a mixture of proteolytic enzymes for the surface treatment of wool and silk) and padded with a chitosan (DD 0.83, viscosity 20 mPas) solution in 1% acetic acid containing a crosslinking agent (glutaraldehyde). The padded fabrics (67.5% WPU) were dried at 90°C for 40 sec and cured at 130°C for 20 sec. Based on the results, it was concluded that enzymatic pretreatment itself greatly improved the shrink-proofing property by partial destruction of the fiber scales, and the post-application of chitosan enhanced the shrink-proofing as well as assured an increased dyeability of wool fabrics by reactive dyes.

5.4. Chitosan and Its Derivatives in Antimicrobial Finishing

Textile goods, especially those made from natural fibers, provide an excellent environment for microorganisms to grow, because of their large surface area and ability to retain moisture. In recent years, consumers' demand has greatly increased for textile products with antimicrobial properties, as their standard of living rises and consumers become aware of the importance of these products.

A number of chemicals have been employed to impart antimicrobial activity to textiles. Those chemicals include inorganic salts, organometallics, iodophors (substances that slowly release iodine), phenols and thiophenols, onium salts, antibiotics, heterocyclics with anionic groups, nitro compounds, ureas and related compounds, formaldehyde derivatives, and amines [131]. Many of these chemicals, however, are

toxic to humans and do not easily degrade in the environment. These facts have facilitated the use of chitosan as a new antimicrobial agent for textiles.

The most commonly used quantitative methods for testing the antimicrobial activity of textiles are the Shake Flask method [132] developed by Dow Corning Corporation and AATCC TM 100 [133]. In the Shake Flask method, fabric swatches are shaken in a suspension of test organism for a desired contact time (usually 1 hr). The suspension both before and after contact is serially diluted and cultured to determine the number of surviving bacteria. For the AATCC TM 100, a suspension of test organism is inoculated to the test fabrics and incubated over the desired contact period. The fabrics are serially diluted and the number of surviving bacteria are counted. The antimicrobial activity, in both methods, is reported in terms of % reduction of bacteria calculated by comparing the numbers of surviving bacteria before and after contact. The most frequently used test organisms are *S. aureus* (gram-positive) and *E. coli* (gram-negative), which are commonly found in the human body [131].

Yoo et al. [134] reported the effect of the DD of chitosan on the antimicrobial activity of chitosan-treated cotton fabrics. For the study, four chitosans of different DD (0.65, 0.78, 0.84, and 0.95) with similar MW (~ 50,000) were used. Cotton fabrics were treated with chitosan in 2% acetic acid by a pad-dry (100°C for 5 min)-cure (150°C for 3 min) method. The antimicrobial activity of the treated fabrics against *S. aureus* evaluated by the Shake Flask method was found to increase with the increase in DD of chitosan. The antimicrobial activity also increased as the amount of chitosan applied increased and reached its maximum value (% reduction: ~ 40% with DD 0.65, ~ 90% reduction with

DD 0.78, and 100% reduction with DD 0.84 and 0.95) at the chitosan concentration of 0.5% owb (on weight of bath), but beyond this concentration, it was not changed significantly. To increase the durability of chitosan on the fabric against repeated launderings, a polyurethane binder or a crosslinker (DMDHEU) at the concentration of 0.1% owb was added to the chitosan solution for the cotton treatment. The antimicrobial activities of the fabrics decreased sharply after 5 laundering cycles. Although the addition of a binder or a crosslinker increased add-on (%), the chitosan-treated fabric without a binder or a crosslinker showed slightly better durability than the ones treated in the presence of a binder or a crosslinker. The authors concluded that the polyurethane binder masked the amino groups of chitosan and that the crosslinker could also react with the amino groups of chitosan resulting in the decreased antimicrobial activity.

Shin et al. [135] examined the MW effect on the antimicrobial activity of chitosan-treated cotton fabrics. Three chitosans with different MW of 1,800, 100,000, and 210,000 having similar DD (0.86-0.89) were applied to cotton fabrics by a pad (100% WPU)-dry (100°C for 3 min)-cure (150°C for 3 min) method. The antimicrobial activity of treated fabrics was found to be increased with the increase in the MW of chitosan and the MW effect was more distinctive at low treatment concentrations. At a higher treatment concentration of chitosan (1.0% owb), all chitosans showed above 90% reduction against *E. coli*, *Proteus Vulgaris*, and *S. aureus*, but they were less effective against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The evaluation of the chitosan-treated fabrics indicated that the increase of MW and treatment concentration of

chitosan led to increases in add-on (%), stiffness, and moisture regain, and a slight decrease in tensile strength.

Shin's research group [136] also examined the application of chitosan oligomer (MW 1,800, DD 0.84) to PP nonwoven fabrics by a pad (100% WPU)-dry (100°C for 3 min) method for diaper and hygienic end-uses. The chitosan-treated PP nonwoven showed over 90% reduction of bacteria against *S. aureus*, *E. coli*, and *P. vulgaris* at 0.01 ~ 0.05% owb of chitosan oligomer. The chitosan oligomer was most effective against *P. vulgaris*, which causes diaper rashes. The addition of a non-ionic wetting agent (Triton X-100) in the finishing bath caused a decrease in the antimicrobial activity of the fabric. For example, the % reduction of *S. aureus* was reduced from 100% to ~ 45% at the chitosan concentration of 0.1% owb by the addition of the non-ionic wetting agent (0.1% owb). The authors concluded that the interactions between the protonated amino groups of chitosan and the weak anionic ethylene oxide groups in the wetting agent reduced the function of the amino groups of chitosan, which relates to its antimicrobial activity. It was reported that the chitosan-treated PP nonwoven showed higher add-on, stiffer handle, higher absorbency, and lower air permeability as the MW and concentration of chitosan increased [137].

Lee et al. [138] applied chitosan (MW 90,000, DD 0.95) to cotton fabrics and 55/45% wood pulp/polyester nonwoven fabrics and then applied a fluoropolymer to give antimicrobial activity as well as blood repellency to the fabrics for use as surgical gown materials. For cotton as a reusable gown material, chitosan solution in 1% acetic acid containing DMDHEU (0.6 molar equivalent to chitosan glucosamine) as a crosslinker for

durability to launderings was applied (85% WPU), dried (110°C for 3 min), cured (160°C for 3 min), and washed. For the nonwoven as single-use gown material, chitosan was applied without DMDHEU. The chitosan-treated cotton and nonwoven were further treated with a fluoropolymer (FC 5102, 3M Co.) by a pad(85% WPU)-cure (160°C for 2 min) method. The antimicrobial activities against *S. aureus* were evaluated by the Shake Flask method. It was found that the dual-finished cotton and nonwoven showed over 95% and 90% reduction of bacteria, respectively, at the concentration of 1.1% owb chitosan and 4% owb fluoropolymer, and the antimicrobial activity of the dual-finished cotton was maintained after 10 laundering cycles. The blood repellency of the dual-finished nonwoven was superior to that of the dual-finished cotton and the blood repellency of the cotton decreased with an increase of the number of launderings. The authors suggested that the nonwoven was more suitable for the blood repellent finishing. It was reported that the increased stiffness of the fabrics by the treatment of chitosan decreased after the fluoropolymer treatment and also that the air permeability of the fabrics decreased slightly.

Chung et al. [139] suggested that incorporation of chitosan with citric acid (CA) provided cotton fabric antimicrobial activity as well as wrinkle-resistance. The chitosan (MW 2,700, DD 0.90) dissolved in aqueous solution of CA (3, 5, 7, and 10% owb) containing a catalyst (sodium hypophosphite) was applied to cotton fabrics by a pad (80±5% WPU)-dry (100°C for 5 min)-cure (180°C for 2 min) method. The authors expected crosslinking of chitosan with cellulose by esterification reactions not only between cellulose and CA, but also between CA and the hydroxyl groups of chitosan.

Another possible proposed interaction was the ionic interaction between free carboxylate groups of citric acid and protonated amino groups of chitosan. The fabric treated with CA/chitosan showed better mechanical properties (tensile and tear strength retention) than the fabric treated with CA alone. The cotton treated with 7% owb CA and 0.8% owb chitosan showed almost 100% reduction of *S. aureus* (by Shake Flask method) and the antimicrobial activity remained over 80% reduction of bacteria after 20 laundering cycles. Also a satisfactory wrinkle resistance was retained. The authors concluded that chitosan and CA are firmly linked to the cotton. However, it is questionable if the antimicrobial activity of the fabric after 20 launderings comes from chitosan or CA, because the authors observed 100% reduction of bacteria from the fabric treated with CA alone before the launderings. Information was not provided on the antimicrobial activity of the fabrics treated with CA alone after repeated launderings.

Kim et al. [140] prepared HTCC (DS 1.01) using chitosan (MW 270,000, DD 0.87) and GTMAC (Figure 6). The aqueous solution of HTCC and chitosan in 1% acetic acid was applied to cotton fabrics by a pad (75% WPU)-dry (60°C for 3 min) method. The antimicrobial assessment of the treated fabrics by the Shake Flask method showed almost 100% reduction of *S. aureus* for HTCC-treated cotton at a HTCC concentration of 0.025% owb, whereas 1% owb of unmodified chitosan showed only about 30% reduction. The poor laundering durability of the HTCC-treated cotton was improved by the incorporation of a nonionic binder (TK Binder-202F) to the HTCC solution and application to cotton by a pad-dry (130°C for 2 min)-cure (150°C for 3 min) method. The cotton treated with 0.5% owb HTCC and the nonionic binder (5% owb) maintained

90% reduction of bacteria after 6 laundering cycles. However, the addition of a crosslinker (DMDHEU) was not effective for the laundering durability. The authors concluded that the crosslinking between HTCC and cellulose was not effective because low MW DMDHEU was in the interior of the fiber but high MW HTCC was on the surface of the fiber.

Seong et al. [141] prepared two different chito-oligosaccharides (COS) (DP 3 and 10) by reacting a fully deacetylated chitosan (DD 0.99) with sodium nitrite in the presence of acetic acid (Figure 23).

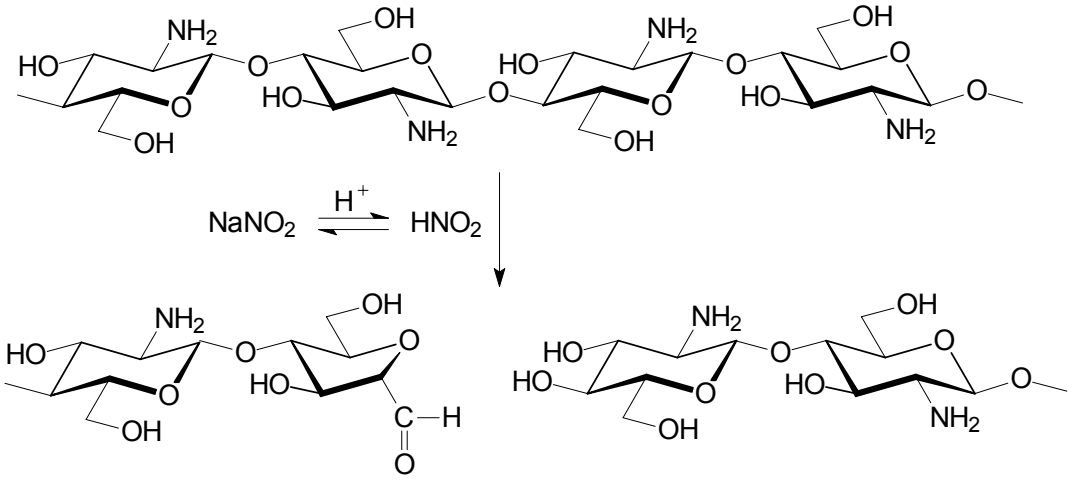

Figure 23. Degradation of chitosan by nitrous acid.

The COS was applied to cotton fabrics by a pad (80% WPU)-dry (60°C for 3 min)-cure (120°C for 3 min). The padding bath included acetic acid (molar equivalent to glucosamine of COS), $MgCl_2 \cdot 6H_2O$ as a catalyst (0.2 molar equivalent to glucosamine of COS), 0.1% Triton X-100, and 0.1% softening agent. The antimicrobial activity evaluated by AATCC TM 100 revealed that the COS-treated cotton fabrics retained over

95% reduction of bacteria (*S. aureus*) after 50 wash cycles when the fabrics were treated with 2.4% owb of COS. The authors suggested that the durability to washing was obtained by the acetal formation between terminal aldehyde groups of COS and cellulose (Figure 24).

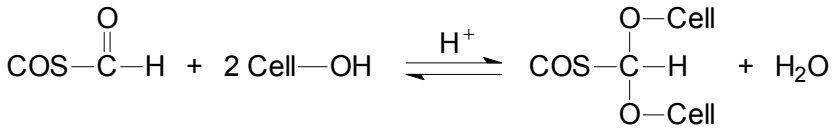

Figure 24. Reaction between COS and cellulose.

The HTCC (Figure 6) was also prepared by Seong et al. [142] by reacting COS (DP 3) with GTMAC at 80°C for 18 hrs in the presence of an acid catalyst (acetic acid). The resulting product was referred to as HTACC (*N*-(2-hydroxy)propyl-3-trimethyl ammonium chito-oligosaccharide chloride). The MIC value against *S. aureus* for HTACC (DS 1.04) was 50 ppm, whereas that of the starting COS was 400 ppm, which means that the HTACC was eight times more effective than the COS. The HTACC was applied to cotton fabrics by a pad (80% WPU)-dry (60°C for 3 min)-cure (120°C for 3 min) method in the presence of a catalyst ($MgCl_2 \cdot 6H_2O$, 0.6 molar equivalent to HTACC). The authors supposed that there would be acetal formation (Figure 24) between the hydroxyl groups of cellulose and terminal aldehyde groups in the HTACC. It was reported that the HTACC-treated fabric showed 100% reduction of bacteria (by AATCC TM 100) after 50 laundering cycles at the concentration of 0.3% owb HTACC, while 2.4% owb of COS was required to get the same effect.

Several attempts to impart durability of COS to cotton fabric by either the addition of crosslinkers such as DMDHEU [143] and 1,2,3,4-butanetetracarboxylic acid (BTCA) [144] or derivatization of COS [145] are reported. Lee et al. [143] reported that the cotton fabric treated with COS (DP 9 and 16) and DMDHEU (0.6 molar equivalent to glucosamine of COS) by a pad (75±3% WPU)-dry (100°C for 3 min)-cure (160°C for 3 min) retained over 80% reduction of *S. aureus* (Shake Flask method) at 20 washings. Seong et al. [144] applied COS (DP 6 (2.4% owb) and 10 (2.0% owb)) and BTCA (1.0 molar equivalent to glucosamine of COS) to cotton fabrics by a pad (80±3% WPU)-dry (100°C for 3 min)-cure (160°C for 3 min). It was reported that the COS-treated cotton retained over 99% and 95% reduction of *S. aureus* (by AATCC TM 100) after 50 laundering cycles.

Lee et al. [145] introduced fiber-reactive groups on COS by reacting COS (DP 9) with *N*-methylolacrylamide (NMA) in an acidic condition (acetic acid) (Figure 25).

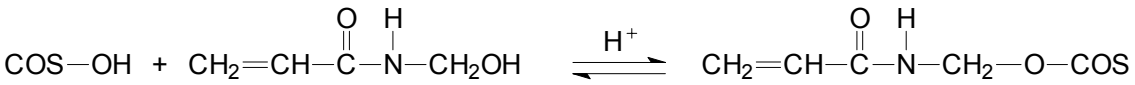

Figure 25. Synthesis of COS-NMA.

The obtained COS-NMA (DS ~ 0.4) was applied to cotton fabrics by a two-step process due to its insolubility at an alkaline condition. In the first step, the COS-NMA (2.5% owb) in dilute acetic acid was applied to cotton by padding (75±3% WPU) and dried at 85°C for 3 min. In the second step, the dried fabric was padded (wet pick-up 75±3%) with Na_2CO_3 aqueous solution and cured at 140°C for 3 min. In this step, the reaction between COS-NMA and cellulose occurs (Figure 26).

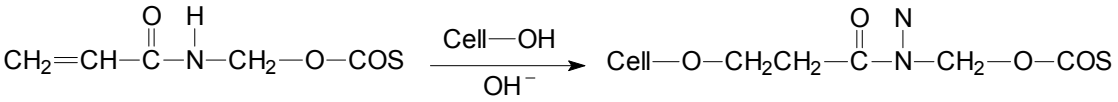

Figure 26. Reaction between COS-NMA and cellulose.

The treated fabric was neutralized with 1% acetic acid and washed. The COS-MNA-treated fabric retained 95% reduction of *S. aureus* (by Shake Flask method) after 20 laundering cycles.

For the works related to COS [141,142,144], the reduction of bacteria for all test samples was retained over 95% after 50 laundering cycles. It can be noticed that the results were obtained by AATCC TM 100 and that they were considerably higher than those obtained by the Shake Flask method. This discrepancy could result from either the different methods of antimicrobial activity assessment or the efficient applications of COS. Unfortunately, in all work related to COS [141-145], the authors did not provide the comparison of durability between test and control samples. It should be kept in mind that the efficiencies of antimicrobial activity of fabrics between different works cannot be directly compared to each other because the result will vary depending on different test parameters, such as the size of test fabrics, number of bacteria used for the test, test temperature, contact time between test organism and fabrics, and so on.

It was reported that the introduction of monochlorotriazinyl group on chitosan improved the durability of chitosan on cotton [146]. The chitosan derivative (CHI-CNC) was prepared by a two step synthesis (Figure 27).

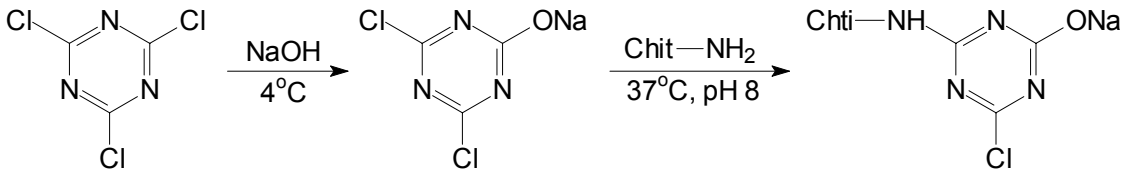

Figure 27. Synthesis of CHI-CNC.

The first step was the preparation of a water-soluble dichlorotriazine sodium salt by reacting cyanuric chloride (CNC) with NaOH at a low temperature. In the second reaction step, the dichlorotriazine solution was slowly added to a chitosan (MW 2,000, DD 0.84) solution while maintaining pH 8. The obtained CHI-CNC (DS 0.12) was applied at the concentration of 1.0% owb to cotton fabrics by the exhaustion method at 75°C for 90 min in the presence of an alkali (Na_2CO_3, 1 g/l).

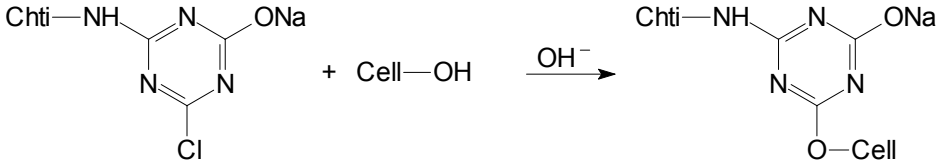

Figure 28. Reaction between CHI-CNC and cellulose.

The proposed reaction between the CHI-CNC and cellulose is shown in Figure 28, which is common between fiber-reactive dye and cotton. For comparison, unmodified chitosan was also applied to cotton by the same method. After treatments, both CHI-CNC- and chitosan-treated fabrics were neutralized in 2% acetic acid and washed. The comparison of the laundering durability of the two fabrics was made in terms of color strength (K/S) (after dyeing with C.I. Acid Red 88), nitrogen content, and % reduction of *S. aureus* (by Shake Flask method) (Table 2).

Table 2. Laundering durability of chitosan- and CHI-CNC-treated fabrics. (From Ref. 146.)

Laundering cycles	K/S value		Nitrogen content (%)		Reduction of bacteria (%)	
	Chitosan	CHI-CNC	Chitosan	CHI-CNC	Chitosan	CHI-CNC
0	2.74	3.36	0.05	0.16	62	100
5	2.41	3.13	0.04	0.15	40	90
10	2.40	3.12	0.03	0.13	36	85
15	2.36	2.90	0.03	0.12	-	-
20	1.80	2.81	0.02	0.12	31	79

The authors concluded that the CHI-CNC-treated cotton showed ~ 80% reduction of bacteria after 20 launderings due to the covalent bonds between cellulose and chitosan, while the chitosan-treated cotton showed a large decreased antimicrobial activity after 5 launderings due to its weak bonding to cellulose, such as by hydrogen bonding and van der Waals' forces.

Park et al. [147] examined the antimicrobial and deodorant activities of chitosan-treated wool fabrics. Chitosan (2% w/v) solutions in 1% acetic acid were applied to wool fabrics with 80% WPU and dried at 120°C for 3 min. All fabrics treated with chitosans having MW range of 30,000 ~ 480,000 and DD over 0.70 showed almost 100% reduction of bacteria (*S. aureus*) by the Shake Flask method. The deodorant activity of the chitosan-treated fabric, assessed using ammonia, was found to increase gradually with DD when MW of chitosan was similar (~ 400,000). Overall the chitosan-treated fabrics showed 30 ~ 40% higher deodorant activity than untreated fabric. It was reported that the chitosan-treated wool fabrics showed a relatively high laundering-durability due to the ionic bonding between chitosan and wool keratin. When the DD of chitosan was the

same (DD 0.82), the MW range of 100,000 ~ 400,000 showed better deodorant activity than MW of 50,000 and 480,000.

5.5. Miscellaneous Uses of Chitosan in Dyeing and Finishing

5.5.1. Chitosan as a dyeing-improving agent for polypropylene (PP)

There are no commercial dyeing methods for unmodified PP fibers due to their hydrophobicity, high crystallinity, and lack of reactive sites. Rochery et al. [148] suggested that the grafting of chitosan on the PP surface could be one of the methods to improve the dyeability of PP. Before the chitosan grafting, a PP film was treated with plasma under different conditions to generate oxidized groups on the PP surface. A 2% chitosan (MW 70,000) solution in 3% acetic acid was applied onto the plasma-treated PP films and then the films were dried at 110°C for 3 min. The authors suggested several possible reactions between chitosan and the functional groups on PP generated by the plasma treatment (Figure 29). When the chitosan grafted-PP films were dyed with an acid dye, the long time (4 hrs) plasma treated film showed a homogeneous dye uptake.

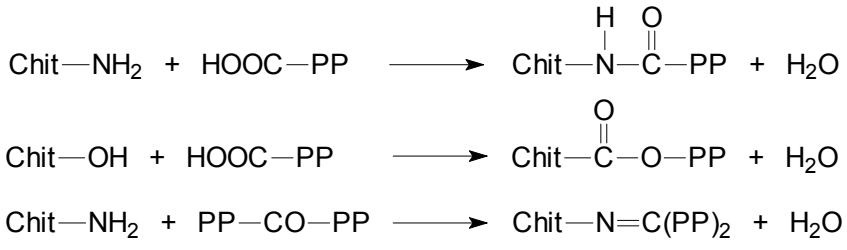

Figure 29. Proposed reactions between chitosan and functional groups on PP.

5.5.2. Chitosan as an antistatic finish

Synthetic fibers such as polyamides, acrylics, and polyesters tend to build static charge due to their hydrophobicity. Treatment of these synthetics with chitosan, a hydrophilic biopolymer, could be one of the methods to dissipate the static charge.

Matsukawa et al. [149] applied chitosan to polyester (PET) fabric to impart antistaticity to PET fabric. The PET fabric was hydrolyzed in NaOH solution before the chitosan treatment to generate carboxyl groups on the PET surface, which then react with the amino groups of chitosan. The PET fabric was treated with chitosan (DP 200, DD 0.80) in 1% acetic acid by a pad-dry-cure method. At the same time, the chitosan films on the fabric was insolubilized by the addition of a dicarboxylic acid to crosslink the chitosan. The electrostatic charge measurement (65% RH at 20°C) revealed that the hydrolyzed PET given a chitosan-treatment showed a considerably lower value (6 volts) compared to those of hydrolyzed PET (2,600 volts) and untreated PET (4,600 volts). It was reported that the decreased strength of the fabric by the NaOH treatment was recovered by the chitosan treatment. Bandyopadhyay et al. [150] reported that polyester and its blended fabrics treated with chitosan solution in dilute acetic acid by a pad-dry method showed decreased static charge development as the amount of applied chitosan increased.

5.5.3. Chitosan in textile printing

The use of chitosan as a combined thickener and binder in pigment printing was examined by Bahmani et al. [151]. The performance of chitosan (MW 171,000) was

evaluated by comparing that of a commercial paste (Alcoprint system). The chitosan printing paste was prepared by dissolving chitosan in dilute acetic acid and a pigment was added and stirred to give a homogeneous dispersion of pigment. PET and PET/cotton fabrics were printed using chitosan printing paste, then dried at room temperature and cured at 150°C for 6 min. It was reported that the printed fabrics using chitosan gave a comparable color fastness to the printed fabrics using the Alcoprint system. The major problems with the chitosan system were the poor color strength (K/S) and the stiffness of the fabrics. The authors suggested that the stiffness of fabric could be minimized by the addition of appropriate auxiliaries and further study would be necessary to improve the poor color strength which resulted from the reduced stability of the pigment dispersion in chitosan paste (pH 4).

5.5.4. Chitosan as a preservative in textile wet processing

Most cotton and synthetic fiber staple yarns need to be sized before weaving to prevent damage on the yarns by abrasion and breakage during the weaving process. Generally, a preservative is added to size mixes before application to yarns or applied on sized yarns to prevent mildew growth. However, a majority of preservatives are toxic and not biodegradable, therefore, the textile effluent causes environmental hazards. The Bombay Textile Research Association (BTRA) developed a new eco-friendly preservative based on chitosan, named 'BTRA-EP' [152]. The BTRA-EP was added to several sizing agents. The evaluation was made in terms of fungus growth (black or

white spots) or drop in viscosity of the sizing pastes. The effect of 1% addition of BTRA-EP compared to control (without BTRA-EP) is summarized in Table 3.

Table 3. The antimildew property of BTRA-EP. (From Ref. 152.)

Pastes	Stability of paste (number of days)	
	without BTRA-EP	1.0% BTRA-EP
Maize Starch (10%)	2	> 47
Tapioca Starch (8%)	1	30
Anilose (10%)	1	no growth even after 30 days
Carboxymethyl Starch (10%)	2	> 60
Cuar Gum (2%)	2	> 20

When BTRA-EP was compared with pentachlorophenol (PCP), which had been one of the best preservatives but was banned by EU countries since 1992 due to its high toxicity, its efficiency was comparable to that of PCP. The authors suggested BTRA-EP as a new preservative in textile wet processing, which is biodegradable, eco-friendly, and effective on different types of gums and starches.

5.5.5. Chitosan as a deodorant agent for textiles

Hasebe et al. [153] synthesized two different chitosan hybrids to use as deodorant agents for textiles. An aqueous solution of chitosan and methacrylic acid (MAA) was mixed with organic solvents, emulsified, and polymerized to give polymer A, which is a porous polymer particle (8-20 μm) and a composite between chitosan and polymethacrylate (PMAA). The unique property of this particle was its possession of numerous basic groups inside the porous structure. Polymer B was synthesized by the

polymerization of the emulsion containing an aqueous solution of chitosan and MAA, and lauryl methacrylate (LMA). The resulting polymer formed a particle (0.1-3.0 μm, suitable for application to fabrics) consisting of a hydrophobic core (poly-laurylmethacrylate (PLMA)) covered with a hydrophilic shell, and which has the same composite structure as polymer A. The polymers A and B were simply composites where chitosan and PMAA or chitosan and PMAA-LMA are physically entangled. Polymer A showed high deodorizing performance due to its amphoteric property, i.e., an acidic substance is absorbed on to an amino group of chitosan and a basic substance is absorbed on the carboxylic acid groups of PMAA. The cotton fabric treated (pad (100% WPU)-cure (120°C for 1 min)) with polymer B containing a binder effectively absorbed acetic acid, isovaleric acid, and ammonia compared with the untreated fabric.

6. PREPARATION OF HIGHLY DEACETYLATED CHITOSAN

Commercial chitosan with a low MW was purchased from Korea Chitosan Co., Ltd. as a fine powder derived from crab shells (α-chitin). To increase the DD and decrease the MW, the chitosan was subject to an alkaline treatment. The evidence of deacetylation was elucidated by FT-IR and ^{1}H NMR. The DD and viscosity average MW were measured by ^{1}H NMR and viscometry, respectively.

6.1. Deacetylation of the Commercial Chitosan

The commercial chitosan (20 g) was charged into a 250 mL three-neck round-bottomed flask containing 200 mL of 10% (w/w) NaOH and 2 g of $NaBH_4$ as an antioxidant [154] to prevent yellowing of chitosan under the severe alkaline reaction conditions. The reaction mixture was refluxed at 110°C for 5 hrs while stirring, filtered over a glass funnel with perforated plate, and washed with distilled water until neutral to pH paper. After the final distilled water wash, the chitosan powder was washed with methanol, then acetone, and air-dried. The deacetylated chitosan was further dried at 70°C for 24 hrs under vacuum for the characterizations and the next step of synthesis.

6.2. FT-IR Spectroscopy

FT-IR, which requires a small amount of sample and little sample preparation time, is one of the useful techniques in polymer characterization. All IR spectra in this work were obtained by using a Nicolet 510P FT-IR spectrophotometer. The data collection parameters employed were as follows unless specified otherwise: Gain: 1, Resolution: 4.0 cm^{-1}, and

Scans: 32. All samples for this work were prepared as KBr pellets and were scanned against a blank KBr pellet background. The FT-IR sample chamber was continually flushed with dry air.

Chitin and chitosan can be differentiated by IR peak analysis. Chitin shows two strong absorption peaks in the range of 1680-1630 cm^{-1} and 1565-1475 cm^{-1} for the C=O stretch and the N−H bending of the secondary amide, respectively. Whereas, highly deacetylated chitosan shows a medium to strong absorption peak in the range of 1650-1580 cm^{-1} for the N−H bending of the primary amine.

Figures 30 and 31 show the difference between the commercial chitosan and the deacetylated chitosan. In the IR spectrum of the commercial chitosan, there were two absorption peaks at 1660 cm^{-1} and 1595 cm^{-1}, which correspond to the C=O stretch of the secondary amide and the N−H bending of the primary amine, respectively. The spectrum of the deacetylated chitosan showed a reduction of the peak at 1660 cm^{-1}, indicating that most of the secondary amide has been further changed to primary amine by the alkaline deacetylation.

6.3. Degree of Deacetylation (DD) by ^{1}H NMR Spectroscopy

^{1}H NMR is one of the simplest and most precise methods to determine the DD of chitosan. Chitosan with a high MW does not give a good NMR spectrum due to its high viscosity. In this study, because the MW of the chitosan was relatively low, ^{1}H NMR was used to determine DD.

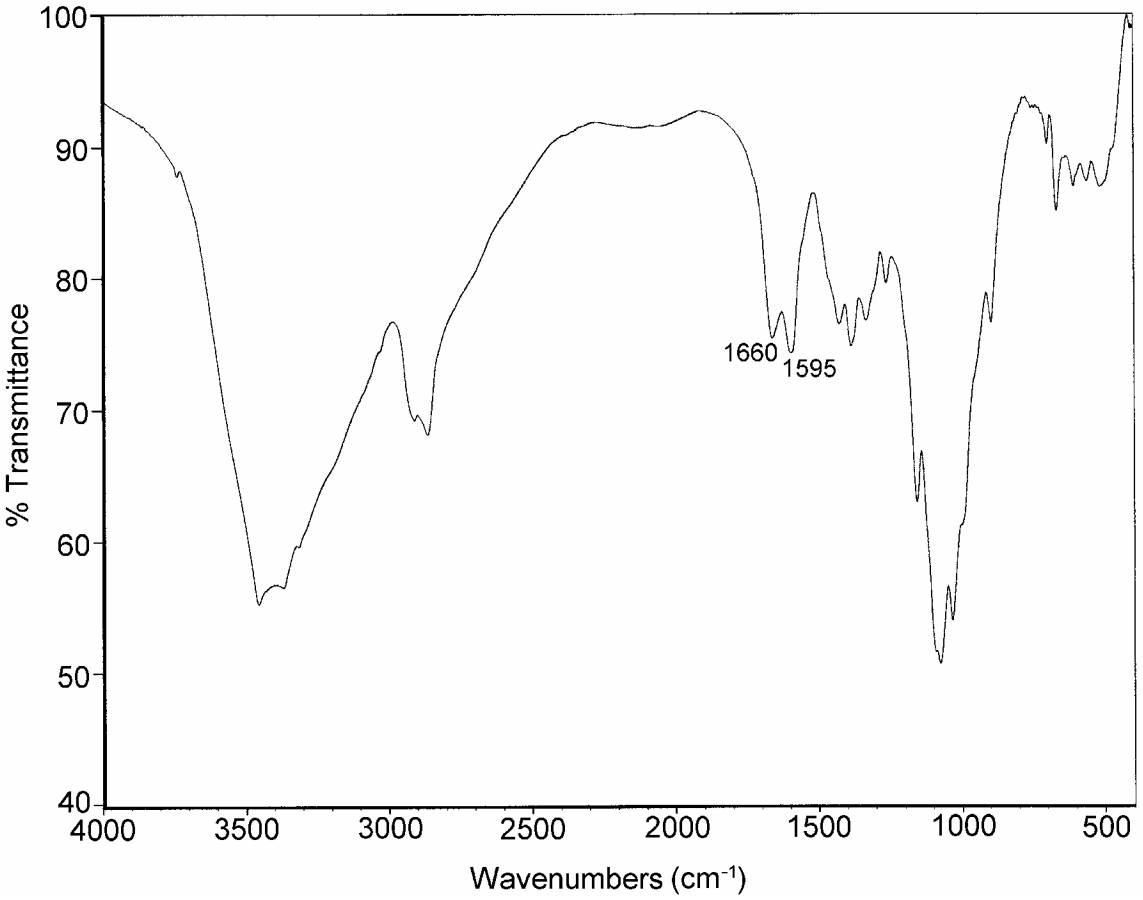

Figure 30. FT-IR spectrum of the commercial chitosan.

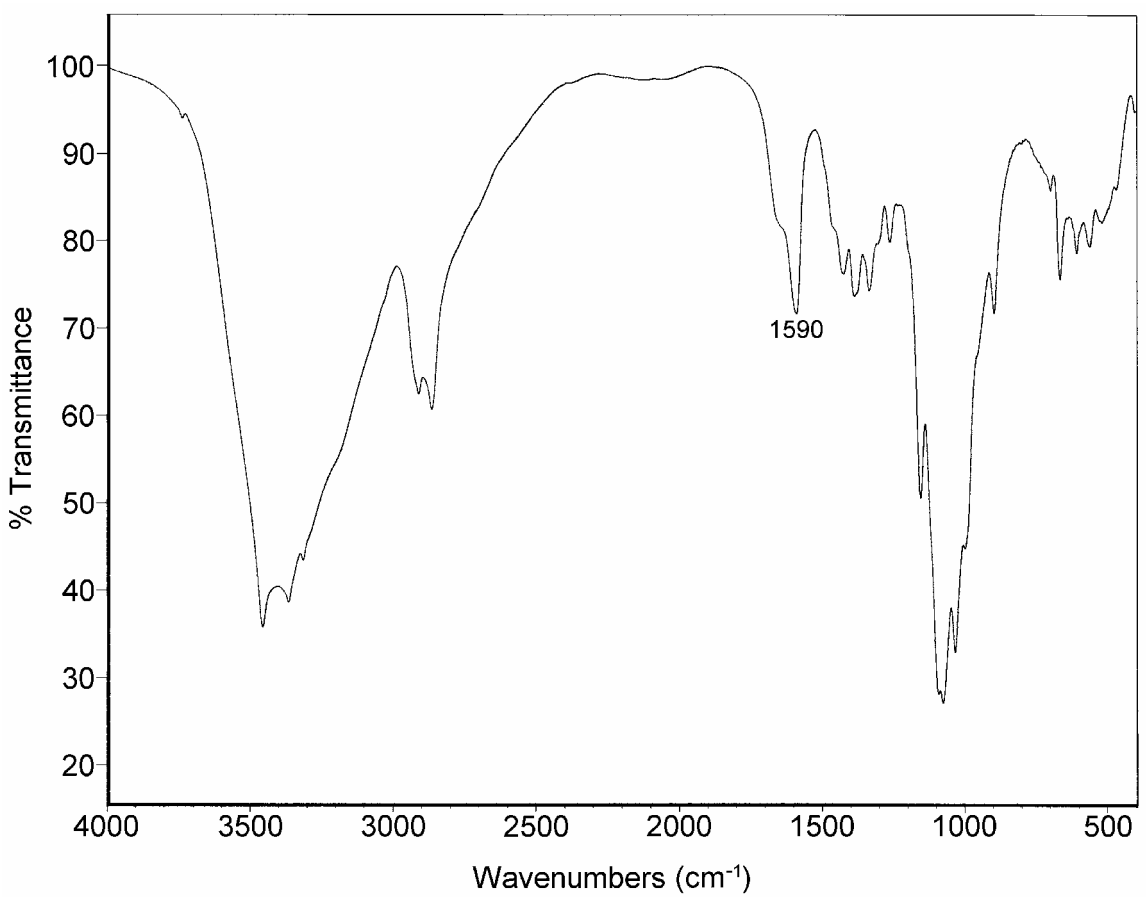

Figure 31. FT-IR spectrum of the deacetylated chitosan.

A known amount (10 mg) of chitosan was dissolved in 1 g of 2% (w/w) DCl/D_2O solution and introduced into a 5 mm NMR tube. ^1H NMR measurement was performed using a GE GN 300Ω NMR spectrometer at room temperature for all samples. As an internal reference, 3-(trimethylsilyl)propionic 2,2,3,3,-d_4 acid sodium salt (TSP) was used. For the data collection, the numbers of acquisition used were 128.

The evidence of deacetylation of the commercial chitosan can be seen by comparing Figures 32 and 33, which are ^1H NMR spectra for the commercial and deacetylated chitosan, respectively. The deacetylated chitosan shows lower intensity in the *N*-acetyl peak at 2.06 ppm than that of commercial chitosan. In both spectra, H1 and H2 appear at 4.88 and 3.19 ppm, respectively. The remaining protons appear as a clustered signal between 4.1 and 3.6 ppm.

The DD of the chitosan was determined based on the integral ratio of peaks by the following equation [23], where I_{CH3} is the integral intensity of *N*-acetyl protons and $I_{H2\text{-}H6}$ is the sum of integral intensities of H2, 3, 4, 5, 6, and 6' protons.

$$DD = 1 - \left(\left(\frac{I_{CH3}}{3} \right) \Big/ \left(\frac{I_{H2-H6}}{6} \right) \right)$$

The values of DD calculated were 0.922 and 0.971 for the commercial and deacetylated chitosan, respectively.

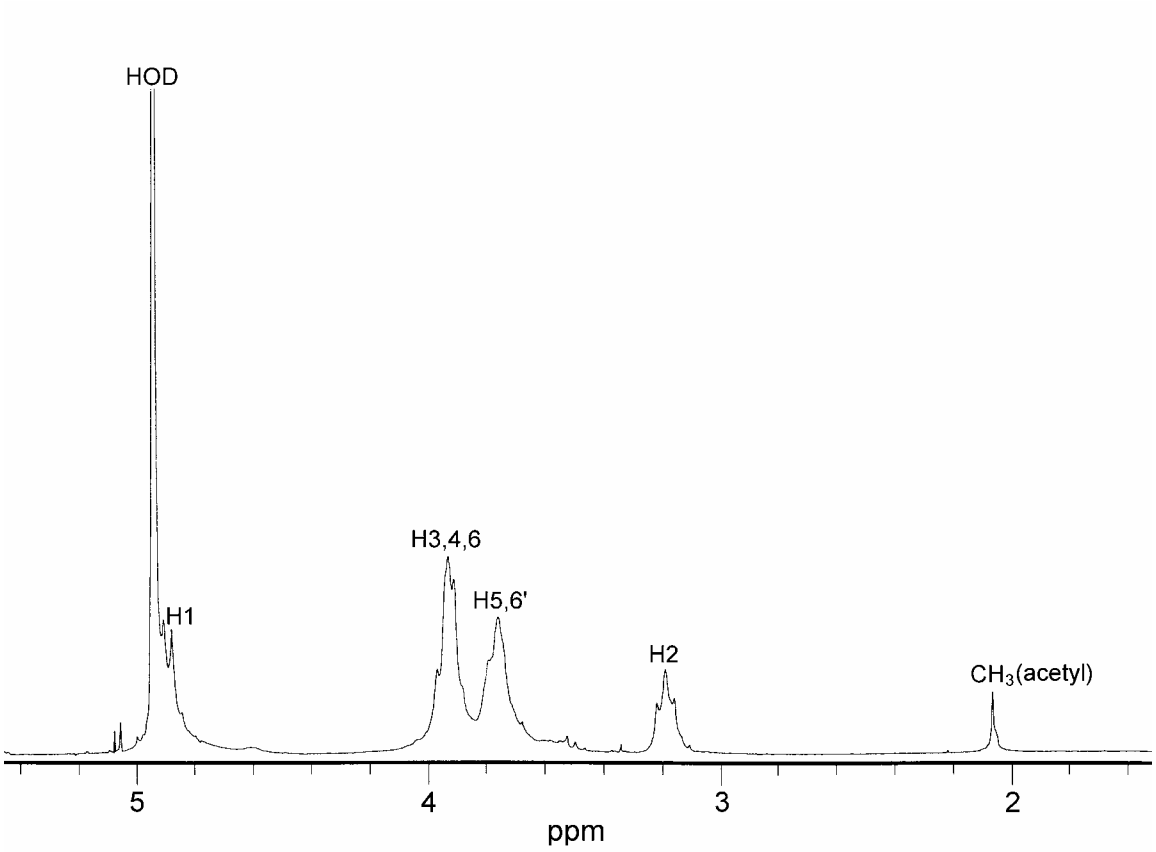

Figure 32. 300 MHz ^{1}H NMR spectrum of the commercial chitosan.

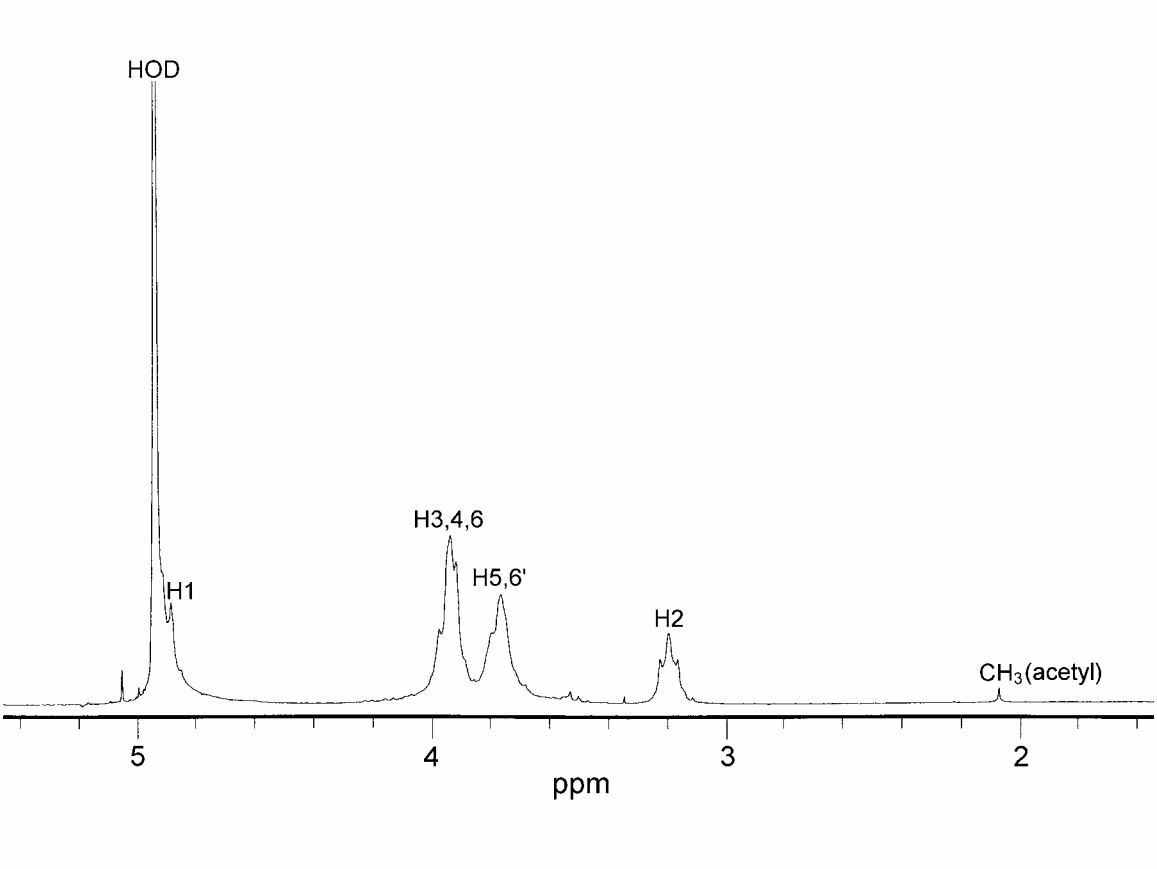

Figure 33. 300 MHz ^{1}H NMR spectrum of the deacetylated chitosan.

6.4. Molecular Weight (MW)

The viscosity average MW ($\overline{M}_v$) of chitosan can be determined by the Mark-Houwink equation, where [η] is intrinsic viscosity determined from a Huggins plot and *k* and α are empirical coefficients dependent on the DD of chitosan.

$$[\eta] = k\overline{M}_v^{\alpha}$$

Wang and coworkers [43] established the functional relationships for *k* and α as a function of % DD of chitosan when chitosan is dissolved in 0.2M CH_3COOH/0.1M CH_3COONa aqueous solution at 30°C.

$$k = 1.64 \times 10^{-30} \times (\%DD)^{14}$$

$$\alpha = -1.02 \times 10^{-2} \times (\%DD) + 1.82$$

The $\overline{M}_v$ chitosan was determined by the method of Wang et al. [43]. A known amount of thoroughly dried chitosan was dissolved in 0.2M CH_3COOH/0.1M CH_3COONa aqueous solution and a series of dilute solutions were prepared. A Cannon-Ubbelohde Semi-micro Viscometer (size 75, No. N177, Viscometer Constant = 0.00745 mm^2/s^2 (cSt/s)) was charged with 3 mL of each solution and equilibrated to 30°C in a water bath. Ten flow times were recorded at each concentration and averaged. Specific viscosity (η_{sp}) were calculated according to the following equation,

$$\eta_{sp} = \frac{t - t_s}{t_s},$$

where t is a sample flow time and t_s is a solvent flow time. The results of viscosity measurement are reported in Tables 4 and 5.

Table 4. Scheme of intrinsic viscosity measurement for the commercial chitosan.

c (g/mL)	0 (solvent)	0.009933	0.007946	0.005960	0.003973	0.001987
Time (sec)	113.57	196.87	177.78	159.55	142.93	127.49
η_{sp}		0.73347	0.56538	0.40486	0.25852	0.12257
η_{sp}/c		73.84	71.15	67.93	65.07	61.69

Table 5. Scheme of intrinsic viscosity measurement of the deacetylated chitosan.

c (g/mL)	0 (solvent)	0.009947	0.007958	0.005968	0.003979	0.001989
Time (sec)	113.57	184.72	168.79	153.61	139.49	126.02
η_{sp}		0.62649	0.48622	0.35256	0.22823	0.10962
η_{sp}/c		62.98	61.10	59.08	57.36	55.11

The intrinsic viscosity [η] was determined by extrapolating the linear regression of plots of η_{sp}/c versus c, where c is concentration of chitosan solution (g/mL), to zero concentration (Figures 34 and 35). The DD obtained by NMR method was used to calculate k and α, which were used together with [η] to calculate $\overline{M}_v$ of chitosan using the Mark-Houwink equation. The properties of the commercial and deacetylated chitosans are provided in Table 6. As a result of the alkaline treatment, a highly deacetylated chitosan with low MW was obtained. The deacetylated chitosan will be used for the synthesis.

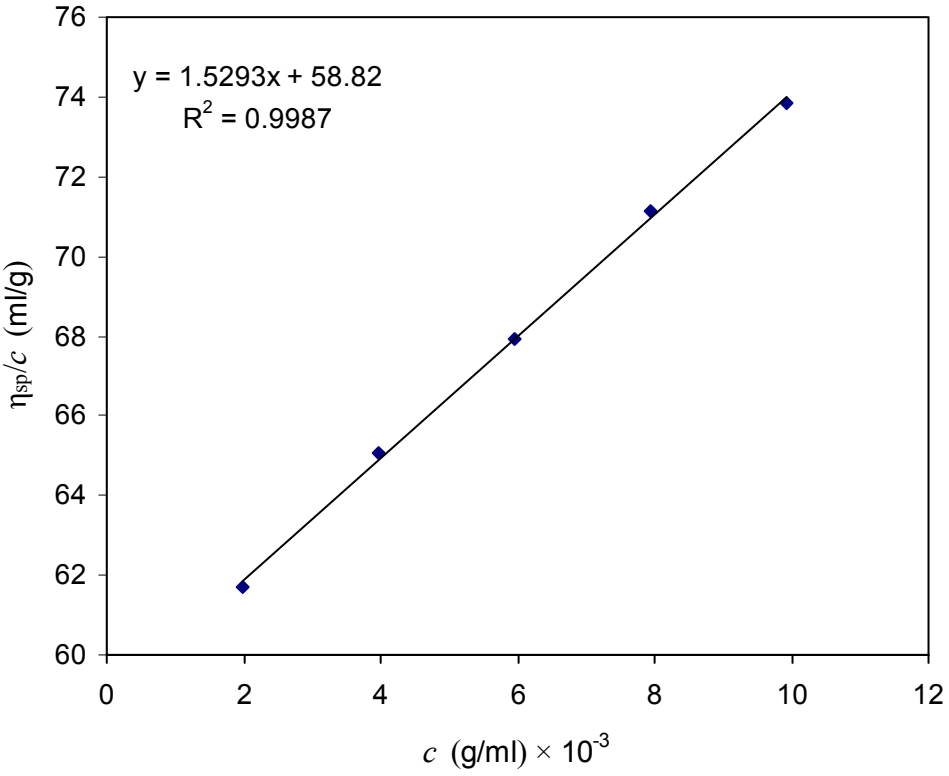

Figure 34. Huggins plot of η_{sp}/c versus c for the commercial chitosan in 0.2M CH_3COOH/0.1M CH_3COONa aqueous solution.

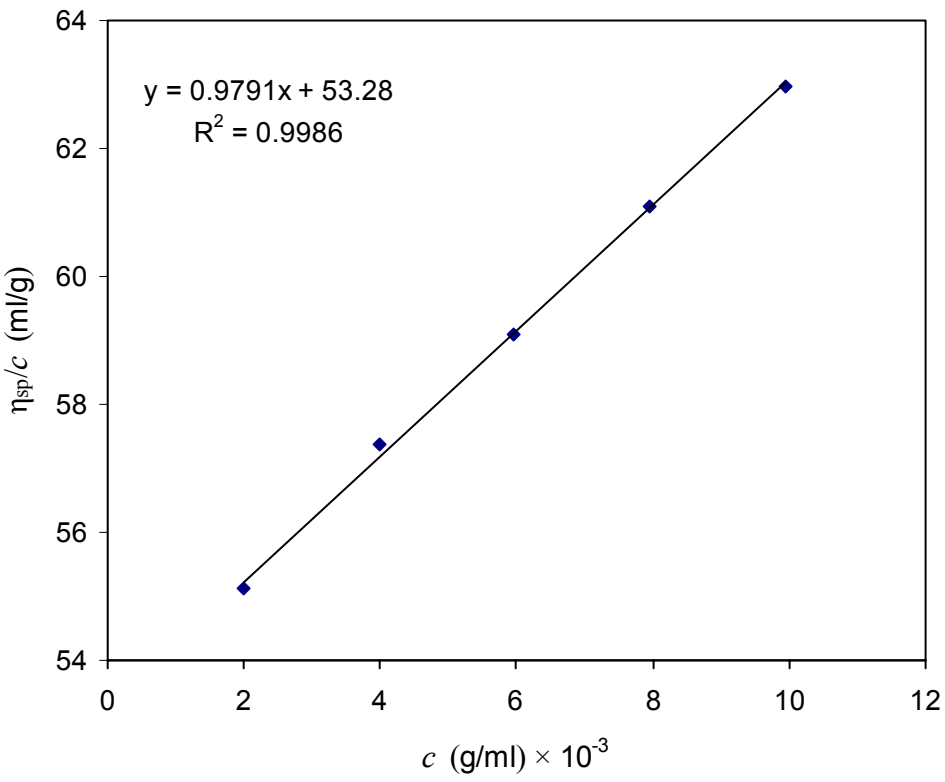

Figure 35. Huggins plot of η_{sp}/c versus c for the deacetylated chitosan in 0.2M CH_3COOH/0.1M CH_3COONa aqueous solution.

Table 6. Properties of the commercial and deacetylated chitosan.

Chitosan	%DD	k	α	[η]	$\overline{M}_v$
Commercial	92.2%	0.005261	0.8796	58.82	40,100
Deacetylated	97.1%	0.01086	0.8296	53.29	28,100

7. PREPARATION OF WATER-SOLUBLE CHITOSAN DERIVATIVE

Polymeric quaternary ammonium compounds have received the most attention as biocidal polymers over the years [77]. However, they have some deficiencies, such as non-biodegradability and no affinity to cotton. Chitosan dissolves in water only in acidic conditions by the protonation of the amino groups and thus its antimicrobial activity is limited to acidic conditions [53]. Therefore, introduction of quaternary ammonium salts onto the chitosan backbone will be one of the best methods to give antimicrobial activity as well as the water solubility over the entire pH range.

Glycidyltrimethylammonium chloride (GTMAC) was chosen as a quaternization reagent because of its ease of reaction with amino groups of chitosan and it is known that the reaction product, *N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (referred to here as HTCC) shows water-solubility as well as an excellent antimicrobial activity [83,140,142]. The HTCC (Figure 36) can be prepared by reacting chitosan with GTMAC in a neutral aqueous condition, in which the hydroxyl groups of chitosan are not sufficiently nucleophilic to induce ring opening of GTMAC, whereas the amino group of chitosan is nucleophilic enough to do that.

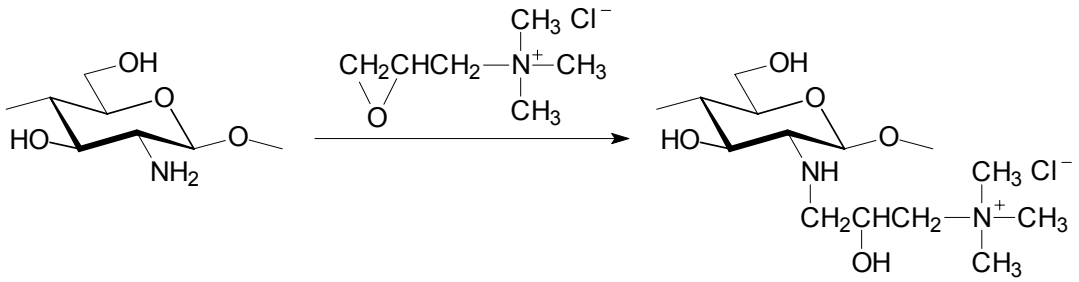

Figure 36. Preparation of HTCC.

7.1. Synthesis of HTCC

The HTCC was prepared by a modified method of Lang et al. [82]. The deacetylated chitosan (6 g) was dispersed in 60 mL of distilled water at 85°C. GTMAC was added three times (one mole equivalent to amino group of chitosan each time) at two hour intervals. After a total 10 hr reaction, the clear and yellowish reaction solution was poured in 200 mL cold acetone while stirring and kept in the refrigerator overnight. The next day, acetone was decanted and the remaining gel-like product was dissolved in 100 mL methanol. The solution was precipitated in a 250 mL acetone:ethanol (4:1) mixture. The white product was collected by filtration and it was further purified by washing with hot ethanol using a Soxhlet extractor for 24 hrs. The final product was dried at 70°C overnight. The weight of the final product was 10.19g and the yield calculated was 87.8%.

7.2. FT-IR Spectroscopy

The IR spectrum of the HTCC is shown in Figure 37. It shows evidence of the quaternary ammonium salt group, at 1480 cm^{-1}, the C-H bending of trimethylammonium group. It should be also noted that the N−H bending (1595 cm^{-1}) of the primary amine disappeared due to the change of the primary amine to the secondary amine (aliphatic) [155]. A new peak at 1650 cm^{-1} was assigned for the C=O stretch of the secondary amide (2.9 % on the deacetylated chitosan), which was a shoulder of the N−H bending peak at 1590 cm^{-1} as shown in Figure 31 in Section 6.2. In addition, the spectrum shows a broad band at around 3400 cm^{-1}, probably due to the increased number of hydroxyl groups.

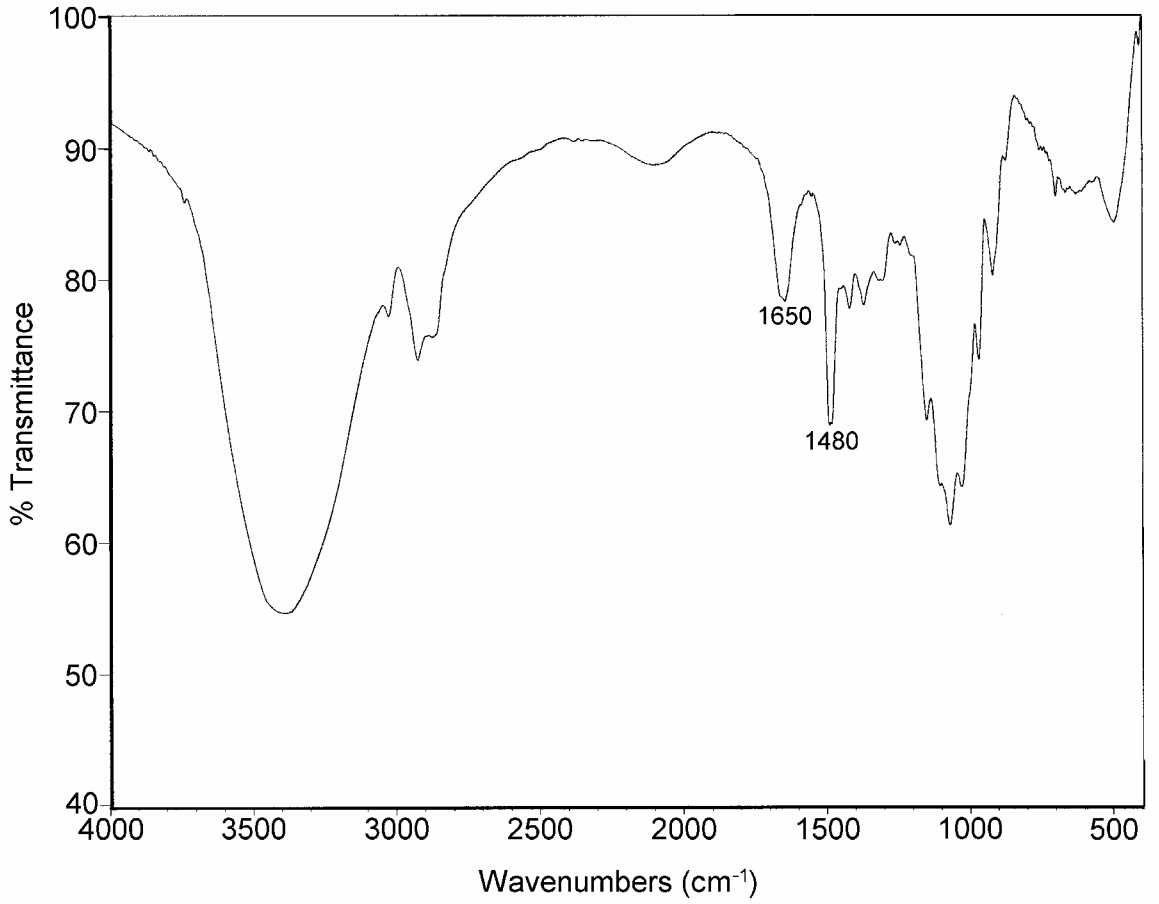

Figure 37. FT-IR spectrum of the HTCC.

7.3. ^{1}H NMR Spectroscopy

A known amount (7.5 mg) of sample was dissolved in 0.75 g of D_2O and TSP was used as an internal reference. The NMR spectrum of the sample is shown in Figure 38. As evidence of the reaction, the methyl group in the quaternary ammonium salt group was observed as a very strong peak at 3.2 ppm.

7.4. Conductometric Titration for Degree of Substitution (DS)

The DS of GTMAC on chitosan was measured by titration of Cl^- with aqueous silver nitrate ($AgNO_3$) solution. Thoroughly dried HTCC (0.1000 g) was dissolved in 100 mL of deionized water and conductometrically titrated with 0.017N $AgNO_3$ aqueous solution using a 10-mL buret. Solution conductivities were monitored with an Orion Benchtop Conductivity Meter (Model 162) equipped with an Orion Conductivity Cell (Model 013030). During the titration, the temperature of the solution was kept constant (20.4 - 20.5 ºC) by using a water bath because the conductivity is a function of temperature.

The titration curve for the HTCC is provided in Figure 39. Before the inflection point, the curve has a negative slope, which corresponds to the decrease in conductivity due to the precipitation of AgCl. The positive slope after the inflection point results from the excess $AgNO_3$. The amount of $AgNO_3$ used (18.62 mL) at the inflection point equals to the amount of Cl^- ions present on the HTCC. Since 1 mL of 0.017N $AgNO_3$ is equivalent to 1 mg NaCl, 0.1000 g of the HTCC contains 3.186×10^{-4} moles of Cl^- ions.

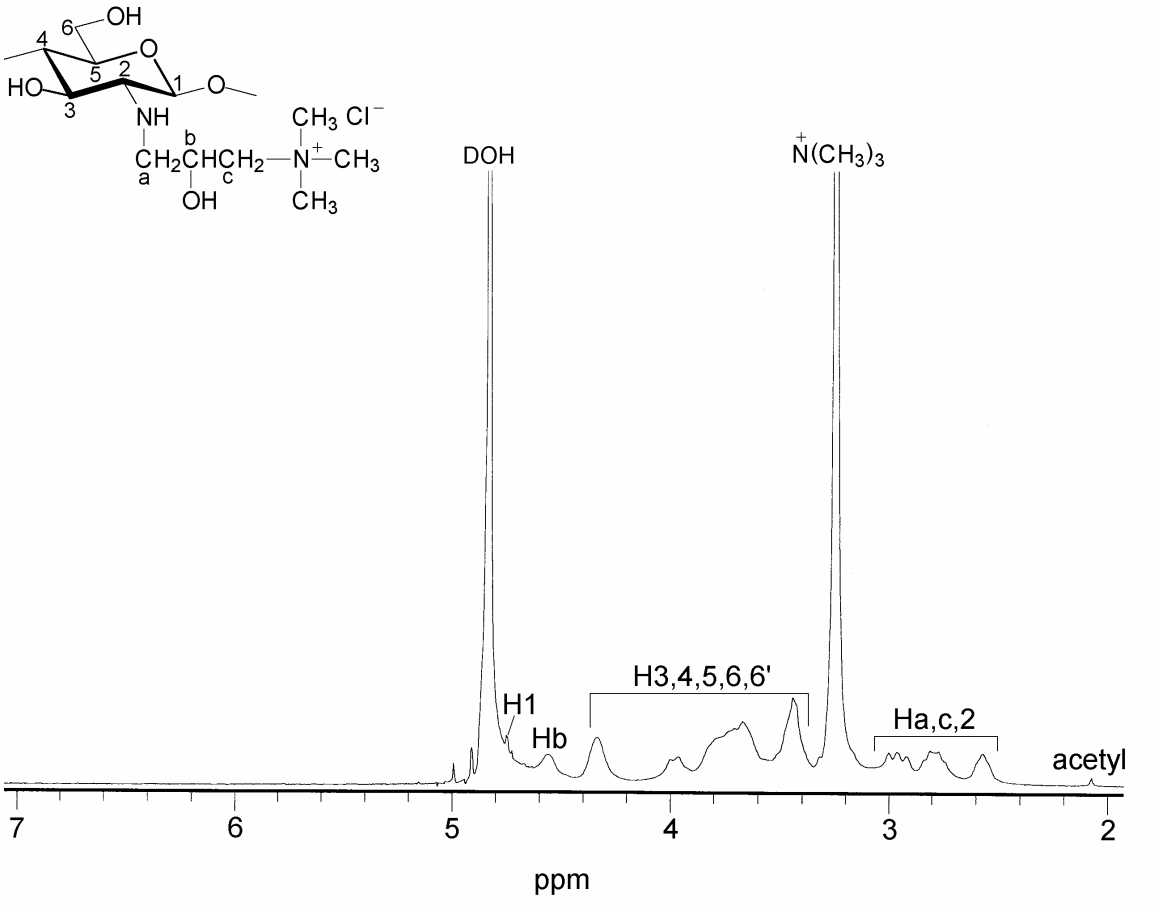

Figure 38. 300 MHz ^{1}H NMR spectrum of HTCC.

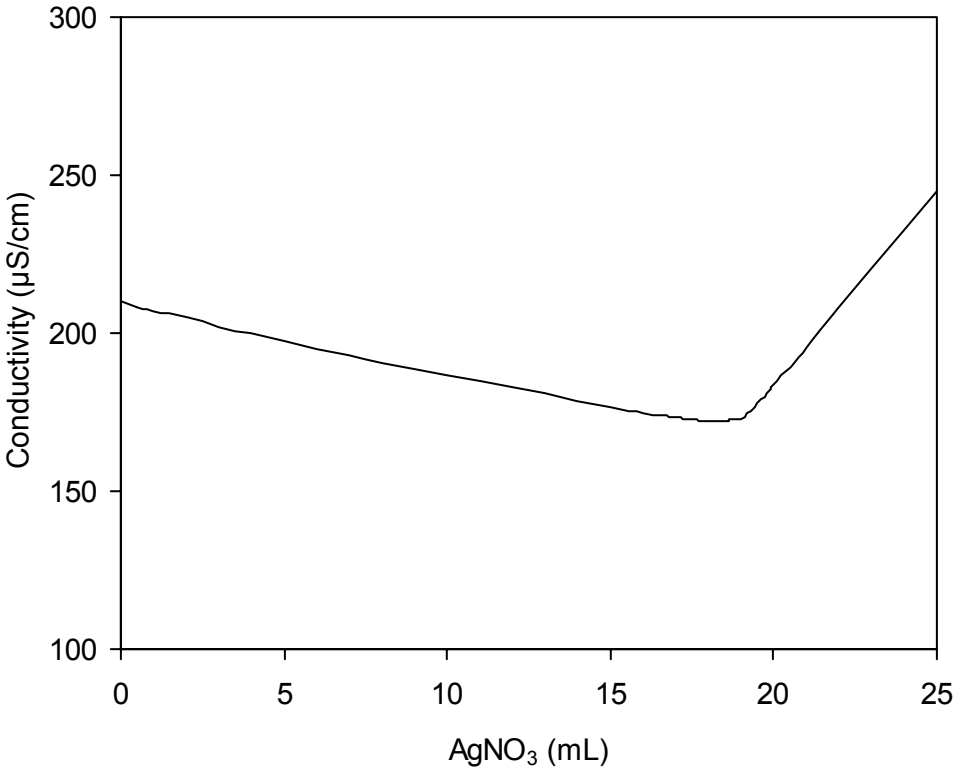

Figure 39. Conductometric titration curve for the HTCC.

The DS of the derivatives can be calculated by the following equation.

$$DS = \frac{M \times N_{Cl}}{m_{dry}}$$

where M is the molecular weight (g/mol) of glucosamine repeat unit when the DS is 1.00 (314.01 g/mol), N_{Cl^-} is the number of moles of Cl^- ions in the samples, and m_{dry} is the mass of dried sample in grams. The value of DS calculated was 1.00.

8. PREPARATION OF FIBER-REACTIVE CHITOSAN DERIVATIVE

The last step of synthesis is the introduction of a fiber-reactive group on the HTCC to overcome the poor laundering durability of chitosan [134,140,146]. *N*-Methylolacrylamide (NMA) was chosen as a reagent to introduce a fiber-reactive group onto the HTCC because it has often been used for crosslinking of cellulose [156-159]. NMA (CH_2=CHCONHCH_2OH) has two reactive groups, which react under different conditions, a *N*-methylol group and a double bond conjugated with a carbonyl group. By reacting NMA with HTCC under acidic conditions, acrylamidomethyl-HTCC (referred to here as NMA-HTCC) is obtained as shown in Figure 40.

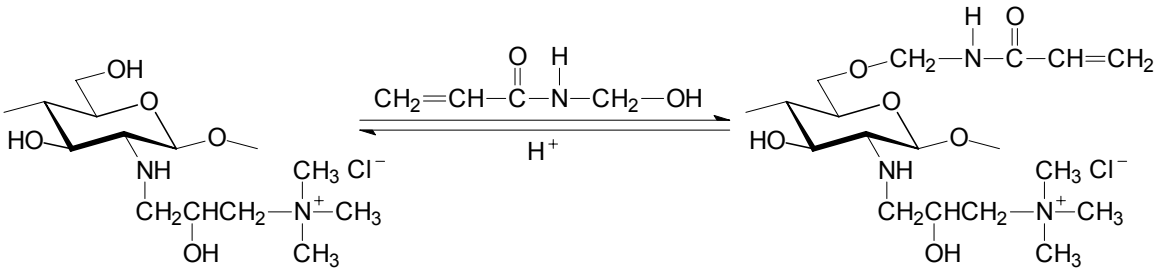

Figure 40. Preparation of NMA-HTCC.

The detailed mechanism of the reaction (acrylamidomethylation) is shown in Figure 41. The proton of the acid catalyst is transferred to the hydroxyl group of NMA and H_2O is eliminated to give a carbonium ion, which is stabilized by resonance structures. This ion reacts with a nucleophilic group, i.e., the hydroxyl group (C-6) of the HTCC, which is a primary alcohol.

As an acid catalyst, ammonium chloride (NH_4Cl) [159] was used, which is a latent acid and generates HCl at an elevated temperature as shown in Figure 42.

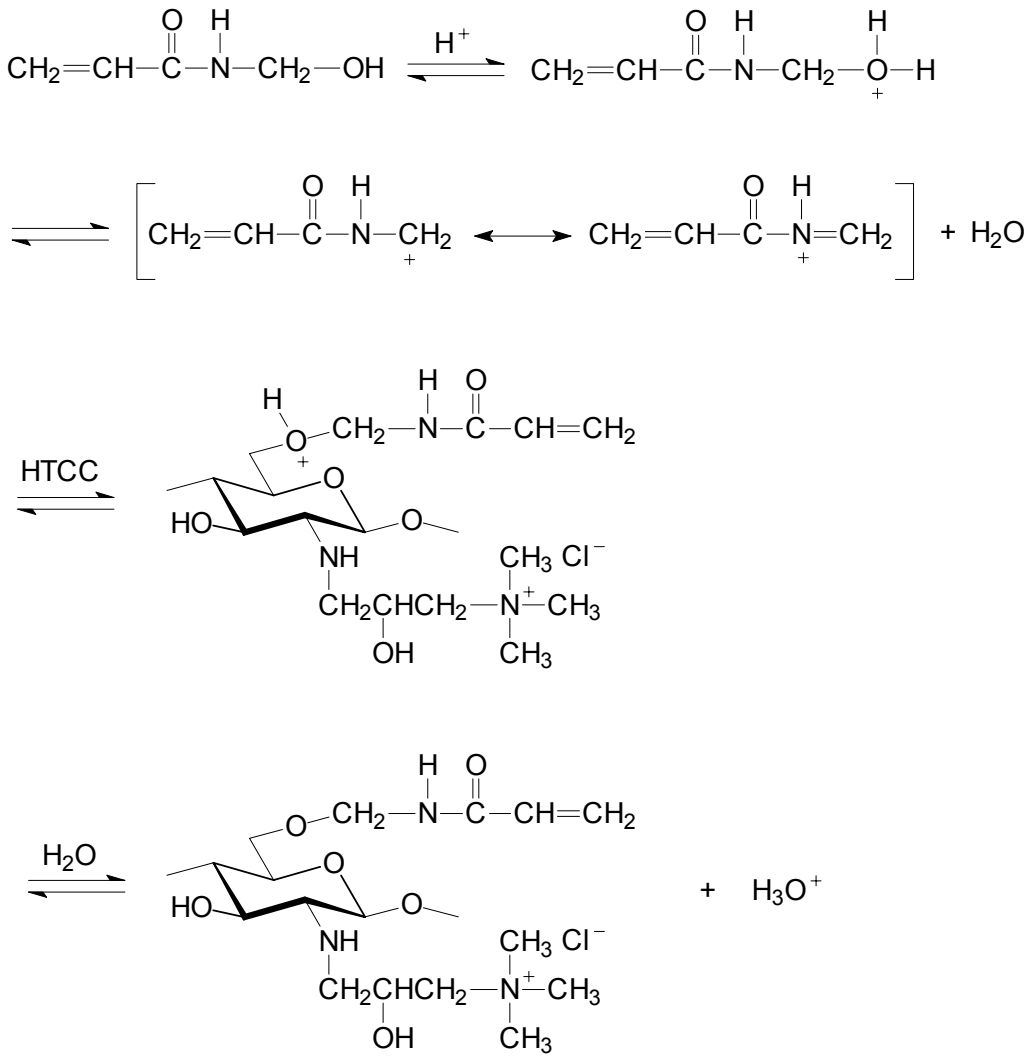

Figure 41. Reaction mechanism between NMA and HTCC.

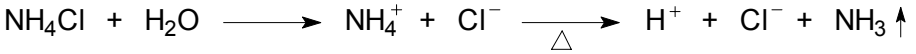

Figure 42. Release of HCl from NH_4Cl.

The released acid can protonate secondary amine groups of HTCC, which prevent the amine groups from acting as a nucleophile. Also, the amine group of the HTCC is in a relatively bulky environment due to the quaternary ammonium salt groups. Therefore, the reaction between NMA and hydroxyl groups on C-6 position (primary alcohol) of HTCC is predominant. The NMA-HTCC has acrylamidomethyl groups as a fiber-reactive group, which has a pendent double bond that can react with the hydroxyl groups of cellulose under alkaline conditions.

8.1. Synthesis of NMA-HTCC

The HTCC (1 g) was dissolved in 5 mL of 48 wt. % aqueous NMA solution (8 mole excess to HTCC) containing a small amount of 4-methoxyphenol (0.2% w/v) as a polymerization inhibitor at room temperature. To the solution, NH_4Cl (1 - 4 mole excess to HTCC) was added and dissolved. The reaction solution was reacted at 140°C for 8 - 16 min. After reaction, 15 mL of methanol was added to the reaction solution and it was stirred for 10 sec. The product was precipitated in 100 mL acetone and washed thoroughly with a mixture of acetone:ethanol (1:1) and finally with ether. The white reaction product was dried at 40°C under vacuum for 2 days. The weights of the products were 1.02-1.16g depending on their reaction conditions.

8.2. Double Bond Content

One of the methods for determining double bonds adjacent to electron-withdrawing groups is to react the compound containing double bonds with a thiol and

then titrate the excess of unreacted thiol with iodine. It is known that aliphatic alcohols do not interfere with the results because the double bonds react much faster with thiols than alcohols [156].

The double bond content of the NMA-HTCC was determined by the method used by Kamel et al. [157]. A known amount of NMA-HTCC (0.3 g) was dissolved in 10 mL deionized water contained in a weighing bottle at room temperature. To the solution, 5 mL of 3% 2-mercaptoethanol($HOCH_2CH_2SH$) aqueous solution and 1 mL 2M NaOH were added and stirred in a closed weighing bottle at room temperature for 20 min to ensure the complete nucleophilic addition of mercaptoethanol to the double bond. The mixture was acidified with 2.5 mL 1N HCl. After the addition of several drops of starch indicator, it was titrated against a 0.1N iodine (I_2) solution until the endpoint at which the first faint blue color that persisted for at least 30 sec. Figure 43 shows the oxidation reaction of thiol with iodine [160]. The endpoint corresponds to the complete oxidation of the remaining thiols and after that point excess iodine shows blue color by interacting with starch indicator. A blank was run in an identical manner.

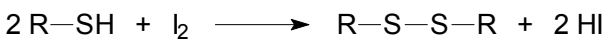

Figure 43. Oxidation of thiol by iodine.

The double bond content was calculated using the following equation,

$$\text{Double bond content (mmol/g NMA} - \text{HTCC)} = \frac{(V_B - V_S) \times 0.1}{W},$$

where W is the weight of sample in grams, V_B and V_S are the amount (mL) of iodine solution used in blank and sample titrations, respectively, and 0.1 is the molarity of the iodine solution.

8.3. Optimal Reaction Conditions for the Acrylamidomethylation

The extent of the acrylamidomethylation, which is a measure of double bond content was examined at different reaction conditions. As variable parameters, the amount of catalyst (NH_4Cl) and reaction time were selected. There were no changes in the amount of NMA (8 mole excess) and reaction temperature (140ºC). The double bond content of the each sample was summarized in Tables 7 and 8.

The effect of the catalyst on the double bond content is provided in Figure 44. The double bond content increased as the mole ratio of catalyst increased from 1 to 3. However, further increase in the amount of catalyst showed an adverse effect. The acrylamidomethylation is a reversible reaction as shown in Figure 41. It is likely that the increased amount of NH_4Cl liberated more HCl, which increased hydrolysis of the ether linkage between the acrylamidomethyl group and the HTCC.

Figure 45 shows the effect of reaction time on the acrylamidomethylation. At the reaction time of 10 min, the double bond content was maximal and considerablely higher than that at 8 min. However, further increase in the reaction time decreased the double bond content. The adverse effect at longer reaction time (12 - 16 min) can be attributed to the increased hydrolysis of the ether linkage between the HTCC and the

acrylamidomethyl group. Thus, the optimal reaction time (10 min) is required to increase the acrylamidomethylation and compensate for the hydrolysis.

Table 7. Double bond content at different concentration of catalyst (NH_4Cl)[a].

Mole ratio of NH_4Cl to HTCC	1	2	3	4
Double bond content (mmol/g NMA-HTCC)	0.84	0.89	0.98	0.95

a. Reaction condition: NMA 8 mole excess, temperature 140ºC, time 10 min.

Table 8. Double bond content at different reaction time[a].

Reaction time (min)	8	10	12	14	16
Double bond content (mmol/g NMA-HTCC)	0.62	0.98	0.95	0.91	0.77

a. Reaction condition: NMA 8 mole excess, NH_4Cl 3 mole excess, temperature 140ºC.

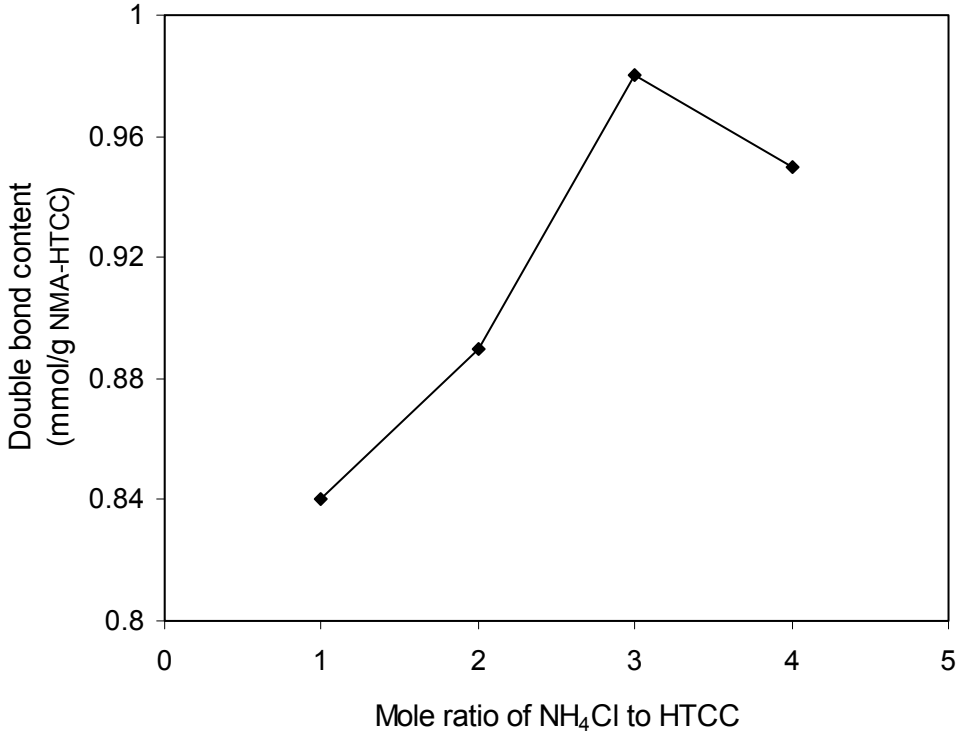

Figure 44. Effect of the amount of catalyst (NH_4Cl) on the double bond content (reaction condition: NMA 8 mole excess, temperature 140ºC, time 10 min).

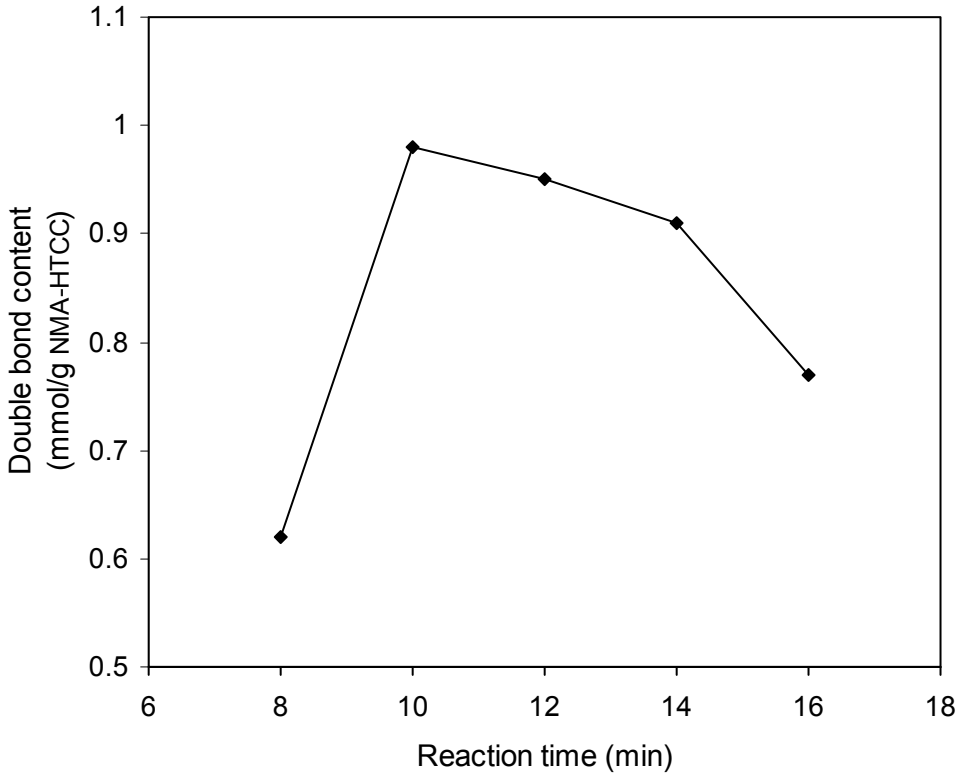

Figure 45. Effect of the reaction time on the double bond content
(reaction condition: NMA 8 mole excess, NH_4Cl 3 mole excess, temperature 140ºC).

Another possible explanation of the decrease in double bond content at higher concentration of catalyst and longer reaction time can be the increased hydrolysis of NMA (reactant) by HCl as shown in Figure 46.

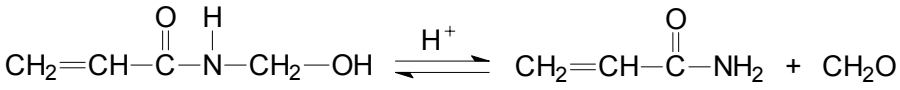

Figure 46. Hydrolysis of NMA by HCl.

From the results, the optimal reaction conditions obtained for the acrylamido-methylation were as follows: MNA: 8 mole excess, NH_4Cl: 3 mole ratio, reaction time: 10 min, and temperature: 140°C. The NMA-HTCC was prepared at this reaction condition and used for the characterization and application.

8.4. FT-IR Spectroscopy

The IR spectrum of the NMA-HTCC, shown in Figure 47, indicates the acrylamidomethylation did occur as the result of the peaks at 1670 and 1545 cm^{-1}. These peaks are most likely due to the C=O stretch and N–H bending of the secondary amide in the acrylamidomethyl group, respectively. As further evidence of the reaction, a new peak appears at 1630 cm^{-1}, which corresponds to the C=C stretch of the conjugated vinyl group.

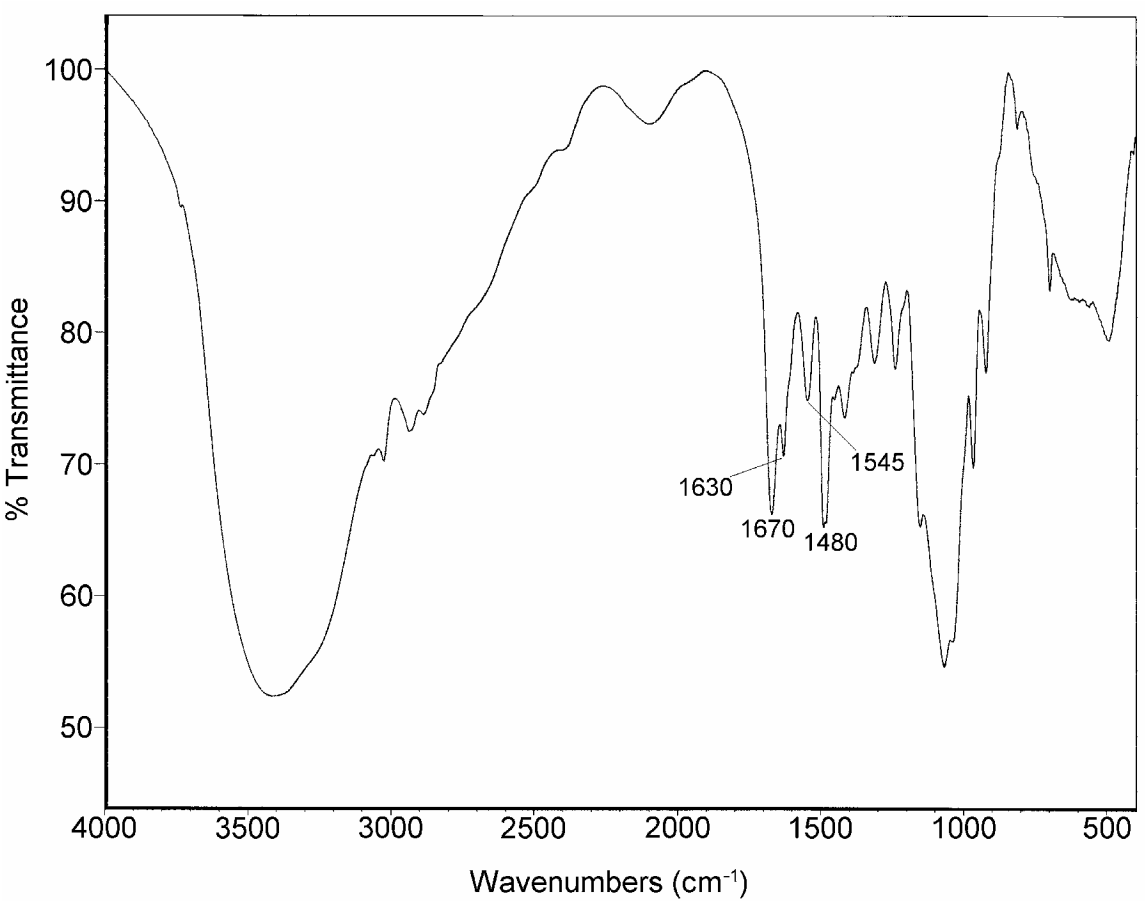

Figure 47. FT-IR spectrum of the NMA-HTCC.

8.5. ^{1}H NMR Spectroscopy

A known amount (7.5 mg) of sample was dissolved in 0.75 g of D_2O and TSP was used as an internal reference. The NMR spectrum of the sample is provided in Figure 48. The acrylamidomethylation was confirmed by the peaks at 6.3 and 5.9 ppm, which corresponds to the vinyl group.

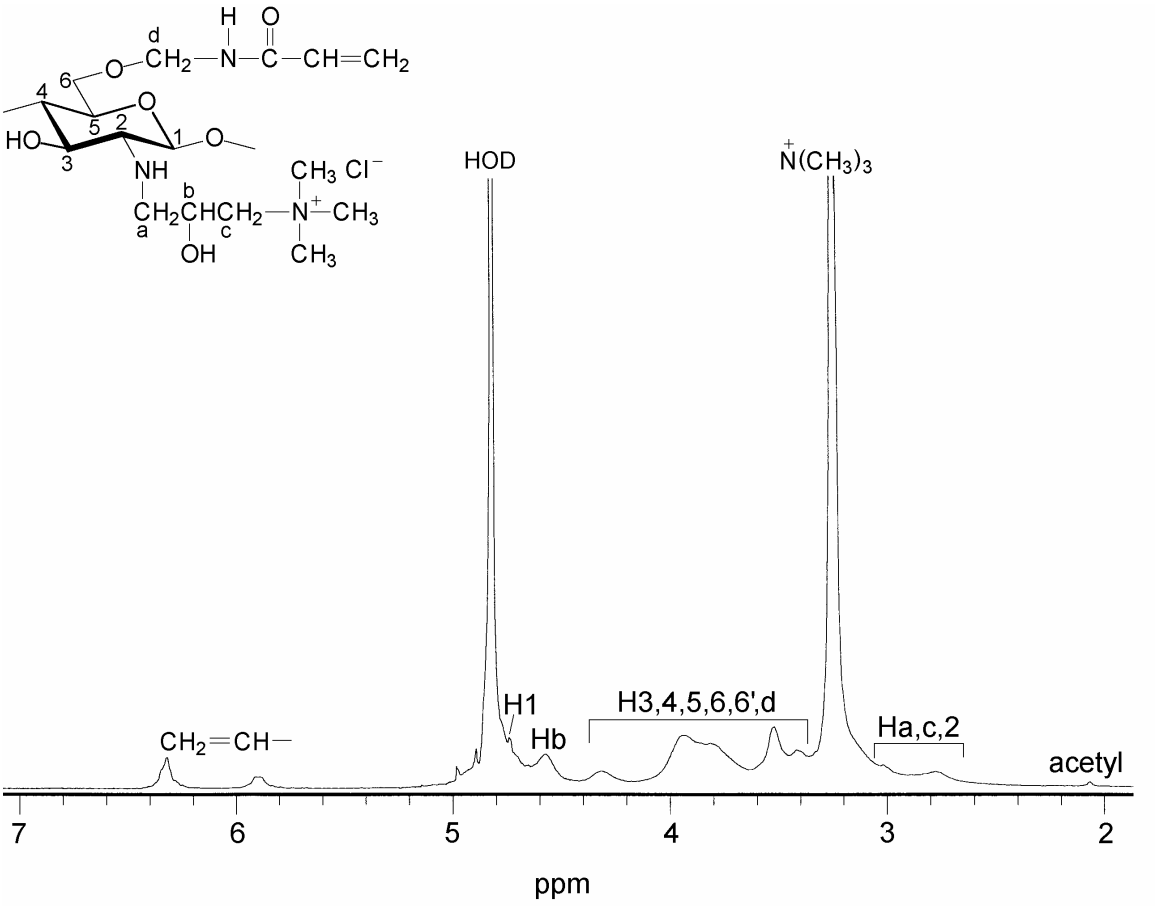

Figure 48. 300 MHz ^{1}H NMR spectrum of the NMA-HTCC.

8.6. Antimicrobial Activity of the NMA-HTCC

Prior to the application of the NMA-HTCC to cotton fabric, its antimicrobial activity was evaluated according to the method used by Ikeda et al. [161]. Antimicrobial activity of the chitosan derivative was examined against *Staphylococcus aureus* (gram positive, ATCC 6538) and *Escherichia coli* (gram negative, ATCC 25922), which were obtained from American Type Culture Collection (ATCC). These two bacteria were selected because they are commonly found in the human body [131].

Each culture was suspended in a small amount of Difco™ nutrient broth, spread on the Bacto™ nutrient agar plate, and incubated at 37ºC for 24 hrs. A single colony was picked off with an inoculating loop, placed in 5 mL nutrient broth, and incubated for 18 hrs at 100 rpm and 37ºC. A final concentration of 1.5-3.0 × 10^6 colony forming units per milliliter (CFU/mL) was prepared by appropriately diluting each culture with a sterile buffer solution (0.3 mM phosphate buffer, pH 7.2), which was used as a diluent in all experiments. These dilute culture solutions were used for the antimicrobial test. The NMA-HTCC was dissolved in the buffer solution at several different concentrations. To the 9.0 mL sample solutions, which were contained in test tubes (17 × 100 mm) and preequilibrated at 37ºC for 1 hr, 1.0 mL of each culture suspension was added. At the same time, 1.0 mL of the same culture was added to 9.0 mL of buffer solution and this was used as a control. The initial cell concentration was enumerated by a standard plate count technique from the control solution. At various contact times at 37ºC and 150 rpm, 0.2 mL portions were taken from each control and sample solution, and diluted by mixing with 9.8 mL buffer solution. From the dilutions, 0.5 mL and 0.15 mL portions for the

sample and control, respectively, were spread on the nutrient agar plates. The inoculated plates were incubated at 37ºC for 30 hrs and the surviving cells were counted. The average values of the duplicates were converted to CFU/mL in the test tube by multiplying the dilution factor.

The results were summarized in Tables 9 and 10. Figures 49 and 50 show log (CFU/mL) versus contact time plots for the NMA-HTCC against *S. aureus* and *E. coli*, respectively. About 1.7 - 2.4 × 10^5 CFU/mL of bacterial cells were contacted to 10, 50, 100, and 200 ppm of the NMA-HTCC in 0.3 mM phosphate buffer (pH 7.2). At the concentrations of 10, 50, and 100 ppm, all *S. aureus* cells were completely killed within 20, 40, and 60 min, respectively. Interestingly, the antimicrobial activity was inversely proportional to the concentration of the NMA-HTCC. The highest antimicrobial activity was observed at the lowest concentration (10 ppm) of the NMA-HTCC. Similarily, Sudarshan et al. [53] observed that the antibacterial activity of chitosan under an acidic condition (pH 5.8) was higher at lower concentration. It was explained by the charge interaction between the protonated chitosan and negatively charged bacterial surface. At lower concentrations, chitosan binds to the negatively charged cell surface, disturbs the cell membrane, and causes death of the cell by inducing leakage of intracellular components. Whereas, at higher concentrations, the protonated chitosan may coat the cell surface and prevent the leakage of intracellular components. In addition, the positively charged bacterial cells repel each other and prevent agglutination.

Table 9. Antimicrobial activity of the NMA-HTCC against *S. aureus*.

Sample Concentration	CFU/mL at various contact times			
	0 min	20 min	40 min	60 min
0 ppm (control)	2.36×10^5	2.48×10^5	2.36×10^5	2.43×10^5
10 ppm	2.36×10^5	0	0	0
50 ppm	2.36×10^5	1.0×10^2	0	0
100 ppm	2.36×10^5	3.8×10^3	5.0×10^2	0
200 ppm	2.36×10^5	1.73×10^4	2.01×10^4	1.36×10^4

Table 10. Antimicrobial activity of the NMA-HTCC against *E. coli*.

Sample Concentration	CFU/mL at various contact times			
	0 min	20 min	40 min	60 min
0 ppm (control)	1.68×10^5	1.76×10^5	1.74×10^5	1.71×10^5
10 ppm	1.68×10^5	0	0	0
50 ppm	1.68×10^5	0	0	0
100 ppm	1.68×10^5	0	0	0
200 ppm	1.68×10^5	0	0	0

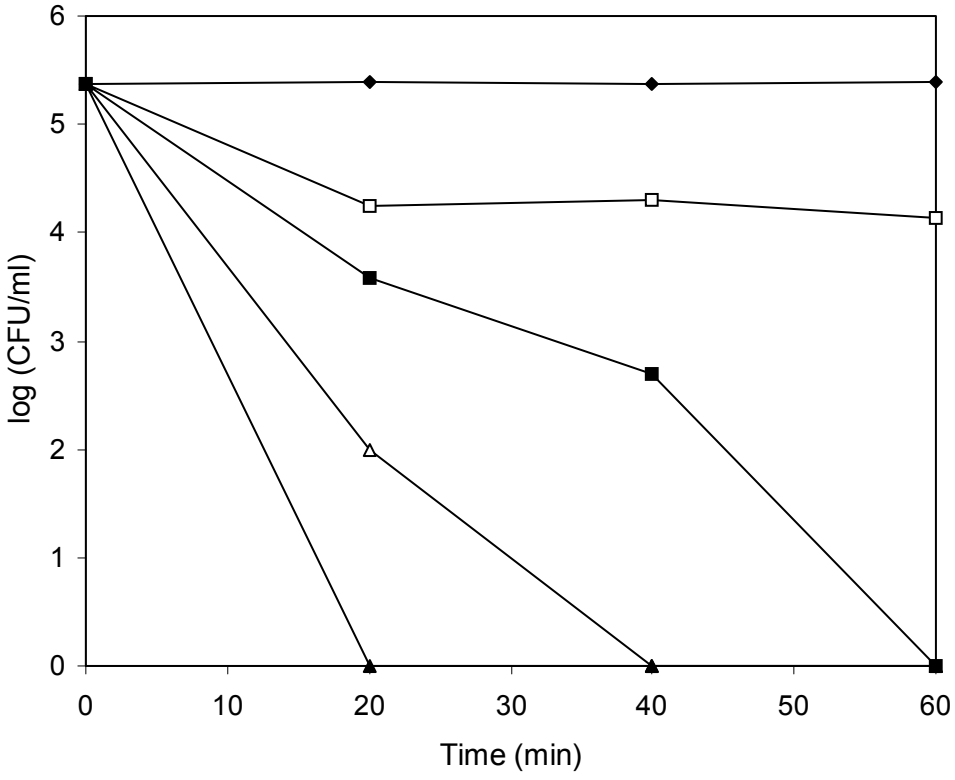

Figure 49. Antimicrobial activity of the NMA-HTCC against *S. aureus* (◆ control (0 ppm), □ 200 ppm, ■ 100 ppm, △ 50 ppm, and ▲ 10 ppm).

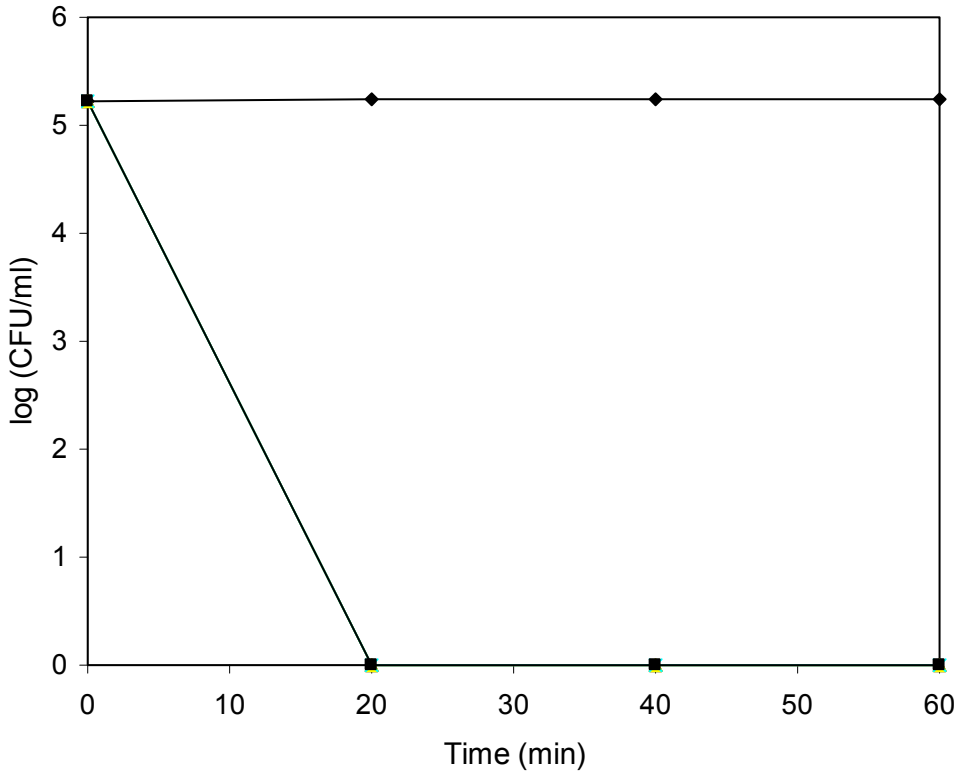

Figure 50. Antimicrobial activity of the NMA-HTCC against *E. coli* (◆ control (0 ppm), □ 200 ppm, ■ 100 ppm, △ 50 ppm, and ▲ 10 ppm).

According to Figure 50, it was found that all four concentrations were effective to kill *E. coli* cells completely within 20 min. The concentration-dependant behavior, which was observed in *S. aureus*, was not observed in *E. coli* at the same concentration ranges employed.

From the test results, it has been confirmed that the NMA-HTCC has an excellent antimicrobial activity against both *S. aureus* and *E. coli* compared to chitosan, which does not dissolve in pH 7.2 and does not show any antimicrobial activity under this condition. Even at a very low concentration (10 ppm), NMA-HTCC completely killed *S. aureus* and *E. coli* within 20 min. Further experiments on lower concentrations than 10 ppm were not performed because the final objective is the application of the NMA-HTCC to cotton fabric.

9. APPLICATION OF NMA-HTCC TO COTTON FABRIC

The NMA-HTCC can react with cellulose under alkaline conditions as shown in Figure 51. Two application methods, pad-dry-cure and pad-batch methods, were employed to apply the NMA-HTCC to cotton fabric. The former one is a common method to apply chemical finishes to textile fabrics. The latter one is one of the methods to dye cotton with reactive dyes. In this work, the two methods were tried and compared in terms of whiteness of the treated cotton fabrics, efficiency, and even distribution of the NMA-HTCC on the fabrics.

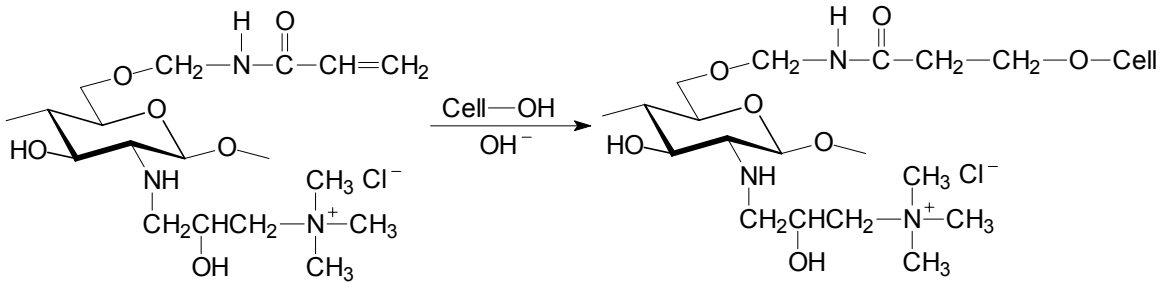

Figure 51. Reaction between NMA-HTCC and cellulose.

9.1. Test Fabric

As a test fabric, 100% cotton print cloth (Style # 400M, 107 g/m^2), which was bleached, desized, and mercerized, was purchased from Testfabrics, Inc., West Pittston, PA. Prior to the application of the NMA-HTCC, the fabric was further purified by washing with warm water in a home laundering machine (Kenmore). Non-ionic detergent (Kierlon NB-MFB, BASF) was added (1 g/L) for the washing. After washing, the fabric was rinsed with warm water three times and dried using a tumble dryer at 70ºC for 10 min.

9.2. Application Methods

9.2.1. Pad-dry-cure method

The pad solutions were prepared by dissolving the NMA-HTCC and an alkaline catalyst in distilled water. As an alkaline catalyst, sodium bicarbonate ($NaHCO_3$, from Fisher) was used because it is a latent alkaline catalyst [162], which is a mild alkali at room temperature but is converted to Na_2CO_3 during the curing as shown in Figure 52. It is expected that the mild alkalinity of $NaHCO_3$ can minimize the hydrolysis (Figure 53) of the NMA-HTCC in a pad solution before application. The formulas of four finish solutions for the pad-dry-cure method were as follows.

NMA-HTCC	1% owb (constant)
$NaHCO_3$	1, 3, or 5% owb

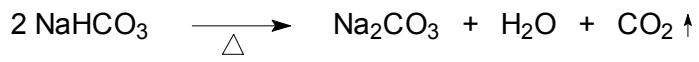

Figure 52. Conversion of $NaHCO_3$ to Na_2CO_3 by heat.

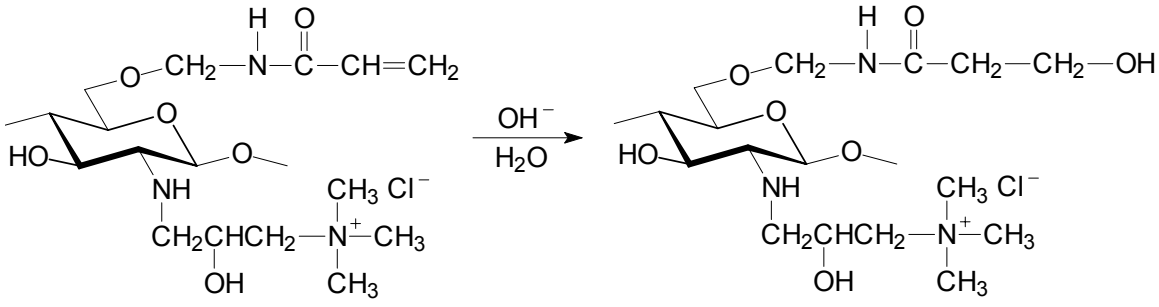

Figure 53. Alkaline hydrolysis of NMA-HTCC.

Cotton samples were padded with the finish solutions at 80% WPU using a laboratory padder (Werner Mathis AG). The padded samples were dried at 70°C for 4 min and then cured in a laboratory oven (Werner Mathis AG) for 5 min at three different temperatures (110, 130, and 150°C). The treated fabrics were washed with tap water until neutral to pH paper and further washed in warm water using a home laundering machine (normal cycle : washing(6 min)-rinsing-spinning) to remove unfixed NMA-HTCC. The fabric was air-dried at room temperature.

9.2.2. Pad-batch method

For the pad-batch application, two alkaline catalysts, NaOH and Na_2CO_3, were chosen because they are commonly used for a pad-batch method. Before the application of the NMA-HTCC, a preliminary experiment was performed, in which two cotton samples were padded with 1% owb NMA-HTCC solutions containing 2% owb of NaOH and Na_2CO_3, respectively. The two treated samples were placed in sealed plastic bags at room temperature for 24 hrs and washed. The fixation of the NMA-HTCC on cotton fabrics can be verified by dyeing the fabrics with an anionic dye because the NMA-HTCC contains quaternary ammonium salt groups. Therefore, two samples and an untreated sample were dyed with C.I. Direct Blue 78 (without adding salt) in a beaker for 30 min at boiling. From the dyeing result, it was found that the shade of the cotton treated in the presence of NaOH was much deeper than that of cotton treated in the presence of Na_2CO_3, which was slightly deeper than that of the untreated cotton. The

preliminary experiment revealed that Na_2CO_3 is not effective as an alkaline catalyst. Therefore, the final choice of an alkaline catalyst was NaOH.

The pad solutions for the pad-batch application were prepared as follows.

NMA-HTCC	1% owb (constant)
NaOH	0, 0.5, 1, 1.5, 2, 3, or 4% owb

Cotton samples were padded with each pad solution at 100% WPU, placed in plastic sample bags, and tightly sealed using Scotch™ tape to prevent air penetration into the bags. All samples were kept at room temperature (22ºC) for 24 hrs, washed with the same method described in Section 9.2.1, and air-dried.

9.3. Evaluation of NMA-HTCC Treated Fabrics

9.3.1. Whiteness

Cellulose is degraded by heat and the degradation involves oxidation and chain scission. The presence of aldehyde groups in oxidized cellulose makes it unstable and causes yellowing. The yellowing is further enhanced by the presence of alkali [163].

The two application methods employed here involve both heat and alkali, or alkali for the pad-dry-cure or pad-batch method, respectively. Therefore, the whiteness of the treated fabrics was evaluated. The CIE-Whiteness Index was measured on each fabric using a Datacolor Spectraflash® SF 300 spectrophotometer. The Shelyn SLI-Form software was used to calculate the whiteness index. The spectrophotometer settings were as follows:

Illuminant : D65-10
Reflectance Mode

Specular Component Included
Port Plate Size : 30 mm (Large Area View)
UV-Filter Out
Measurement : Polychromatic

Each fabric was folded twice and the whiteness was measured four times at different portions of the fabric surface. The average value was recorded.

The results of the CIE-Whiteness Index measurement are listed in Tables 11 and 12, and plotted in Figures 54 and 55. Figure 54 shows the effect of the curing temperature and amount of alkali on the whiteness of the fabrics treated by a pad-dry-cure method. For the fabric treated without an alkaline catalyst, the whiteness decreased slightly as temperature increased. However, the fabrics treated with an alkaline catalyst showed more significant reduction in whiteness. At lower temperatures (110 and 130°C), the whiteness decreased similarly regardless of the amount of alkali. Whereas, at a higher temperature (150°C), the higher amounts (3 and 5% owb) of alkali decreased the whiteness of fabrics compared to that of the fabric treated with a lower amount (1% owb) of alkali. The general trend was that the whiteness decreased by the presence of alkali and the increase of the curing temperature.

As shown in Figure 55, the whiteness of the fabrics treated by a pad-bath method decreased very slightly compared to that of fabrics treated by a pad-dry-cure method. The whiteness decreased slightly as the amount of alkali (NaOH) increased up to 1% owb. At the concentration of NaOH over 1% owb, there was no further decrease in whiteness.

Table 11. CIE-Whiteness Index of the fabrics treated by a pad-dry-cure method[a,b].

Curing temperature (ºC)	**Alkaline catalyst ($NaHCO_3$ % owb)**	**CIE-Whiteness Index[c]**
110	0	70.20
	1	62.68
	3	63.69
	5	64.42
130	0	68.77
	1	58.22
	3	59.10
	5	59.94
150	0	65.05
	1	50.21
	3	44.27
	5	44.23

a. All samples were dried at 70ºC for 4 min before the curing.
b. All dried samples were cured for 5 min.
c. CIE-Whiteness Index of the untreated fabric : 72.86.

Table 12. CIE-Whiteness Index of the fabrics treated by a pad-batch method[a].

Alkaline catalyst (NaOH, % owb)	0	0.1	0.5	1	1.5	2	3	4
CIE-Whiteness Index[b]	72.64	70.64	70.29	68.00	67.79	67.94	69.08	68.02

a. All samples were batched at room temperature for 24 hrs.
b. CIE-Whiteness Index of the untreated fabric : 72.86.

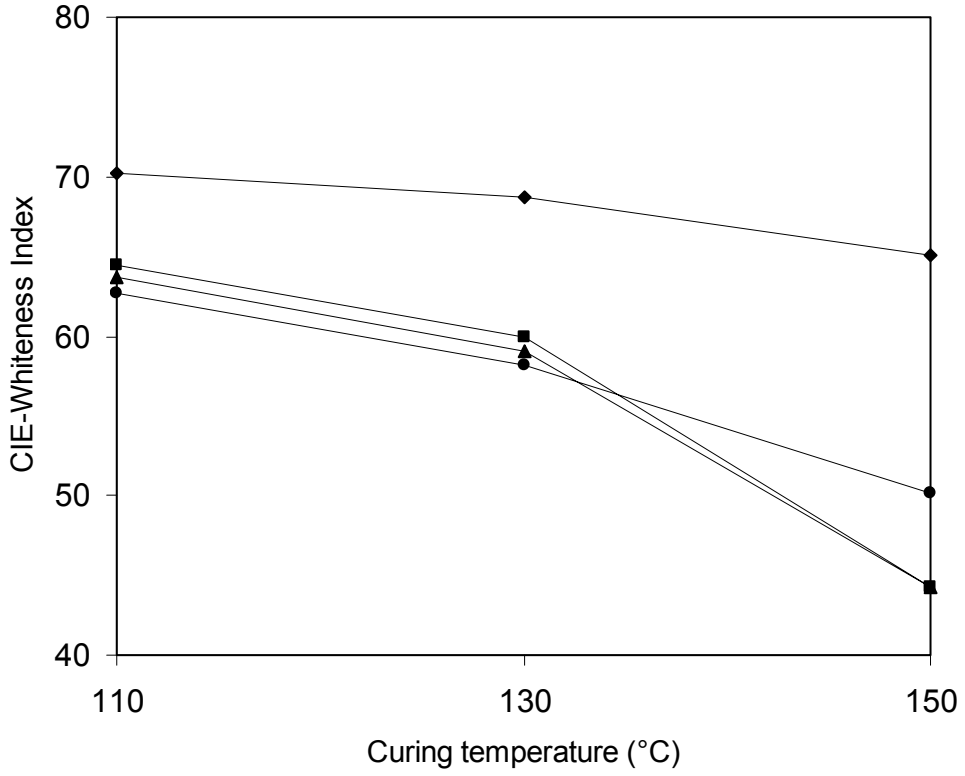

Figure 54. CIE-Whiteness Index for the fabrics treated by a pad-dry-cure method. (amount of alkali ($NaHCO_3$): ◆ no alkali (0% owb), ● 1% owb, ▲ 3% owb, and ■ 5% owb), (CIE-Whiteness Index of the untreated fabric : 72.86).

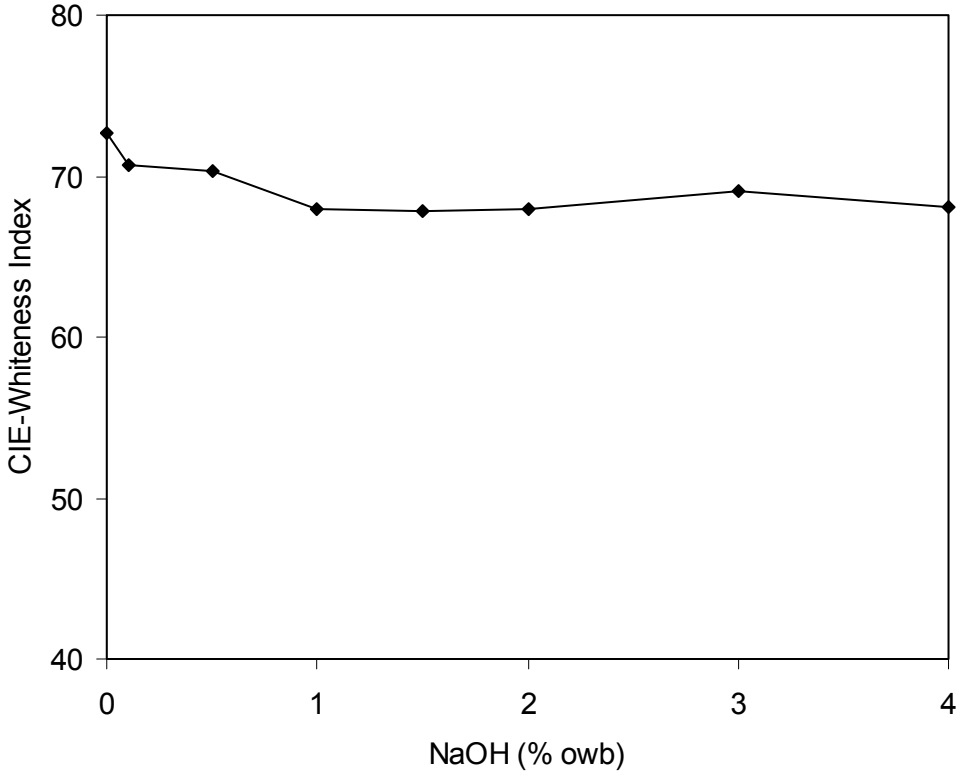

Figure 55. CIE-Whiteness Index for the fabrics treated by a pad-batch method. (CIE-Whiteness Index of the untreated fabric : 72.86).

9.3.2. Quantitative analysis of the amount of NMA-HTTC on fabrics

The amount of NMA-HTCC was measured by a stoichiometric dye adsorption method [164,165] using C.I. Acid Orange 7 as a dye indicator. In this method, the NMA-HTCC treated fabrics are dyed with excess amount of C.I. Acid Orange 7, which is an anionic dye and makes an ionic interaction with the quaternary ammonium salt groups on the NMA-HTCC. From the dyed fabrics, the dyes are extracted and the amount of dye is measured as a measure of the amount of NMA-HTCC fixed on the fabrics.

9.3.2.1. Purification of C.I. Acid Orange 7

The dye, C.I. Acid Orange 7, was purchased from Aldrich® with a purity of 87%. Before the dye adsorption experiment, the dye was further purified by recrystallization [166]. The dye was dissolved in boiling 80% aq. ethanol [165] to give a saturated solution. The dye solution was filtered through a glass funnel with filter paper (Whatman® No.54), which was preheated by placing over an Erlenmeyer flask containing boiling 80% aq. ethanol. The filtered dye solution was cooled in an ice bath and the dye crystals were filtered over a filter paper by suction. The dye crystals were resuspended and washed with cold 80% aq. ethanol in the suction filter three times. The dye was air-dried overnight, collected, and further dried in a vacuum oven at 70°C for 2 days.

9.3.2.2. Verification of the purity of the recrystallized C.I. Acid Orange 7

As a method to verify the purity of the recrystallized dye, the extinction coefficient (ε) of the dye in 0.1M acetic acid was measured and compared with a

reference value. A stock dye solution in 0.1M acetic acid was prepared to give absorbance less than 2 at λ_{max}. From the stock solution, five more dilute solutions were prepared. The absorbance at λ_{max} (= 484 nm) of each solution was measured using a Varian Cary 3 UV-VIS spectrophotometer. The data collection parameters are listed below and those were also used for all absorbance measurements in the remaining part of the work.

SBW (Slit Beam Width)	2.0 nm
Signal Averaging Time	0.10 sec
Data Interval	1.0 nm
Scan Rate	600 nm/min

A calibration curve was constructed, as shown in Figure 56, using the concentration (c, mol/L) of each dye solution and its absorbance (A). Based on the Beer-Lambert Law ($A = c\varepsilon l$, where l is cell path length (1 cm)), the slope of the calibration curve is the value of ε. The value of ε obtained was 22,400 L/mol·cm, which was almost the same as the reference value of 22,500 L/mol·cm [165]. This confirmed that the purity of the recrystallized dye is sufficient. Therefore, no further verification, such as elemental analysis, was attempted.

9.3.2.3. Extinction coefficient (ε) of C.I. Acid Orange 7 in 25% aq. pyridine

In the dye adsorption experiment, the dye on the dyed fabric was extracted using 25% aq. pyridine. Therefore, ε of C.I. Acid Orange 7 in 25% aq. pyridine was needed to calculate the amount of dye extracted from the fabrics. The calibration curve (Figure 57)

for C.I. Acid Orange 7 in 25% aq. pyridine was constructed by the same method used in section 9.3.2.2. The value of ε obtained at λ_{max} (= 490 nm) was 20,500 L/mol·cm.

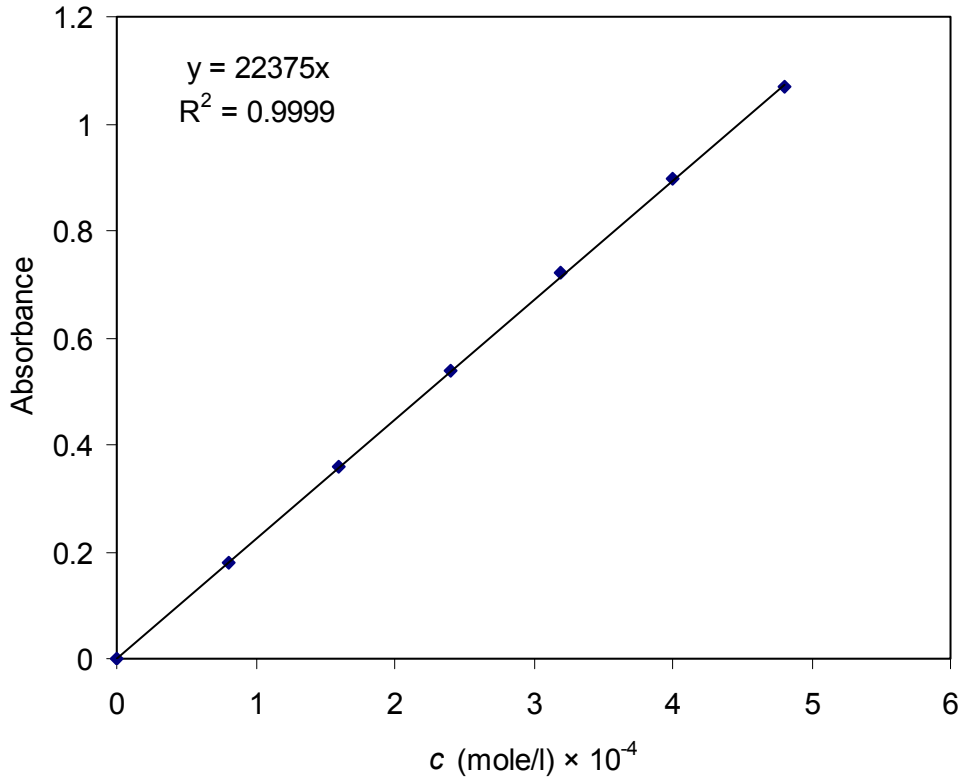

Figure 56. Calibration curve for C.I. Acid Orange 7 in 0.1M AcOH.

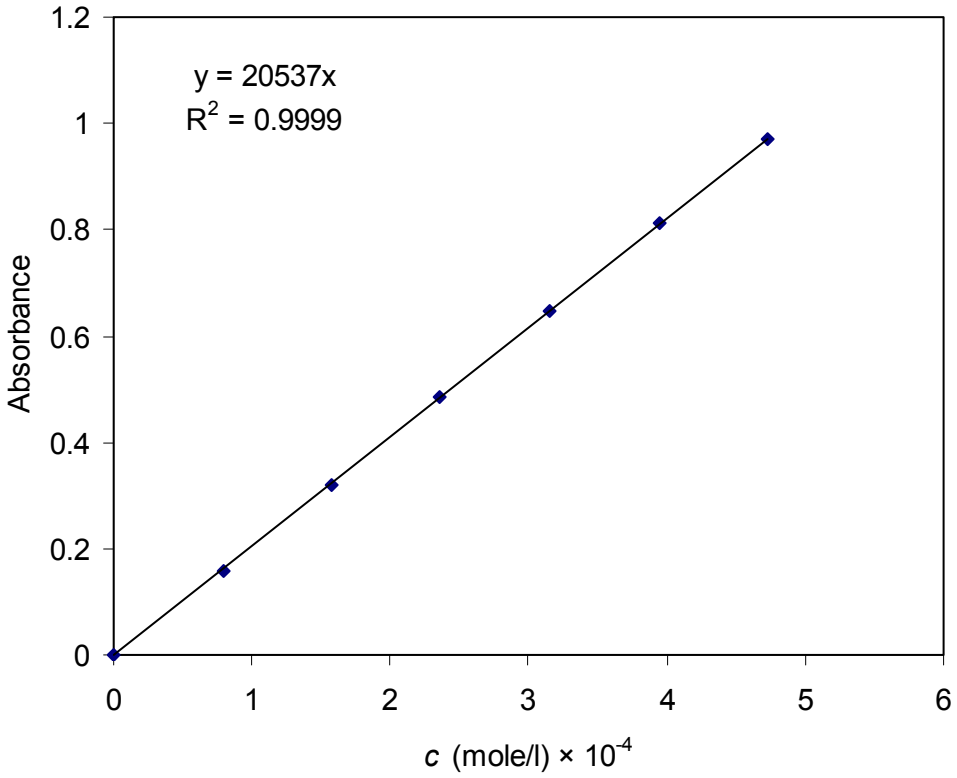

Figure 57. Calibration curve for C.I. Acid Orange 7 in 25% aq. pyridine.

9.3.2.4. Stoichiometric dye adsorption experiment

Each NMA-HTCC treated cotton fabric swatch (0.4 g) was transferred into a 250-mL Erlenmeyer flask with a screw cap. An 80 mL aq. dye (C.I. Acid Orange 7) solution (0.5 g/L) was added to each flask. The flasks were capped tightly and placed in a shaker incubator. All fabrics were dyed for 24 hrs at 50°C and 120 rpm. The dyed fabric swatches were thoroughly washed in tap water until the wash water did not show any color.

The dyed samples were dried at 60°C for 2 hrs and then conditioned at room temperature for 2 days before dye extraction. Each sample was cut to a small swatch (0.025 ~ 0.0450 g) and transferred into a vial (23 × 85 mm, from Fisher). To the vial, 10 mL of 25% aq. pyridine was added. All vials were tightly sealed with screw caps, placed in a shaker incubator, and stirred at 60°C and 150 rpm for 3 hrs. All sample swatches were removed from the vials. The vials were capped again and the extracted dye solutions were cooled down to room temperature. The absorbance of each dye solution was measured at λ_{max} (= 490 nm). Based on the absorbance (A), the concentration (c, mol/L) of the solution was calculated using the Beer-Lambert Law ($A = c\varepsilon l$), where ε is 20,500 L/mol·cm and l is 1 cm. The calculated concentration was converted to the amount of dye (mmol/kg fiber) adsorbed on the NMA-HTCC treated fabric by the following equation, where c is concentration (mol/L) of extracted dye solution and w is weight of fabric in grams.

$$\text{Amount of dye (mmol/kg fiber)} = (c/w) \times 10^4$$

The amount of dye extracted was used as a direct measure of the amount of quaternary ammonium groups on the NMA-HTCC treated fabric by the following two assumptions:

(1) There exists a 1:1 stoichiometry between the dye and the quaternary ammonium group of the NMA-HTCC.

(2) Under the dyeing condition (50ºC, 24 hrs), the quaternary ammonium groups on the NMA-HTCC treated fabric are completely saturated with the dye molecules.

The amount of quaternary ammonium groups for each sample was corrected by subtraction of a control (0.024 mmol/kg), which is a cotton not treated with the NMA-HTCC. The results are listed in Tables 13 and 14.

Figure 58 shows the amount of quaternary ammonium groups on the fabrics treated by a pad-dry-cure method. The general trend was that the amount of quaternary ammonium groups increased as the curing temperature and the amount of alkaline catalyst ($NaHCO_3$) increased. From the plot, it seems that 3% owb $NaHCO_3$ is the most effective because the quaternary ammonium content increased linearly as the temperature increased. It should be noted that there is some fixation of NMA-HTCC on cotton without an alkaline catalyst. The fixation increased linearly as the curing temperature increased. The fixation should be from hydrogen bonding and van der Waals' forces due to the presence of hydroxyl groups on cellulose and NMA-HTCC, and the similar chemical structure between cellulose and chitosan backbone, respectively.

Table 13. Quaternary ammonium content on the fabrics treated by a pad-dry-cure method[a,b].

Curing temperature (ºC)	Alkaline catalyst ($NaHCO_3$ % owb)	Quaternary ammonium (mmol/kg fiber)
110	0	0.249
	1	2.278
	3	2.612
	5	2.373
130	0	0.393
	1	4.081
	3	4.337
	5	4.126
150	0	0.557
	1	4.446
	3	5.505
	5	4.179

a. All samples were dried at 70ºC for 4 min before the curing.
b. All dried samples were cured for 5 min.

Table 14. Quaternary ammonium content on the fabrics treated by a pad-batch method[a].

Alkaline catalyst (NaOH, % owb)	0	0.1	0.5	1	1.5	2	3	4
Quaternary ammonium (mmol/kg fiber)	0.065	0.261	1.048	1.492	3.276	2.263	0.978	0.459

a. All samples were batched at room temperature for 24 hrs.

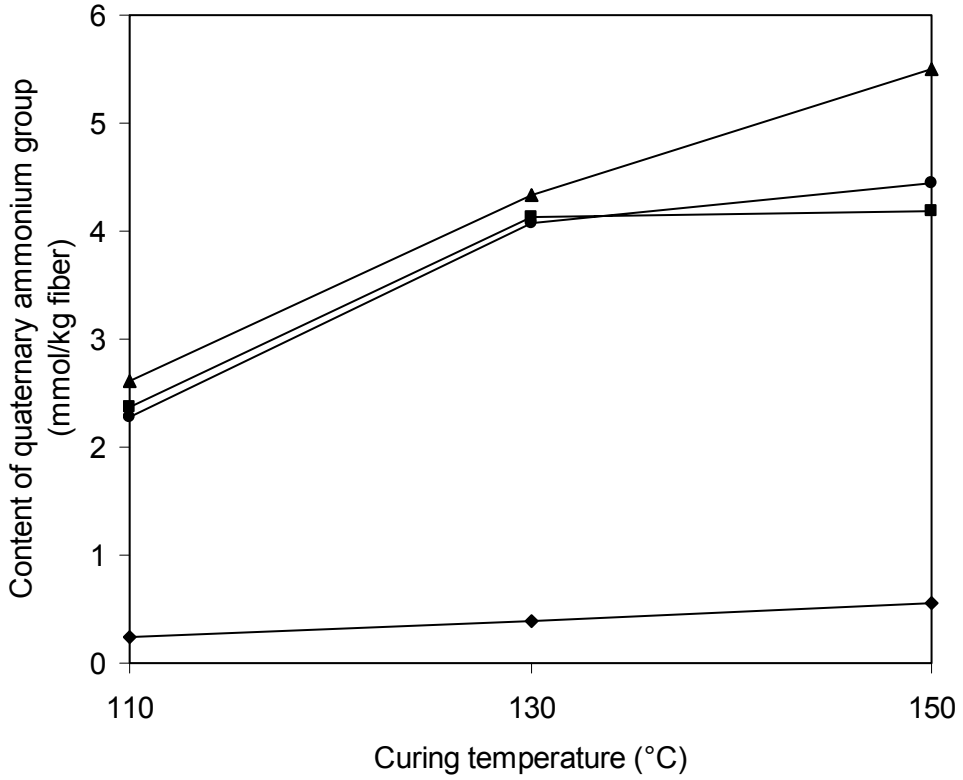

Figure 58. Quaternary ammonium content on the fabrics treated by a pad-dry-cure method. (amount of alkali ($NaHCO_3$): ◆ no alkali (0% owb), ● 1% owb, ▲ 3% owb, and ■ 5% owb).

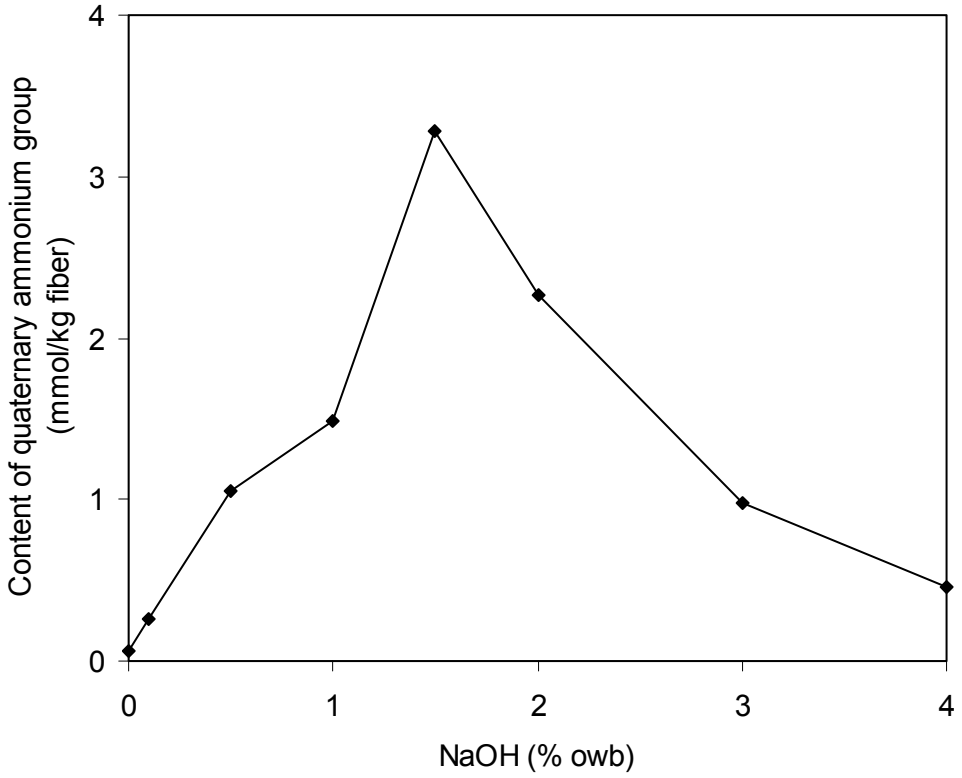

Figure 59. Quaternary ammonium content on the fabrics treated by a pad-batch method.

Figure 59 shows the amount of quaternary ammonium groups on the fabrics treated by a pad-batch method. The amount increased as the amount of alkaline catalyst (NaOH) increased up to 1.5% owb. However, it decreased as the amount of NaOH increased over 1.5% owb. The reaction between NMA-HTCC and cotton (Figure 51) is a competitive reaction with the alkaline hydrolysis of the NMA-HTCC (Figure 53). It seems that the increase in the amount of alkali over a certain point (1.5% owb here) increases the hydrolysis of NMA-HTCC rather than the reaction with cotton.

The quaternary ammonium content on the cotton treated with NMA-HTCC without addition of the alkaline catalyst was 0.065 mmol/kg fiber, which is 3.8 to 8.6 times less than those of fabrics treated by a pad-dry-cure method without alkaline catalyst. This indicates that the heat is an important factor that induces the hydrogen bonding or van der Waals' forces between cotton and MMA-HTCC.

9.4. Optimal Application Condition

Two application methods of NMA-HTCC to cotton fabrics, pad-dry-cure and pad-batch methods, were compared to choose an optimal application condition. Several factors were considered, such as whiteness of the treated fabrics, the amount of fixation of NMA-HTCC on the fabric, and the even distribution of NMA-HTCC.

In terms of the fixation, the pad-dry-cure method was more effective than the pad-batch method by 1.68 times at their maximal fixation conditions. However, severe yellowing was observed on the fabric treated by the pad-dry-cure method. From the comparison of the dyed fabrics with C.I. Acid Orange 7, all samples treated by the pad-

dry-cure method in the presence of an alkaline catalyst showed very uneven distribution of dye on the fabrics and different depth of shade between front and back of the fabrics due to migration.

As a result, the pad-batch method is desirable for the application of the NMA-HTCC to cotton fabrics and the optimal concentration of the alkaline catalyst obtained was 1.5% owb when 1.0% owb NMA-HTCC was used.

9.5. Concentration of NMA-HTCC vs. Amount of Quaternary Ammonium Group

Cotton fabrics were treated by the pad-batch method described in Section 9.2.2, to evaluate the effect of the concentration of the NMA-HTCC on the amount of fixation of the NMA-HTCC on cotton fabrics. The pad solutions were prepared as follows.

NMA-HTCC	0.25, 0.5, 1.0, or 2.0% owb
NaOH	1.5% owb (constant)

The effect of concentration of the NMA-HTCC on the quaternary ammonium content was summarized in Table 15 and plotted in Figure 60. As seen in the figure, the fixation of the NMA-HTCC on the cotton fabrics increased linearly as the amount of the NMA-HTCC applied increased.

Table 15. Quaternary ammonium content on the fabrics treated at different concentration of the NMA-HTCC[a,b].

NMA-HTCC (% owb)	0.25	0.5	1	2
Quaternary ammonium (mmol/kg fiber)	0.883	2.102	3.276	6.791

a. All samples were prepared by a pad-batch method at room temperature for 24 hrs.
b. The amount of an alkaline catalyst (NaOH) was constant (1.5% owb).

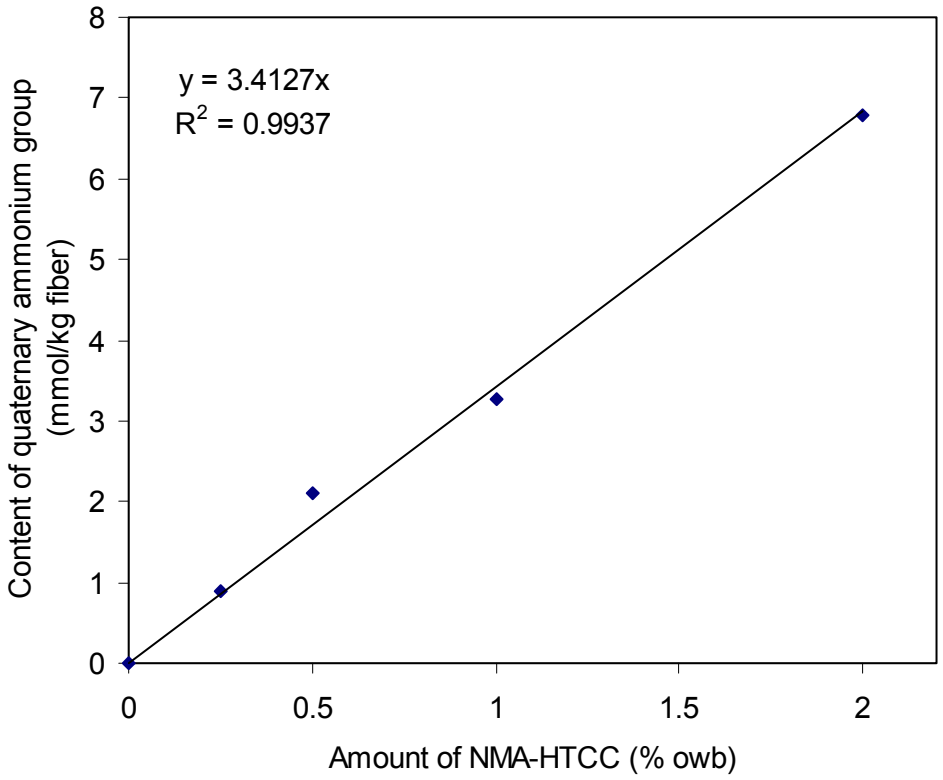

Figure 60. Quaternary ammonium content on the fabrics treated at different concentration of the NMA-HTCC.

10. NMA-HTCC AS AN ANTIMICROBIAL FINISH FOR COTTON FABRIC

The term “antibacterial finish” was introduced in 1940 [167]. After that there has been significant research on the development of antibacterial agents and their applications to textiles. In recent years, market demand for antimicrobial textile products has greatly increased as consumers’ standard of living has risen and they became aware of the importance of these products. As a result, there are a growing number of companies that develop and produce antimicrobial textile products.

The great concerns on the antimicrobial agents used for textiles are their safety towards the human body and durability against launderings. There are two types of antimicrobial finishes. One is a leaching type and the other is a non-leaching type. The fabrics treated with a leaching type finish kill bacteria by releasing the active substances from the fabrics. However, the antimicrobial property will be lost eventually after repeated launderings. Moreover, this type of finishes contact human skin, when used in fabrics, therefore, they can kill useful organisms or cause allergic reactions and rashes. It is also known that microorganisms have the chance to adapt and become resistant to the finish because these finishes are mobile [168].

Non-leaching type finish is bound to fabrics through chemical reactions, such as grafting or polymerization. The treated fabrics show a high durability against launderings and overcome most of the limitations of leaching type antimicrobial finishes.

The excellent antimicrobial activity of the NMA-HTCC was reported in Section 8.6. The NMA-HTCC is a non-leaching type finish, which can form covalent bonds with

cellulose. Here the antimicrobial activities of the NMA-HTCC treated cotton fabrics and their durability were evaluated.

10.1. Antimicrobial Properties of NMA-HTCC Treated Cotton Fabrics

The antimicrobial properties of the NMA-HTCC treated fabrics were evaluated by ASTM E2149-01 [169], which is a quantitative antimicrobial test method performed under dynamic contact conditions. This test method was used for all remaining antimicrobial fabric testing. As a test organism, *Staphylococcus aureus* was used. The test culture was suspended in a 5 mL nutrient broth, spread on a nutrient agar plate, and incubated at 37ºC for 24 hrs. Two single colonies were picked off with an inoculating loop, suspended in a 5 mL nutrient broth, and incubated for 18 hrs at 100 rpm and 37ºC. A final concentration of 1.5 - 3.0 × 10^5 CFU/mL was prepared by diluting the culture solution with a sterilized 0.3 mM phosphate buffer (pH 7.2). This solution was used as a working bacterial dilution.

The cotton fabrics treated with NMA-HTCC at three different concentrations by the pad-batch method in the presence of an alkaline catalyst (NaOH) and untreated cotton fabric (control) were used for the test. Each fabric (1.00 g) was cut into small pieces (1 × 1 cm) and transferred to a 250 mL Erlenmeyer flask containing 50 mL of the working bacterial dilution (1.5 - 3.0 × 10^5 CFU/mL). All flasks were capped loosely, placed on a shaking incubator, and shaken at 37ºC and 120 rpm for 1 hr. A series of dilutions were made using the buffer solution and each 1 mL of the dilution was plated in nutrient agar. The inoculated plates were incubated at 37ºC for 2 days and surviving cells were counted.

The average values of the duplicates were converted to CFU/mL in the flasks by multiplying by the dilution factor.

The antimicrobial activity was expressed in terms of % reduction of the organism after contact with the test specimen compared to the number of bacterial cells surviving after contact with the control. The % reduction was calculated using the following equation,

$$\% \text{ Reduction} = \frac{B - A}{B} \times 100,$$

where A and B are the surviving cells (CFU/mL) for the flasks containing test samples (NMA-HTCC treated cotton) and the control (blank cotton), respectively, after 1 hr contact time.

The antimicrobial activities of the NMA-HTCC treated cotton fabrics are listed in Table 16. All NMA-HTCC treated fabrics showed very high activities with over 99.99% reduction. The activity increased as the amount of NMA-HTCC applied increased and 100% reduction was obtained at the concentration of 1.0% owb.

Table 16. Antimicrobial test results against *S. aureus* before launderings.

Concentration of NMA-HTCC applied (% owb)	Surviving cells (CFU/mL)	% Reduction
0 (control)	2.24×10^5	
0.25	15	99.9933
0.5	1	99.9996
1.0	0	100

10.2. Durability of the NMA-HTCC Treated Fabrics

The durability of the antimicrobial activities of the NMA-HTCC treated fabrics was evaluated according to AATCC Test Method 61(2A)-1996 [133] using an ATLAS Launder-Ometer®. One cycle of laundering by this method is considered equivalent to five home machine launderings at medium or warm setting at the temperature range of 38±3°C. The fabric size used was 10×15 cm instead of 5×15 cm, which was specified in the test method, because at least 1 g of fabric is required for the antimicrobial test. As a detergent, 1993 AATCC Standard Reference Detergent WOB was used. All NMA-HTCC treated fabrics were subject to 10 consecutive launderings either with or without the detergent. At the end of the 10th cycle, all fabrics were rinsed with warm water using a home laundering machine and air-dried.

The antimicrobial properties of the fabrics after launderings are listed in Tables 17 and 18. As shown in Table 17, the antimicrobial activities of the fabrics after 10 launderings without the detergent were almost intact compared to those of the fabrics before launderings (see Table 16). At lower concentrations (0.25 and 0.5% owb) of NMA-HTCC applied, there were very slight decreases in the activities. This can be attributed to the removal of NMA-HTCC loosely fixed on the fabric by van der Waals' forces or hydrogen bonding. However, at a higher concentration (1.0% owb), complete reduction of bacteria was observed.

Table 18 shows the antimicrobial activities of the tested fabrics in the presence of the detergent. Further decreases in the activities were observed compared to those tested without the detergent. However, still over 99% of bacterial reduction was maintained at

the concentration of 1.0% owb. From the results, it can be assumed that the detergent has some interaction with NMA-HTCC.

The NMA-HTCC is a water-soluble chitosan derivative. Therefore, it should be almost completely removed from the fabric after a severe agitation of 10 launderings, if there were no strong bonds with fabrics. The test results show that the NMA-HTCC was fixed on the cotton fabrics covalently.

Table 17. Antimicrobial test results against *S. aureus* after 10 launderings[a] (without AATCC detergent).

Concentration of NMA-HTCC applied (% owb)	Surviving cells (CFU/mL)	% Reduction
0 (control)	2.32×10^5	
0.25	268	99.88
0.5	29	99.99
1.0	0	100

a. 10 launderings by AATCC Test Method 61(2A) are equivalent to 50 home machine launderings.

Table 18. Antimicrobial test results against *S. aureus* after 10 launderings[a] (with AATCC detergent).

Concentration of NMA-HTCC applied (% owb)	Surviving cells (CFU/mL)	% Reduction
0 (control)	2.32×10^5	
0.25	1.62×10^4	93.02
0.5	2.91×10^3	98.75
1.0	1.77×10^3	99.24

a. 10 launderings by AATCC Test Method 61(2A) are equivalent to 50 home machine launderings.

For the further confirmation, cotton fabric was treated with 1% owb NMA-HTCC solution without an alkaline catalyst (NaOH) by the pad-batch method described in Section 9.2.2. The antimicrobial activity of the fabric was evaluated before and after one laundering. As shown in Table 19, the fabric lost its antimicrobial activity almost completely after one laundering, whereas the 1% NMA-HTCC fabric, which was prepared in the presence of the alkaline catalyst, maintained its antimicrobial activity over 99% even after 10 launderings (see Table 18). This shows that the NMA-HTCC forms covalent bonds with cotton fabric in the presence of the alkaline catalyst (Figure 51) and the bonds are durable against repeated launderings.

Table 19. Antimicrobial activity of the 1% NMA-HTCC cotton fabric prepared without an alkaline catalyst against *S. aureus*.

Number of launderings[a]	Surviving cells[b] (CFU/mL)	% Reduction
0	1.09×10^4	94.76
1	1.86×10^5	10.58

a. One laundering by AATCC TM 61(2A) is equivalent to 5 home machine launderings. AATCC detergent was used for the test.
b. Surviving cells of control (blank cotton) was 2.08×10^5 CFU/mL.

10.3. Effect of Detergent on Antimicrobial Activity

Detergents are very complex formulations containing several different types of substances. The 1993 AATCC Standard Reference Detergent WOB has a typical composition of commercial laundry product except a few differences [133] and is composed of the following substances [170].

Linear alkyl benzene sulfonate, sodium salt (C11, LAS)	18.00 %
Sodium aluminium silicate solids	25.00 %
Sodium carbonate	18.00 %
Sodium silicate solids	0.50 %
Sodium sulfate	22.13 %
Polyethylene glycol	2.76 %
Sodium Polyacrylate	3.50 %
Silicone, Anti-foaming agent	0.04 %
Moisture	10.00 %
Miscallaneous	0.07 %

The main component of the detergent is linear alkylbenzene sulfonate (LAS), which is an anionic surfactant. The LAS has sulfonate groups and there is a possibility of them to interact with the quaternary ammonium groups of the NMA-HTCC.

To elucidate the effect of the LAS on the antimicrobial activity of the NMA-HTCC treated fabrics, the 1% NMA-HTCC fabrics were treated with sodium dodecylbenzenesulfonate (from Aldrich, Figure 61).

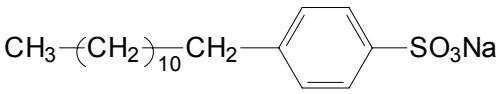

Figure 61. Structure of sodium dodecylbenzenesulfonate.

To the three 250 mL flasks containing 150 mL distilled water, different amounts of sodium dodecylbenzenesulfonate were dissolved, such as 0, 0.041, and 0.41 g, respectively. The amount of 0.041 g is equivalent to that of LAS in the AATCC

detergent used for one laundering by AATCC TM 61(2A). After equilibrating the temperature of the solutions to 50°C in a shaking incubator, each 1% NMA-HTCC fabric (1.5 g) was transferred to the flask. The flasks were stirred at 50°C and 150 rpm for 45 min. All fabrics were rinsed thoroughly in warm tap water, air-dried, and subjected to the antimicrobial test against *S. aureus*.

Table 20 shows the antimicrobial activities of the 1% NMA-HTCC cotton fabrics treated with LAS at different concentrations. The fabrics treated with LAS showed a slight decrease in antimicrobial activity and showed similar % reduction to the 1% NMA-HTCC fabric (99.24% reduction) subjected to the AATCC TM 61(2A) laundering test (see Table 18). However, the NMA-HTCC fabric, which was not treated with LAS, maintained 100% of bacterial reduction. From the result, it was confirmed that LAS affects the antimicrobial activity of the NMA-HTCC treated fabric.

Table 20. Antimicrobial test results of 1% NMA-HTCC fabrics contacted with LSA.

Amount (g) of LAS	Surviving cells[a] (CFU/mL)	% Reduction
0	0	100
0.041	1.73×10^3	99.12
0.41	2.37×10^3	98.79

a. Surviving cells after contact with the control (blank cotton) was 1.96×10^5 CFU/mL.

The antimicrobial activity of NMA-HTCC treated fabric comes from its quaternary ammonium groups on the fabric, which interact with the negatively charged bacterial cell surface. It can be thought that the anionic groups of LAS interact ionically

with the cationic groups of NMA-HTCC and reduce the chance of NMA-HTCC to interact with bacterial cells.

When it comes to the concentrations of LAS, it seems that the concentration is not an important factor to affect the antimicrobial activity because the difference of the antimicrobial activities of the fabrics treated at low and high concentrations are not so much different. This can be explained in that only a certain amount of LAS remains on the fabrics after rinsing, which removes the excess of LAS.

10.4. Antimicrobial Activity of Chitosan Treated Cotton Fabric

As a comparison, chitosan was applied to cotton fabric and its antimicrobial activity was compared to that of the NMA-HTCC treated one. Chitosan with DD 97.1% and MW 28,100, which is a starting material for the NMA-HTCC, was applied to cotton fabric by a pad-dry-cure method, which gives better durability against laundering than that by a pad-dry method [171]. Chitosan was dissolved in 2% acetic acid at a concentration of 1% owb. Cotton fabric was padded with the chitosan solution at 100% WPU, dried at 100°C for 3 min, and cured at 150°C for 3 min. The treated fabric was rinsed thoroughly in warm tap water and air-dried. The chitosan treated fabric was subject to launderings according to AATCC TM 61(2A) in the presence of 1993 AATCC Standard Reference Detergent WOB.

The antimicrobial activities of the chitosan treated fabrics against *S. aureus* after different numbers of launderings were summarized in Table 21 and Figure 62. The antimicrobial activity of chitosan treated fabric was considerably decreased after 2

launderings but there was no further decrease after 4 washing cycles, which agreed with Shin's result [171]. One interesting observation was that the activity increased after one laundering compared to that before laundering. This can be attributed to the relatively high amount of chitosan on the fabric before laundering, which may reduce the antimicrobial activity as proposed by Sudarshan et al. [53], in which a large amount of protonated chitosan may coat the bacterial cell surface and prevent the leakage of intracellular components. The similar behavior of the NMA-HTCC against *S. aureus* was previously observed and reported in Section 8.6.

Table 21. Antimicrobial activity of chitosan treated fabrics after launderings[a].

Number of launderings	Surviving cells[b] (CFU/mL)	% Reduction
0	9.10×10^3	96.40
1	2.31×10^2	99.91
2	1.74×10^5	31.23
4	1.75×10^5	30.83

a. One laundering by AATCC TM 61(2A) is equivalent to 5 home machine launderings.
b. Surviving cells of control (blank cotton) was 2.53×10^5 CFU/mL.

Figure 62 shows the antimicrobial activities of chitosan treated and NMA-HTCC treated fabrics. When the antimicrobial activity of chitosan treated fabric is compared to that of the NMA-HTCC treated one by the pad-batch method, the chitosan treated fabric lost much of its antimicrobial activity after only 2 launderings, whereas the NMA-HTCC treated one kept over 99% bacterial reduction even after 10 launderings. The poor durability of chitosan treated fabric is due to the lack of strong binding between chitosan

and cotton fabrics. It can be concluded that the chitosan treatment on cotton fabric cannot be used as a durable antimicrobial finishing, because the fabric needs at least 80% bacterial reduction against *S. aureus* to be considered as an antimicrobial textile product [172].

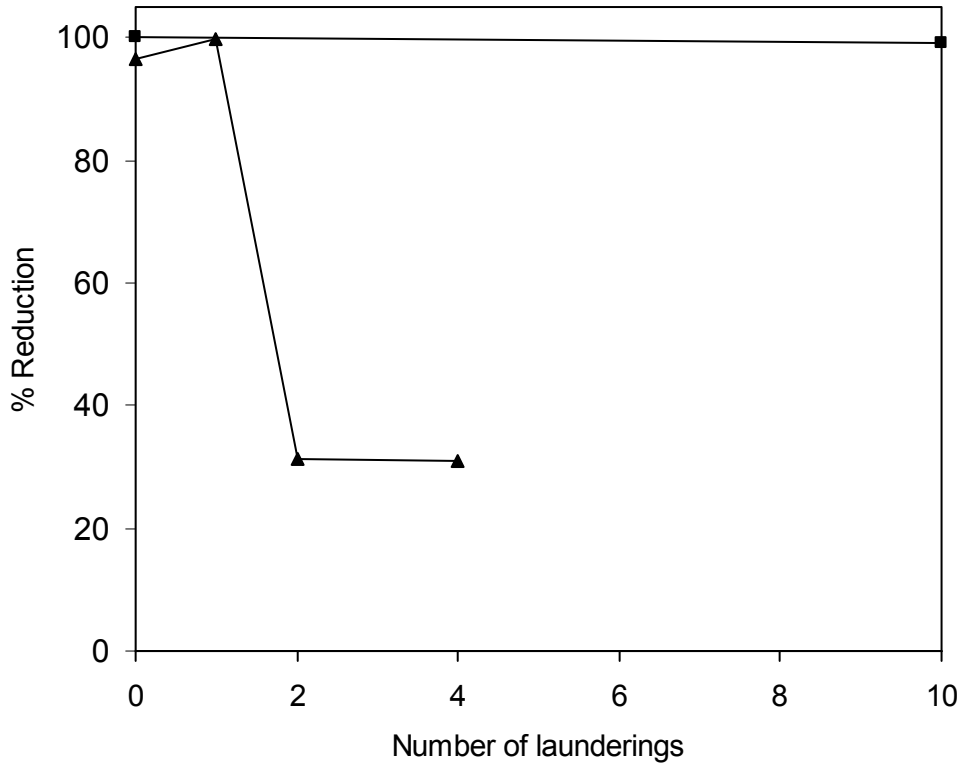

Figure 62. Antimicrobial activities of chitosan and NMA-HTCC treated fabrics (■ 1% NMA-HTCC treated cotton, ▲ 1% chitosan treated cotton).

11. NMA-HTCC AS A DYEING IMPROVING AGENT FOR COTTON FABRIC

Direct and reactive dyes have been widely used for dyeing cotton fabrics. These dyes are anionic dyes, which have sulfonate groups ($-SO_3^-$) as water-solubilizing groups. Therefore, these dyes have a low affinity to the cotton fiber since cotton fibers develop anionic surface charge in water. The charge repulsion between dyes and cotton can be overcome by adding an electrolyte like sodium chloride or sodium sulfate, which screens the surface charge of cotton. However, the large amount of salts required in dyeing can cause pollution of rivers and streams where the biological equilibrium depends to a large extent on the salt content of the water [104]. In addition, the highly colored dyebath discharge, due to inadequate dyebath exhaustion, also causes environmental problems.

As an attempt to reduce the use of salts as well as increase dyebath exhaustion, a number of researchers have cationized cotton fibers through chemical modifications with compounds containing cationic groups [105,173-180]. However, the majority of the chemicals used for the cationization of cotton are not safe environmentally [105]. Since the NMA-HTCC synthesized in this study is based on chitosan, a biopolymer, and the HTCC was reported as a composition of a hair product [82], it is believed that the NMA-HTCC will be safe to the environment. Here the NMA-HTCC treated cotton fabrics were dyed with direct and reactive dyes and their dyeability was evaluated.

11.1. Dyeing of Untreated and NMA-HTCC Treated Cotton Fabrics

Two direct dyes, C.I. Direct Blue 78 and Red 80, and two reactive dyes, C.I. Reactive Blue 21 and Orange 107 were used for the dyeing experiment. As test fabrics,

untreated cotton and NMA-HTCC treated cotton at the concentrations of 0.25, 0.5, and 1.0% owb were used. All dyeings were carried out using a laboratory dyeing machine (Ahiba Spectradye Plus, Datacolor International) at a liquor ratio (LR) of 30:1. Two different dye concentrations were used for the untreated cotton at 3.0, and 4.0% on weight of fabric (owf) and the dye concentration used for the NMA-HTCC treated fabrics was 3.0% owf.

11.1.1. Dyeing with direct dyes

The dyebath was prepared by adding the dye, wetting agent (Triton X-100, 0.5 g/L), and NaCl (40 g/L) to distilled water at room temperature. For the dyeing of the NMA-HTCC treated fabrics, no electrolyte was added. Wet fabric was added to each dyebath and dyeing was commenced after running 5 min at room temperature. The temperature was raised to 100ºC at 1.5°C/min. Dyeing was continued at 100ºC for 50 min and then cooled to 70ºC at -3°C/min. The dyed fabrics were rinsed thoroughly in hot tap water. The untreated cotton fabrics were further rinsed in NaCl solution (5g/L) two times and finally rinsed in cold tap water until clear rinse. All dyed fabrics were air-dried.

11.1.2. Dyeing with reactive dyes

The dyebath was prepared by adding dye, NaCl (60g/L), and Na_2CO_3 (7g/L) to distilled water at room temperature. For the dyeing of the NMA-HTCC treated cotton, no electrolyte was added. Wet fabric was added to each dyebath and dyeing was

commenced after running 5 min at room temperature. The temperature was raised to 60ºC at 1°C/min. Dyeing was continued at 60ºC for 50 min. All dyed fabrics were rinsed thoroughly in hot tap water and soaped in a solution containing 1 g/L of nonionic surfactant (Trition X-100) at 90ºC for 20 min at a RL of 30:1 using the laboratory dyeing machine. All fabrics were taken out, rinsed thoroughly in hot tap water, and air-dried.

11.2. Color Measurement of Dyed Fabrics

Measurement of K/S values of dyed fabrics is a measure of dye concentration on the fabrics. K/S is defined by the following equation, where R is the reflectance value of fabric.

$$K/S = \frac{(1-R)^2}{2R}$$

K/S value for each fabric was determined at the λ_{max} of each dye at which the reflectance value is the lowest. The spectrophotometer settings were identical to those in Section 9.3.1.

The K/S values for the dyed fabrics are listed in Tables 22 and 23, and plotted in Figures 63-66. As can be seen, the color yields of NMA-HTCC cotton fabrics increased as the concentration of NMA-HTCC applied increased. All 1% NMA-HTCC fabrics showed higher color yields than those of untreated fabrics dyed with 3% owf dye. Moreover, the 1% NMA-HTCC fabrics dyed with C.I. Direct Blue 78 and C.I. Reactive Blue 21 produced higher color yield than those of untreated fabrics dyed with 4% owf dye. The higher color yields obtained for the NMA-HTCC cotton fabrics without adding

electrolyte is attributed to the formation of ionic bonds between the quaternary ammonium groups of NMA-HTCC and anionic groups of dyes. In the case of the 1% NMA-HTCC cotton dyed with C.I. Direct Blue 78, a complete dyebath exhaustion was observed at the end of dyeing. The high dyebath exhaustion of the NMA-HTCC cotton without adding salt is greatly beneficial in terms of the reduction of the pollution caused by the large amount of salt and remaining dyes in the dyehouse effluent.

Table 22. K/S values for direct dyes.

Samples[a,b]	K/S of C.I. Direct	
	Blue 78 (λ_{max} = 610 nm)	Red 80 (λ_{max} = 530 nm)
0.25% NMA-HTCC cotton	7.68	10.41
0.5% NMA-HTCC cotton	10.23	13.14
1.0% NMA-HTCC cotton	13.99	16.87
Cotton[1]	11.40	16.38
Cotton[2]	13.39	18.32

a. All NMA-HTCC cotton fabrics were dyed at the dye concentration of 3% owf.
b. Cotton[1] and cotton[2] were dyed at the dye concentrations of 3% and 4% owf, respectively.

Table 23. K/S values for reactive dyes.

Samples[a,b]	K/S of C.I. Reactive	
	Blue 21 (λ_{max} = 680 nm)	Orange 107 (λ_{max} = 430 nm)
0.25% NMA-HTCC cotton	14.17	6.97
0.5% NMA-HTCC cotton	18.62	8.55
1.0% NMA-HTCC cotton	22.38	12.57
Cotton[1]	14.89	12.28
Cotton[2]	17.40	15.14

a. All NMA-HTCC cotton fabrics were dyed at the dye concentration of 3% owf.
b. Cotton[1] and cotton[2] were dyed at the dye concentrations of 3% and 4% owf, respectively.

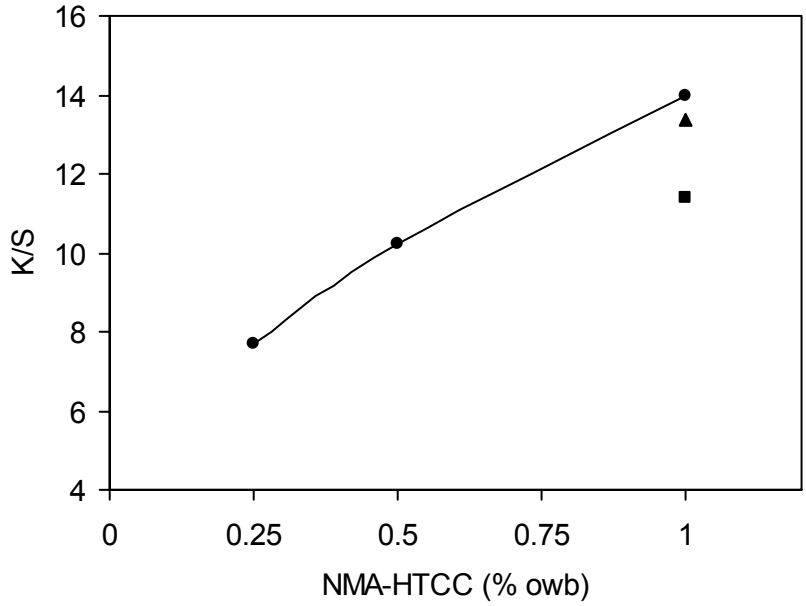

Figure 63. K/S values vs. the amounts of NMA-HTCC applied to cotton fabrics for C.I. Direct Blue 78 (● NMA-HTCC cotton fabrics dyed at 3% owf, ■ cotton dyed at 3% owf, ▲ cotton dyed at 4% owf).

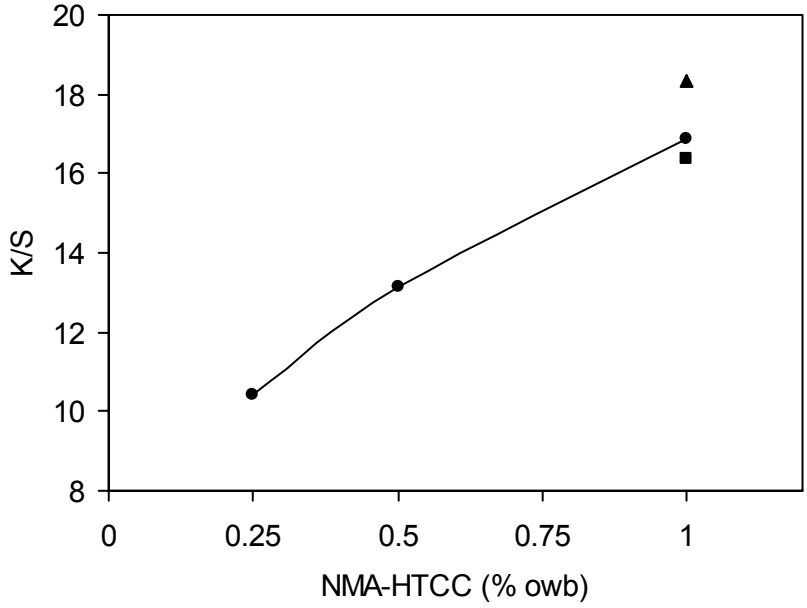

Figure 64. K/S values vs. the amounts of NMA-HTCC applied to cotton fabrics for C.I. Direct Red 80 (● NMA-HTCC cotton fabrics dyed at 3% owf, ■ cotton dyed at 3% owf, ▲ cotton dyed at 4% owf).

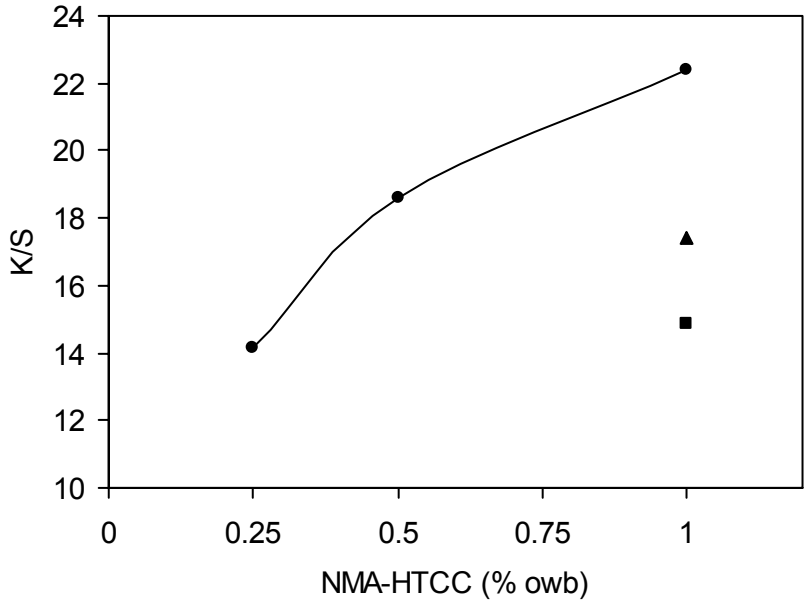

Figure 65. K/S values vs. the amounts of NMA-HTCC applied to cotton fabrics for C.I. Reactive Blue 21 (● NMA-HTCC cotton fabrics dyed at 3% owf, ■ cotton dyed at 3% owf, ▲ cotton dyed at 4% owf).

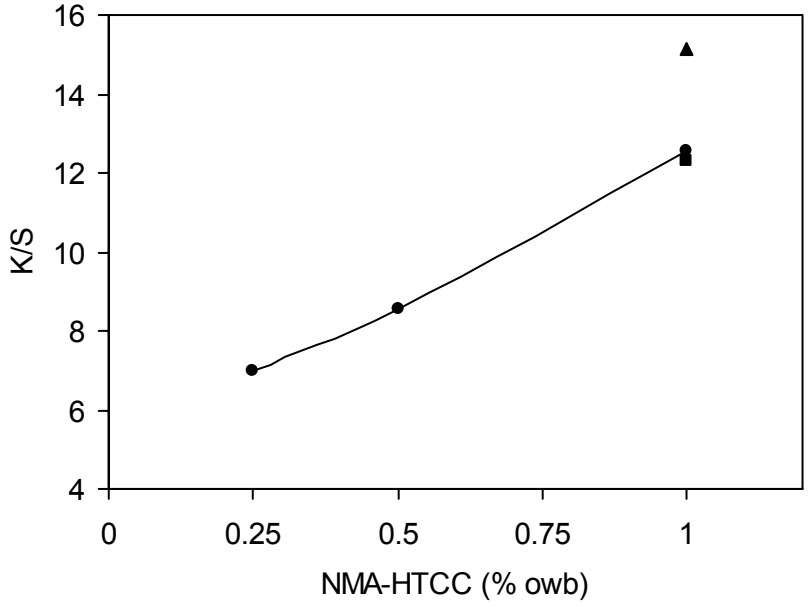

Figure 66. K/S values vs. the amounts of NMA-HTCC applied to cotton fabrics for C.I. Reactive Orange 107 (● NMA-HTCC cotton fabrics dyed at 3% owf, ■ cotton dyed at 3% owf, ▲ cotton dyed at 4% owf).

When the shade of the dyed fabrics was compared visually, the 1% NMA-HTCC cotton dyed with C.I. Direct Red 80 showed a noticeable shade difference compared to the untreated cotton counterpart. To compare shade difference between NMA-HTCC cotton and untreated cotton, CIELAB L^*(lightness), a^*, b^*, and C^*(chroma) values were measured using the spectrophotometer at the identical settings used in Section 9.3.1. Color difference (ΔE^*) and hue difference (ΔH^*) were calculated by the following equations [181].

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$\Delta H^* = \sqrt{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2}$$

Each 1% NMA-HTCC cotton was compared to the untreated cotton with similar color yield (K/S). The 1% NMA-HTCC cotton dyed with C.I. Reactive Blue 21 was not compared because the cotton sample with similar K/S was not available.

Table 24 shows the colorimetric data for the 1% NMA-HTCC cotton and untreated cotton. C.I. Direct Red 80 showed the highest ΔH^* value, whereas C.I. Direct Blue 78 showed almost no change in the hue. The NMA-HTCC cotton dyed with C.I. Direct Red 80 was brighter and yellower than untreated cotton counterpart. In case of C.I. Reactive Orange 107, the NMA-HTCC cotton was duller and redder when compared to its counterpart. The shade difference observed between the NMA-HTCC cotton and untreated cotton may be due to the different arrangement of dye molecules on the fiber caused by the interaction between the NMA-HTCC and dye molecules.

Table 24. Colorimetric data for the 1% NMA-HTCC cotton and untreated cotton.

Dye	Sample	K/S	L^*	A^*	b^*	C^*	ΔE^*	ΔH^*
C.I. Direct Blue 78	1% NMA-HTCC[a]	13.99	28.67	-1.39	-22.56	22.60	1.10	0.07
	Cotton[b]	13.39	29.40	-1.46	-23.38	23.42		
C.I. Direct Red 80	1% NMA-HTCC[a]	16.87	41.32	56.26	22.93	60.75	5.78	3.87
	Cotton[c]	16.38	38.45	54.61	18.19	57.56		
C.I. Reactive Orange 107	1% NMA-HTCC[a]	12.57	69.04	32.33	75.97	82.56	4.60	2.73
	Cotton[d]	12.28	71.80	30.72	79.28	85.03		

a. All 1% NMA-HTCC cotton were dyed at the dye concentration of 3% owf.
b. Dyed at the dye concentration of 4% owf.
c. Dyed at the dye concentration of 3% owf.
d. Dyed at the dye concentration of 3% owf.

11.3. Fastness Properties of Dyed Fabrics

The colorfastness properties of the dyed fabrics against laundering and light were evaluated according to AATCC Test Methods [133]. As test samples, 1% NMA-HTCC cotton fabrics dyed with 3% owf dye and untreated cotton fabrics dyed with 3% and 4% owf dye were used. Note that the color yield (K/S) of the 1% NMA-HTCC cotton fabrics were between those of cotton dyed with 3% and 4% owf dye or were higher than that of cotton dyed with 4% owf dye.

11.3.1. Washfastness

Fastness to laundering was evaluated according to AATCC TM 61 (2A) using an ATLAS Launder-Ometer®. Multifiber Fabric 1 was used for evaluation of staining on cotton during laundering.

11.3.2. Lightfastness

Fastness to light was evaluated according to AATCC TM 16E using an ATLAS Ci 3000^{+} Xenon Weather-Ometer. All samples were exposed to 20 AATCC Fading Units, which corresponds to 21.5 hrs of continuous exposure under a xenon lamp at the irrandiance power of $1.1 W/m^2/nm$ at 420 nm.

11.3.3. Evaluation of color change and staining

The evaluation of color change and staining was made by instrumental measurement instead of visual evaluation. A Datacolor Spectraflash® SF 300 spectro-photometer was used for the evaluation using the following settings,

Illuminant : D65-10
Reflectance Mode
Average 2
Specular Component Included
Port Plate Size : 6.6 mm (Ultra Small Area View)
UV-Filter Out
Measurement : Polychromatic

The Shelyn SLI-Form software was used to calculate the CMC(2:1) color-difference ($\Delta E_{CMC(2:1)}$), Gray Scale (GS) grade for color change, and GS grade for staining by comparing the color of a sample before and after testing. The GS grade is assigned from 1 to 5 in half step increments and a grade of 5 means no color change for the colorfastness test or no color transfer for the staining test.

11.3.4. Colorfastness test results

Color change of dyed fabrics after laundering was summarized in Tables 25 and 26 in terms of the $\Delta E_{CMC(2:1)}$ and a GS grade. In all cases, the 1% NMA-HTCC cotton fabrics showed better washfastness as indicated by less color change. Especially, for direct dyes, much better fastness properties were observed compared to reactive dyes. This can be explained by the fact that the ionic bonds formed between the dyes and the NMA-HTCC cotton are much stronger than the hydrogen bonds and van der Waals' forces between the dyes and untreated cotton. As seen in Table 27, the NMA-HTCC cotton produced less staining on the adjacent undyed cotton for direct dyes, since less dyes were removed from the NMA-HTCC cotton during laundering.

Tables 28 and 29 shows the lightfastness properties of the dyed fabrics. In all cases, the 1% NMA-HTCC cotton showed reduction in the lightfastness compared to its untreated cotton counterpart. This adverse effect on the lightfastness property was previously reported by other researchers in their work for pretreatment of cotton with polymeric amine or amide compounds to improve its dyeability [176,177,182-185]. Compared to others, the NMA-HTCC cotton dyed with C.I. Reactive Blue 21 showed relatively better lightfastness property, which is close to the untreated cotton counterpart. A careful dye selection may reduce the lightfastness problem.

Table 25. Washfastness of dyed fabrics for direct dyes.

Samples[a,b]	C.I. Direct Blue 78		C.I. Direct Red 78	
	$\Delta E_{CMC(2:1)}$	GS grade	$\Delta E_{CMC(2:1)}$	GS grade
1% NMA-HTCC cotton	1.00	4	1.20	3.5
Cotton[1]	3.98	2	2.86	2.5
Cotton[2]	4.49	2	3.74	2

a. 1% NMA-HTCC cotton was dyed at the dye concentration of 3% owf.
b. Cotton[1] and cotton[2] were dyed at the dye concentrations of 3% and 4% owf, respectively.

Table 26. Washfastness of dyed fabrics for reactive dyes.

Samples[a,b]	C.I. Reactive Blue 21		C.I. Reactive Orange 107	
	$\Delta E_{CMC(2:1)}$	GS grade	$\Delta E_{CMC(2:1)}$	GS grade
1% NMA-HTCC cotton	1.17	4	0.13	5
Cotton[1]	1.42	3	0.36	5
Cotton[2]	1.44	3	0.34	5

a. 1% NMA-HTCC cotton was dyed at the dye concentration of 3% owf.
b. Cotton[1] and cotton[2] were dyed at the dye concentrations of 3% and 4% owf, respectively.

Table 27. Cotton staining by dyed fabrics.

Samples[a,b]	GS grade for C.I. Direct		GS gade for C.I. Reactive	
	Blue 78	Red 80	Blue 21	Orange 107
1% NMA-HTCC cotton	3	3	4.5	5
Cotton[1]	2.5	2.5	4.5	5
Cotton[2]	2.5	2	4.5	5

a. 1% NMA-HTCC cotton was dyed at the dye concentration of 3% owf.
b. Cotton[1] and cotton[2] were dyed at the dye concentrations of 3% and 4% owf, respectively.

Table 28. Lightfastness of dyed fabrics for direct dyes.

Samples[a,b]	C.I. Direct Blue 78		C.I. Direct Red 78	
	$\Delta E_{CMC(2:1)}$	GS grade	$\Delta E_{CMC(2:1)}$	GS grade
1% NMA-HTCC cotton	2.20	3.5	2.45	3
Cotton[1]	0.88	4.5	0.94	4
Cotton[2]	0.84	4.5	0.88	4.5

c. 1% NMA-HTCC cotton was dyed at the dye concentration of 3% owf.
d. Cotton[1] and cotton[2] were dyed at the dye concentrations of 3% and 4% owf, respectively.

Table 29. Lightfastness of dyed fabrics for reactive dyes.

Samples[a,b]	C.I. Reactive Blue 21		C.I. Reactive Orange 107	
	$\Delta E_{CMC(2:1)}$	GS grade	$\Delta E_{CMC(2:1)}$	GS grade
1% NMA-HTCC cotton	1.62	3.5	3.91	3
Cotton[1]	1.14	4	0.95	4.5
Cotton[2]	1.21	4	0.85	4.5

c. 1% NMA-HTCC cotton was dyed at the dye concentration of 3% owf.
d. Cotton[1] and cotton[2] were dyed at the dye concentrations of 3% and 4% owf, respectively.

11.4. Antimicrobial Properties of Dyed Fabrics

The 1% NMA-HTCC cotton showed 100% of bacterial reduction before dyeing as shown in the previous chapter. To examine the effect of dyeing on the antimicrobial activity of the NMA-HTCC cotton, all dyed 1% NMA-HTCC fabrics were subjected to the antimicrobial test according to ASTM E2149-01 as described in Section 10.1. Untreated cotton fabrics dyed with similar color yield to those of the NMA-HTCC cotton were also tested to check whether the dye itself has antimicrobial activity.

The results were summarized in Tables 30 and 31. From the results of the untreated fabrics, all fabrics showed antimicrobial activities less than 20%, which means that dye itself has only a negligible antimicrobial activity.

The 1% NMA-HTCC cotton fabrics dyed with direct dyes showed almost no antimicrobial activities against *S. aureus*, whereas the fabrics dyed with reactive dyes maintained 62.56 and 41.71% reduction for C.I. Reactive Blue 21 and C.I. Reactive Orange 107, respectively.

Table 30. Antimicrobial activity of fabrics dyed with direct dyes against *S. aureus*.

C.I. Direct	Samples[a]	K/S	Surviving cells[b] (CFU/mL)	% Reduction
Blue 78	1% NMA-HTCC cotton	13.99	1.93×10^5	6.76
	Cotton[c]	13.39	1.69×10^5	18.36
Red 80	1% NMA-HTCC cotton	16.87	1.99×10^5	3.86
	Cotton[d]	16.38	1.98×10^5	4.35

a. 1% NMA-HTCC cotton fabrics were dyed at the dye concentration of 3% owf.
b. Surviving cells of control (blank cotton) was 2.07×10^5 CFU/mL.
c. Dyed at the dye concentration of 4% owf.
d. Dyed at the dye concentration of 3% owf.

Table 31. Antimicrobial activity of fabrics dyed with reactive dyes against *S. aureus*.

C.I. Reactive	Samples[a]	K/S	Surviving cells[b] (CFU/mL)	% Reduction
Blue 21	1% NMA-HTCC cotton	22.38	7.90×10^4	62.56
	Cotton[c]	17.40	1.72×10^5	18.48
Orange 107	1% NMA-HTCC cotton	12.57	1.23×10^5	41.71
	Cotton[d]	12.28	1.78×10^5	15.64

a. 1% NMA-HTCC cotton fabrics were dyed at the dye concentration of 3% owf.
b. Surviving cells of control (blank cotton) was 2.11×10^5 CFU/mL.
c. Dyed at the dye concentration of 4% owf.
d. Dyed at the dye concentration of 3% owf.

The chemical structures of C.I. Direct Blue 78 and C.I. Direct Red 80 are shown in Figures 67 and 68. The structures of the two reactive dyes are not shown here because the dye manufacturers kept them confidential. In general, direct dyes are linear and have higher molecular weights than reactive dyes because the dye fixation of direct dyes on cotton depends on the hydrogen bonds and van der Waals' forces with cotton. Direct dyes require more water-solubilizing groups ($-SO_3^-$) than reactive dyes due to their higher molecular weights. As described earlier in Section 3, the antimicrobial activity of a cationic polymer comes from its cationic charges, which interact with negatively charged bacterial cell surface and cause cell surface alterations. Based on these facts, the better antimicrobial activities of NMA-HTCC fabrics dyed with reactive dyes can be explained by the fact that the direct dyes fixed ionically on the NMA-HTCC cotton block the interaction of the cationic groups on the NMA-HTCC with bacteria as well as repel them with larger numbers of anionic groups on the dyes than the reactive dyes do.

Although the NMA-HTCC fabrics dyed with reactive dyes have better antimicrobial activities than those dyed with direct dyes, the activities are less than the standard (at least 80%) required for antimicrobial textiles. Therefore, the NMA-HTCC cotton fabrics dyed both direct and reactive dyes cannot be considered as efficient antimicrobial textiles.

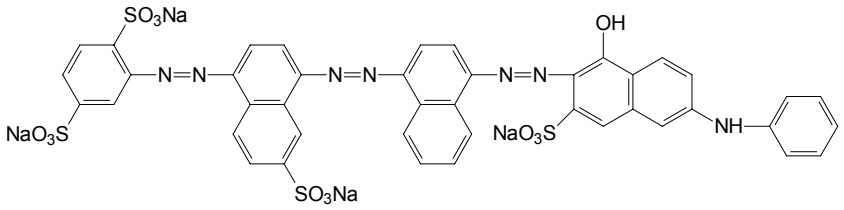

Figure 67. Chemical structure of C.I. Direct Blue 78.

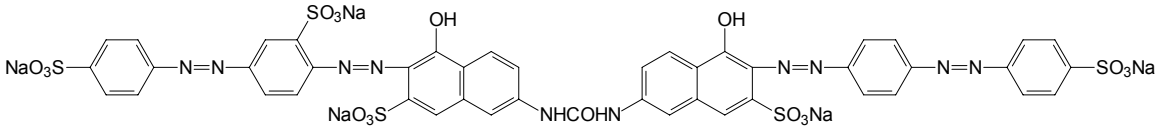

Figure 68. Chemical structure of C.I. Direct Red 80.

12. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

A fiber-reactive chitosan derivative (NMA-HTCC) was successfully prepared, which overcame the drawbacks of chitosan, such as limited solubility and antimicrobial activity, and poor laundering durability when applied on cotton fabrics. The HTCC, a water-soluble chitosan derivative with an excellent antimicrobial activity and a precursor for the NMA-HTCC, was synthesized by reacting chitosan with glycidyltrimethyl-ammonium chloride (GTMAC) in a neutral aqueous condition. The complete substitution of quaternary ammonium groups on the amino groups of chitosan was obtained without difficulty. The preparation of the NMA-HTCC involved reaction of HTCC with *N*-methylolacrylamide (NMA) in the presence of an acid catalyst (NH_4Cl). Since the reaction is reversible and NMA can be hydrolyzed by the acid catalyst, the reaction time and the amount of NH_4Cl were important factors for the reaction. The maximal double bond content was obtained at the reaction time of 10 min and 3 mole excess of NH_4Cl when the HTCC was reacted with 8 mole excess of NMA at 140°C. The NMA-HTCC showed complete reduction of bacteria within 20 min at the concentration of 10 ppm, when contacted with *S. aureus* and *E. coli* ($1.5 - 2.5 \times 10^5$ CFU/mL).

The NMA-HTCC was applied to 100% cotton fabric in the presence of an alkaline catalyst to evaluate its use as an antimicrobial textile finish. Two application methods, a pad-dry-cure and a pad-batch method, were evaluated. The amount of the NMA-HTCC fixed on cotton was measured by a stoichiometric dye adsorption method using purified C.I. Acid Orange 7. Although the pad-dry-cure method produced higher

fixation (up to 1.68 time at each maximal condition) of the NMA-HTCC on cotton, the method caused severe yellowing of the fabric and uneven distribution of the NMA-HTCC on the cotton fabric. Therefore, the pad-batch method was desirable as an application method. For the pad-batch application, the optimal amount of an alkaline catalyst (NaOH) was required to get the highest fixation because a high amount of the catalyst over a certain point decreased the fixation by hydrolyzing the NMA-HTCC. The optimal concentration of the catalyst obtained was 1.5% owb when 1% owb NMA-HTCC was used.

The antimicrobial activity of NMA-HTCC treated cotton fabrics were evaluated quantitatively against *S. aureus*. The 1% NMA-HTCC cotton showed 100% of bacterial reduction and the antimicrobial activity was maintained over 99% even after 50 home laundering. The slight decrease in the activity after launderings resulted from the interaction of the NMA-HTCC with anionic surfactant in the detergent rather than the removal of the NMA-HTCC from cotton. In contrast, 1% chitosan treated cotton showed about 30% reduction after 10 home launderings due to lack of a strong bonding between chitosan and cotton. From the result, it was concluded that the NMA-HTCC is a very efficient antimicrobial textile finish that overcame the poor laundering durability of chitosan. For the laundering test, 1993 AATCC Standard Reference Detergent WOB was used, which has a typical composition of commercial detergent except for a few differences. For example, commercial detergent contains enzyme, perfume, and other ingredients, which are not in the AATCC detergent. It is recommended to test a variety of commercial laundering detergents to see if they affect the antimicrobial activity of the

NMA-HTCC cotton. As a test organism for the antimicrobial test, only *S. aureus* was used. However, more extensive study is suggested using other bacteria that are commonly found in human body.

The dyeing properties of the NMA-HTCC cotton were evaluated using direct and reactive dyes. The 1% NMA-HTCC cotton produced a higher color yield without the addition of salt than untreated cotton dyed in the presence of a large amount of salt. For C.I. Direct Blue 78, the 1% NMA-HTCC cotton showed a complete dyebath exhaustion at the dye concentration of 3% owf and showed a higher color yield than untreated cotton dyed at 4% owf dye. The higher color yield shown by the NMA-HTCC cotton was the result of the formation of ionic bonds between the cationic groups of the NMA-HTCC and the anionic groups of dye molecules. The high dyebath exhaustion of the NMA-HTCC cotton without the aid of salt will be greatly beneficial in terms of the pollution reduction. When compared to the washfastness of the dyed fabrics, the NMA-HTCC cotton showed better fastness than untreated cotton for all dyes tested. Especially, for direct dyes, the NMA-HTCC cotton had a significantly increased fastness due to the strong ionic bonds between the NMA-HTCC and dye molecules. However, the lightfastness of the NMA-HTCC cotton was inferior to that of untreated cotton. Compared to other dyes, C.I. Reactive Blue 21 showed relatively better lightfastness. Future work is required to improve the lightfastness of the NMA-HTCC cotton. As an alternative, a careful dye selection may minimize the lightfastness problem. The antimicrobial activity of the 1% NMA-HTCC cotton was almost lost after dyeing. This

was probably due to the occupation of the cationic groups of the NMA-HTCC with anionic dye molecules.

The overall conclusion is that the NMA-HTCC cotton can be successfully used as an antimicrobial finish for the undyed cotton fabrics, such as underwear, T-shirts, socks, and so on. The NMA-HTCC also can be used as a dyeing improving agent for cotton but the NMA-HTCC cotton cannot keep its antimicrobial activity after dyeing. Therefore, future research is required to add antimicrobial activity to dyed fabrics.

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