DETERMINATION ON NITROGEN CONTENT OF ANTIBACTERIAL COTTON FABRIC TREATED WITH CHITOSAN

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ABSTRACT

The study aims to evaluate the nitrogen content on antibacterial cotton fabrics treated with chitosan using four different quantitative determination methods: field-emission scanning electron microscopy with energy dispersive X-ray spectroscopy (FESEM-EDX), dyeability of antibacterial treated samples based on color strength (K/S), elemental analyses via combustion tests, and antibacterial ability under dynamic contact conditions or the dynamic shake flask test. The nitrogen content was analyzed under the effects of molecular weight (MW) of chitosan (2.6 kDa and 187 kDa), crosslinkers including citric acid (CA) and dimethylol dihydroxyethyleneurea (DMDHE), and washing cycles. The findings from these measurements not only determined the nitrogen content of antibacterial cotton fabric but also elucidated the bonding mechanism between cellulose and chitosan in the presence of crosslinking compounds. Consequently, the antibacterial activity of cotton fabrics treated with chitosan could be indirectly assessed through the nitrogen content obtained from the amine groups of finishing compounds.

KEYWORDS

Nitrogen content; Antibacterial activity; Crosslinker; Chitosan; Cotton fabric; Dyeability.

INTRODUCTION

Chitosan is a natural linear polysaccharide produced by the deacetylation of chitin, which is derived from shrimp shells, insect cuticles, and cell walls of fungi and algae. These are abundant biopolymers second only to cellulose derivatives [1-4]. Notably, commercial chitosan samples, deacetylated chitin from crustacean sources, are soluble in aqueous acidic solutions with a pH lower than 6.5, such as acetic acid, formic acid, and lactic acid [5-8]. The solubility of chitosan is a crucial for its chemical conversion into thin films and yarns. Chitosan contains more active groups than chitin, especially amine groups, which can inhibit bacterial growth of bacteria due to their positive charge [3]. In acidic solution, amine groups in chitosan transform into quaternary ammonium cations, enabling it to prevent both gram-negative and gram-positive bacteria [4]. Outstanding properties of chitosan include nontoxicity, biocompatibility, and biodegradation, offering benefits such as antibacterial ability, anticancer properties, and antibody abilities [5, 9]. The main application fields of commercial chitosan are biotechnology, pharmaceutics, wastewater treatment, cosmetics, agriculture, food science, and textiles [2]. Chitin is insoluble in diluted acetic acid but if its degree of N-acetylation is higher than 60%, it transforms into chitosan, which is soluble in acidic solutions [4, 10, 11]. In addition, molecular weight of chitosan, dependent on its origin and acetylation conditions (time, temperature and sodium hydroxide concentration), is significant.

In textile finishes, chitosan is primarily used for antibacterial treatments on cotton fabrics due to high economic efficiency and excellent human health protection [5]. The antibacterial properties of cotton fabrics treated with chitosan were attributed to the interaction between cationic chitosan and anionic bacterial and fungal surfaces, which alters cell wall absorption or causes cytoplasmic leakage [12-14]. Chitosan with positive charges and low molecular weight can bind to DNA in bacterial and fungal cells, inhibiting biosynthesis [15]. Studies have shown that the antibacterial effectiveness of cotton fabric treated with chitosan is influenced by factors such as molecular weight, degree of acetylation, pH value, temperature, concentration and additives. Some researchers have demonstrated that lower molecular weight chitosan results in higher antibacterial effectiveness [5, 16] and lower moisture or water absorption [17]. However, the relationship between

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antibacterial ability and molecular weight of chitosan is still debated due its natural variability and range of acetylation degrees, making its effects difficult to determine. Chitosan with a higher degree of acetylation possesses more quaternary amine cations, thus enhancing its antibacterial activity [18]. Additionally, the antibacterial efficacy of chitosan is optimal at pH value around 5.0 [13, 19, 20]. The best antibacterial effectiveness is observed when treating samples with chitosan at temperature between 25 ºC and 37 ºC, while it decreases below 25 ºC [21]. To enhance the linkage of chitosan to cotton fabrics, crosslinkers such as citric acid (CA), 1,2,3,4-butanetetracarboxylic acid (BTCA), glutaraldehyde and dimethylol dihydroxyethyleneurea (DMDHEU) have been used in previous researches [22, 23].

Some methods for evaluating the antibacterial ability of fabrics treated with chitosan include dynamic contact methods according to ASTM E2149-01, AATCC 100 and AATCC 147 standards, commonly testing gram-positive bacteria (e.g., Staphylococcus aureus) and gram-negative bacteria (e.g., Escherichia coli) [5]. In addition, advanced techniques such as scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), X-Ray diffraction analysis (XRD are also applied to confirm the presence of chitosan in fabric structure. Some methods like dyeing, mass comparing and nitrogen content determination were reported [9, 24-26]. In this study, the authors will determine the nitrogen (N) content on antibacterial treated cotton fabrics with chitosan, as this quantitative evaluation allows for assessing antibacterial ability. Many studies have shown that amine groups in chitosan interact with anionic bacterial cell to inhibit microbial growth.

EXPERIMENTS

Materials and chemicals

Pretreated cotton fabrics were provided by Nam Dinh Textile Garment JSC (Vietnam). The cotton fabrics were specified as 2/1 twill with a weft count 16 (Ne), a warp count 34 (Ne), and 175 and 410 threads per centimeter (TPC) in crosswise and lengthwise directions, respectively, and a weight of 230 grams per square meter (GSM).

Chitosan with deacetylated degree (DD of about 75% was purchased from Vietnam Chitosan Co. Ltd, and was extracted from shrimp shells. The molecular weight of the chitosan was reduced using gamma irradiation. Two types chitosan with different molecular weights, 6.9 kDa (CTS1) and 187 kDa (CTS2), were determined through solution viscosity and calculated using the Mark–Houwink equation [27].

Since chitosan can not form covalent bonds with cellulose, citric acid (CA) and dimethylol dihyroxyethyleneurea (DMDHEU) were used as crosslinking agents. Acid dyes, commercially named Lanaset® Yellow 2R, were purchased from Huntsman Corporation, and applied at pH of 4.5. Additionally, acetic acid, ethanol, sodium hypophosphite (SHP) for CA agent, catalyst NKC for DMDHEU agent and a nonionic wetting agent (Hostapal MRN) were used as additives.

Instrument and methods

Cotton fabrics were washed, desized and bleached according to AATCC 187-2013 and stored in a conditioner (M250-RH, Mesdan) for 24 hours. Chitosan was dissolved in citric acid (7%) or acetic acid (2 g/l) using a magnetic stirrer (Starlet), and the dyeing medium was controlled by a pH meter (Mettler Toledo). The prepared solution (chitosan, crosslinker, wetting agent, catalyst, etc.) was applied to the cotton fabrics using a padding mangle (Roaches machine) and a setting drier (Hisaka). All treated samples were cleaned in a washing machine (Quickwash Plus) to evaluate color fastness and chemical retention.

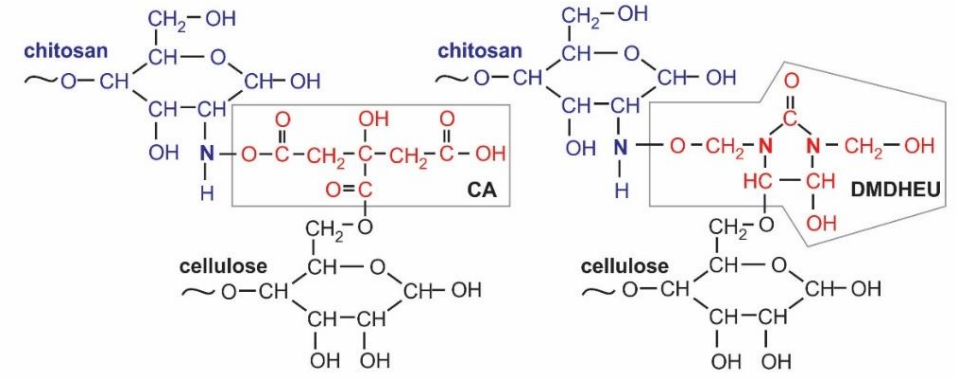
A recipe with liquor ratio of 1/50, 0.5% on weight of fabric (owf) of acid dyes, and 2g/l acetic acid was used to dye the treated cotton fabrics with chitosan and crosslinkers at 98 ºC in 130 minutes using exhaust dyeing equipment (Colorstar) and an infrared dyeing machine (Starlet-2). The color strength (K/S) of dyed samples was measured using a UV-vis 1601PC and a UV spectrophotometer (Gretag Color-Eye 2180, daylight D65). Furthermore, an elemental analysis method (Elementar Analysensysteme GmbH) and a field emission scanning electron microscope with energy dispersive X-ray spectroscopy (FESEM-EDX, JSM 7600) were used to determine and evaluate the nitrogen content of antibacterial treated cotton fabric with chitosan and crosslinkers (CA and DMDHEU).

Escherichia coli (E. coli), a type of gram-negative bacteria stored at minus 40 ºC, provided by Proteomics lab, was used to examine the antibacterial activity of treated samples according to ASTM E2149-01 test method under dynamic contact conditions. The bacterial reduction was evaluated after 60 minutes and 2 minutes of exposure.

RESULTS AND DISCUSSION

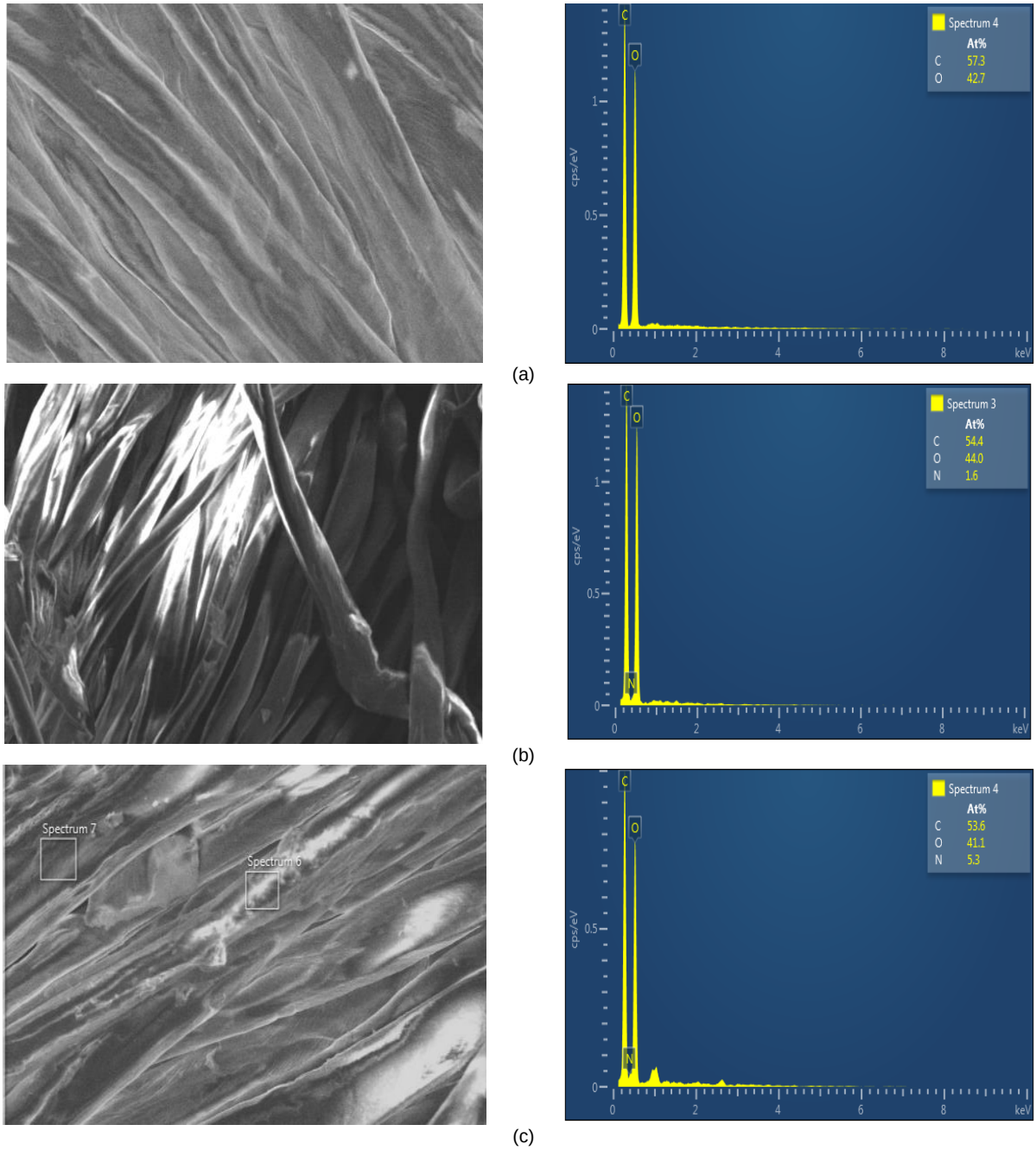
Determination on nitrogen content of treated fabrics based on FESEM images coupled with EDX spectra

In previous works, chitosan was cross-linked by citric acid (CA) via an amidation reaction (i.e., amide group replaces a hydrogen atom on amino group) at an elevated temperature [28]. CA agent can dissolve chitosan completely. DMDHEU is also known as an excellent anti-wrinkle finishing agent on cotton fabric. Both CA and DMDHEU can react with hydroxyl groups and amine groups in cellulose and chitosan as illustrated in the proposed mechanism.



(a) (b)

Figure 1. Proposed bonding mechanism of cellulose and chitosan in the presence of crosslinkers (CA and DMDHEU): (a) Chitosan – CA – Cellulose, (b) Chitosan – DMDHEU - Cellulose.



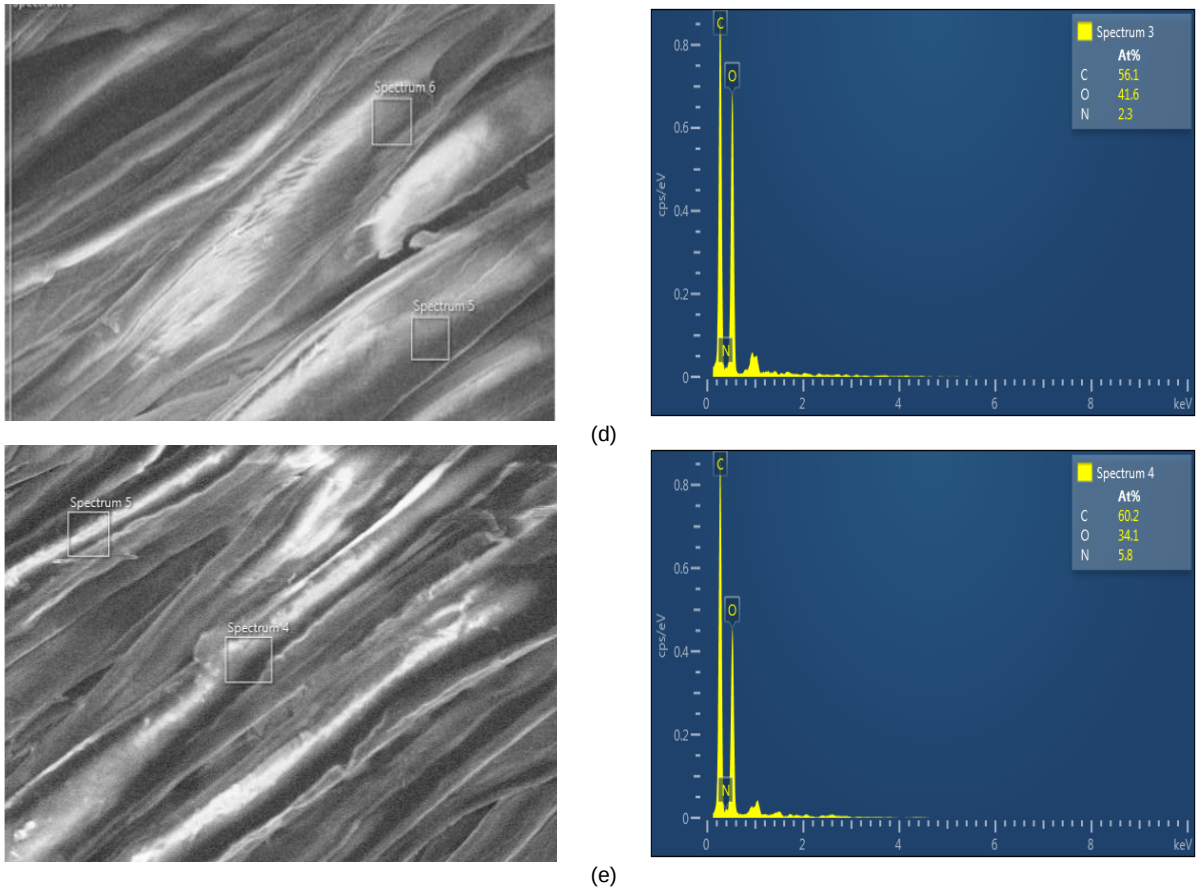


Figure 2. FESEM photos and EDX spectra of (a) untreated sample, treated with b) CTS1 and CA, c) CTS2 and DMDHEU, d) CTS1 and CA, and e) CTS2 and DMDHEU.

Table 1. Total content of carbon (C), oxygen (O) and nitrogen (N) atoms for untreated sample and treated samples with CTS1-CA, CTS2-CA, CTS1-DMDHEU, and CTS2-DMDHEU based on EDX spectra.

Sample	Molecular weight (kDa)	Content (%)		
		Carbon	Oxygen	Nitrogen
Untreated	-	57.3	42.7	0.0
CTS1-CA	2.6	54.4	44.0	1.6
CTS2-CA	187	56.1	41.6	2.3
CTS1-DMDHEU	2.6	53.6	44.1	5.3
CTS2-DMDHEU	187	60.2	34.1	5.8

For the antibacterial treatment of cotton fabrics with chitosan, two types of molecular weight of chitosan (2.6 kDa and 187 kDa, referred to as CTS1 and CTS2) and two types of crosslinker (CA and DMDHEU) were used. As shown in Figure 2 and Table 1, FESEM images and EDX spectra demonstrated that nitrogen atoms were not detectable in untreated sample with chitosan in the presence of CA. However, nitrogen atoms were present in all antibacterial samples treated with CTS1 and CTS2. For the same crosslinker, the nitrogen content of samples treated with CTS2 was higher than that of those treated with CTS1 (specifically, being 2.3 % vs. 1.6 % for CA, being 5.8 % vs. 5.3% for DMDHEU, respectively). Moreover, for the same chitosan type, the nitrogen content of antibacterial treated cotton fabrics crosslinked with CA was significantly lower than those crosslinked with DMDHEU due to absence of nitrogen atoms in the chemical structure of citric acid (specifically, 1.6% vs. 5.3 % for CA and 2.3 % vs. 5.8 % for DMDHEU). In other words, a significant nitrogen content was added to the cotton fabrics due to the nitrogen atoms present in both chitosan and DMDHEU, as illustrated in Figure 1.

Not only N atoms but also O and C atoms were detected in EDX spectra. When replacing CA with DMDHEU, the C content decreases from 54.4% to 53.6% for CTS1 and increases from 56.1% to 60.2% for CTS2 while O content decreases from 44.0% to 41.1% for CTS1 and from 41.6 to 34.1% for CTS2. Besides, as shown in **Figure 2**, FE-SEM photos provide further evidence of morphological variations in antibacterial treated cotton samples compared to untreated sample, with a small amount of finishing agent scattered across the surface of cotton fibers.

Evaluation on nitrogen content of treated fabrics based on dyeability

It is known that cellulose fibers cannot be dyed with acid dyes, but antibacterial treated cotton fibers with chitosan possess amine groups that may bond to acid dyes. Hence, nitrogen content could be determined through the content of acid dyes. The content of absorbed acid dyes was equivalent to the amount of amine groups inside treated cotton fabrics [29].

Antibacterial treated fabrics were dyed with acid dyes, and their dyeability was evaluated by color strength (K/S). This is because K/S is proportional to the content of chitosan linked to amine groups, allowing nitrogen content be determined indirectly. To ensure objectively K/S values of treated samples, an investigation was conducted to detect tthe maximum wavelength in the range from 360 to 700 cm^{-1} in various acid concentrations (including 1, 5, 10, 20, 25, 50 and 100 g/l) through UV-vis measurements. The experimental results indicated that the most suitable wavelength was 440 cm-1, at which the highest absorbance (1.1906 au) was found, as shown in Figure 3.

At this wavelength, the relationship between the absorbance (A) of antibacterial treated samples with chitosan and dye concentration (C) is expressed by the following linear calibration equation

$$A = 0.0084C + 0.0033,\ R^2 = 0.9999 \qquad (1)$$

Acid dyes could be removed from cotton fabrics because of relatively weak physical interactions (i.e., no chemical bonds). Therefore, the content of acid dyes was measured over several washing cycles, as presented in **Figure 4**. It can be observed that after 0, 5, 10, 15 and 20 of washing cycles, the contents of acid dyes on samples treated with CTS1 and CTS2 were slightly decreased, being 83.97, 79.78, 71.76 and 64.13 % for CTS1 and 90.73, 86.54, 84.99 and 80.35% for CTS2, respectively. Clearly, acid dyes formed a relatively durable linkage with textile fibers due to strong interactions between amine groups of chitosan. However, dyed samples with CTS1 exhibited less washing durability than those with CTS2. In other words, the amine groups of higher molecular weight chitosan on antibacterial treated cotton fabrics were bonded to dyestuffs more effectively [16].

Additionally, the decrease in K/S value of treated samples with CTS1 and CTS2 over washing cycles was represented as a linear function (as shown in Figure 5). The negative slopes of these lines were -0.006 and -0.012 for treated samples with CTS1 and CTS2, respectively, indicating that the content of acid dyes on antibacterial treated cotton with high molecular weight chitosan decreases more quickly with washing cycles. It can be affirmed that the higher the content of acid dyes, the darker the color on the fabric, and the higher the K/S value.

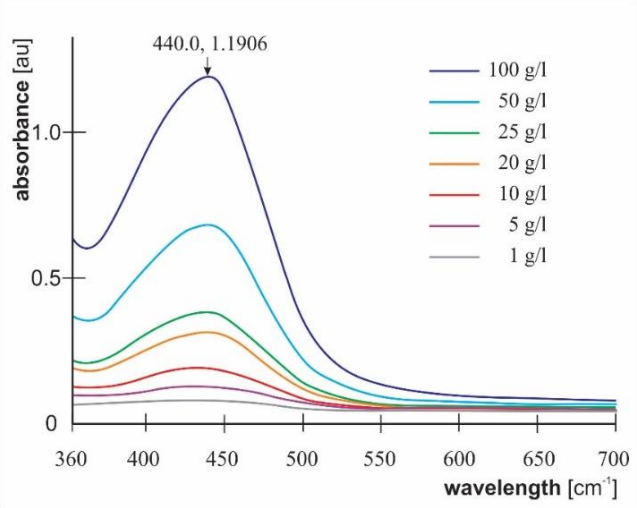


Figure 3. UV-vis spectra of antibacterial treated samples with 0.1% owf of chitosan at various concentrations of acid dyes (1, 5, 10, 20, 25, 50 and 100 g/l).

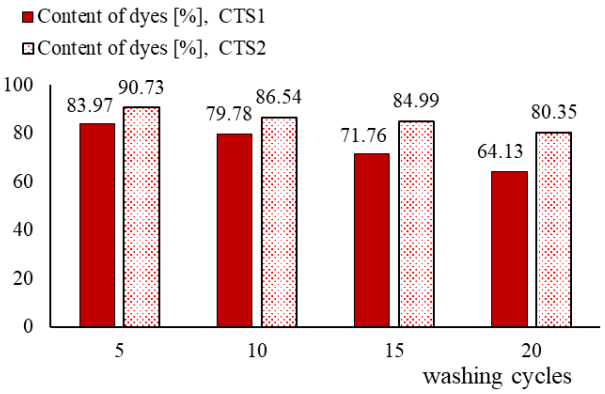


Figure 4. Decrease in content of acid dyes on antibacterial cotton fabrics treated with CTS1 and CTS2 after 5, 10, 15 and 20 washing cycles.

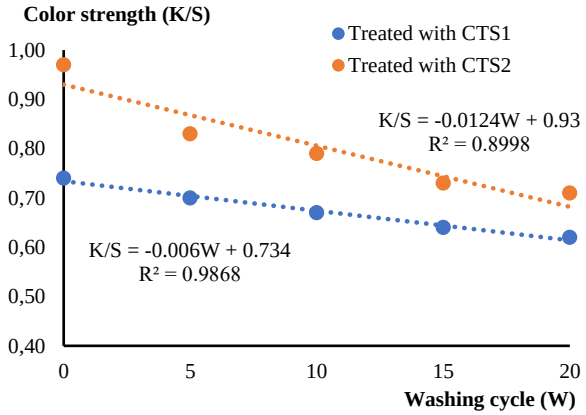


Figure 5. Curve of color strength (K/S) versus washing cycle (W) for antibacterial treated cotton fabrics with CTS1 and CTS2.

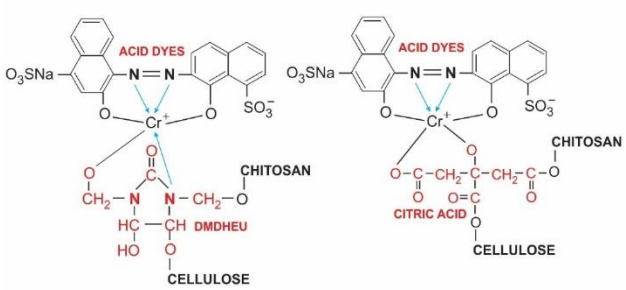


Figure 6. Proposed bonding mechanism of acid dyes with CA and DMDHEU owing to metal complex formation on antibacterial treated cotton fibers with chitosan.

Figure 7. Content of acid dyes on antibacterial treated cotton fabrics with CTS2, CA and DMDHEU after 0, 5, 10, 15 and 20 washing cycles.

The role of crosslinkers, including CA and DMDHEU, in dyeing cotton fabrics with chitosan was also examined under washing conditions. A bonding mechanism among acid dyes, metal complexes, CA/DMDHEU, CTS1/CTS2 and cellulose was proposed, as illustrated in Figure 6. Metal ions played an important role in forming complexes to coordinate acid dyes with CA and DMDHEU.

The content of acid dyes fixed with CA/DMDHEU due to metal complex formation on antibacterial treated fabrics with CTS2 is shown in Figure 7. The nitrogen content absorbed into cotton fabrics (M_{abs}) is calculated based on the following equation:

$$M_{abs} = M_{pre} - M_{post} - M_{wash} \quad (2)$$

where M_{pre} and M_{post} are the masses of dyes in pre-dyed and post-dyed solution, respectively, and M_{wash} is the mass of dyes in the washing solution after various cycles.

Accordingly, the content of acid dyes bonded with amine groups (M_{amine}) is determined as follows

$$M_{amine} = M_{abs(treated)} - M_{abs(untreated)} \quad (3)$$

where $M_{abs(treated)}$ is the content of acid dyes on untreated sample with chitosan and $M_{abs(untreated)}$ is the content of acid dyes on treated sample with chitosan.

Based on the obtained results, it is demonstrated that the content of acid dyes on treated fabrics with DMDHEU is much higher than that with CA after all washing cycles. This may be because DMDHEU possesses hydroxyl groups and azo groups which promote more metal complex formation with acid dyes, while CA possesses hydroxyl groups only. Consequently, the content of acid dyes on all treated samples with CA was much lower than that with DMDHEU after several washing cycles.

To evaluate the chemical retention of antibacterial treated cotton fabrics with CTS1 in the presence of CA and DMDHEU through the amount of acid dyes bonded to amine groups, the reduction in the content of acid dyes was investigated after 5, 10, 15 and 20 washing cycles, as reported in Table 3. The results indicated that the content of acid dyes on samples crosslinked with DMDHEU seemed to be reduced more rapidly than those crosslinked with CA after the first washing cycles, but these reductions became similar after 20 washing cycles.

In summary, the nitrogen content of antibacterial cotton fabrics treated with chitosan can be quantitatively determined based on the amount of acid dyes using the calibration curve of absorbance versus dye concentration, as represented in Equation (1).

Table 3. The content of acid dyes in antibacterial treated cotton fabrics with CTS1 crosslinked with CA and DMDHEU after 0, 5, 10, 15 and 20 washing cycles (denoted as CTS1-CA00, CTS1-CA05, CTS1-CA10, CTS1-CA15, CTS1-CA20, CTS1- DMDHEU00, CTS1-DMDHEU05, CTS1- DMDHEU10, CTS1- DMDHEU15, and CTS1-DMDHEU20, respectively) (M_{pre} is 10mg).

Sample	Washing cycle	M_{post} [mg]	M_{wash} [mg]	M_{amine} [mg]	Reduction [%]
CTS1-CA00	0	4.4606	3.2478	2.2920	-
CTS1-CA05	5	4.7668	2.9698	2.2600	1.4
CTS1-CA10	10	4.1051	3.7886	2.1063	8.1
CTS1-CA15	15	4.1973	3.8178	1.9840	13.44
CTS1-CA20	20	4.3797	3.8870	1.7330	24.39
CTS1-DMDHEU00	0	3.9822	3.2771	2.7410	-
CTS1-DMDHEU05	5	4.1885	3.3502	2.4610	6.8
CTS1-DMDHUE10	10	4.2178	3.3502	2.4320	14.4
CTS1-DMDHEU15	15	3.9808	3.6574	2.3600	18.33
CTS1-DMDHEU20	20	4.3377	3.4965	2.1660	24.14

Table 4. Nitrogen content of antibacterial treated cotton samples with CTS1-CA and CTS2-CA after 0, 5, 10, 15 and 20 washing cycles.

Washing cycles	Nitrogen content [%]			
	CTS1-CA		CTS2-CA	
	Content [%]	Reduction [%]	Content [%]	Reduction [%]
0	0.1458	-	0.1770	-
5	0.1393	4.40	0.1500	15.25
10	0.1394	4.38	0.1319	25.48
15	0.1238	15.01	0.1315	25.70
20	0.1052	27.85	0.1364	22.90

Evaluation on total nitrogen content of treated fabrics using elemental analysis method

The Dumas method, also known as combustion method, is an elemental analysis technique used to quantitively determine total nitrogen content in chemical substances, particularly for assessing crude protein concentration in food specimens). In this method, a specimen of known weight is combusted at extremely high temperature in the presence of oxygen, releasing carbon dioxide (CO_2), water (H_2O) and nitrogen (N_2). A special column containing potassium hydroxide (KOH) absorbs all molecules of CO_2 and H_2O, allowing nitrogen content to be measured. The determination results of nitrogen content using elemental analysis are presented in Table 4.

In an initial investigation, an insignificant nitrogen content about 0.0083 % was detected in untreated samples with chitosan due to the minimal nitrogen presence in cotton cultivation. Conversely, all treated samples with CTS1 and CTS2 had significantly higher nitrogen content than untreated samples, by at least 12.67 times. Table 4 presents the nitrogen content of antibacterial treated cotton fabrics obtained from two different types of molecular weight (i.e., 2.6 kDa and 187 kDa) and after 5, 10, 15 and 20 washing cycles. In general, the nitrogen content of samples treated with both CT1-CA and CTS2-CA significantly decreased with washing cycles. Notably, the elemental analysis method concluded that the nitrogen content in samples treated with CTS2 decreased more rapidly with washing cycles compared to those treated with CTS1. For examples, after 5 washing cycles, the nitrogen content dropped to 15.25 % and 4.4 % for treated sample with CTS2 and CTS1, respectively.

Evaluation of nitrogen content on treated fabrics based on antibacterial ability

Since antibacterial agent is not easy to diffuse into an aqueous solution, it may cause suspension issues. Accordingly, the antibacterial activity of treated cotton fabrics with chitosan should be examined under dynamic contact conditions. These tests were conducted with a predominant gram-negative bacterium, Escherichia coli, following the E2149–01 standard method. The antibacterial ability of immobilized agents on antimicrobial treated cotton fabrics was quantitatively determined through bacterial reduction after 60 and 2 contact minutes. The percent reduction of bacteria in the solution after contacting treated samples, compared to the initial solution without a sample (R_o) and with an untreated control sample (R_c), is calculated using the following equations.

$$\boldsymbol{R_o}\ [\%] = \mathbf{100}\frac{\boldsymbol{N_o - N_n}}{\boldsymbol{N_o}} \quad (4)$$

$$\boldsymbol{R_c}\ [\%] = \mathbf{100}\frac{\boldsymbol{N_c - N_n}}{\boldsymbol{N_c}} \quad (5)$$

where N_o is the number of colonies in the initial bacterial solution [CFU/ml], and N_c and N_n are the numbers of colonies in the bacterial solution (CFU/ml) after incubating the untreated sample and treated sample with chitosan for a given time under standard conditions.

The evaluation results of antibacterial activity on treated cotton fabrics with CTS1 or CTS2, which were crosslinked with CA or DMDHEU under various washing conditions, are reported in Table 5 and Figure 8. It can be concluded that the antibacterial activity of all treated samples with chitosan decreased with washing cycles. It rapidly reduced from 0 to 5 washing cycles and gradually reduced from 5 to 20 washing cycles. At the same washing cycle, the antibacterial activity of treated samples crosslinked with DMDHEU was always lower than that crosslinked wth CA. Furthermore, the antibacterial fastness of samples treated with DMDHEU was also much better than those treated with CA. For instance, after 15 washes, the bacterial reduction on antibacterial treated sample with CTS1 and CA in 60 minutes of exposure was 59%, while it was 38% for those treated with CTS1 and DMDHEU. Similarly, the reduction was 67.4% for sample treated with CTS2 and CA, while it was 49.2% for those treated with CTS2 and DMDHEU. The investigation results also suggested that using chitosan with a higher molecular weight on cotton fabrics ensures better antibacterial ability and maintains its antibacterial durability through washing conditions. These results are consistent with the evaluations and explanations in previous sections (i.e., results of image analysis, dyeability, elemental analysis).

Table 5. Bacterial reduction (R_c) of cotton fabrics treated with CTS1/CTS2 and CA/DMDHEU after 0, 5, 10, 15 and 20 washing cycles.

Sample	Washing cycles	R_c [%]	
		2 min	60 min
CTS1-CA00	0	56.0	100.0
CTS1-CA05	5	16.7	69.7
CTS1-CA10	10	13.6	62.0
CTS1-CA15	15	9.0	59.0
CTS1-CA20	20	6.0	57.6
CTS1-DMDHEU00	0	48.0	80.0
CTS1-DMDHEU05	5	16.6	53.7
CTS1-DMDHEU10	10	11.1	48.1
CTS1-DMDHEU15	15	7.4	38.0
CTS1-DMDHEU20	20	3.7	35.0
CTS2-CA00	0	60.0	100.0
CTS2-CA05	5	47.8	74.0
CTS2-CA10	10	39.0	71.7
CTS2-CA15	15	30.4	67.4
CTS2-CA20	20	30.4	63.0
CTS2-DMDHEU00	0	48.0	84.0
CTS2-DMDHEU05	5	16.6	60.0
CTS2-DMDHEU10	10	11.1	56.9
CTS2-DMDHEU15	15	7.4	49.2
CTS2-DMDHEU20	20	3.7	40.0

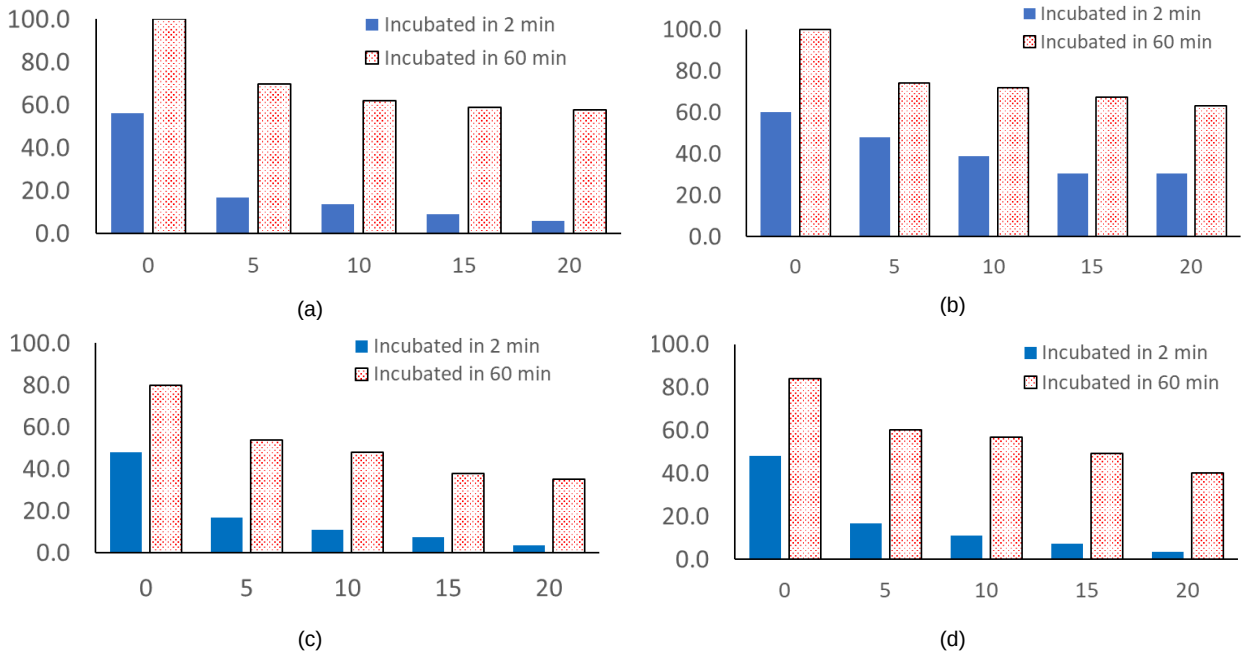


Figure 8. Bacterial reduction *Re* [%] of treated cotton fabrics with (a) CTS1 and CA, (b) CTS2 and CA, (c) CTS1 and DMDHEU and (d) CTS2 and DMDHEU after 0, 5, 10, 15 and 20 washing cycles.

CONCLUSIONS

This work used four different methods including electron microscopy, additional dyeability, elemental analysis through combustion test and antibacterial experiments under dynamic contact conditions to determine N content, thereby evaluating antibacterial activity of treated fabrics with chitosan. Besides, two types of chitosan (CTS1 and CTS2) and two types of crosslinkers (CA and DMDHEU) were applied to clarify bonding mechanism and effectiveness of antibacterial treated fabrics. Actually, the relationship between antibacterial ability and molecular weight of chitosan as well as washing durability is still being debated because it is a naturally occurring polymer with a wide range of degrees of acetylation, making it

difficult to determine its effects. The authors attempted to conduct this work to demonstrate that the nitrogen content in chitosan with low/high molecular weight and crosslinkers could be determined in four different ways, so that the antibacterial ability of treated fabrics could be evaluated both directly and indirectly.

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