


Article

Adsorption Studies of Ammonia, Protein, and Phytic Acid Using Chitosan Fiber Coated with Oxidized Cellulose Nanofiber

Duangkamol Dechojarassri ^{1,2}, Kensuke Nishida ¹, Ryousuke Ozakiya ¹, Tetsuya Furuike ^{1,2} and Hiroshi Tamura ^{1,2,*}

¹ Faculty of Chemistry, Materials and Bioengineering, Kansai University, 3-3-35 Yamate-cho, Osaka 564-8680, Japan

² Organization for Research and Development of Innovative Science and Technology (ORDIST), Kansai University, 3-3-35 Yamate-cho, Osaka 564-8680, Japan

* Correspondence: tamura@kansai-u.ac.jp; Tel.: +81-6-6368-0871

Abstract: Herein, chitosan (CS) fibers coated with TEMPO-oxidized cellulose nanofibers (CS/TOCN fibers) were successfully prepared using a wet spinning technique; CS was dissolved in acetic acid to obtain a CS doping solution. The tensile strength and ammonia adsorption percentages increased with increasing TOCN concentration. The maximum ammonia adsorption percentage (41.39%, 8.3 mg/g) was obtained when 2% NaOH and 0.01% TOCN were used as the coagulation solution. Additionally, the adsorption of model proteins, including lysozyme (Lz), cytochrome C (Cyt C), and bovine serum albumin (BSA), were studied. In water, the CS/TOCN fibers with negative charges adsorbed more Lz with positive charges than CS fibers containing positive charges. Contrastingly, CS fibers adsorbed more Cyt C and BSA containing negative charges in phosphate-buffered saline solutions than CS/TOCN fibers. Furthermore, the adsorption percentage of phytic acid using the CS/TOCN fibers reached 64.18% (288 mg/g) within 60 min. Thus, TOCNs improved the tensile properties of CS fibers and preferred positively charged materials contaminated in water, such as ammonia, Lz, and phytic acid.

Keywords: adsorption; Chitosan; TOCN



Citation: Dechojarassri, D.; Nishida, K.; Ozakiya, R.; Furuike, T.; Tamura, H. Adsorption Studies of Ammonia, Protein, and Phytic Acid Using Chitosan Fiber Coated with Oxidized Cellulose Nanofiber. *Fibers* **2023**, *11*, 32. <https://doi.org/10.3390/fib11040032>

Academic Editors: Catalin R. Picu and Lucian Lucia

Received: 2 March 2023

Revised: 24 March 2023

Accepted: 29 March 2023

Published: 30 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Chitosan (CS) is a high-molecular-weight polysaccharide that is biocompatible, biodegradable, biodegradable, non-toxic, and has high mechanical strength. Thus, CS can be applied in various fields. Particularly, CS can be used as adsorptive materials for dye, heavy metals, and several types of proteins [1,2]. In addition, CS can be modified as a magnetic material for bovine serum albumin (BSA) adsorption, which can be easily removed after adsorption [3,4].

Recently, 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-oxidized cellulose nanofibers (TOCNs) prepared from cellulose using TEMPO-mediated oxidation have attracted significant interest, as they can be used as reinforcing filler in polymer composites. Additionally, TOCNs can improve tensile strength, flexural strength, optical transparency, and toughness [5–8]. Because there is a negative charge on the surface of TOCNs, they can be used in several applications, such as medicine, cement, and catalysis, including antimicrobial applications [9–13]. Moreover, they can react with several polymers to form hydrogels [14–17]. In addition, many researchers have reported that TOCNs and CS can form CS/TOCN composites owing to electrostatic attraction. The TOCNs can improve the mechanical and barrier properties of CS films [18,19]. The TOCN composites are present not only as beads, hydrogels, and films, but can also be coated on the fiber surface of polymers. Coating TOCNs on poly(vinyl alcohol) (PVA)-drawn fibers can dramatically improve the tensile modulus compared to that of a commercial PVA-drawn fiber [20]. Another interesting application of the polymer/TOCN composite is its use as a protein adsorbent. Cellulose triacetate modified with TOCN can be used as an ultrafiltration membrane. To investigate

the absorption abilities of protein, several types of proteins with different sized molecules, including trypsin (20 kDa), pepsin (39 kDa), egg albumin (EA, 45 kDa), and bovine serum albumin (69 kDa), were chosen to represent different sizes of particles. However, only the size of protein molecules was considered in terms of molecular weight cut-off, while the attraction and surface charges between TOCN and protein were not addressed [21]. Therefore, the effect of the surface charges of different proteins on the adsorption abilities of TOCN/polymer composites should be studied.

Ammonia, which is present in cigarette smoke and released during cement manufacturing, has spread into the environment, affecting human health [22,23]. Many materials can adsorb ammonia, such as coal ash, carboxymethyl starch-g-polyvinylpyrrolidone, carbon-silica composites, and Fe nanoparticles [22–25]. Because ammonia can protonate H^+ in water, it is possible to use TOCN, which has a negative charge on its surface, to adsorb ammonia in water.

Additionally, in recent times, the price of fishmeal used in aquaculture has increased. As soybean meal contains high protein content and is inexpensive, it is pertinent to use it instead of the typical fishmeal. However, phytic acid in soybean meal inhibits mineral absorption, which may promote mineral deficiency. Tripolyphosphate/genipin co-crosslinked CS beads can be used as an adsorbent for phytic acid from a soybean whey solution, with an adsorption percentage of 30.23% [26].

In this study, a CS fiber coated with TOCN (CS/TOCN fiber) using the wet spinning technique was successfully prepared for the first time. A commercial TOCN, Rheocrysta[®], obtained from DKS Co. Ltd., was used in this experiment. To understand the attraction between proteins and TOCNs, three types of proteins, namely, BSA (negative charge), cytochrome C (Cyt C, zero charge), and Lysozyme (Lz, positive charge), were chosen as the protein models. In addition, the adsorption of ammonia from water (positive charge) and phytic acid from water using CS/TOCN fibers was also investigated.

2. Materials and Methods

2.1. Materials

The CS (grade FL-80, DAC: 84.7, 5 mPa·s) was supplied by Koyo Chemical Co., Ltd. (Osaka, Japan); TOCN, Reocrysta I-2SX (solid content 2.3%, pH 7.1, viscosity 64,000 mPa·s) was provided by Dai-ichi Kogyo Seiyaku Co., Ltd. (DKS Co., Ltd., Kyoto, Japan). Acetic acid (99.9%), sodium hydroxide (NaOH), methanol (MeOH), hydrochloric acid (HCl), nitric acid, phosphate-buffered saline (PBS) powder (0.01 mol/L, pH 7.2–7.4), and Cyt C (derived from bovine heart, untreated TCA) of analytical grade were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). BSA (lyophilized powder, essentially globulin free, essentially protease free, (agarose gel electrophoresis)) and Lz (from chicken egg white) were obtained from Sigma-Aldrich. All chemicals were used without any treatment.

2.2. Preparation of CS Fiber

The wet spinning apparatus setup was similar to that in our previous work [27]. However, the conditions of wet spinning were varied and optimized according to the material. First, acetic acid (2.5 g) was added to distilled water (137.5 g) to obtain a 1% acetic acid solution. Then, CS powder (10 g) was added to the 1% acetic acid solution and mixed until the drop solution was homogeneous to obtain a CS solution with a concentration of 4 wt%. The CS drop solution was then de-foamed until the air bubbles completely disappeared. The dope solutions were subsequently spun using a wet spinning technique to obtain CS fibers, as shown in Figure 1. A 2% NaOH solution was used as the coagulant for CS fiber production. After spinning, the fibers were neutralized by replacing 2% NaOH solution with MeOH and then stirred for 24 h. Subsequently, fresh MeOH was replaced repeatedly every 4 days until the pH value reached 7. Finally, the products were dried at room temperature.

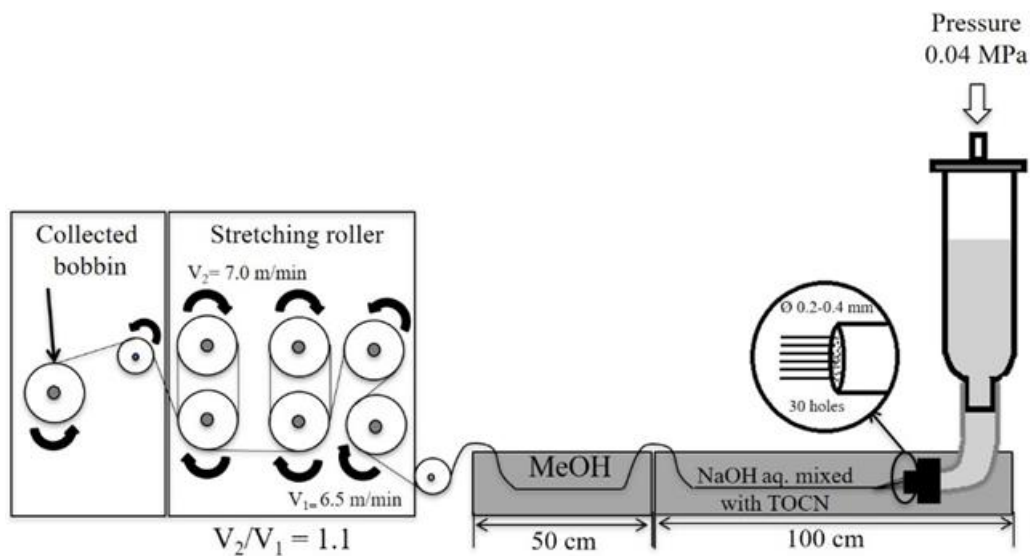


Figure 1. Wet-spinning apparatus and spinning conditions.

2.3. Preparation of CS/TOCN Composite Fiber with Various TOCN Concentration

To investigate the effect of the TOCN content on the tensile properties and spinning ability, the NaOH concentration was fixed at 2%, while the TOCN concentrations were varied as 0, 0.001, 0.0075, 0.01, 0.05, and 0.1%. The chemical structure of CS and the cross-sectional structure of TOCN are shown in Figure 2.

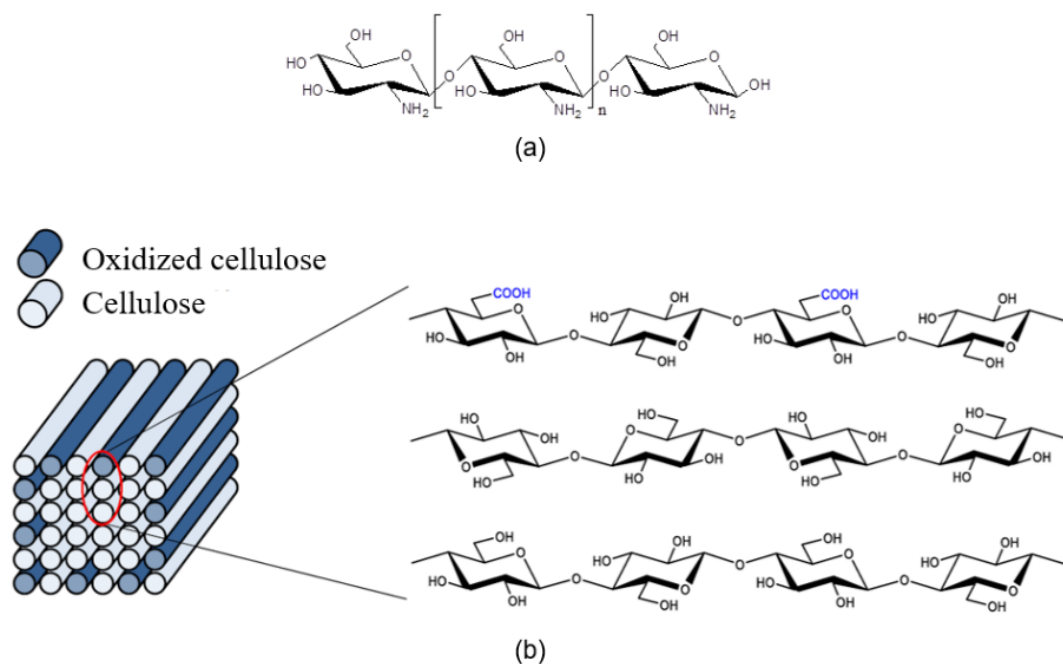


Figure 2. (a) Chemical structure of CS; (b) cross-sectional structure of TOCN.

2.4. Preparation of CS/TOCN Composite Fiber with Various Concentrations of NaOH Solution

To investigate the effect of NaOH concentration on the tensile properties and spinning ability, the TOCN concentration was fixed at 0.01%, while the NaOH concentrations were varied as 1, 2, 4, and 6%.

2.5. Preparation of CS/TOCN Composite Fiber by Washing with a Mixture Solution of MeOH and Water

The CS doping solution was prepared using the method described in Section 2.2. For the spinning process, 150 mL of 0.1% TOCN was dispersed in 1350 mL NaOH aqueous solution to obtain the final TOCN and NaOH concentrations of 0.01% and 2%, respectively. The fibers were then washed using the same method as described in Section 2.2, using mixed solutions of MeOH and water in the ratios of 10:0, 4:6, and 0:10.

2.6. Tensile Property of CS and CS/TOCN Fibers

To measure the tensile properties of the CS/TOCN composite fiber (change in TOCN concentration, NaOH concentration, and washing conditions), first, the fiber diameter was measured. Subsequently, the tensile properties were measured using a universal testing machine (STA-1150; A&D Co., Ltd., Michigan, USA), and the average value reported here was calculated using 30 samples. A fiber sample with width, thickness, and length of 1.00, 0.10, and 10 mm, respectively, was used for the measurements.

2.7. Morphology of CS and CS/TOCN Fibers

The morphologies of the dried CS and CS/TOCN fibers were observed using a scanning electron microscope (SEM, JEOL JSM-6700F, Japan). Each sample was vacuum-dried at room temperature and coated with platinum for 200 s before observation.

2.8. Swelling Tests

The samples were vacuum-dried at 35 °C for 24 h, weighed, and finally immersed in distilled water at room temperature for 24 h. Subsequently, the samples were removed from distilled water, and excess water was removed. The weight of the fiber in its swollen state was measured using an electronic balance. The swelling ratio was determined using Equation (1):

$$\text{Swelling ratio} = (W_s - W_i)/W_i, \quad (1)$$

where W_i (g) and W_s (g) are the initial and swollen weights, respectively.

2.9. Fourier Transform Infrared Spectroscopy Measurement

Fourier transform infrared spectroscopy (FTIR) (JASCO FT/IR-4200) measurements were performed for qualitative analysis (change in TOCN concentration) of CS/TOCN composite fibers using the KBr method. Before measurement, the finely chopped fibers were dried in a vacuum oven for 1 day. For measurement, CS powder and 0.001, 0.0075, and 0.01% CS/TOCN composite fibers were used. A measurement range of 4000–400 cm^{-1} , accumulation count of 16, and resolution of 4.0 cm^{-1} were used for the measurements.

2.10. Measurement of Zeta Potential of Fiber Surfaces

In this study, polystyrene latex (particle size ~ 500 nm) with hydroxypropyl cellulose (Mw = 30,000) was used as the standard monitoring particle. Water was used as the solvent for the monitoring solution. The measurement temperature was 25 °C and the refractive index of the solvent was 1.3328. The viscosity of the monitoring solution was 0.8878 mPa·s, whereas that of the solvent was 78.6 mPa·s. A zeta-potential (ZP) and particle size analyzer (ELSZ-1000, OTSUKA Electronics Co., Ltd., Osaka, Japan) was used to measure the ZP of the fiber surface.

To prepare the samples for measuring of the ZP of fiber surfaces, the spun CS fibers and 0.01% CS/TOCN fibers were washed with MeOH five times, followed by washing with pure water to remove salt from the fibers. Then, the fibers were washed twice with pure MeOH and dried at room temperature. The dried fibers were knotted as single-line-knotted fibers by using a braiding machine (Kokubun Ltd., Hamamatsu, Japan) before measurement, as shown in Figure S1. The sample was then placed in a solid sample cell unit. The values reported here were obtained using the average of three values. Fourier transform infrared spectroscopy (FTIR) (JASCO FT/IR-4200) measurements were performed for qualitative

analysis (change in TOCN concentration) of CS/TOCN composite fibers using the KBr method. Before measurement, the finely chopped fibers were dried in a vacuum oven for 1 day. For measurement, CS powder and 0.001, 0.0075, and 0.01% CS/TOCN composite fibers were used. A measurement range of 4000–400 cm^{-1} , accumulation count of 16, and resolution of 4.0 cm^{-1} were used for the measurements.

2.11. Ammonia Adsorption Test

The dried fiber sample (50 mg) was soaked in 100 mL of ammonia-contaminated water (10 mg/mL, pH 7.6). The mixture was then shaken at 25 °C in a shaking bath at 195 rpm for 60 min. The initial and remaining ammonia contents in the contaminated water were measured using a weak base and a strong acid titration method. In this study, 0.01 M HCl was used as the strong acid. The values reported here are the average value obtained from three titrations.

2.12. Protein Adsorption Test

This study chose Lz, Cyt C, and BSA as the model proteins. For the adsorption test, 50 mg of various types of fibers was added to 25 mL of protein solution and shaken at 195 rpm and 25 °C for 60 min. The remaining protein in the solution was determined using ultraviolet-visible (UV-vis) spectroscopy (Hitachi U-2910, Hitachi High-Tech Corporation, Tokyo, Japan) with scanning wavelengths of 260–300 nm, 300–500 nm, and 260–340 nm for Lz, Cyt C, and BSA, respectively. The properties of protein solutions in water or PBS and the maximum UV wavelength for each protein are shown in Table 1.

Table 1. Properties of the protein solutions in water or PBS, and the maximum UV wavelength for each protein.

Protein Types	M.W. (Da)	ZP (mV)		Maximum Wavelength (nm)
		In Water	In PBS Solution	
Lz	14,307	14.25 ± 5.40 (pH 6.2)	−11.41 ± 2.50 (pH 6.8)	280
Cyt C	12,384	12.87 ± 1.85 (pH 6.2)	−0.34 ± 0.25 (pH 6.8)	490
BSA	66,000	−7.86 ± 1.35 (pH 5.9)	−12.4 ± 1.83 (pH 6.8)	278

Protein adsorption percentage and adsorption capacity are defined by Equations (2) and (3), respectively:

$$\text{Protein adsorption percentage (\%)} = (C_0 - C_e)/C_e \times 100, \quad (2)$$

where C_0 (g) and C_e (g) are the protein concentration (mg/L) at the initial stage and equilibrium, respectively.

$$q = ((C_0 - C_t) \times V)/w, \quad (3)$$

where q is the adsorption capacity (mg/g), C_t is the remaining protein concentration (mg/L), V (mL) is the volume of the solution, and w (g) is the weight of fiber.

2.13. Phytic Acid Adsorption Test

The dried fiber sample (50 mg) was soaked in 30 mL of water-contaminated phytic acid (750 mg/mL). The mixture was then shaken at 25 °C in a shaking bath at 195 rpm for 60 min. Subsequently, the fibers were removed from the solution. The initial and remaining phytic acid content was measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Shimadzu ICPS-7510, Shimadzu Corporation, Kyoto, Japan). The phytic acid adsorption percentage and adsorption capacity were calculated according to Equations (2) and (3), respectively.

3. Results and Discussion

3.1. Effect of Different Coagulants on Fiber Production, and Their Tensile Properties and Fiber Diameter

In this study, the effects of concentration of either TOCN or NaOH as a coagulant and washing conditions on the diameters and tensile properties of the obtained fibers were investigated.

First, the effect of TOCN concentration on fiber production was studied. For TOCN concentrations of 0.01–0.1%, coagulation of TOCN can be observed during fiber production, as shown in Figure 3. These results suggest that the TOCN concentration in the coagulated bath of 0.01% is the maximum concentration that can be used in this wet spinning method.

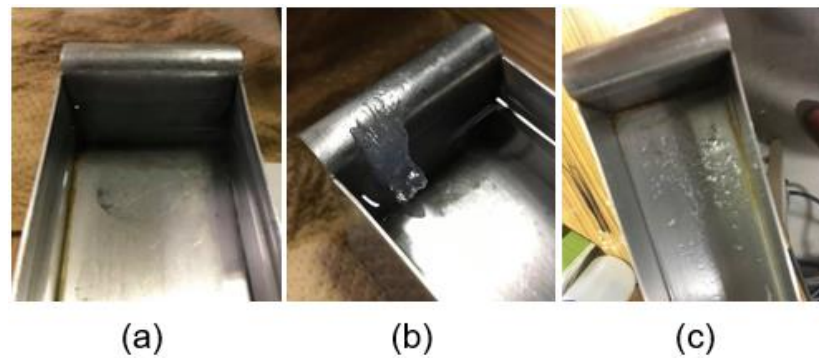


Figure 3. Coagulation of TOCN during fiber production using different concentrations of TOCN: (a) 0.01%, (b) 0.05%, and (c) 0.1%.

The fiber diameters of CS/TOCN composite fiber (change in TOCN concentration, NaOH concentration, and washing conditions) are shown in Table 2. The fiber diameter of pristine CS fibers was $77 \pm 6 \mu\text{m}$. For CS/TOCN composite fibers, the diameter tended to be smaller, and the fiber diameter at 0.01% TOCN was $72 \pm 4 \mu\text{m}$. These results can be attributed to contraction owing to the attachment of TOCN to CS via electrostatic interaction. By contrast, the fibers gradually thicken at concentrations higher than 0.01%. The fiber diameter at 0.1% TOCN was $80 \pm 4 \mu\text{m}$; this increased thickness of the fiber can be attributed to the excessive adhesion of TOCN.

Table 2. Physical and mechanical properties of various types of CS/TOCN.

NaOH Solution (%)	TOCN Conc. (%)	Ratio of MeOH:Water	Diameter of Obtained Fiber (μm)	Tensile Strength (MPa)	Percentage of Elongation at Break (%EL)
2	0	10:0	77 ± 6	107.5 ± 19.7	10.7 ± 2.5
	0.001		74 ± 7	140.3 ± 27.1	8.4 ± 1.8
	0.0075		73 ± 7	151.9 ± 17.5	8.1 ± 1.8
	0.01		72 ± 4	158.0 ± 17.0	7.7 ± 2.2
	0.05		75 ± 1	122.1 ± 21.7	12.0 ± 2.3
	0.1		80 ± 4	107.5 ± 21.2	14.0 ± 2.7
1	0.01	10:0	81 ± 4	125.8 ± 30.9	8.5 ± 1.4
2			72 ± 4	158.0 ± 24.1	7.7 ± 2.1
4			71 ± 4	117.0 ± 19.8	14.4 ± 2.5
6			94 ± 4	106.5 ± 14.2	10.7 ± 3.5
2	0.01	10:0	72 ± 4	158.0 ± 24.1	7.7 ± 2.1
		4:6	107 ± 11	96 ± 20	23 ± 5
		0:10	93 ± 13	92 ± 22	18 ± 4

The tensile strength increased with increasing TOCN concentration, reaching a maximum at a TOCN concentration of 0.01%. Beyond this concentration, the strength decreased with increasing TOCN concentration (Table 2). These results correspond to the maximum concentration of TOCN in the coagulation bath. The self-aggregation of the TOCNs beyond 0.01% TOCN concentration directly affected the tensile strength of the obtained fibers. The

percentage of elongation at break (%EL) of the CS/TOCN fibers was lower than that of CS fibers. However, when the TOCN concentration was higher than 0.01%, %EL increased with increasing TOCN concentration. These results can be attributed to the lower flexibility on account of the higher strength, which leads to a lower %EL. In this study, the enhancement of the tensile strength of CS fiber by TOCN is similar to the improvement of the tensile strength of PVA-drawn fiber by TOCN [20].

To determine the effect of NaOH concentration as a coagulant, a mixture of TOCN and NaOH was prepared by fixing the TOCN concentration at 0.01% and varying the NaOH concentration as 1, 2, 4, and 6%. The results revealed that a NaOH concentration of 2% yielded the highest tensile strength of the obtained fibers. The tensile strengths decreased at 4% and 6% NaOH concentrations due to the TOCN self-aggregation. In the experiment, when acetic acid from CS dope solution reacts with NaOH, sodium acetate with a cylindrical rod shape will be formed, and the rods of sodium acetate could interact with TOCN, leading to TOCN self-aggregates. Fukuzumi et al. (2014) investigated the effect of NaCl addition with the various NaCl concentrations on the aggregation behavior of 0.1% TOCN in water. It was found that the TOCN dispersion was still homogeneous when adding 50 mM NaCl, but the aggregated gel could be confirmed when adding 100 mM. This phenomenon could be explained by the interaction potential energy between two cylindrical rods based on the Derjaguin–Landau–Verwey–Overbeek theory [28].

When the washing conditions were changed, the fiber strength decreased as the proportion of water in the mixture of MeOH and water increased. When a mixture with a MeOH-to-water ratio of 0:10 was used, the observed value of tensile strength was 92 ± 22 MPa. During fiber preparation, the molecules in the fiber are oriented by stretching. However, after washing with the mixture, MeOH was replaced with water, disturbing the molecular orientation. Therefore, the fiber strength was considered to decrease. Based on the fiber strength results, it seems more appropriate not to use water for washing the fibers.

3.2. Morphology, Swelling Ratio, and FTIR Spectra of CS and CS/TOCN Fibers under Various Conditions

The SEM analysis was performed to investigate the morphological changes on the fiber surface corresponding to the TOCN content, NaOH concentration, and washing conditions. Figure 4a–h show the SEM images of the CS/TOCN composite fibers with varying TOCN content. The SEM images with 250- and 15,000-times magnification confirmed that the fibers became thinner with increasing TOCN concentration. As the TOCN concentration increased, the fiber surface became smoother, and fewer cracks were observed. This can be attributed to the densification of the CS matrix owing to the electrostatic interaction between the TOCN and CS, leading to shrinkage of the fiber surface. Figure 4i–p show the SEM images of the CS/TOCN composite fibers with various NaOH concentrations. The SEM images indicate that for the NaOH concentration of 4 and 6%, many cracks were observed on the fiber surface. Again, as mentioned in Section 3.1, the NaOH concentration more than 2% as a fiber coagulant was considered to be too high. Figure 4q–v show the SEM images of the CS/TOCN composite fiber for different washing conditions; the smoothest fiber was obtained after washing with a mixture with a MeOH–water ratio of 10:0.

A swelling test of the CS/TOCN composite fibers under varying washing conditions was performed in pure water and PBS; the results of the swelling tests are shown in Figure 5. After the swelling test, the fibers retained their morphology and remained strong enough not to break unless pulled strongly. There was no significant change in the swelling ratios of the CS fibers and CS/TOCN composite fibers in pure water (Figure 5a). However, at higher TOCN concentrations in PBS, the swelling ratio and the difference between the swelling ratios in pure water and PBS decreased (Figure 5b). This can be attributed to the interaction between the fibers and salts owing to the electrostatic cross-linking of TOCN and CS. Considering the change in the washing conditions, the swelling ratio decreased as the proportion of water during washing increased. Salt was removed by washing with water, and the effect of osmotic pressure on the salt could be considered the main reason

for the decrease in the swelling ratio. When the Na content in the fiber was reduced, it became difficult for water to enter the fiber (Figure 5c).

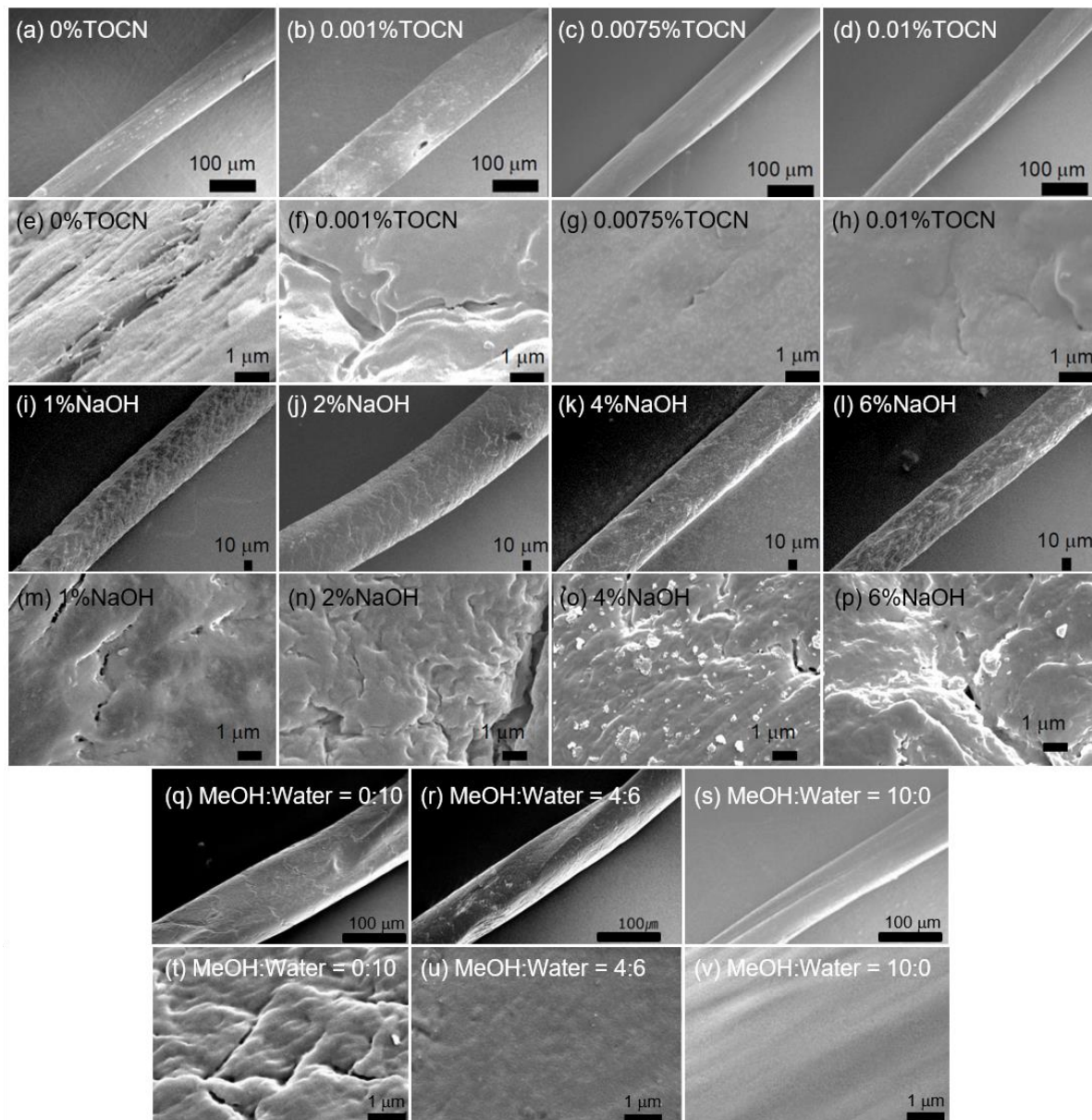


Figure 4. SEM micrographs of CS/TOCN fibers ($\times 250$) with various TOCN contents (a–d), ($\times 15,000$) with various TOCN contents (e–h), ($\times 250$) with various percentages of NaOH (i–l), ($\times 15,000$) with various percentages of NaOH (m–p), ($\times 250$) with various ratios of MeOH to water (q–s), and ($\times 250$) with various ratios of MeOH to water (t–v).

Based on the tensile properties and SEM images, the optimal spinning conditions for the CS/TOCN composite fibers were a NaOH concentration of 2% and a TOCN concentration of 0.01%.

The FTIR measurements of CS powder along with 0.001, 0.0075, and 0.01% CS/TOCN composite fibers were performed to confirm the presence of TOCN (Figure 6). FTIR was unable to confirm the presence of TOCN in any of the samples because the spectra obtained had the same shape as that obtained for the CS powder. The lack of change in the FTIR spectra can be attributed to the extremely small amount of TOCN on the CS surface.

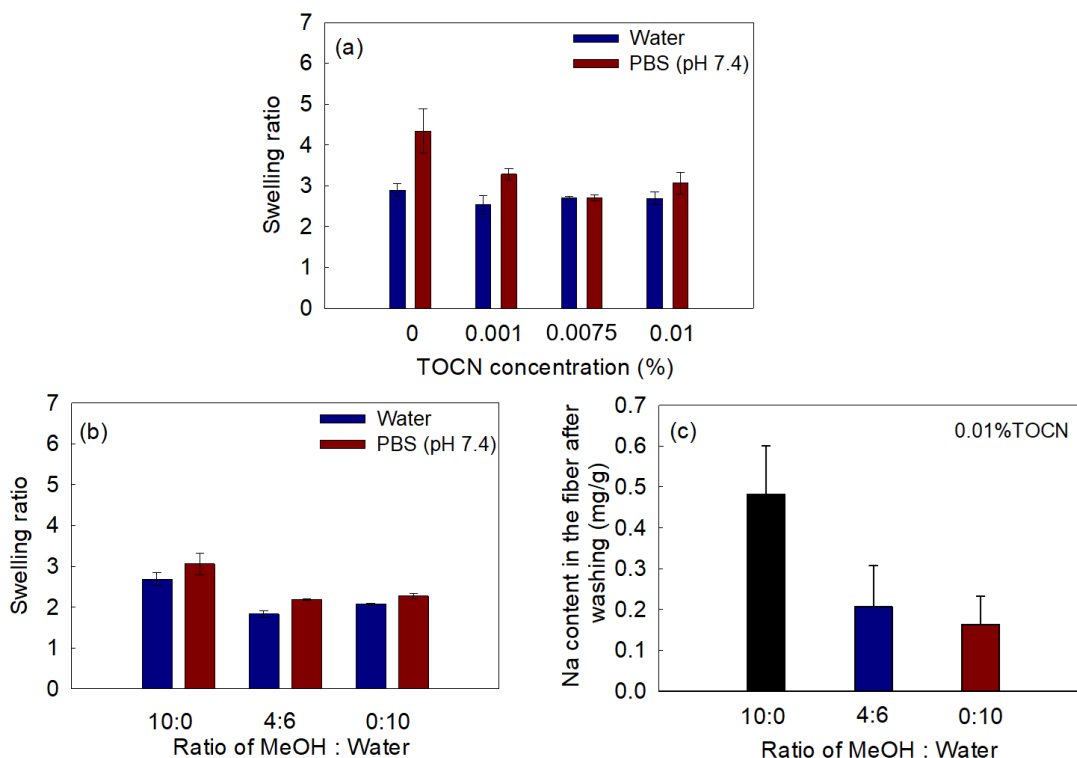


Figure 5. Results of the swelling test of fibers (a) at various TOCN concentrations (MeOH:water = 10:0) and (b) at various ratios of MeOH to water as the washing agent (0.01%TOCN), and (c) Na content in the fibers after washing at various ratios of MeOH to water (0.01%TOCN).

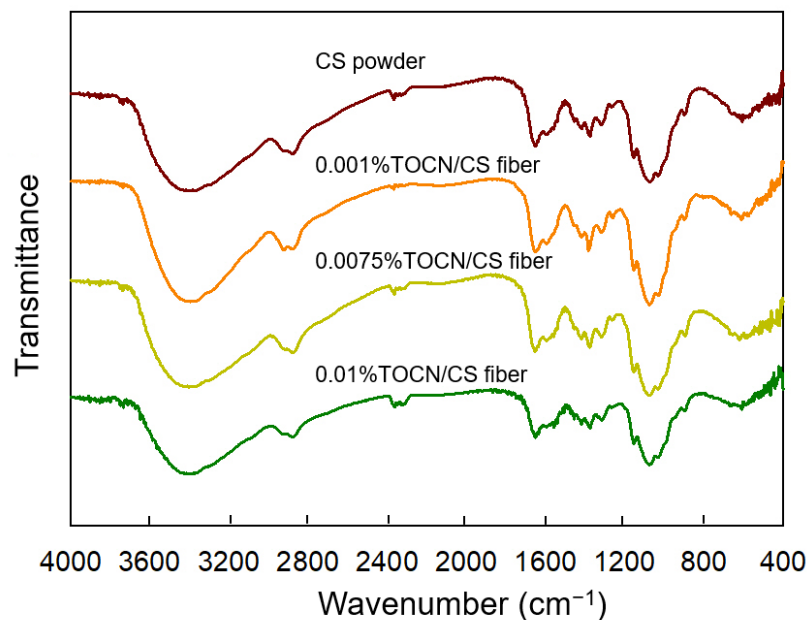


Figure 6. FTIR spectra for CS powder and CS/TOCN composite fibers.

3.3. ZP of Fiber Surfaces

The results of ZP measurements for varying TOCN contents, NaOH concentrations, and washing conditions are shown in Figure 7a–c, respectively. The ZP values of the samples with TOCN contents of 0, 0.001, 0.0075, and 0.01% were 6.5, 3.0, −1.7, and −3.2 mV, respectively. As the TOCN content increased, the ZP became more negative, thereby increasing the amount of TOCN with a negative charge in the CS/TOCN fiber. These

results correspond to the fact that CS can protonate cations (positive charge) from NH_2 in the CS molecule, whereas TOCN has a carboxylate functional group (negative charge) in its molecule [29]. When the NaOH concentrations were 1%, 2%, 4%, and 6%, the ZP values were 3.8, -3.2 , 3.9, and 1.3 mV, respectively. Thus, the trend in ZP values corresponding to the varying NaOH concentrations could not be confirmed. Although the degree of coagulation of the fiber and the surface morphology depended on the NaOH concentration, it did not affect the amount of TOCN or salt content. When the ratios of MeOH to water were 10:0, 4:6, and 0:10, the ZP values were -3.4 , -56.0 , and -55.0 mV, respectively. At MeOH-to-water ratios of 4:6 and 0:10, the ZPs of both samples exhibited almost the same value. As the proportion of water increased, the positively charged Na was removed, and the negative charge increased compared to that obtained for washing with pure MeOH. In addition, the surface ZP values for 0.01% CS/TOCN fiber at the initial, middle, and final stages of spinning production were also determined. The surface ZP values were similar at all stages, implying that the TOCN concentration of 0.01% in the NaOH solution was sufficient to produce the same quality of 0.01% CS/TOCN fibers (Figure 7d).

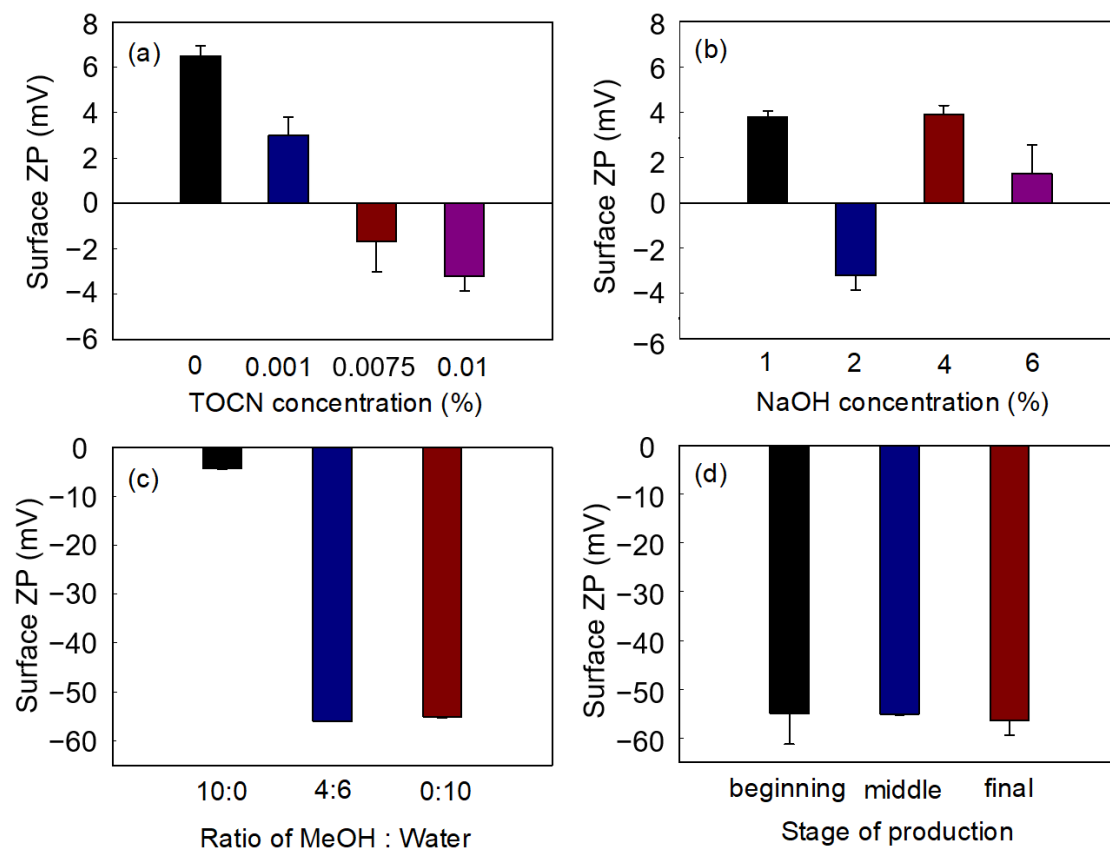


Figure 7. ZP values of fiber surfaces (a) at various TOCN concentrations using 2% NaOH as coagulation solution, (b) at various NaOH concentrations using 0.01% TOCN mixture as the coagulation solution, (c) at various ratios of MeOH to water as the washing agent, and (d) at different stages of production using 0.01% TOCN and 2% NaOH solution as coagulation solution.

3.4. Ammonia Adsorption

The ammonia adsorption of CS/TOCN fiber from ammonia-contaminated water with the initial ammonia concentration of 10 mg/mL was carried out using CS/TOCN fiber amount of 0.5 g/L, a temperature of 25 °C, and a shaking speed of 195 rpm, 60 min. The ammonia adsorption results are shown in Figure 8. A higher absorption NH_4^+ was observed for a higher concentration of TOCN in the coagulation solution, presumably owing to the presence of a higher amount of TOCN on the fiber surface. Because the charges on TOCN are negative, more negative charges on CS/TOCN could react with more

NH_4^+ , increasing the NH_4^+ adsorption percentage. In addition, the results of ammonia adsorption percentages demonstrate a trend similar to that for the strength of CS/TOCN fibers, which exhibited the maximum value of 41.39% (8.3 mg/g) when 2% NaOH was used along with 0.01% TOCN as the coagulation solution. Long et al. (2008) reported that the activated carbon from coconut with a specific area of $1050 \text{ m}^2/\text{g}$ could adsorb ammonia 12.96 mg/g for the initial ammonia concentration of 1.7 mg/mL using 120 mesh-activated carbon, an activated carbon amount of 33.3 g/L, $40 \text{ }^\circ\text{C}$, and stirring speed of 200 rpm for 60 min. In addition, the adsorption capacity increased with higher temperatures, and a smaller amount of active carbon was also reported [30]. Even though the ammonia adsorption capacity of CS/TOCN fiber could not be compared to the result from Long et al. (2008) directly, because the experimental conditions were not similar, this result still shows that composite fibers have the potential to be used as an ammonia adsorbent.

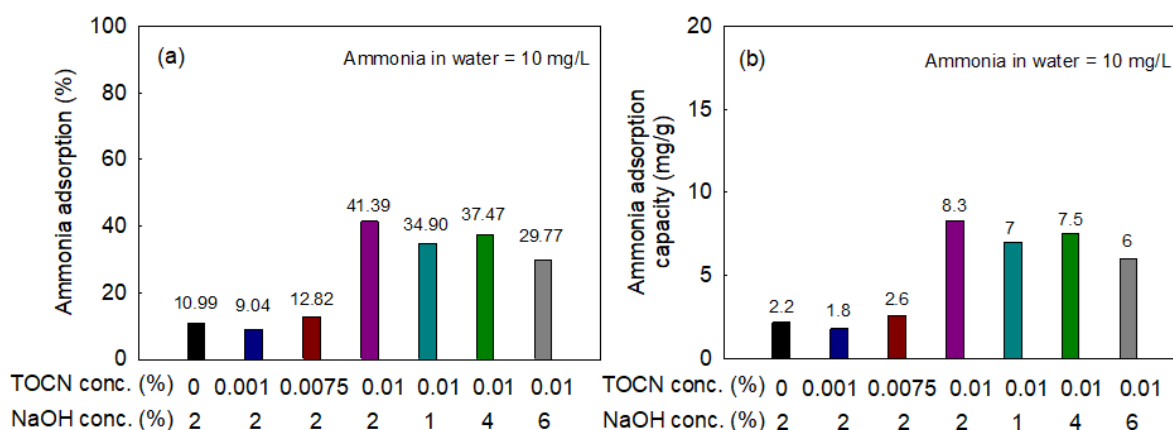


Figure 8. Ammonia adsorption percentage (a) and ammonia adsorption capacity (b) for various TOCN and NaOH concentrations.

3.5. Protein Adsorption

In this study, Lz, Cyt C, and BSA were chosen as the model proteins. The amount of protein was determined using UV-vis spectrophotometer. The properties of protein solutions, either in water or in PBS, are shown in Table 1. The remaining protein content in the solutions and the protein adsorption percentages after the adsorption for 60 min using different types of fibers are shown in Figure 9. For the adsorption test of Lz in PBS solution, two peaks were obtained using the UV-vis spectrophotometer. This may be because the Lz molecule was denatured in the PBS solution, changing the charge on the surface of Lz from positive to negative (Table 1). Considering Cyt C and BSA in water, the calibration could not be performed accurately because of the self-aggregation of either Cyt C or BSA molecules in water.

For the adsorption of Lz in water, the Lz adsorption percentage increased when the TOCN concentration was increased. As the Lz in water was positively charged, a maximum adsorption percentage of 9.09% was observed for the 0.01% CS/TOCN fibers containing a negative charge that could adsorb Lz. Furthermore, when 2% NaOH along with 0.01% TOCN was used as the coagulation solution, the Lz adsorption percentage was higher than those obtained using 1%, 4%, and 6% NaOH solutions, which corresponds to a similar trend as that observed for their strength. By contrast, BSA in PBS solution was negatively charged, and CS-containing cationic material (positive charge) could adsorb BSA in PBS solutions with a maximum of 20.4%. In addition, the BSA adsorption percentage decreased with increasing TOCN concentration owing to the increasing negative charge on the fiber surfaces. Considering the adsorption of Cyt C in PBS, although the ZP value of Cyt C in PBS solution was negative, it was almost zero; therefore, the adsorption percentages using all types of fibers were also close to zero. These results may be attributed to the presence of charges on the surface of either CS or CS/TOCN fibers, which prevented them from reacting well with the molecules that had no net electrical charge. In addition, the

adsorption of Cyt C in the PBS solution decreased slightly when the TOCN concentration increased. It should be noted that the adsorption of Lz in water using 0.01% CS/TOCN fibers reported here is the 60 min-adsorption result. Thus, to understand the Lz adsorption mechanism of CS/TOCN fibers, other factors such as the initial concentration of Lz, the pH of the Lz solution, the adsorption temperature, the number of fibers, the adsorption kinetics, and the adsorption isotherms should be further investigated in the future, as they are essential for explaining the Lz adsorption mechanism of CS/TOCN fibers.

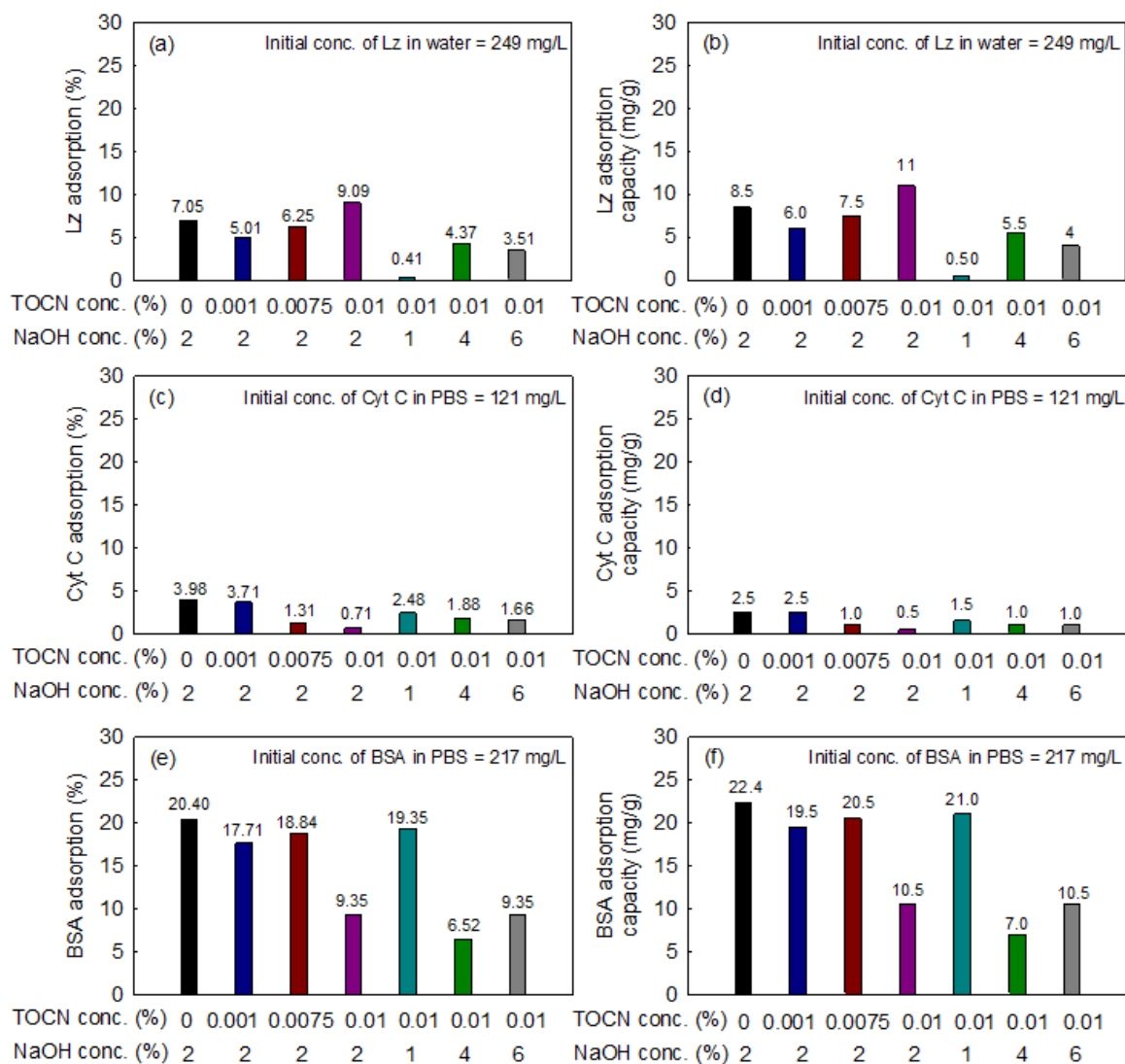


Figure 9. Protein adsorption percentages (a,c,e), and protein adsorption capacities (b,d,f) for various types of fibers. (a,b) Lz; (c,d) Cyt C; (e,f) BSA.

3.6. Phytic Acid Adsorption

The effect of washing conditions on the phytic acid adsorption test using CS/TOCN fibers is shown in Figure 10. Corresponding to the MeOH-to-water ratios of 10:0, 4:6, and 0:10, at an initial phytic acid concentration of 750 mg/L, the adsorption capacities of water were 61.06% (274 mg/g), 63.07% (264.8 mg/g), and 64.18% (288.8 mg/g) within 60 min; the adsorption capacity increased as the proportion of water increased. These results suggest that the amino groups on the surface were exposed when salt was removed from the water. Yang et al. (2011) reported that tripolyphosphate/genipin co-crosslinked CS beads could adsorb phytic acid at an initial concentration of 270 mM (1782 mg/L) from a soybean whey solution with 30.23% adsorption within 24 h [26]. Although the initial concentration of

phytic acid used in that study differs from that used in the present study, it can be concluded that CS/TOCN composite fibers have a high potential for phytic acid absorption.

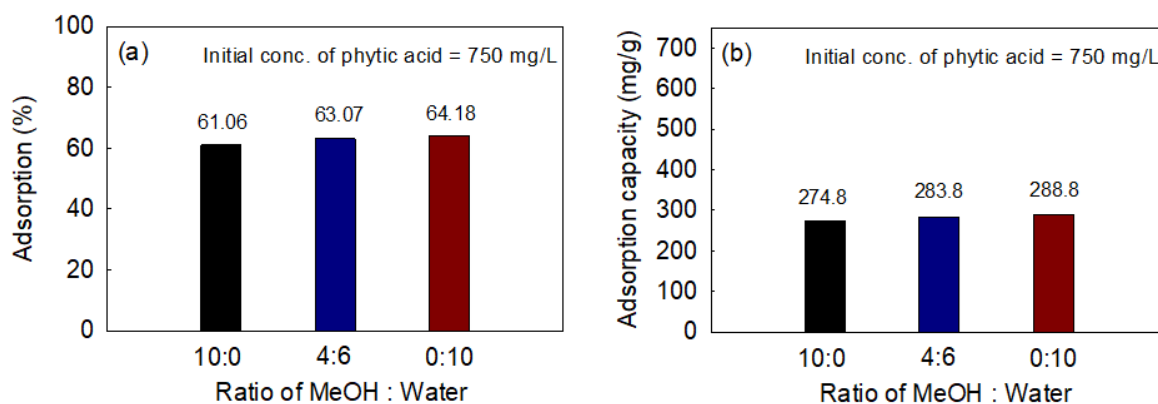


Figure 10. Phytic acid adsorption results: (a) phytic acid adsorption percentage and (b) phytic acid adsorption capacity.

4. Conclusions

In this study, CS fibers coated with TOCN were successfully prepared using the wet spinning technique. With the increase in the TOCN concentration, the amount of negative charge on the surface of CS fibers increased. In addition, the concentration of NaOH as a coagulant in CS fibers coated with TOCNs affected their properties because the sodium acetate cylindrical rods were formed at high NaOH concentration, leading to the TOCN self-aggregation. The optimum NaOH and TOCN concentrations for coagulation were 2% and 0.01%, respectively. In conclusion, CS/TOCN fibers can be used as an adsorbent for removing not only ammonia from contaminated water, and some types of proteins such as Lz, which has a positive charge, but also phytic acid.

5. Patents

- (1) Hashimoto, M. Kitamura, T. Goto, T. Tamura, H. Furuike, T. Nishida, K. Dechojarassri, D. (2021). Composite fiber and method for producing same, and adsorbent (Japan Patent No. 6853551). Japan Patent Office. <https://www.jpo.go.jp/e/index.html>. accessed on 2 March 2023.
- (2) Kitamura, T. Goto, Morita, Y. Hashimoto, M. T. Tamura, H. Furuike, T. Ozakiya, R. Sahara, A. (2021). Composite fiber and method for producing same, and adsorbent (Japan Patent No. 2021123831). Japan Patent Office. <https://www.jpo.go.jp/e/index.html>. accessed on 2 March 2023.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fib11040032/s1>, Figure S1. The single-line-knotted fiber for surface ZP measurement.

Author Contributions: D.D. contributed to the methodology, investigation, writing—original draft, review and editing. K.N. and R.O. contributed to the methodology and investigation. T.F. and H.T. contributed to conceptualization, funding acquisition, resources, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the Private University Research Branding Project: Matching Fund Subsidy from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) Japan (2016–2020).

Data Availability Statement: Data will be made available on request.

Acknowledgments: The authors would like to thank DKS Co., Ltd. for providing TOCN.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Salehi, E.; Daraei, P.; Shamsabadi, A.A. A Review on Chitosan-Based Adsorptive Membranes. *Carbohydr. Polym.* **2016**, *152*, 419–432. [[CrossRef](#)]
2. Zeng, X.; Ruckenstein, E. Cross-Linked Macroporous Chitosan Anion-Exchange Membranes for Protein Separations. *J. Membr. Sci.* **1998**, *148*, 195–205. [[CrossRef](#)]
3. Wang, Y.; Wang, X.; Luo, G.; Dai, Y. Adsorption of Bovin Serum Albumin (BSA) onto the Magnetic Chitosan Nanoparticles Prepared by a Microemulsion System. *Bioresour. Technol.* **2008**, *99*, 3881–3884. [[CrossRef](#)] [[PubMed](#)]
4. Mahdavinia, G.R.; Soleymani, M.; Etemadi, H.; Sabzi, M.; Atlasi, Z. Model protein BSA Adsorption onto Novel Magnetic Chitosan/PVA/Laponite RD Hydrogel Nanocomposite Beads. *Int. J. Biol. Macromol.* **2018**, *107*, 719–729. [[CrossRef](#)] [[PubMed](#)]
5. Fukuzumi, H.; Saito, T.; Isogai, A. Influence of TEMPO-Oxidized Cellulose Nanofibril Length on Film Properties. *Carbohydr. Polym.* **2013**, *93*, 172–177. [[CrossRef](#)]
6. Uribe, B.E.B.; Chiromito, E.M.S.; Carvalho, A.J.F.; Arenal, R.; Tarpani, J.R. TEMPO-Oxidized Cellulose Nanofibers as Interfacial Strengtheners in Continuous-Fiber Reinforced Polymer Composites. *Mater. Des.* **2017**, *133*, 340–348. [[CrossRef](#)]
7. Fujisawa, S.; Saito, T.; Kimura, S.; Iwata, T.; Isogai, A. Comparison of Mechanical Reinforcement Effects of Surface-Modified Cellulose Nanofibrils and Carbon Nanotubes in PLLA Composites. *Compos. Sci. Technol.* **2014**, *90*, 96–101. [[CrossRef](#)]
8. Hamou, K.B.; Kaddami, H.; Dufresne, A.; Boufi, S.; Magnin, A.; Erchiqui, F. Impact of TEMPO-Oxidation Strength on the Properties of Cellulose Nanofibril Reinforced Polyvinyl Acetate Nanocomposites. *Carbohydr. Polym.* **2018**, *181*, 1061–1070. [[CrossRef](#)]
9. Carlsson, D.O.; Hua, K.; Forsgren, J.; Mhraryan, A. Aspirin Degradation in Surface-Charged TEMPO-Oxidized Mesoporous Crystalline Nanocellulose. *Int. J. Pharm.* **2014**, *461*, 74–81. [[CrossRef](#)]
10. Bakkari, M.E.; Bindiganavile, V.; Goncalves, J.; Boluk, Y. Preparation of Cellulose Nanofibers by TEMPO-Oxidation of Bleached Chemi-Thermomechanical Pulp for Cement Applications. *Carbohydr. Polym.* **2019**, *203*, 238–245. [[CrossRef](#)]
11. Shefa, A.A.; Amirian, J.; Kang, H.J.; Bae, S.H.; Jung, H.; Choi, H.; Lee, Y.S.; Lee, B. In Vitro and in Vivo Evaluation of Effectiveness of a Novel TEMPO-Oxidized Cellulose Nanofiber-Silk Fibroin Scaffold in Wound Healing. *Carbohydr. Polym.* **2017**, *177*, 284–296. [[CrossRef](#)] [[PubMed](#)]
12. Liu, S.; Liang, H.; Sun, T.; Yang, D.; Cao, M. A Recoverable Dendritic Polyamidoamine Immobilized TEMPO for Efficient Catalytic Oxidation of Cellulose. *Carbohydr. Polym.* **2018**, *202*, 563–570. [[CrossRef](#)] [[PubMed](#)]
13. Lal, S.S.; Mhaske, S.T. AgBr and AgCl Nanoparticle Doped TEMPO-Oxidized Microfiber Cellulose as a Starting Material for Antimicrobial Filter. *Carbohydr. Polym.* **2018**, *191*, 266–279. [[CrossRef](#)] [[PubMed](#)]
14. Wei, J.; Chen, Y.; Liu, H.; Du, C.; Yu, H.; Zhou, Z. Thermo-Responsive and Compression Properties of TEMPO-Oxidized Cellulose Nanofiber-Modified PNIPAm Hydrogels. *Carbohydr. Polym.* **2016**, *147*, 201–207. [[CrossRef](#)] [[PubMed](#)]
15. Wei, J.; Chen, Y.; Liu, H.; Du, C.; Yu, H.; Ru, J.; Zhou, Z. Effect of Surface Charge Content in the Tempo-Oxidized Cellulose Nanofibers on Morphologies and Properties of Poly(N-Isopropylacrylamide)-Based Composite Hydrogels. *Ind. Crops Prod.* **2016**, *92*, 227–235. [[CrossRef](#)]
16. Li, N.; Chen, W.; Chen, G.; Tian, J. Rapid shape memory TEMPO-Oxidized Cellulose Nanofibers/Polyacrylamide/Gelatin Hydrogels with Enhanced Mechanical Strength. *Carbohydr. Polym.* **2017**, *171*, 77–84. [[CrossRef](#)] [[PubMed](#)]
17. Nguyen, T.H.M.; Abueva, C.; Ho, H.V.; Lee, S.Y.; Lee, B.T. In Vitro and in Vivo Acute Response towards Injectable Thermosensitive Chitosan/TEMPO-Oxidized Cellulose Nanofiber Hydrogel. *Carbohydr. Polym.* **2018**, *180*, 246–255. [[CrossRef](#)]
18. Soni, B.; Hassan, E.B.; Schilling, M.W.; Mahmoud, B. Transparent Bionanocomposite Films Based on Chitosan and TEMPO-Oxidized Cellulose Nanofibers with Enhanced Mechanical and Barrier Properties. *Carbohydr. Polym.* **2016**, *151*, 779–789. [[CrossRef](#)]
19. Tang, R.; Yu, Z.; Renneckar, S.; Zhang, Y. Coupling Chitosan and TEMPO-Oxidized Nanofibrillated Cellulose by Electrostatic Attraction and Chemical Reaction. *Carbohydr. Polym.* **2018**, *202*, 84–90. [[CrossRef](#)]
20. Endo, R.; Saito, T.; Isogai, A. TEMPO-Oxidized Cellulose Nanofibril/Poly(Vinyl Alcohol) Composite Drawn Fibers. *Polymer* **2013**, *54*, 935–941. [[CrossRef](#)]
21. Kong, L.; Zhang, D.; Shao, Z.; Han, B.; Lv, Y.; Gao, K.; Peng, X. Superior Effect of TEMPO-Oxidized Cellulose Nanofibrils (TOCNs) on the Performance of Cellulose Triacetate (CTA) Ultrafiltration Membrane. *Desalination* **2014**, *332*, 117–125. [[CrossRef](#)]
22. Zhao, Y.; Yang, W.; Zhou, J.; Wang, Z.; Liu, J.; Cen, K. Experimental Study on Ammonia Adsorption by Coal Ashes. *J. Fuel Chem. Technol.* **2015**, *43*, 266–272. [[CrossRef](#)]
23. Haroon, M.; Wang, L.; Yu, H.; Ullaha, R.S.; Abdin, Z.U.; Khan, R.U.; Chen, Q.; Liu, J. Synthesis of Carboxymethyl Starch-G-Polyvinylpyrrolidones and Their Properties for the Adsorption of Rhodamine 6g and Ammonia. *Carbohydr. Polym.* **2018**, *186*, 150–158. [[CrossRef](#)] [[PubMed](#)]
24. Furtado, A.M.B.; Wang, Y.; LeVan, M.D. Carbon Silica Composites for Sulfur Dioxide and Ammonia Adsorption. *Microporous Mesoporous Mater.* **2013**, *165*, 48–54. [[CrossRef](#)]
25. Otero, G.S.; Pascucci, B.; Branda, M.M.; Miotto, R.; Belevi, P.G. Evaluating the Size of Fe Nanoparticles for Ammonia Adsorption and Dehydrogenation. *Comput. Mater. Sci.* **2016**, *124*, 220–227. [[CrossRef](#)]
26. Yang, C.; Hsu, C.; Tsai, M. Effect of Crosslinked Condition on Characteristics of Chitosan/Tripolyphosphate/Genipin Beads and Their Application in the Selective Adsorption of Phytic Acid from Soybean Whey. *Carbohydr. Polym.* **2011**, *86*, 659–665. [[CrossRef](#)]

27. Dechojarassri, D.; Omote, S.; Nishida, K.; Omura, T.; Yamaguchi, H.; Furuike, T.; Tamura, H. Preparation of Alginate Fibers Coagulated by Calcium Chloride or Sulfuric Acid: Application to the Adsorption of Sr^{2+} . *J. Hazard. Mater.* **2018**, *355*, 154–161. [[CrossRef](#)]
28. Fukuzumi, H.; Tanaka, R.; Saito, T.; Isogai, A. Dispersion Stability and Aggregation Behavior of TEMPO-Oxidized Cellulose Nanofibrils in Water as a Function of Salt Addition. *Cellulose* **2014**, *21*, 1553–1559. [[CrossRef](#)]
29. Kuramae, R.; Saito, T.; Isogai, A. TEMPO-Oxidized Cellulose Nanofibrils Prepared from Various Plant Hemicelluloses. *React. Funct. Polym.* **2014**, *85*, 126–133. [[CrossRef](#)]
30. Long, X.; Cheng, H.; Xin, Z.; Xiao, W.; Li, W.; Yuan, W. Adsorption of Ammonia on Activated Carbon from Aqueous Solutions. *Environ. Prog.* **2008**, *27*, 225–233. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.