Spherical and rod-like dialdehyde cellulose nanocrystals by sodium periodate oxidation: Optimization with double response surface model and templates for silver nanoparticles

F-F. Lu¹, H-Y. Yu^{1,2*}, Y. Zhou¹, J-M. Yao²

¹The Key Laboratory of Advanced Textile Materials and Manufacturing Technology of Ministry of Education, College of Materials and Textiles, Zhejiang Sci-Tech University, 310018 Hangzhou, China

²National Engineering Lab for Textile Fiber Materials & Processing Technology, Zhejiang Sci-Tech University, 310018 Hangzhou, China

Received 12 April 2016; accepted in revised form 30 June 2016

Abstract. A novel double response surface model is used first time to optimize a regioselective process to prepare spherical dialdehyde cellulose nanocrystals (SDACN) and rod-like dialdehyde cellulose nanocrystals (RDACN) via one-step sodium periodate (NaIO_4) oxidation. The influence of four preparation factors (solid-liquid ratio, NaIO_4 concentration, reaction time and temperature) on the yields and aldehyde contents of the final products were evaluated. For comparison, rod-like cellulose nanocrystals (CN-M and CN-S) were prepared by hydrochloric/formic acid hydrolysis and sulfuric acid hydrolysis, respectively. The RDACN shows high crystallinity of 82%, while SDACN presents low crystallinity due to the high degree of oxidation. Thus, SDACN has poorer thermal stability than RDACN and CN-M, but higher than CN-S. Compared to CN-M, SDACN with higher aldehyde contents as templates is beneficial to deposit more Ag nanoparticles with diameters of 30 ± 4 nm and the resultant nanohybrids exhibit good antibacterial activities against both Gram-negative *E. coli* and Gram-positive *S. aureus*.

Keywords: nanomaterials, dialdehyde cellulose nanocrystals, NaIO_4 oxidation, response surface model, antibacterial activity

1. Introduction

Recently, cellulose nanocrystals (CNs) with various morphologies (rod-like or spherical) isolated from the most abundant cellulosic materials have gained increasing attention in some fields such as antimicrobial packagings [1, 2], template for metallic nanoparticles [3], aerogels for wastewater treatment [4], drug delivery [5], and protein immobilization [6] due to their excellent properties, including easily modified functional groups, high specific area, aspect ratios, and outstanding stiffness. As the nanofillers, the requirement of CNs surface group varies with the different applications. The traditional methods like acid

hydrolysis [4, 7], enzyme-assisted hydrolysis [8] and mechanical treatments [9] can extract the CNs with hydroxyl groups, which are usually modified into other functional groups for further applications. Recently, CNs with functional groups (carboxyl or aldehyde groups) have been developed by 2,2,6,6-tetramethylpiperidiny-1-oxyl (TEMPO) [10, 11], ammonium persulfate (APS) [12], two-step approach combined acid hydrolysis and NaIO_4 oxidations [13–15], and one-step NaIO_4 oxidation [16]. The TEMPO and APS oxidations consume for a long time and the oxidizing agents are toxic [10, 12]. So that, there are growing interests in the preparation of dialdehyde

*Corresponding author, e-mail: phdyu@zstu.edu.cn
© BME-PT

cellulose nanocrystal (DACN) by two-step approach NaIO_4 oxidation, in which DACN with 50–376 nm in length and 3–18 nm in diameter were prepared by the sulfuric acid hydrolysis of fibers (cotton fibers and bleached softwood pulp) and then NaIO_4 oxidation [13–15]. However, the two-step approach NaIO_4 oxidation was complicated and needed environmental unfriendly acid hydrolysis process that leads to the low yield of DACN. More recently, one-step NaIO_4 oxidation was used to fabricate DACN efficiently [16]. Theoretically, the NaIO_4 oxidation can selectively convert hydroxyl groups on the cellulose into aldehyde groups and form CNs by oxidative cleavage between at C_2 and C_3 bonds of the glucose repeat units [17, 18]. The aldehyde groups on the DACN surfaces can be used as the binding site for the fluorescent bioimaging [19] and nanohybrid templates [1, 2, 20]. Yang *et al.* [16] obtained the rod-like DACN with length of 100–200 nm and diameter of 5 nm by NaIO_4 oxidation of sheet from kraft softwood pulp after soaking in the water for 2 days, but the oxidation process needed long reaction time of 96 h with stirring speed of 105 rpm. From above, these mentioned oxidation methods were inefficient and the influence model of reaction conditions on the morphology, yield and aldehyde content of DACN has not been established.

In this work, a novel double response surface model on yield and aldehyde content was established to optimize the regioselective extraction of spherical and rod-like DACN from microcrystalline cellulose by a one-step NaIO_4 oxidation method without additives within a shorter reaction time. The morphologies, microstructures and properties of the resultant DACN were investigated, and their potential application as a template for nanohybrids was also evaluated.

2. Experimental

2.1. Materials

Commercial microcrystalline cellulose (MCC, rubbery-shaped fragments with particle size of 15–30 μm) was purchased from Shanghai Chemical Reagents (Shanghai, China). Sodium periodate (NaIO_4), silver nitrate (AgNO_3), hydrochloric acid (HCl), formic acid (HCOOH), sulfuric acid (H_2SO_4), ammonia solution ($\text{NH}_3 \cdot \text{H}_2\text{O}$) and hydroxylamine-hydrochloride ($\text{NH}_2\text{OH} \cdot \text{HCl}$) were purchased from Hangzhou Mike Chemical Agents Co. Ltd., China. All the chemical reagents and materials were in used as-received without further purification, and the water used in experimental procedures was deionized water (Millipore Milli-Q purification system).

2.2. Preparation of DACN

It is well known that the NaIO_4 oxidation can selectively convert hydroxyl groups on the cellulose into aldehyde groups and formed CNs by oxidative cleavage between at C_2 and C_3 bonds of the glucose repeat units. The yield and the aldehyde content of DACN are considered as the important criteria to judge the effectiveness of this method. Thus, a novel double response surface models and an orthogonal experiment with four factors and three levels ($L_{16}(3)^4$ array) were carried out to optimize the preparation for DACN. In detail, the DACN was fabricated by NaIO_4 oxidation under different conditions (solid-liquid ratio range of 1:25–1:100 [g/mL], NaIO_4 solution concentration of 0.1–0.5 M, time of 0.5–1.5 h, and temperature of 30–60 $^\circ\text{C}$, shown in Table 1). The detailed process was as follows: 1 g of MCC was put into NaIO_4 solution in 100 mL flask with continuous stirring. After desired reaction time, the product was collected by centrifugation and freeze-dried for 48 h.

Table 1. The factorial experiments of solid-liquid ratio, NaIO_4 concentration, reaction time and temperature

(A: solid-liquid ratio; B: NaIO_4 concentration; C: reaction time; D: temperature; Y: yield; ADC: aldehyde group content; DO: degree of oxidation; X_c : crystallinity)

Sample NO.	A [g/mL]	B [M]	C [h]	D [$^\circ\text{C}$]	Y [%]	ADC [mmol/g]	DO	X_c [%]
1	1:100	0.1	0.5	30	93.2	0.5	0.04	72
2	1:100	0.25	1	45	74.3	3.2	0.26	81
3	1:100	0.5	1.5	60	68.4	9.8	0.80	79
4	1:50	0.1	1	60	84.1	3.0	0.24	80
5	1:50	0.25	1.5	30	78.4	1.6	0.13	72
6	1:50	0.5	0.5	45	97.7	3.8	0.31	60
7	1:25	0.1	1.5	45	86.0	1.2	0.10	67
8	1:25	0.25	0.5	60	90.8	0.7	0.06	74
9	1:25	0.5	1	30	91.5	2.5	0.20	72

For comparison, cellulose nanocrystals were prepared by acid hydrolysis from MCC using 64 wt% H₂SO₄ (CN-S) and HCl/HCOOH mixture (CN-M), respectively, as previously reported [1, 2, 7].

2.3. Determination of the aldehyde group content and degree of oxidation

The aldehyde content was determined by pH titration of forming HCl obtained from oxime reaction between the NH₂OH·HCl and aldehyde groups of DACN [21]. Firstly, 0.01 M HCl was added to 20 mL NH₂OH·HCl solution (0.3 M), and a pH meter was used to monitor the pH of the solution simultaneously, then a curve for pH vs. HCl volume (V_{HCl}) was plotted. Next, dried samples (approx. 0.1 g) were added to the same solution which was described above, and the mixture was sufficiently stirred for 24 h at the room temperature, then the pH of the mixture was measured by the pH meter at the room temperature. Finally, the V_{HCl} [mL] of the reaction was determined from the pH vs. V_{HCl} curve, and the aldehyde group content of the sample [mmol/g] was calculated as shown in Equation (1):

$$[\text{CHO}] = V_{\text{HCl}} \cdot \frac{c_{\text{HCl}}}{m_0} \quad (1)$$

where [CHO] is the aldehyde content [mmol/g], V_{HCl} is the HCl volume, c_{HCl} is the concentration of the HCl aqueous solution, m_0 is the mass of dried DACNs [g]. The degree of oxidation (DO) of DACNs refers to the average number of hydroxyls oxidized by NaIO₄ on the anhydrous glucose unit (AGU) and was determined by Equation (2):

$$\text{DO} = \frac{[\text{CHO}]}{\frac{2 \cdot m_0}{162}} \quad (2)$$

where DO is the degree of oxidation, m_0 is the mass of dried DACNs [g], 162 is the molar mass [g/mol] of AGU, and the value of 2 is the theoretical DO value.

2.4. Preparation of CN-Ag nanoparticles

The synthesized procedures of CN-Ag nanohybrids were according to the previously described method [1, 2]. In general, the aqueous solution of silver ammonia (silver ammonia aqueous solution was prepared by adding 1 mL of 25% ammonia to 100 mL

AgNO₃ (0.85 g) solution. The final stage of ammonia addition was performed dropwise to give a clear solution [1, 2, 22]) was mixed with the CNs suspensions (approx. 1.0 g dry sample), and then heated to 90 °C for about 20 min. After cooling to the room temperature, the precipitates were washed with deionized water by successive centrifugation to remove the residual silver ammonia and the reaction byproducts. Moreover, the CN-M with formate esters, RDACN and SDACN with aldehyde groups were selected to synthesize CN-Ag nanohybrids. The samples were dried and denoted as CN-M-Ag-0.85, SDACN-Ag-0.85 and RDACN-Ag-0.85, respectively, and the FE-SEM photos were shown below (Figures 7a, 7b and 7c).

2.5. Characterization

The morphologies of the CNs were observed using field-emission scanning electron microscopy (FE-SEM, JSM-5610; JEOL, Japan) at an acceleration voltage of 1.0 kV at room temperature. Chemical structure of samples was measured on a Nicolet 5700 Fourier transform infrared (FT-IR) spectra and the crystal structure was performed on an X-ray powder diffractometer (XRD, ARL X'RA; Thermo Electron Corp.). The thermal stability test was conducted on samples by using a thermogravimetric analyzer (TGA, Pyris Diamond I; PerkinElmer Corp.) under a nitrogen atmosphere with a heating rate of 20 °C/min from room temperature to 600 °C. Particle size of aqueous CN suspension was tested by the Nano ZS Malvern Zetasizer. *E. coli* as the model Gram-negative bacteria and *S. aureus* as the model Gram-positive bacteria were chosen to investigate the antimicrobial activities of CN-M-Ag-0.85, SDACN-Ag-0.85 and RDACN-Ag-0.85 nanohybrids as reported in previous literatures [1, 2, 20].

3. Results and discussion

3.1. Model fitting and optimization

The orthogonal experiment (L₁₆ (3)⁴ array) was carried out with four factors (Table 1), 'A', 'B', 'C', and 'D' stand for the solid-liquid ratio, NaIO₄ concentration, reaction time and temperature, respectively. From range analysis results of Table 2 and Table 3, it is found that the influencing order of the factors on the yield is C>D>A>B, while that on the aldehyde content is B>A>D>C. It suggests that the re-

action time plays an important role in the yield of DACN, while NaIO_4 concentration has a significant

Table 2. Analyses of the factorial experiments for yields [%] (A: solid-liquid ratio; B: NaIO_4 concentration; C: reaction time; D: temperature)

	A [g/mL]	B [M]	C [h]	D [°C]
K ₁	78.7	81.5	87.7	81.4
K ₂	86.7	87.5	89.6	92.3
K ₃	89.4	85.9	77.6	81.1
R	10.7	6.0	12.0	11.2

Priority factor C>D>A>B

Optimal combination A₃B₂C₂D₂

$K = (\sum \text{yield after oxidation of single-factor})/3$.

$R = K_{\max} - K_{\min}$.

Table 3. Analyses of the factorial experiments for aldehyde group contents [mmol/g] (A: solid-liquid ratio; B: NaIO_4 concentration; C: reaction time; D: temperature)

	A [g/mL]	B [M]	C [h]	D [°C]
K ₁	10.3	1.6	1.7	1.6
K ₂	2.8	1.9	2.9	2.8
K ₃	1.5	11.2	10.1	10.3
R	8.8	9.6	8.4	8.7

Priority factor B>A>D>C

Optimal combination A₁B₃C₃D₃

$K = (\sum \text{aldehyde content after oxidation of single-factor})/3$.

$R = K_{\max} - K_{\min}$.

effect on the formation of aldehyde groups. According to the results of the factorial experiments, the optimal combination A₃B₂C₂D₂ was obtained for maximum yields of 97.7% (the DACN with rod-like shape (see below in Figure 3c) in this condition was denoted as RDACN). Similarly, the optimal combination A₁B₃C₃D₃ was the oxidation conditions for the highest content of aldehyde groups of 9.8 mmol/g with the highest DO value (about 0.8) (the DACN with spherical shape (see below in Figure 3b) in this condition was denoted as SDACN). However, when the NaIO_4 concentration was increased (over 0.5 M), the obtained product was becoming completely amorphous (without crystalline region), the XRD spectrum was shown in Figure 4c, which was similar to previous work [23]. In addition, in order to confirm the complete conversion of DACN, the sample 1 (shortest reaction time of 0.5 h, lowest aldehyde content of 0.5 mmol/g) was tested in Figure 3d. The results of FE-SEM image and particle size distribution indicated the nanoscale sizes of DACN were achieved even under a short reaction time (0.5 h).

The mathematical model representing the yield and aldehyde content of DACN against the function of the independent variables within the range under investigation is expressed Equations (3) and (4):

Figure 1. Response surface plots for maximum yield and aldehyde content of DACN: effects of temperature and reaction time on the yield [%] (a), effects of NaIO_4 concentration and solid-liquid ratio on the aldehyde content [mmol/g] of DACN (b)

$$Y_1 = 135.81 - 12.97X_1 - 0.88X_2 - 41.20X_3 - 1.30X_4 - 8.88 \cdot 10^{-13}X_1X_2 + 3.70 \cdot 10^{-14}X_1X_3 + 2.46 \cdot 10^{-1}X_1X_4 - 2.22 \cdot 10^{-14}X_2X_3 - 8.88 \cdot 10^{-16}X_2X_4 - 0.25X_3X_4 + 259.30X_1^2 + 1.46X_2^2 + 18.93X_3^2 + 0.02X_4^2 \quad (3)$$

$$Y_2 = 2.80 + 40.15X_1 + 17.60X_2 - 3.67X_3 - 0.18X_4 - 666.67X_1X_2 - 1.48 \cdot 10^{-14}X_1X_3 - 2.96 \cdot 10^{-15}X_1X_4 - 1.27 \cdot 10^{-1}X_2X_3 - 2.83 \cdot 10^{-16}X_2X_4 - 4.44 \cdot 10^{-17}X_3X_4 + 1648.15X_1^2 + 14.90X_2^2 + 1.83X_3^2 + 2.04 \cdot 10^{-3}X_4^2 \quad (4)$$

where Y_1 and Y_2 are the yield and aldehyde content of DACN, whereas X_1 , X_2 , X_3 and X_4 are the coded variables for solid-liquid ratio, NaIO₄ concentration, reaction time and temperature, respectively. In general, poor or misleading results may be produced by the exploration and optimization of a fitted response surface unless the model exhibited good fitness [24, 25]. Since the P-value of the model was less than 0.0001, the model's fitness was good. The effects of the variables on the yield and aldehyde content of

DACN are shown in Figure 1. Based on the prediction of the computing program and taking the practical operating condition into consideration, the optimal conditions to obtain the highest yields are solid-liquid ratio of 1: 50, NaIO₄ concentration of 0.5 M, reaction time of 0.5 h, and temperature of 45 °C, while the optimal conditions to obtain the highest aldehyde contents are solid-liquid ratio of 1:100, NaIO₄ concentration of 0.5 M, reaction time of 1.5 h and temperature of 60 °C. Thus, the experimental conditions should be chosen moderately according to the double response surface model, and the RDACN and SDACN could be obtained using this model under controllable conditions.

3.2. Morphology of DACN

In order to observe the morphologies of SDACN and RDACN, their FE-SEM images are clearly shown in Figure 2, in which the morphologies for different kinds of CNs are compared. MCC displayed rubbery-shaped fragments with particle size of 15–30 μm (Figure 2a). The CN-M (Figure 2b) and CN-S (Figure 3a) both exhibited rod-like shape with average

Figure 2. FE-SEM images of MCC (a), CN-M (b), rod-like DACN and the particle size distribution (a', b')

Figure 3. FE-SEM images of CN-S (a), RDACN (b), SDACN (c), rod-like DACN (d) and the particle size distribution (a', b', c', d')

length of 270.5 ± 5.6 , 250.6 ± 4.9 nm, respectively, while the width for both samples was about 8 nm. The similar dimension agreed well with previously reported work [7]. In particular, a good dispersion of CN-S was found due to electrostatic repulsion of sulfate groups after the hydrolysis of sulfuric acid. In Figure 3b, SDACN showed spherical nanoparticles with a diameter of 25–35 nm, while RDACN showed rod-like shape with average length of 230.5 ± 2.3 nm and width of 9 nm, respectively (Figure 3c). Furthermore, the particle sizes (freshly dispersed and kept in the room temperature for 3 days) of SDACN (insert in Figure 3b) and RDACN (insert in Figure 3c) were tested by the Nano ZS Malvern Zetasizer, and the results indicated the nanoscale size of SDACN and RDACN were obtained. Thus, the above mentioned results indicated that with the help of the oxidizing agent (NaIO_4), rod-like or spherical DACN can be regioselectively prepared by modulating the degree of oxidation. The NaIO_4 oxidation as acid-free method showed great potential to prepare the CNs, which can replace common acid-hydrolyzed approach.

3.3. Chemical structure of DACN

FT-IR measurement was performed to determine the variation of surface chemical groups of different kinds of CN samples, and the FT-IR spectra are shown in Figure 4a. Some characteristic peaks of cellulose at 3414, 2909, 1639, 1032–1056 and 895 cm^{-1} were assigned to O–H, symmetric C–H, absorbed moisture, C–O–C stretching vibration and β -glycosilic linkages between the sugar units, respectively [14, 18, 26, 27]. For CN-S, a unique absorption band at 1205 cm^{-1} corresponded to S=O stretching from sulfate groups was observed [7]. Because of the esterification reaction between hydroxyl groups on the surface of commercial MCC and HCOOH , an obvious carbonyl (C=O) peak at 1719 cm^{-1} was found for CN-M due to its surface formate groups (each formate group contained an aldehyde structure) [2, 20], while the DACN samples show new C=O peak at 1725 cm^{-1} assigned to their aldehyde groups. Further, compared to CN-M, the DACN exhibited higher normalized intensity of C=O peak, indicating more aldehyde groups were introduced into DACNs by NaIO_4 oxidation than mixed acid hydrolysis. Similar

Figure 4. FT-IR spectra (normalization intensity of carbonyl peak at 1719 and 1725 cm^{-1}) of CNs and MCC (a), FT-IR spectra (b) of nine orthogonal experiment groups (c), and XRD patterns of CNs and MCC

phenomenon was observed in NaIO_4 oxidation of CNs (natural cellulose fibers) [14]. When the skeleton band of cellulose at $800\text{--}900\text{ cm}^{-1}$ was fixed, normalization intensity of aldehyde C=O peak for DACNs can be used to qualitatively comparison analysis. Moreover, the SDACN showed the higher normalized intensity for C=O peak than that of RDACN, suggesting that spherical nanoparticles with high specific surface area were more susceptible to be attacked by NaIO_4 , leading to the formation of more aldehyde groups. Meanwhile, the FT-IR spectra, normalized intensity for C=O peak at 1725 cm^{-1} and corresponding OD values of DACNs (9 samples from orthogonal experiment groups) prepared by NaIO_4 oxidation were shown in Figure 4b. The results of normalized intensity for C=O peak for CNs can support above results. Additionally, the DACNs prepared by NaIO_4 oxidation exhibited as good suspension stability as CN-M and CN-S samples (insert in Figure 4a).

3.4. Crystal structure and crystallinity of DACN

The XRD spectra for MCC and CN samples were presented in Figure 4c. MCC and the other CNs possessed typical peaks of cellulose I with main peaks at $2\theta = 14.5, 16.5$, and 22.6° , which assigned to the crystalline planes of (1 $\bar{1}$ 0), (110) and (200), respectively [1, 2, 14, 18]. By using Segal's method [28], the crystallinity of MCC, CN-S and CN-M were 76, 84 and 88%, respectively. Compared to CN-S and CN-M, a slight reduction in crystallinity (82%) was found for RDACN and SDACN (79%). The results showed that all CNs have higher crystallinity than

MCC, which indicating that parts of MCC residual amorphous phase have been dissolved by various treatments (mixed acid (HCl/HCOOH), sulfuric acid, NaIO_4 oxidation). For acid hydrolysis, the fabricating process for CNs was aimed at breaking the β -1, 4-glycosidic bonds on the amorphous region of MCC, while the oxidation process would open glucopyranose chains in crystalline or amorphous regions and increase the solubility (Figure 5), which resulting in lower degree of crystallinity than acid hydrolysis process. The decreased crystallinity was ascribed to the higher opened glucopyranose rings (higher DO values) and the higher destruction of ordered CNs structure, under violent condition of NaIO_4 oxidation, and the nanocrystals with the selective orientation causing a reduction in diffraction peak intensity from some Bragg planes [18]. Generally, the higher the DO value was, the lower the crystallinity was. Moreover, the higher crystallinity of CN-M was attributed to only amorphous regions of cellulose materials attacked by acid hydrolysis and esterification reactions, without opened glucopyranose rings in crystalline regions.

3.5. Thermal stability of DACN

TGA and DTG curves (Figure 6) revealed the thermal stability for MCC and CN samples. Comparing with MCC (onset degradation temperature (T_0) of 325.3°C), the CN-M owned the higher T_0 of 346.9°C , the other CNs samples had lower T_0 values. Especially, CN-S showed very low T_0 value of 208.1°C because of the existence of sulfate groups on cellulose surface [7, 28], while the RDACN and SDACN had higher T_0 value of 281.6 and 222.1°C , respec-

Figure 5. NaIO_4 oxidation and acid hydrolysis mechanism schema of CNs

Figure 6. TGA (a) and DTG (b) curve of CN-S, CN-M, RDACN, SDACN and MCC

tively. A similar trend was found for maximum degradation temperature ($T_{\max}$) of the samples. For clear observation, the thermal stability data (T_0 , $T_{\max}$, T_f (complete degradation temperature)) were shown in Table 4. In addition, the first derivative of the weight loss curve with respect to temperature (DTG) was shown in Figure 6b. It can be observed that MCC, CN-M and RDACN owned the single step maximum degradation peaks at 357.3, 377.8 and 344.7 °C, respectively. However, CN-S and SDACN started to degrade at lower temperature and exhibited with two main peaks. For CN-S with sulfate groups, the degradation occurred within a wider temperature range and showed three well separated pyrolysis processes: the first one appeared around 196.9–275.0 °C, the second one was around 277.0–324.7 °C, and the other occurred around 325.0–443.9 °C. The multiple degradation peaks exhibited in CN-S curve were attributed to the wide size distribution and the introduction of sulfate groups during sulfuric acid hydrolysis of MCC with possible different sulfonation degrees [7, 28]. On the other hand, SDACN showed two degradation peaks at 235.3 and 267.2 °C. This was due to that the dialdehyde modified SDACNs chains did not form the hemiacetal linkage during the first (195.5–259.8 °C) degradation process. The second degradation process (259.8–411.4 °C) was ascribed to the degradation of cellulose skeleton for the SDACN. Therefore, the CNs prepared by NaIO_4 oxidation had better thermal stability than that for H_2SO_4 hy-

drolyzed one, but poorer than that of CN-M. In addition, RDACN showed the higher degradation temperature than that of SDACN, probably resulting from low degree of oxidation, surface morphology and crystallinity.

3.6. Properties for CN-Ag nanohybrids

In general, the more aldehyde groups on the CNs surface is beneficial to anchor more metal nanoparticles via redox reaction, thus the SDACN with highest aldehyde content, RDACN and CN-M with low aldehyde content were selected to investigate the morphology and antibacterial activity for resulting CN-Ag nanohybrids. It is found that more Ag nanoparticles (29.6%) with diameter of 30 ± 4 nm on the SDACN-Ag-0.85 are observed, whereas the less Ag nanoparticles with diameter of 33 ± 4 and 35 ± 5 nm were fixed on the RDACN-Ag-0.85 (23.9%) and CN-M-Ag-0.85 (21.6%) surfaces, respectively, shown in Figure 7d. The XRD patterns (Figure 7e) of CN-M-Ag-0.85, RDACN-Ag-0.85 and SDACN-Ag-0.85 showed four diffraction peaks at $2\theta = 38.3$, 44.3 , 64.5 and 77.5° assigning to the (111), (200), (220) and (311) planes of cubic Ag nanoparticles [1, 20]. Further, the antibacterial activities for CN-Ag nanohybrids against *S. aureus* and *E. coli* by formation of zone of inhibitions are shown in Figure 7f. All the nanohybrids gave extremely strong activities against both the Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) species. Moreover, the SDACN-Ag-0.85

Table 4. Thermal stability data (T_0 , $T_{\max}$, T_f) of MCC, CN-M, CN-S, RDACN and SDACN

	MCC	CN-M	CN-S	RDACN	SDACN
T_0 [°C]	325.3	346.9	208.1	281.6	222.1
$T_{\max}$ [°C]	357.3	377.8	245.3/294.1/374.6	344.7	235.3/267.2
T_f [°C]	373.4	390.3	363.9	367.1	341.5

Figure 7. FE-SEM images of CN-M-Ag-0.85 (a), SDACN-Ag-0.85 (b), RDACN-Ag-0.85 nanohybrids (c) and their TGA curves (d), XRD patterns (e), antibacterial abilities against *S. aureus* and *E. coli* (f)

presented the largest inhibition zone against *S. aureus* and *E. coli* (4.6 and 2.8 mm) than RDACN-Ag-0.85 (3.2 and 2.3 mm) and CN-M-Ag-0.85 (2.1 and 2.0 mm), suggesting that the large amount of aldehyde groups on SDACN reduced more Ag^+ into Ag^0 nanoparticles, leading to the high antibacterial activity [1, 2, 20].

4. Conclusions

Spherical and rod-like DACN can be optimized and regioselectively extracted from MCC via one-step NaIO_4 oxidation using a novel double response surface model. Both SDACN with the high aldehyde content of 9.8 mmol/g and RDACN with relatively low aldehyde content of 3.8 mmol/g possessed cellulose I crystal structure. Moreover, RDACN and SDACN showed the better thermal stability than CN-S (H_2SO_4 hydrolysis), but lower than CN-M (hydrochloric/formic hydrolysis) due to less destruction of the ordered cellulose structure. In addition, SDACN can act as the reductant for anchoring more Ag nanoparticles, and thus the resultant SDACN-Ag-0.85 nanohybrids demonstrated the highest antibacterial activity against both Gram-negative *E. coli* and Gram-positive *S. aureus*. Thus, one-step NaIO_4 oxidation in this study as acid-free route showed more advantages than acid hydrolysis methods, and the DACN may have potential applications in functional papers, sensors, and bioimaging for large-scale production of cellulose-based nanocomposites.

Acknowledgements

The work was financially supported by the National Natural Science Foundation of China (51403187), the public technology research plan of Zhejiang Province, China under Grant No. 2015C33111, and ‘521’ Talent Project of Zhejiang Sci-Tech University and Open fund in Top Priority Discipline of Zhejiang Province in Zhejiang Sci-Tech University (2015YXQN04, 2016YXQN07, 2015YXQN11).

References

- [1] Yu H., Sun B., Zhang D., Chen G., Yang X., Yao J.: Reinforcement of biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with cellulose nanocrystal/silver nanohybrids as bifunctional nanofillers. *Journal of Materials Chemistry B*, **2**, 8479–8489 (2014). DOI: [10.1039/C4TB01372G](https://doi.org/10.1039/C4TB01372G)

- [2] Yu H.-Y., Qin Z.-Y., Sun B., Yan C. F., Yao J.-M.: One-pot green fabrication and antibacterial activity of thermally stable corn-like CNC/Ag nanocomposites. *Journal of Nanoparticle Research*, **16**, 2202/1–2202/12 (2014). DOI: [10.1007/s11051-013-2202-4](https://doi.org/10.1007/s11051-013-2202-4)
- [3] Padalkar S., Capadona J. R., Rowan S. J., Weder C., Moon R. J., Stanciu L. A.: Self-assembly and alignment of semiconductor nanoparticles on cellulose nanocrystals. *Journal of Materials Science*, **46**, 5672–5679 (2011). DOI: [10.1007/s10853-011-5518-4](https://doi.org/10.1007/s10853-011-5518-4)
- [4] Yang X., Cranston E. D.: Chemically cross-linked cellulose nanocrystal aerogels with shape recovery and superabsorbent properties. *Chemistry of Materials*, **26**, 6016–6025 (2014). DOI: [10.1021/cm502873c](https://doi.org/10.1021/cm502873c)
- [5] Wang H. R., He J. L., Zhang M. Z., Tam K. C., Ni P. H.: A new pathway towards polymer modified cellulose nanocrystals via a ‘grafting onto’ process for drug delivery. *Polymer Chemistry*, **6**, 4206–4209 (2015). DOI: [10.1039/C5PY00466G](https://doi.org/10.1039/C5PY00466G)
- [6] Cao S.-L., Li X.-H., Lou W.-Y., Zong M.-H.: Preparation of a novel magnetic cellulose nanocrystal and its efficient use for enzyme immobilization. *Journal of Materials Chemistry B*, **2**, 5522–5530 (2014). DOI: [10.1039/C4TB00584H](https://doi.org/10.1039/C4TB00584H)
- [7] Yu H., Qin Z., Liang B., Liu N., Zhou Z., Chen L.: Facile extraction of thermally stable cellulose nanocrystals with a high yield of 93% through hydrochloric acid hydrolysis under hydrothermal conditions. *Journal of Materials Chemistry A*, **1**, 3938–3944 (2013). DOI: [10.1039/C3TA01150J](https://doi.org/10.1039/C3TA01150J)
- [8] Satyamurthy P., Vigneshwaran N.: A novel process for synthesis of spherical nanocellulose by controlled hydrolysis of microcrystalline cellulose using anaerobic microbial consortium. *Enzyme and Microbial Technology*, **52**, 20–25 (2013). DOI: [10.1016/j.enzmictec.2012.09.002](https://doi.org/10.1016/j.enzmictec.2012.09.002)
- [9] Li M.-C., Wu Q., Song K., Qing Y., Wu Y.: Cellulose nanoparticles as modifiers for rheology and fluid loss in bentonite water-based fluids. *ACS Applied Materials and Interfaces*, **7**, 5006–5016 (2015). DOI: [10.1021/acsami.5b00498](https://doi.org/10.1021/acsami.5b00498)
- [10] Montanari S., Roumani M., Heux L., Vignon M. R.: Topochemistry of carboxylated cellulose nanocrystals resulting from TEMPO-mediated oxidation. *Macromolecules*, **38**, 1665–1671 (2005). DOI: [10.1021/ma048396c](https://doi.org/10.1021/ma048396c)
- [11] Paralakar S. A., Simonsen J., Lombardi J.: Poly(vinyl alcohol)/cellulose nanocrystal barrier membranes. *Journal of Membrane Science*, **320**, 248–258 (2008). DOI: [10.1016/j.memsci.2008.04.009](https://doi.org/10.1016/j.memsci.2008.04.009)
- [12] Cheng M., Qin Z. Y., Liu Y., Qin Y., Li T., Chen L., Zhu M.: Efficient extraction of carboxylated spherical cellulose nanocrystals with narrow distribution through hydrolysis of lyocell fibers by using ammonium persulfate as an oxidant. *Journal of Materials Chemistry A*, **2**, 251–258 (2014). DOI: [10.1039/C3TA13653A](https://doi.org/10.1039/C3TA13653A)

- [13] Dash R., Ragauskas A. J.: Synthesis of a novel cellulose nanowhisker-based drug delivery system. *RSC Advances*, **2**, 3403–3409 (2012).
DOI: [10.1039/C2RA01071B](https://doi.org/10.1039/C2RA01071B)
- [14] Sun B., Hou Q., Liu Z., Ni Y.: Sodium periodate oxidation of cellulose nanocrystal and its application as a paper wet strength additive. *Cellulose*, **22**, 1135–1146 (2015).
DOI: [10.1007/s10570-015-0575-5](https://doi.org/10.1007/s10570-015-0575-5)
- [15] Huang R., Liu Z., Sun B., Fatehi P.: Preparation of dialdehyde cellulose nanocrystal as an adsorbent for creatinine. *The Canadian Journal of Chemical Engineering*, **94**, 1435–1441 (2016).
DOI: [10.1002/cjce.22523](https://doi.org/10.1002/cjce.22523)
- [16] Yang H., Chen D., van de Ven T. G. M.: Preparation and characterization of sterically stabilized nanocrystalline cellulose obtained by periodate oxidation of cellulose fibers. *Cellulose*, **22**, 1743–1752 (2015).
DOI: [10.1007/s10570-015-0584-4](https://doi.org/10.1007/s10570-015-0584-4)
- [17] Yang H., Tejado A., Alam N., Antal M., van de Ven T. G. M.: Films prepared from electrosterically stabilized nanocrystalline cellulose. *Langmuir*, **28**, 7834–7842 (2012).
DOI: [10.1021/la2049663](https://doi.org/10.1021/la2049663)
- [18] Yang H., Alam M. N., van de Ven T. G. M.: Highly charged nanocrystalline cellulose and dicarboxylated cellulose from periodate and chlorite oxidized cellulose fibers. *Cellulose*, **20**, 1865–1875 (2013).
DOI: [10.1007/s10570-013-9966-7](https://doi.org/10.1007/s10570-013-9966-7)
- [19] Grate J. W., Mo K-F., Shin Y., Vasdekis A., Warner M. G., Kelly R. T., Orr G., Hu D., Dehoff K. J., Brockman F. J., Wilkins M. J.: Alexa fluor-labeled fluorescent cellulose nanocrystals for bioimaging solid cellulose in spatially structured microenvironments. *Bioconjugate Chemistry*, **26**, 593–601 (2015).
DOI: [10.1021/acs.bioconjchem.5b00048](https://doi.org/10.1021/acs.bioconjchem.5b00048)
- [20] Drogat N., Granet R., Sol V., Memmi A., Saad N., Korkamp C. K., Bressollier P., Krausz P.: Antimicrobial silver nanoparticles generated on cellulose nanocrystals. *Journal of Nanoparticle Research*, **13**, 1557–1562 (2011).
DOI: [10.1007/s11051-010-9995-1](https://doi.org/10.1007/s11051-010-9995-1)
- [21] Kim U-J., Wada M., Kuga S.: Solubilization of dialdehyde cellulose by hot water. *Carbohydrate Polymers*, **56**, 7–10 (2004).
DOI: [10.1016/j.carbpol.2003.10.013](https://doi.org/10.1016/j.carbpol.2003.10.013)
- [22] Wu M., Kuga S., Huang Y.: Quasi-one-dimensional arrangement of silver nanoparticles templated by cellulose microfibrils. *Langmuir*, **24**, 10494–10497 (2008).
DOI: [10.1021/la801602k](https://doi.org/10.1021/la801602k)
- [23] Kim U-J., Kuga S., Wada M., Okano T., Kondo T.: Periodate oxidation of crystalline cellulose. *Biomacromolecules*, **1**, 488–492 (2000).
DOI: [10.1021/bm0000337](https://doi.org/10.1021/bm0000337)
- [24] Tang L-R., Huang B., Ou W., Chen X-R., Chen Y-D.: Manufacture of cellulose nanocrystals by cation exchange resin-catalyzed hydrolysis of cellulose. *Bioresource Technology*, **102**, 10973–10977 (2011).
DOI: [10.1016/j.biortech.2011.09.070](https://doi.org/10.1016/j.biortech.2011.09.070)
- [25] Sun B., Yu H-Y., Zhou Y., Huang Z., Yao J-M.: Single-step extraction of functionalized cellulose nanocrystal and polyvinyl chloride from industrial wallpaper wastes. *Industrial Crops and Products*, **89**, 66–77 (2016).
DOI: [10.1016/j.indcrop.2016.04.040](https://doi.org/10.1016/j.indcrop.2016.04.040)
- [26] Visanko M., Liimatainen H., Sirviö J. A., Heiskanen J. P., Niinimäki J., Hormi O.: Amphiphilic cellulose nanocrystals from acid-free oxidative treatment: Physicochemical characteristics and use as an oil–water stabilizer. *Biomacromolecules*, **15**, 2769–2775 (2014).
DOI: [10.1021/bm500628g](https://doi.org/10.1021/bm500628g)
- [27] Keshk S. M. A. S.: Homogenous reactions of cellulose from different natural sources. *Carbohydrate Polymers*, **74**, 942–945 (2008).
DOI: [10.1016/j.carbpol.2008.05.022](https://doi.org/10.1016/j.carbpol.2008.05.022)
- [28] Kargarzadeh H., Ahmad I., Abdullah I., Dufresne A., Zainudin S. Y., Sheltami R. M.: Effects of hydrolysis conditions on the morphology, crystallinity, and thermal stability of cellulose nanocrystals extracted from kenaf bast fibers. *Cellulose*, **19**, 855–866 (2012).
DOI: [10.1007/s10570-012-9684-6](https://doi.org/10.1007/s10570-012-9684-6)