

Tailor the Deacetylation for High Yield Preparation of Carboxyl β -Chitin Nanofibers from Squid Pen by TEMPO Oxidation

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Abstract

2,2,6,6-tetramethylpiperidine-1-oxyl-radical (TEMPO) oxidized is a classical method to obtain carboxyl chitin nanomaterials, but when the traditional TEMPO/NaBr/NaClO (TBN) oxidation system was oxidizing β -chitin, it was only suitable for β -chitin with a degree of deacetylation (DD) of 1%. Herein, the high yield carboxyl β -chitin nanofibers (CO-ChNFs) were successfully prepared from squid pen β -chitin with partially deacetylated (DD: 5–30%) using the TEMPO/NaClO/NaClO₂ (TNN) oxidation system. The results show that the DD of purified β -chitin has a great impact on the yield of CO-ChNFs, and the yield is as high as 98% when the DD of β -chitin was 11%. XRD results showed that β -chitin undergoes slight of β - α transformation after TNN TEMPO oxidation, and the 010 crystal planes are arranged more closely. AFM characterization suggests that thus prepared CO-ChNFs have a diameter of 2–6 nm and a length in the micrometer range. The proposed preparation method breaks through the previous limitation of TEMPO oxidation in the application of β -chitin, and will facilitate the in-depth research and application of carboxyl β -chitin nanofibers.

Introduction

With the development of nanomaterials and the deepening of environmental awareness, biological nanomaterials have attracted extensive attention (Jin et al. 2021; Thomas et al. 2018). Chitin is the naturally derived amino biological polysaccharide found from crustacean shells, squid pens and insect cuticles in the form of crystalline nanofibers surrounded with the aid of a sheath of protein (Anitha et al. 2014; Pillai et al. 2009). Due to the high intrinsic strength, controllable surface group, and unique biocompatibility, chitin nanomaterials have a wide range of promising applications in films, biomedicine, and other fields (Fang et al. 2020; Huang et al. 2017). To achieve the well dispersion chitin nanomaterials, electrostatic repulsive forces could be introduced by the strategy of surface chemical modification to overcome the hydrogen bond interactions among chitin (Yang et al. 2020).

2,2,6,6-tetramethylpiperidine-1-oxyl-radical (TEMPO) oxidation is a classical and effective method to obtain carboxyl chitin nanomaterials that can be properly dispersed in alkaline conditions (Lasseguette et al. 2008; Wu et al. 2021; Ye et al. 2021). For example, in a pioneering work, Fan et al. (Fan et al. 2008a) successfully prepared anionic chitin nanocrystals (COO⁻, length 340 nm, width 8 nm) using α -chitin from crab shell by TEMPO/NaBr/NaClO (TBN) oxidation system, the hydroxyl group on C6 of chitin is firstly selectively oxidized to the aldehyde group and finally to the carboxyl group. But in subsequent work, they applied this method to β -chitin found that only the β -chitin from tubeworm can be used to produce carboxyl chitin nanofibers (CO-ChNFs), while the β -chitin from squid pen failed under any conditions (Fan et al. 2009). This is because when the hydroxyl group is oxidized to an aldehyde group, the amino group on the β -chitin from squid pen (degree of deacetylation (DD): 10%) binds with the aldehyde group in large amounts to form polychitouronic acid, while there is almost no amino group on the β -chitin from tubeworms (DD: 1%). In our previous work (Pang et al. 2017), found that the TEMPO/NaClO/NaClO₂ (TNN) oxidation system could circumvent the defects of the TBN oxidation system, and the zwitterionically chitin nanocrystals containing both amino and carboxyl groups were successfully

prepared by using TNN oxidation system from partially deacetylated α -chitin. In the TNN system, NaClO first initiated the TEMPO reagent, then the TEMPO reagent oxidized the hydroxyl group to the aldehyde group on chitin C6, and finally NaClO₂ rapidly oxidized the aldehyde group to the carboxyl group avoids the reaction between the amino group on chitin and the aldehyde group (Saito et al. 2009; Ye et al. 2020). However this work has not received attention in the beta chitin, to date, the TEMPO oxidation only suitable a DD of 1% when oxidation β -chitin, and most sources β -chitin have a DD greater than 1% (such as the DD of squid pen \approx 10%), which limits the research of β -chitin. Considering that there is different chain packing modes, resulting in various inter-molecular hydrogen interaction in α and β crystal from chitin sources. Therefore, it has great significance in breaks through the previous limitation of TEMPO oxidation in the application of β -chitin and promoting the application research of β -chitin that the chitin nanomaterials with different morphology or surface groups might be obtained for partially deacetylated β -chitin by TNN oxidation.

In this study, the CO-ChNFs have been successfully extracted by TNN oxidation using β -chitin from squid pens with different DD, the DD of β -chitin was closely related to the yield of CO-ChNFs. The CO-ChNFs were characterized by atomic force microscopy, FT-IR and XRD. At the same time, the effects of oxidation time and dosage of NaClO₂ on the yield and carboxyl content of CO-ChNFs were investigated. The results of zeta potential and ninhydrin color reaction showed that only a small amount of amino groups remained in CO-ChNFs after TNN oxidation, and the corresponding mechanism was speculated in the paper. Finally, TNN oxidation was compared with other oxidation methods for preparing CO-ChNFs. The proposed preparation method has a high yield and a wider range of applications, laying the foundation for its large-scale application.

Experimental

Materials

Squid pen were collected from the local seafood market. Purified β -chitin was extracted from squid pen after the removal of minerals and proteins, according to previous literature reports (Fan et al. 2008b; Wu et al. 2019; Yokoi et al. 2017). Briefly, squid pen were first demineralized using 1 M HCl for several hours, followed by overnight stirring in alcohol to remove lipids, and finally 1 M NaOH overnight stirring to remove proteins. The degree of deacetylation of purified β -chitin was 5% that subjected by potentiometric titration. TEMPO reagent, NaClO, NaClO₂, NaBH₄, NaOH, and HCl all with analytical grade were purchased from commercial sources in China, and were used without further purification. Deionized water was utilized in the whole work.

Alkali treatment of β -chitin

The purified β -chitin and 10 wt% NaBH₄ to prevent alkali-induced depolymerization was added to 10 wt% NaOH solution reacted at different times (0, 1, 5, 12, and 24 h) to partial deacetylation in 40°C. At the end

of the reaction, the alkali was removed by filtration using deionized water and dried to obtain β -chitin samples with different degree of deacetylation.

Carboxyl β -chitin nanofiber preparation

β -chitin (1 g), TEMPO (0.016 g), and NaClO_2 (80%, 1.13 g) were sequentially added into 0.05 M sodium phosphate buffer (90 mL). Then, NaClO solution (0.532 g) was added dropwise to initiate the oxidation of chitin at 60°C and 600 rpm (Pang et al. 2017; Saito et al. 2009). The carboxyl chitin slurry was obtained after the reaction ended, wash to neutral. The slurry was dispersed in deionized water (800mL), adjusted to pH = 11 with NaOH, and the dispersion was ultrasonically treated by a numerically controlled ultrasonic cleaning machine (700 W, JZ2000) for 20 min. Finally, a transparent β -chitin nanofiber suspension (CO-ChNFs-X, X represents the degree of deacetylation of the β -chitin used) was obtained after the centrifugation (8000 rpm, 5 min).

Procedure for cationic chitin nanofibers

The cationic amino β -chitin nanofiber (NH-ChNFs) was prepared by following the previous paper (Fan et al. 2010). In simple terms, β -chitin with degree of deacetylation = 11% was dispersed in deionized water, adjusted to an acidic pH, and then sonicated to obtain NH-ChNFs.

Characterization methods

Degree of deacetylation was determined by an electrical potential titration method as previously reported (Tolaimate et al. 2000). The specific operation is as follows: β -chitin dried powder (0.1 g) was added to deionized water (50 mL), adjusted to pH = 1–2 with 0.1 M HCl. The suspension was uniformly dispersed by stirring overnight. Then added 0.1 M NaOH to the suspension, recorded the NaOH consumption and the corresponding pH value, and ended the titration when the pH value is in the range of 11–12. The titration curve is shown in Fig. S1.

The principle of determining the carboxyl content is the same as that for the determination of the degree of deacetylation. The potential of the CO-ChNFs suspension was first adjusted to -270 mV using 0.1 M NaOH. Then 0.1 M HCl was added to the suspension, the consumption of HCl and the corresponding potential were recorded, and the titration was terminated when the potential of the suspension was in the range of 230–250 mV. The titration curve is shown in Fig. S4, Fig. S5.

The FT-IR spectra of purified chitin and ChNFs were collected on a FTIR spectrometer (Bruker, Germany) with a resolution of 4 cm^{-1} . The CO-ChNFs were dried at 60°C to form a film, then the film was immersed in 0.1 M HCl to convert sodium carboxylate into carboxyl groups, and finally dried at 60°C again. Then the sample was mixed and ground well with KBr, and finally pressed into a film for measurements.

The XRD patterns were collected using an X-ray diffractometer (Rigaku, Japan) with Cu K α radiation ($\lambda = 0.154\text{ nm}$) in the 2θ range of 5°–35° and the scan rate of 5 °/min at 40 KV. As previously reported (Wu et al. 2021), the crystallinity index (CI) of purified chitin and ChNFs was estimated based using the relation:

$$CI = \left(\frac{I_{110} - I_{am}}{I_{110}} \right) \times 100\%$$

The $I_{(110)}$ and I_{am} denote the maximum values of the crystalline peak at $2\theta = 19 \sim 20^\circ$ and the amorphous peak at $2\theta = 12 \sim 14^\circ$, respectively.

The Zeta potentials of CO-ChNFs were conducted by a Dynamic Light Scattering (DLS, NanoBrook Omni, Brookhaven, USA). The CO-ChNFs samples (0.05 wt%) were scanned three times at ambient conditions (25°C), and zeta potential data were recorded.

AFM images of CO-ChNFs were acquired utilizing ScanAsyst mode (Dimension Icon, Bruker, USA). The sample preparation was as follows: 0.02 wt% CO-ChNFs suspension was dropped on mica flakes, then spin-coated at 2000 rpm for 1 min, and dried at 60°C before measurement.

Results And Discussions

Morphological structure of CO-ChNFs

The key steps in the experimental process are depicted in Fig. 1. According to the previous work (Fan et al. 2009), the β -chitin in the squid pen has a certain degree of deacetylation (DD), and the traditional TBN oxidation cannot proceed when the DD reaches 10%. The purified β -chitin was first deacetylated obtained partially deacetylated slurries (DE-chitin) with different DD to prove the TNN oxidation can break through the limitation of DD. Afterwards, purified chitin and DE-chitin were oxidized by TNN to obtain carboxyl chitin slurry (CO-chitin). Finally, the CO-chitin was suspended in alkaline deionized water, a transparent β -chitin nanofiber (CO-ChNFs) suspension was achieved after ultrasonication and centrifugation. The physical changes of β -chitin and expected changes of the functional groups on the β -chitin molecular chain are reflected in the illustration.

As seen in Fig. 2a, the transparent CO-ChNFs suspension was successfully prepared from various DD of β -chitin. Since the suspension is transparent, the Tyndall phenomenon is used to explain the existence of nano-chitin more intuitively. The CO-ChNFs were successfully prepared that because in the TNN oxidation system, the NaClO_2 immediately oxidize the aldehyde group to carboxyl group, avoiding the reaction between the aldehyde group oxidized by the TEMPO reagent and the amino group (Saito et al. 2009). In order to further investigate the influence of pH on the stability of nanofibers, the zeta potential of CO-ChNFs suspensions was measured as a function of pH, as shown in Fig. 2b. Obviously, all CO-ChNFs share the same trend. Under alkaline conditions (8–11), all CO-ChNFs have higher zeta potentials which means that CO-ChNFs have better dispersibility under alkaline conditions. However, the pH of CO-ChNFs suspensions is adjusted to an acidic, the absolute values of zeta potential are less than 30 mV and tend to zero. This means that no functional groups in CO-ChNFs provide a positive charge and the only source of positive charge in CO-ChNFs is the amino group obtained by alkali treatment. In order to further verify the amino groups changes on CO-ChNFs, it found that ninhydrin is a reagent commonly used to detect

amino groups. When amino groups are present, ninhydrin will react with amino groups to produce purple or dark blue substances (Ruhemann purple) (Gonzalez-Gonzalez et al. 2011). As indicated in Fig. S2, all the chitin nanofiber films were transparent at first. With a longer reaction time, the color reaction of amino chitin nanofiber film (NH-ChNFs) deepened, but almost no color reaction occurred on the other all CO-ChNFs film, indicating that the amino on CO-ChNFs was almost absent.

The morphologies of CO-ChNFs prepared from β -chitin with different DD were characterized by AFM. Fig.s 2c-2e respectively show AFM images and corresponding height curve of CO-ChNFs prepared from β -chitin with 5, 11 and 29% DD, respectively. It can be clearly seen, all CO-ChNFs exhibit nanofiber morphology with a length of several microns, and the diameters are 2–6 nm from corresponding height curves. The morphology are similar to those β -chitin nanofibers prepared from squid pen under other preparation methods (Fan et al. 2009; Ma et al. 2019; Wu et al. 2021). During the preparation process, it was found the conditions of alkali treatment had an important effect on the morphology of the oxidation products. When the concentration of alkali treatment NaOH solution was 30 wt%, the β -chitin molecular chains was depolymerized, and finally a chitin nanosphere was obtained (Jin et al. 2020), as shown in Fig. S3. Therefore, the alkali treatment was carried out in 10 wt% NaOH solution at 40°C, and the fibrous morphology of β -chitin was preserved.

Effect of different factors on the yield of CO-ChNFs

The yield of CO-ChNFs was found to be closely related to the DD of β -chitin in the experiment. Figure 3a shows the relationship between the DD of β -chitin and the alkali treatment time. When the alkali treatment time is 0, 1, 5, 12 and 24 h, the DD is 5, 8, 11, 22 and 29%, respectively. The DD increases with the prolongation of deacetylation time, the DD was obtained by the potentiometry method (Fig. S1). Figure 3b shows the yields of CO-ChNFs obtained by TNN oxidation using β -chitin with different DD. When the DD was 5, 8, 11, 22, and 29%, the yield of CO-ChNFs was 74.79, 87.14, 96.5, 82.46, and 74.9%. The yield of CO-ChNFs first increased then decreased with the increase of DD. When the DD of β -chitin = 11%, the yield of CO-ChNFs reached the maximum of 96.5%. The increased yield of CO-ChNFs at low DD (less than 11%) that because with the increase of amino content, the hydrogen bonds between the chitin molecular chains are destroyed, and the chitin is more easily oxidized, so the yield is increased. But when the DD in β -chitin exceeds 11%, the stronger hydrogen bonding also tends to occur between nanofibers, and the side reaction between the amino group and TNN system are more likely to occur. (Pang et al. 2017). Therefore, the highest yields of CO-ChNFs were obtained at DD = 11%.

Therefore, the optimum DD for preparing CO-ChNFs is 11%. The effects of oxidation reaction time and the amount of NaClO₂ on the carboxyl content and yield of CO-ChNFs were studied in β -chitin DD = 11%. The effects of oxidation reaction time on the carboxyl content and yield of CO-ChNFs are plotted in Fig. 3c. Unexpectedly, when the oxidation reaction time is 1 h, the yield of CO-ChNFs reaches 90%, and the carboxyl content reaches 0.76 mmol/g. With the prolongation of reaction time, the yield reached the maximum (96.5%) when the reaction time is 4 h, and the carboxyl group content increased to a maximum of 1.06 mmol/g (8h). Therefore, the oxidation time of 4 h was selected to explore the influence of the

amount of NaClO_2 on the carboxyl content and yield of CO-ChNFs. The results show that when the dosage of NaClO_2 is 5–20 mmol/g chitin, the yield is more than 95% and the highest is 98%. When the dosage of NaClO_2 is 5, 10, 15 and 20 mmol/g chitin, the carboxyl content is 0.58, 0.98, 1.08 and 1.08 mmol/g, respectively. The carboxyl content mentioned above is determined by potentiometric titration and calculated by relevant formulas, titration curves are shown in Fig. S4 and Fig. S5. Therefore, when the DD = 11% of β -chitin, a high yield of CO-ChNFs (> 90%) can be obtained and the carboxyl content can be adjusted by controlling the reaction time and the amount of NaClO_2 .

Physicochemical characteristics of CO-ChNFs

The chemical structure of the purified β -chitin and CO-ChNFs were analyzed by FT-IR and the results are given in Fig. 4a. The IR absorption band at 1640 cm^{-1} , 1560 cm^{-1} are the characteristic peaks of chitin, which correspond to the C = O and N-H tensile vibrations of amide I and the N-H bending vibration and C-N tensile vibrations of amide II, respectively (Xu et al. 2018). After TNN oxidation, a new peak appears in the 1730 cm^{-1} band that is the C = O stretching vibrations of protonated carboxyl groups, confirming the success of the TNN oxidation (Yang et al. 2015). Moreover, a slight split occurs around the amide I band at 1630 cm^{-1} , which may be a slight β - α crystal transformation (Jin et al. 2016; Ma et al. 2019).

The crystal structures of purified chitin and CO-ChNFs were investigated by employing XRD measurements (Fig. 4b). The β -chitin has two main diffraction peaks, and the peak position of purified chitin is consistent with previous research (Fan et al. 2008b; Wu et al. 2019). The diffraction peak at $2\theta = 19.6^\circ$ corresponds to the (110) crystal plane of the β -chitin crystal, and there is almost no change at the (110) crystal plane of CO-ChNFs after TNN oxidation. The peak $2\theta = 8.2^\circ$ corresponds to the (010) crystal plane of the β -chitin crystal and shifts to 8.9° after TNN oxidation, the characteristic peaks of the (010) crystal plane have shifted toward α -chitin (Bogdanova et al. 2016; Fan et al. 2008b). Moreover, CO-ChNFs show a slight characteristic diffraction peak at $\approx 23^\circ$ of 2θ diffraction angle, which belongs to the characteristic peak of α -chitin (Ma et al. 2021). These results indicate that a small proportion of β -chitin undergoes β - α transformation after TNN oxidation. The (010) crystal plane generally shows the degree of close packing of molecular chains (Ye et al. 2021). The distance between the crystal plane layers and the crystal size of the (010) crystal plane were calculated respectively by the Bragg equation and the Scherrer formula (Chen et al. 2021). The calculation results show that after the TNN oxidation, the interfacial spacing of the (010) crystal plane changes from the original 1.07 nm to 1.00 nm. The crystal sizes before and after oxidation were 3.62 nm and 3.63 nm, respectively. This indicates that the molecular chains of CO-ChNFs are more compactly packed compared with purified β -chitin. Finally, the crystallinity (CI) of purified chitin and CO-ChNFs are calculated by formulas, which are 75.69% and 74.2%, respectively, which means the crystal structures of β -chitin are not damaged.

Mechanism of oxidation

In our previous work, the zwitterionically chitin nanocrystals were obtained from partial deacetylation α -chitin by TNN oxidation, but in this paper, solely carboxyl chitin nanofibers were obtained from partial

deacetylation β -chitin. Therefore, the presumed oxidation mechanism is shown in Fig. 5. The α -chitin molecular chains are arranged antiparallel and have strong intermolecular interactions. Although deacetylation disrupts some of the interactions, the retained intermolecular interactions are still very strong, so the oxidation occurs around the surface of crystallites (Ye et al. 2020). Therefore, the amino groups on them can still be protected when TNN oxidation is performing, and finally obtain zwitterionically chitin nanocrystals.

The molecular chains of β -chitin are arranged in parallel and the intermolecular chain forces are mainly intramolecular hydrogen bonding. This means that the oxidation can occur in the inner part of crystallites when TNN oxidation is carried out. In addition, the intramolecular forces in β -chitin could not provide protection for the amino group like α -chitin, while the amino group itself has high reactivity. Therefore, after TNN oxidation, the amino groups on α -chitin are slightly lost, and the amino groups in β -chitin are not retained. (Jiang et al. 2018; Pang et al. 2017; Ye et al. 2020). The amino group might also be transformed to the nitro in the presence of strong oxidizing agents (Ma et al. 2019). Finally, only carboxyl β -chitin nanofibers were obtained.

Considering that this is a new preparation method, we compared TNN oxidation with other oxidation methods for preparing carboxyl β -chitin nanomaterials. As shown in Table 1, the current oxidation method for preparation carboxyl chitin only TBN oxidation (Fan et al. 2009; Wu et al. 2021), ammonium persulfate (APS) oxidation (Ma et al. 2019) and our work TNN oxidation.

Table 1
The conditions and properties of carboxyl β chitin prepared by different oxidation methods were compared

Methods	Material	DD (%)	Yield	Height (nm)	Zeta potential (mv)	CC (mmol/g)	Cl (%)
TBN oxidation	Tubeworm	0	70%	~	-60	0.25	60
	Squid pen	10	cannot be oxidized by TBN oxidation				
TBN oxidation	Squid pen (<i>Illex Argentinus</i>)	0.1	21%	2–8	-25.3	0.17	83.3
APS oxidation	Squid pen	9	18%	2–4	-35	0.8	~
TNN oxidation	Squid pen	5–30	98%	2–6	-42	1.08	74.2

DD: degree of deacetylation; CC: carboxyl content; Cl: crystallinity

The TBN oxidation system has a long oxidation time and requires to keep the pH = 10 during the oxidation process, which is too cumbersome. And the DD of chitin limits the application range of TBN oxidation. APS oxidation is a preparation method previously developed by our team that can one-step

oxidation of raw squid pen into carboxyl β -chitin nanofibers without purification treatment. The yield of APS oxidation can reach 18%, while the β -chitin content in raw Squid pen is generally 30% (Hunt and Nixon 1981; Suenaga et al. 2016), which means that the yield is around 50% and is not affected by the concentration of APS. However, the amount of oxidant used to oxidation 1 g chitin by APS oxidation is as high as 45 g, and the author did not investigate the effect of DD on oxidation. Compared with the previous two oxidation methods, TNN oxidation has a wider application range that can be oxidized the β -chitin with the DD range between 5–30%. The yield of TNN oxidation is also very high, and high yield products can be obtained within 1 h. In short, this work breaks through the previous limitation of TEMPO oxidation limited by DD, and can quickly obtain high-yield carboxyl β -chitin nanofibers.

Conclusions

The β -chitin nanofibers containing only carboxyl groups were successfully prepared from Squid pens by TNN oxidation. Results showed only a small amount of amino groups remained in CO-ChNFs after TNN oxidation, that because the parallel-arranged β -chitin molecular chains with mainly intramolecular hydrogen bonds cannot protect the highly active amino groups, and the amino groups may be oxidized to nitro.

This method has a wide application range that can be oxidized the β -chitin with the DD range between 5–30%, and the DD of β -chitin was closely related to the yield of CO-ChNFs. The highest yield (98%) was reached when the DD of β -chitin is 11%, and high yields (90%) of CO-ChNFs could be obtained quickly (1 h) by adjusting the oxidation time. Compared with TBN oxidation and APS oxidation method, the TNN oxidation method has higher yield, faster oxidation rate and wider application. This work breaks the previous limitation that TEMPO oxidation is only applicable to β -chitin with a degree of deacetylation \approx 1%, and provides a new way to prepare carboxyl β -chitin nanofibers, laying the foundation for its large-scale application.

Declarations

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Declaration of competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Conceptualization: [Daying Liu]; Methodology: [Daying Liu]; Investigation : [Daying Liu], [Shasha Huang], [Hao Wu]; Data curation: [Daying Liu]; Writing - original draft preparation: [Daying Liu]; Writing - review and editing: [Jianming Zhang]; Supervision: [Jianming Zhang]

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Figures

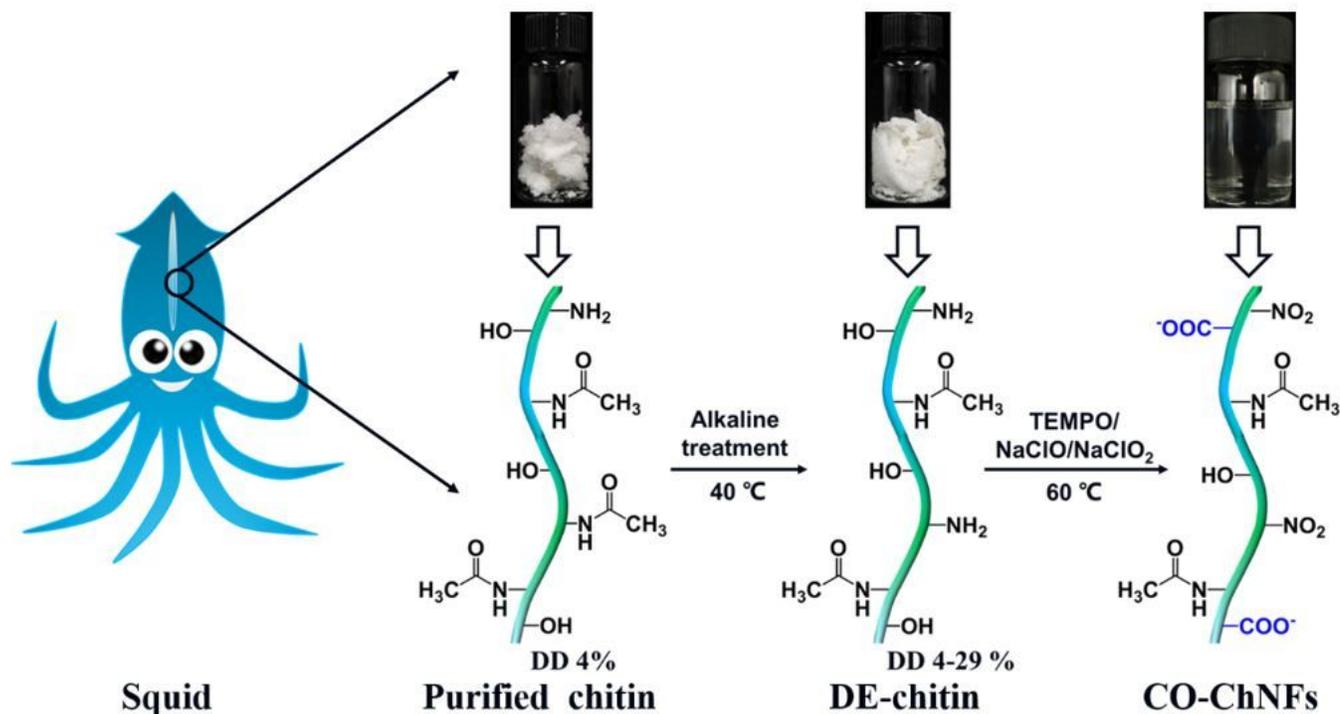


Figure 1

Illustration for the preparation of the CO-ChNFs from the squid pen. The digital photo illustrations show chitin solids or suspension.

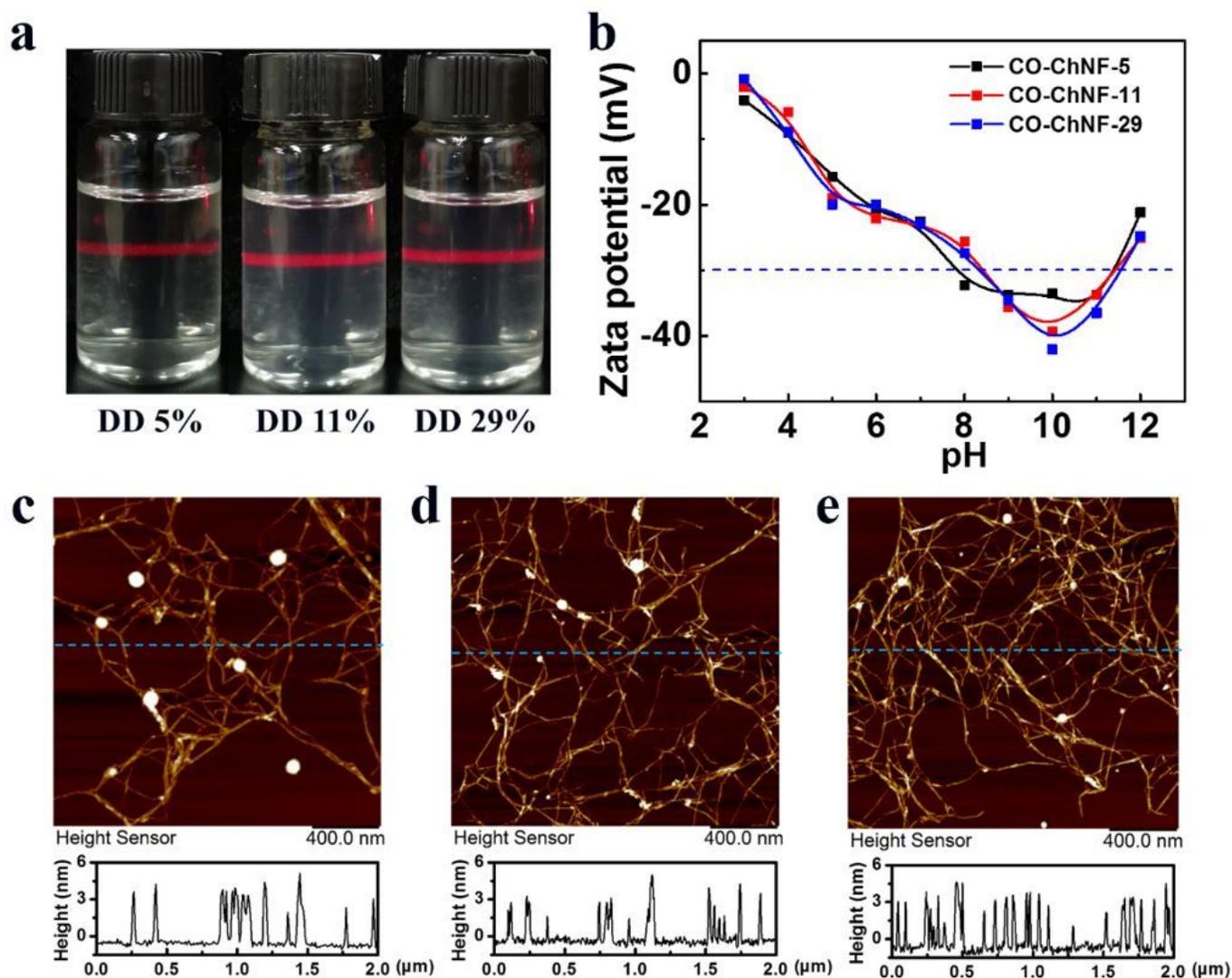


Figure 2

The digital photos of CO-ChNFs suspensions (**a**), The pH dependence of the Zeta potential of CO-ChNFs-X (**b**) (X represents the DD value of the β -chitin used during TEMPO oxidation), AFM images and corresponding height curve of chitin nanofibers prepared from β -chitin with different DD 5% (**c**), 11% (**d**), 29% (**e**)

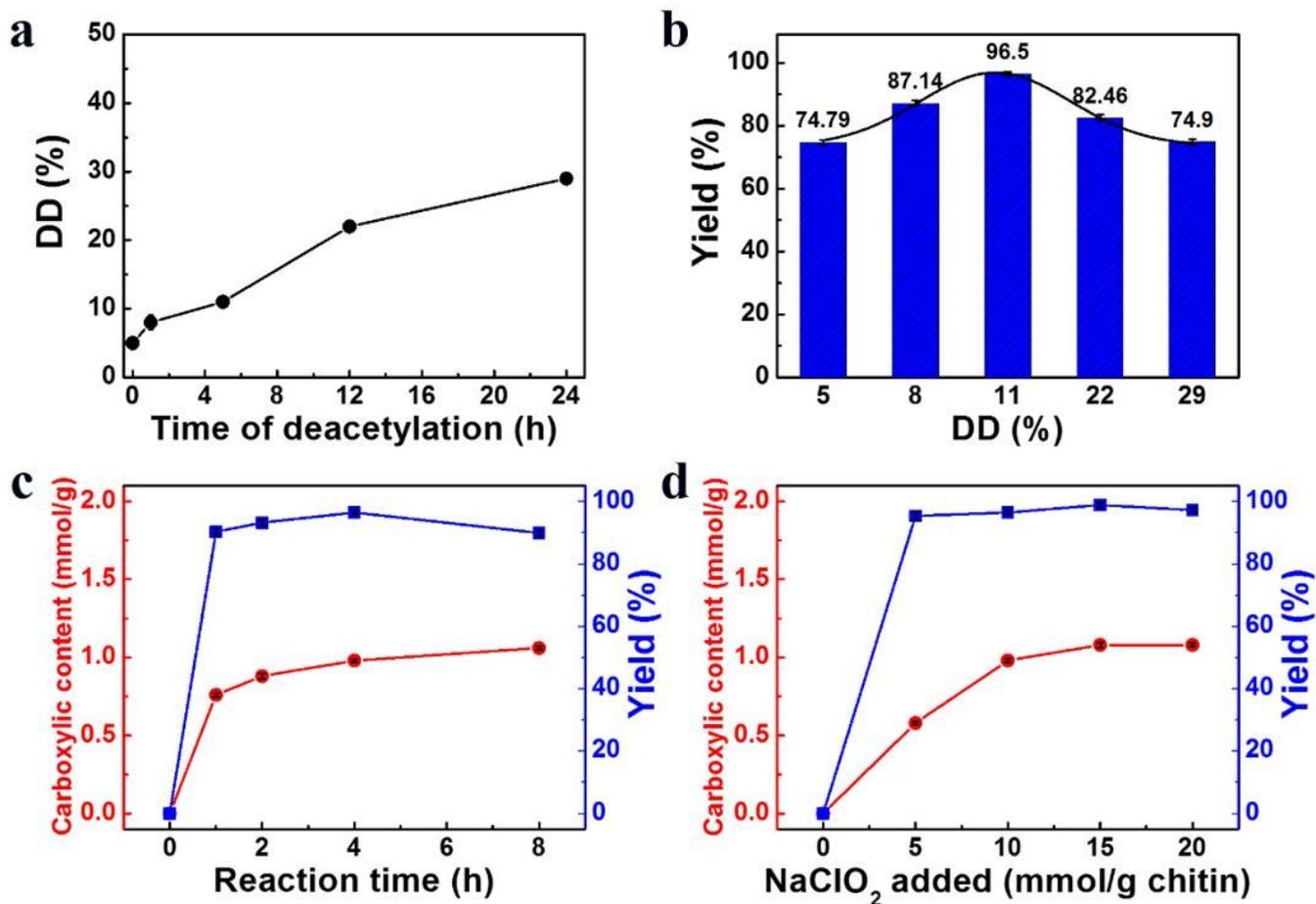


Figure 3

The relationship between the DD of β -chitin with alkali treatment time (**a**), the yield of CO-ChNFs and β -chitin DD (**b**), The relationship between the reaction time of TNN oxidation (**c**), the NaClO₂ added (**d**), and carboxyl content and the corresponding yield

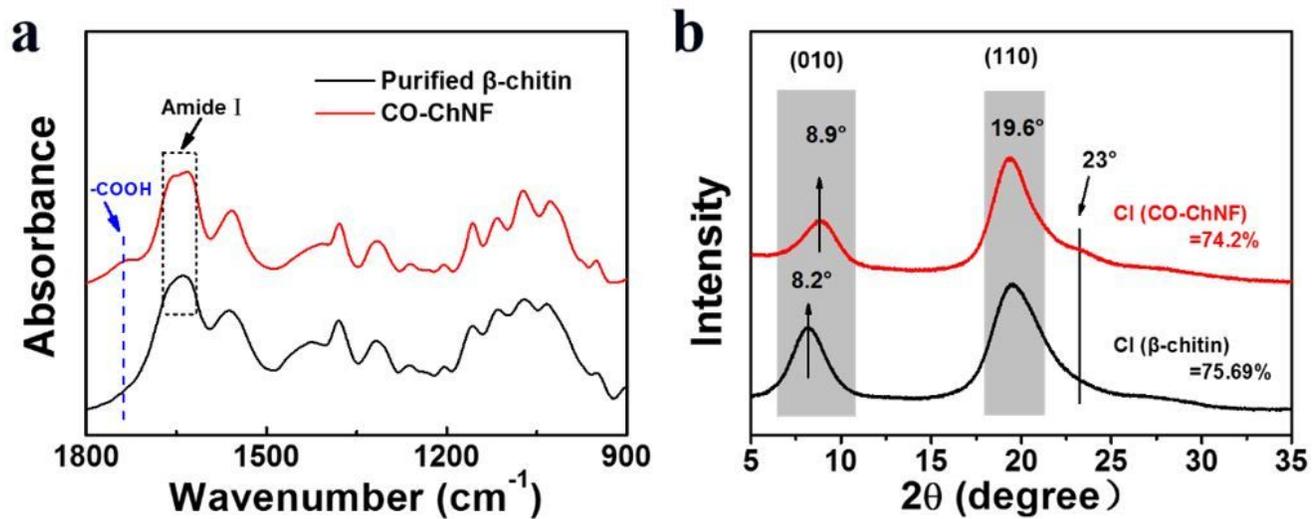


Figure 4

FTIR spectra (a), XRD spectra (b), of the purified chitin and CO-ChNFs

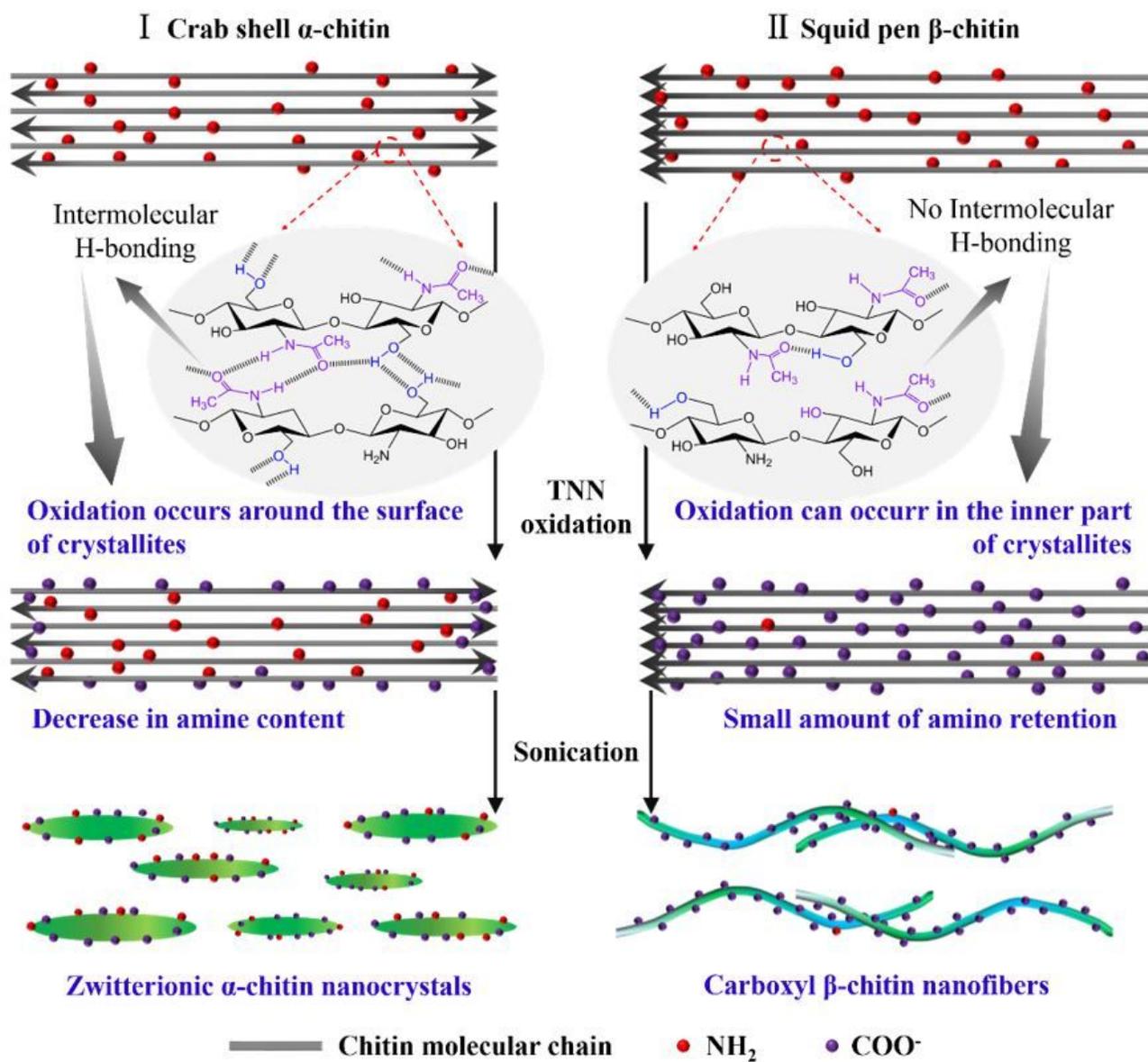


Figure 5

Speculation on the mechanism of action of TNN oxidation on β -chitin different from α -chitin

Supplementary Files

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