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Choline chloride-zinc chloride deep eutectic solvent mediated preparation of partial O-acetylation of chitin nanocrystal in one step reaction

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Highlights:

- One step hydrolysis and O-acetylation of chitin in deep eutectic solvent (DES).
- DES acted as non-volatile solvent and catalyst.
- Low concentration of acid is needed for the reaction.
- The obtained chitin is easy nanofibrillation and form nanocrystal with ultrasonication.

Abstract: An efficient and one step production of acetylated and esterified chitin nanocrystals (CNCs) was successfully achieved using choline chloride-zinc chloride deep-eutectic solvent (ChCl-ZnCl₂ DES). In this method, ChCl-ZnCl₂ DES with a mole ratio of 1:2, was used for the esterification and O-acetylation of chitin at 90 °C for 3 h and 6 h. This strategy consisted in using ChCl-ZnCl₂ DES as a green and non-volatile solvent for chitin under heterogeneous condition that, upon addition of acetic anhydride or acetic acid, simultaneous acetylation or esterification and hydrolysis occurred. The DES act as an efficient catalysis of the functional reaction. The acetylation and hydrolysis proceeded efficiently and the yield of partial acetylated CNCs was ca. 61.6% with DS (degree of substitution) of 0.23 under the optimized conditions. Acetic acid was used to substituted acid anhydride to produce CNCs. The yield of acetic acid induced CNCs was ca. 62.0% with higher DS of 0.34. A thorough investigation of the physicochemical characteristics changes of chitin pointed out that the main skeletal structure of obtained CNCs was intact. The thermal stability of CNCs decreased after treated by ChCl/ZnCl₂. In addition, thermal stability of CNCs functionalized with acetic acid is lower than the ones functionalized with acetic anhydride. DES showed low expenditure and toxicity. This approach provides a novel method for production of functional chitin nanocrystals.

Key words: Chitin, Deep eutectic solvent, Nanocrystals, Lewis acid

1. Introduction

Chitin is one of the most abundant renewable biological resources found in nature. It is the second largest natural polysaccharide biopolymer source after cellulose, with a yearly estimated production of approximately 10¹⁰–10¹² tons (Roberts, 1992). Chitin is a linear polymer of $\beta(1\rightarrow4)$ -linked 2-amino-2-deoxy-D-glucan and 2-acetamido-2-deoxy-D-glucan and is found

extensively in crustacean shells, insect cuticles, some mushrooms envelopes, and the cell walls of many fungi and some green algae (Croisier & Jérôme, 2013; Martin, 1998). Natural chitin possesses a high degree of crystallinity and displays three types of crystal structures: α , β , and γ allomorphs (Pereira, Muniz & Hsieh, 2014). Alpha-chitin is the most common form of chitin and is found mainly in the cuticles of arthropods and in the cell walls of mushrooms. It consists of two opposite parallel chains that show strong hydrogen bond interactions. Alpha-chitin is arranged as 2.5 nm to 25 nm diameter nanofibers embedded in a protein matrix (Goodrich & Winter, 2007; Ifuku, 2014).

The commercially available chitin powder is poorly soluble in most solvents and precipitates immediately (Ifuku, 2014), which limits its utilization. However, chitin nanofibers can be dispersed in many solvents for use as a reinforcement in polymers, where they enhance the polymer properties at even low nanofiller loading (Arias, Heuzey, Huneault, Ausias & Bendahou, 2015; Mariano, El Kissi & Dufresne, 2014; Siró & Plackett, 2010). Chitin has been processed in the form of nanofibers (CNFs) or nanocrystals (CNCs) by various techniques, including acid hydrolysis (Goodrich et al., 2007; Morin & Dufresne, 2002), TEMPO mediated oxidation (Fan, Saito & Isogai, 2008) and ultrasonication (Zhao, Feng & Gao, 2007). The CNCs are rod-like or whisker-shaped nanomaterials that have drawn substantial interest in many areas, due to their extremely large and active surface area, excellent mechanical toughness, biocompatibility, biodegradability, renewability, low density, and easy modification (Duan, Huang, Lu & Zhang, 2018; Fan et al., 2008; Zeng, He, Li & Wang, 2012). CNCs have been used in the fabrication of hybrid materials, polymer nanocomposites, and other functional materials; in food and biomedical applications; and in water treatment (Duan et al., 2018; Fan et al., 2008; Zeng et al., 2012).

Recently, ionic liquids (ILs), a class of sustainable solvents, were found to dissolve chitin (King, Shamshina, Gurau, Berton, Khan & Rogers, 2017; Prasad, Murakami, Kaneko, Takada, Nakamura & Kadokawa, 2009). Moreover, 1-allyl-3-methylimidazolium bromide (AMIMBr) has been used as a solvent for the pre-treatment of chitin to fabricate chitin nanocrystals

(Hatanaka, Yamamoto & Kadokawa, 2014; Kadokawa, Takegawa, Mine & Prasad, 2011). However, ILs have some disadvantages, such as their high cost and toxicity, which limit their application in many areas (Sharma, Mukesh, Mondal & Prasad, 2013). By contrast, deep eutectic solvents (DESs) consist typically of a hydrogen bond donor and hydrogen bond acceptor pair. The composition of a DES corresponds to a eutectic, self-associated mixture that forms through hydrogen bonding; consequently, the resulting system is a liquid at low temperatures (below 100 °C) (Morales, Sánchez-Leija, Carranza, Pojman, Monte & Luna-Bárcenas, 2017; Smith, Abbott & Ryder, 2014). DESs are often considered a new class of ionic liquid analogues, and they can typically be derived from inexpensive, commercially available raw materials (Abbott, Boothby, Capper, Davies & Rasheed, 2004; Del Monte, Carriazo, Serrano, Gutierrez & Ferrer, 2014). Consequently, DESs can potentially be less costly and more environmentally benign when compared with traditional ionic liquids (Del Monte et al., 2014; Hong et al., 2016).

A recent report indicated that acidic deep eutectic solvents could be used as hydrolytic media for cellulose nanocrystal production (Sirviö, Visanko & Liimatainen, 2016). A DES composed of choline chloride (ChCl) and oxalic acid dihydrate was able to achieve the partial dissolution of the non-crystalline parts of cellulose and liberated cellulose nanocrystals after mechanical treatment. The similarity of the structures of chitin and cellulose suggests that acidic deep eutectic solvents may also be useful as hydrolytic media for chitin nanocrystal production. This hypothesis is also supported by the recent discovery that DESs can potentially dissolve and modify chitin (Feng et al., 2019; Sharma et al., 2013). However, the previous efforts have mainly focused on DES use as a solvent and catalyst for functionalization of cellulose (Abbott, Bell, Handa & Stoddart, 2005; Selkala, Sirvio, Lorite & Liimatainen, 2016; Sirvio & Heiskanen, 2017). For example, urea-LiCl DES was applied as an efficient medium for the synthesis of succinylated cellulose, a promising adsorbent for an ionizable pharmaceutical, salbutamol (Selkala et al., 2016; Selkala et al., 2018). An effective O-

acetylation of cellulose was also studied using a Lewis acid DES of ChCl-ZnCl₂ (Abbott et al., 2005).

Inspired by these previous findings, we hypothesized that the ChCl-ZnCl₂ DES could be used as a novel medium to produce O-acetylated chitin nanocrystals. The introduction of acetyl groups as hydrophobic functional groups on chitin could benefit its applications by improving its dispersibility in nonpolar solvents, reducing its hygroscopicity, and enhancing its adhesion properties within hydrophobic matrices in composite materials (Ifuku, 2014; Kurita, 2001).

Figure 1. Schematic illustrations of the one-step fabrication of partially O-acetylated and esterified chitin nanocrystals (CNCs).

This work reports the use of a DES composed of zinc chloride and choline chloride as a non-volatile solvent and catalyst for a one-step heterogeneous production of acetylated chitin nanocrystals. Ultrasonication was subsequently applied for mechanical exfoliation to disperse the chitin nanocrystals. A schematic illustration of the one-step fabrication of partially O-acetylated chitin nanocrystals is shown in **Figure 1**. To the best of our knowledge, previous work on chitin has involved only a DES of ChCl-thiourea, used as a swelling solvent for the regeneration of dissolved chitin for production of chitin nanofibers (Mukesh, Mondal, Sharma & Prasad, 2014) by a self-assembly (bottom-up) route (Kadokawa, 2013). In the present work, DES was

simultaneously used as the solvent and the catalyst under heterogeneous conditions to liberate CNCs by a top-down approach.

2. Materials and methods

2.1 Materials

Choline chloride (ChCl), urea, thiourea and zinc chloride were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Acetic anhydride, hydrochloric acid (37%, HCl), and acetic acid were purchased from Nanjing Chemical Reagent Co., Ltd. The α -chitin was chemically isolated from shrimp shells and purchased from Aladdin Industrial Corporation. All chemicals were of analytical grade and were used without further purification.

2.2 Preparation of DES

The hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) were mixed in an Erlenmeyer flask (the molar ratio of HBA to HBD was shown in Table 1). The mixture was then transferred to an oil bath at 90 °C and stirred with a magnetic stirrer until the mixture became transparent and homogeneous, as described previously (Hong et al., 2016). The mixture was stored in a desiccator before until used for the pre-treatment of chitin.

Table 1

Preparation of DESs and modification of chitin in DESs.

DES	HBA	HBD	Molar ratio	Weight (g)	Chitin (g)	Acetic anhydride (g)
CZ	ChCl	ZnCl ₂	1:2	40	2	4.736
CU	ChCl	Urea	1:2	40	2	4.736
CT	ChCl	Thiourea	1:2	40	2	4.736
ZU	ZnCl ₂	Urea	1:3.5	40	2	4.736

2.3 Modification of chitin with DES

A 2 g sample of chitin was added to 40 g DES, followed by addition of acetic anhydride (0 g, 2.368 g or 4.736 g), acetic acid (0 g or, 5.572 g), and deionized water (0 g, 0.5 g, or 1 g), Table 2. The samples were mixed in an oil bath with magnetic stirring at 90 °C for 3 h or 6 h. After the reaction, the chitin was separated by centrifugation and washed with 100 ml of distilled water and 100 ml of ethanol. Subsequently, the modified chitin was washed until the conductivity was close to that of distilled water. All the experiments were done in triplicate. The chitin was diluted to a concentration of 0.5% and stored at 5 °C.

Table 2

Chitin nanocrystals prepared under different conditions (2 g chitin was dissolved in 40 g DES, - = not used).

Sample	Water (g)	Time (h)	Acetic anhydride (g)	Acetic acid (g)
DA1	-	3	2.368	-
DA2	-	3	4.736	-
DA3	-	6	4.736	-
DA4	0.5	3	2.368	-
DA5	0.5	3	4.736	-
DA6	0.5	6	4.736	-
DA7	1	3	2.368	-
DA8	1	3	4.736	-
DA9	1	6	4.736	-
DA10	-	3	-	5.572
DA11	-	6	-	-

2.4 Ultrasonication Process

The DES pre-treated chitin samples were subjected to ultrasonication with an ultrasonic generator (ATPIO-1200D, Nanjing Xianou Instruments Manufacture Co. Ltd. Nanjing, China) equipped with a 20 mm cylindrical titanium alloy probe. The ultrasonication was conducted

with an output power of 600 W and 2/2 s on/off pulses for 45 min. Powdered chitin samples were prepared by lyophilization of their aqueous solutions.

2.5 Characterization of chitin

FTIR spectra were recorded on a Nicolet 380 FTIR spectrometer (Nicolet, US) at room temperature, with samples as KBr pellets. All spectra were recorded in the range of 4000–400 cm^{-1} (4 cm^{-1} resolution).

The crystalline structures of the chitin samples were characterized using a Bruker D8 ADVANCE instrument with $\text{CuK}\alpha$ radiation ($\lambda=1.5406$) (40 kV, 30 mA). The chitin samples were scanned from a 2θ angle of 5° to 55° with a scanning speed of 4° per minute. The crystalline index (CrI; %) was calculated according to Eq. (1).

$$CrI_{110} = \left[\frac{I_{110} - I_{am}}{I_{110}} \right] \times 100 \quad (1)$$

where I_{110} is the maximum intensity at $2\theta \cong 19.2^\circ$ and I_{am} is the intensity of amorphous diffraction at $2\theta \cong 16^\circ$ (Focher, Beltrame, Naggi & Torri, 1990).

A droplet of 0.05% w/v chitin nanocrystal solution was coated onto monocrystalline silicon and dried in an oven at 60°C . The obtained film-like chitin samples were coated with a thin Au layer under vacuum using a sputter coater and imaged by field emission scanning electron microscopy (FE-SEM, HITACHI SU8220). The chitin nanocrystal morphology was observed by AFM (Bruker Dimension Icon AFM). The chitin suspension was sonicated for 10 min before use and then diluted to 0.005% w/v with water. The samples were dropped onto mica substrates and vacuum-dried before use. The images were acquired in tapping mode with silicon nitride cantilever tips. Images were processed and analyzed with the NanoScope Analysis 1.8 software.

The TG and DTG analyses were carried out with a NETZSCH STA 449C apparatus. About 3 mg of each sample was put onto an aluminum pan and heated from 35 to 600°C with a temperature ramping of $10^\circ\text{C min}^{-1}$ under a nitrogen atmosphere.

The chitin samples were characterized by ^{13}C cross-polarization/magic angle spinning (^{13}C CP/MAS). The spectra were acquired with a Bruker AVANCE 400WB spectrometer operating at 100.7 MHz for ^{13}C . The spinning rate was 7.0 kHz, with a recycle delay of 5 s. The sample was acquired using a cptppm2 pulse program using 90° pulses with a strength of 4.0 μs for matched spin-lock cross-polarization transfer (Ramírezwong et al., 2016) with a contact time of 1000 μs and 1024 scans.

3. Results and discussion

3.1 Chitin modification with different DESs

We first study the reaction of chitin in the above synthesized DESs with addition of acetic anhydride, as shown in **Table 1**. The reactions were all conducted at 90 °C for 3 h. **Figure S1** showed the appearance of the four pre-treated chitin solution (0.1%) before and after ultrasonication treatment. As it is clearly shown that chitin obtained after CZ and UZ treatment showed flocculent like while the other two were particle like and is easy form sediment. This may ascribe the existence of Lewis acid which help accelerating O-acetylation reaction (Abbott et al., 2005). After sonication treatment, the chitin obtained from CZ treatment form homogeneous and showed blue light suspension. However, the other three are still flocculent like. Although, UZ contains Lewis acid, urea is not stable under relative high temperature. It may release NH_3 that could react with acetic anhydride and reduce its absolute content which may affect the reaction between chitin. Therefore, ChCl-ZnCl_2 DES were used for further study.

3.2 Effect of reaction condition on the yield, stability and DS of chitin

The original aim of this work was to conduct O-acetylation of chitin for the production of CNFs in a DES. Conventional methods for O-acetylation of chitin involve the use of pyridine, perchloric acid, Lewis acid, and sodium acetate as a catalyst and mixing with an excess of acetic anhydride (Abbott et al., 2005; Dasgupta, Singh & Srivastava, 1980; Ifuku, Morooka,

Morimoto & Saimoto, 2010; Schlubach & Repenning, 1959). In this study, the ChCl-ZnCl_2 DES served both as the non-volatile solvent and as the catalyst for the O-acetylation of chitin. ChCl-ZnCl_2 DES with mole ratio of 1:2 showed high viscosity at low temperature which may hinder the dispersion of chitin in DES according to previous study (Abbott, Capper, Davies & Rasheed, 2004). The high temperature may in turn cause the over hydrolysis of chitin which significantly affect the yield of chitin nanocrystals. Previous work already found that the reaction temperature of 90 °C could be effective for O-acetylated cellulose (Abbott et al., 2005). Therefore, the temperature studied here was fixed at 90 °C. The purchased chitin was first added to the DES and then reacted with acetic anhydride or acetic acid. After the desired reaction time (3 h or 6 h, respectively), the mixture was washed with ethanol and deionized water to obtain a neutral chitin suspension (pH=7.0). Here, a DA5 suspension was selected as the representative sample for comparison with an unmodified chitin suspension. **Figure S2** shows the chitin suspension before and after the DES treatment. Note that the DA5 suspension had a cloudy appearance, while the untreated chitin suspension was very transparent. The partially acetylated chitin sediment, in turn, presented an aggregated structure instead of individual particles. This phenomenon is similar to the findings of a previous report on O-acetylation of cellulose using a zinc based ionic liquid (Abbott et al., 2005). We also tried to use the same DES and reaction conditions to treat cellulose pulp and microcrystalline cellulose (Avicel®), but we were unable to produce well-dispersed suspensions after mechanical treatment.

Figure 2. Photos of the 0.5 w/v% (left) and 0.1 w/v% (right) chitin nanocrystal aqueous suspensions after ultrasonication (top row) and after sitting undisturbed for two months at room temperature (bottom row).

The reaction conditions for chitin nanocrystal fabrication in DES are presented in **Table 2**. Different reaction conditions were studied (i.e., the water content of the DES, the reaction time, and the acetic anhydride mass ratio) on the yield, degree of substitution (Zou & Khor, 2005) (DS) and related characteristics of CNCs. We first studied the role of the reaction time and acetic anhydride content in determining the yield and stability of the CNCs suspension. The yield of CNCs decreased as a function of the acetic anhydride content (DA1 and DA2, DA4 and DA5, DA7 and DA8). The stability of the CNCs suspensions (**Figure 2**) was significantly influenced by the acetic anhydride content. This may be ascribed to the increased content of acetyl groups in the CNCs, which promoted the dispersion of the CNCs (**Figure 3** and **Figure S3**). As hydrophobic groups, the acetyl groups introduced into chitin can serve as a steric barrier that blocks hydrogen bonding between the chitin chains (Klemm et al., 2011). The chitin structure may also be hydrolyzed under more severe reaction conditions (as indicated by the yield values). A similar trend to that seen in response to the acetic anhydride content was also observed with increasing reaction time (DA2 and DA3, DA5 and DA6, DA8 and DA9). Water content can have a significant role in this DES reaction system. This is because acetic anhydride can be hydrolyzed into acetic acid, which may, in turn, affect the acetylation of chitin and promote the hydrolysis of chitin. Here, we noted that additional water increased the stability of the CNCs suspensions (DA2, DA5, DA8), as visually evaluated after two months (**Figure 2**). Since acetic acid is readily available and less expensive than acetic anhydride, we next replaced acetic anhydride with acetic acid to explore the possibility of using acetic acid for the production of acetylated CNCs. In these experiments, acetic acid was successfully applied for the production of CNCs (**Table 2**, DA10). The obtained CNCs suspension also remained stable after two months. The yield of DA10 was also very close to that of DA2, DA5, and DA8. Moreover, compared with the CNC obtained from hydrolysis with HCl according to

the procedure described by previous report (the yield is about 58.71%) (Araki & Kurihara, 2015), this CNCs showed wider adaptability in water under various pH (**Figure S4**).

Figure 3. The yield of chitin or chitin nanocrystals and the degree of substitution (DS, the detailed acetyl group content was calculated according to the FTIR results. N.D., Nondetectable, because of the low intensity of the absorption of ester at 1734 cm^{-1}).

As depicted in **Figure 3**, the yield of CNCs decreased with increases in the reaction time, the dosage of acetic anhydride, and the water content in the DES. When taking the stability of the CNCs suspension into account, the best reaction condition in this study at $90\text{ }^{\circ}\text{C}$ was 3 h with a 1:20 mass ratio of chitin to DES. The reference sample, DA11 (chitin pre-treated with DES without any addition of acetic anhydride or acetic acid), formed an unstable suspension that sedimented immediately and contained particles visible to the naked eye. The yield of DA11 reached 97.40%, indicating that the chitin structure may be intact after the treatment with DES. The role of DES treatment in chitin structural modification was further analyzed using DA2, DA5, DA8, and DA10.

3.3 Morphology of chitin nanocrystals

The CNCs solutions were spread onto monocrystalline silicon and coated with gold for scanning electron microscopy (SEM) analysis. The morphologies of the CNCs are depicted in **Figure 4**. All four samples had a rod-like structure with nanoscale dimensions. Although the CNCs stacked together, individual CNCs were clearly visible (the high-resolution SEM images

are shown in **Figure S5**). In order to compare the morphology changes of chitin, the morphology of pristine chitin was observed by SEM and is showed in **Figure S6**, which presents bundles of long fibers. The CNCs were then subjected to atomic force microscopy (AFM) to obtain an accurate morphology and size distribution. The AFM findings also confirmed (**Figure 4**) that the CNCs had a rod-like structure and that even the specimen formed in acetic acid (DA10) had similar structural characteristics. The diameters and lengths of the individual CNCs ranged from 30–80 nm and 200–700 nm for DA2, 30–60 nm and 100–650 nm for DA5, 20–60 nm and 150–50 nm for DA8, and 20–50 nm and 100–400 nm for DA10, respectively. These dimensions are very close to those for CNCs fabricated using the HCl hydrolysis method (e.g., a length of 100–650 nm and diameter of 10–80 nm for CNCs from crab shell chitin (Lu, Weng & Zhang, 2004) and a length of 200–560 nm and width of 18–40 nm for CNCs from shrimp shell chitin (Phongying, Aiba & Chirachanchai, 2007)). This result revealed that acetylation of chitin in the ChCl-ZnCl₂ DES simultaneously accelerated the hydrolysis reaction of chitin and promoted the surface functionalization of the resulting CNCs. Therefore, this procedure represented a new one-step production approach for the fabrication of acetylated chitin nanocrystals.

Acetylated cellulose nanocrystals have been previously synthesized by treating cellulose raw materials with a mixture of an inorganic acid (sulfuric acid or hydrochloric acid) and acetic acid (Braun & Dorgan, 2009; Tang et al., 2013). The inorganic acid acts as a catalyst for the esterification and promotes the hydrolytic cleavage of the glycosidic bonds for the formation of rod-like nanocellulose. This approach may also be extended to the fabrication of acetyl chitin nanocrystals. The use of the ChCl-ZnCl₂ DES alone is insufficient for the hydrolysis of chitin, but it is an efficient solvent for the catalysis of the hydrolytic reaction of chitin in the presence of acetic anhydride or acetic acid. When compared with inorganic acids, the ChCl-ZnCl₂ used in this approach is a milder reaction medium for the fabrication of acetylated chitin nanocrystals.

Figure 4. Scanning electron microscopy (SEM) (top row) and atomic force microscopy (AFM) (bottom row) images of chitin nanocrystals obtained under different treatments: a) anhydrous, 3h, 10.59 wt% acetic anhydride, b) 1.11 wt% water, 3h, 10.47 wt% acetic anhydride, c) 2.14 wt% water, 3h, 10.13 wt% acetic anhydride, d) anhydrous, 3h, no acetic anhydride, 12.23 wt% acetic acid.

3.4 Chemical structure changes before and after DES treatment

The chemical structure of the chitin nanocrystals, as studied by Fourier Transform Infrared (FTIR) spectroscopy, is shown in **Figure 5 (a)**. The chitin peaks are assigned according to previous reports (Brugnerotto, Lizardi, Goycoolea, Argüelles-Monal, Desbrières & Rinaudo, 2001; Bulut, Sargin, Arslan, Odabasi, Akyuz & Kaya, 2017; Chen, Deng, Yang, Yang, Ye & Li, 2018; Chen, Li, Yano & Abe, 2019; Chen, Wang, Yang, Pan, Ye & Li, 2018; Kaya et al., 2017; Zhu, Gu, Hong & Lian, 2017). The split bands around 1661 cm^{-1} and 1623 cm^{-1} are assigned to the amide I band. The values of the amide II band and amide III band are 1554 cm^{-1} and 1331 cm^{-1} , respectively. After pre-treatment with DES, the obtained chitin samples were all in the α form. After acetylation, DA2, DA5, DA8, and DA10 exhibited a new absorption band at 1734 cm^{-1} , which is due to the C=O stretching of the ester linkage (Hirayama, Yoshida, Yamamoto & Kadokawa, 2018; Ifuku et al., 2010). The intensity of the absorption at 1734 cm^{-1} of DA10 is slightly higher than the intensity observed for the other specimens. The degree of substitution (DS) of the acetylated chitin was calculated by

comparing the absorption ratio of ester at 1734 cm^{-1} and the amide I at the 1661 cm^{-1} bands (Zou et al., 2005). The DSs of the acetyl groups of DA2, DA5, DA8, and DA10 are 0.23, 0.25, 0.27, and 0.34, respectively. A plausible reason for the high DS of sample DA10 may be the high acidity of acetic acid, which promoted the hydrolysis of chitin and led to the formation of small particulates with high specific surface areas. As a result, the acetylation reaction could proceed more rapidly than it could with the other samples. Note also that the peak width of the O-H stretching at 3451 cm^{-1} decreased after the pre-treatment with DES and that the intensity of the bands at 1069 cm^{-1} and 1014 cm^{-1} increased sharply (these are attributed to C-O-C and C-O stretching (Du et al., 2014; Kaya, Sofi, Sargin & Mujtaba, 2016) respectively), indicating the successful acetylation of chitin. No other additional peaks appeared, indicating that no side reactions had occurred.

Figure 5. FT-IR spectra (a) of chitin and CNC and solid-state ^{13}C NMR spectra (b) of chitin samples.

The solid-state nuclear magnetic resonance (NMR) spectra of chitins are presented in **Figure 5 (b)**. The characteristic chemical shift is assigned to the carbonyl carbon in N-acetylamine (C=O, 173.7 ppm), C-1 (104 ppm), C-4 (83.4 ppm), C-5 (75.9 ppm), C-3 (73.6 ppm), C-6 (61 ppm), C-2 (55.2 ppm), and CH₃ (22.8 ppm). No extra peaks were detected, which means that the skeletal structural of chitin remained intact after acetylation and nanofibrillation in the ChCl-ZnCl_2 DES and ultrasonication treatment. Although the DA2, DA5, DA8, and DA10 samples were acetylated, no related peaks were observed in the NMR spectra. The

chemical shift of carbon in the C=O and CH₃ of the acetyl group should appear at 170–180 ppm and 18–22 ppm, respectively (Wu et al., 2018); therefore, a plausible explanation is that the signals derived from the carbon of acetyl group overlapped with those of chitin.

3.4 XRD analysis

The crystal structure of chitin before and after acetylation and nanofibrillation was investigated using X-ray diffraction (XRD) (**Figure 6**). All XRD patterns of chitin exhibited typical crystalline peaks associated with chitin at the 2 theta values of 9.2° (020), 12.6° (021), 19.2° (110), 20.6° (120), 23.3° (130), and 26.3° (013) (Goodrich et al., 2007; Huang, Zhao, Guo, Xue & Mao, 2018; Kumirska et al., 2010; Sikorski, Hori & Wada, 2009). No additional peaks appeared, which indicated that the chitins obtained were pure and intact. This result was in accordance with the FTIR analysis. The diffraction peak at the 2 theta value of 12.6° of DA10 almost disappeared, in agreement with a previous report on esterified chitin after an ultrasonication treatment (Wang, Yan, Chang, Ren & Zhou, 2018).

Figure 6. X-ray diffraction patterns of chitin powder.

The CrI value of the pristine chitin was 86.48%, but this value varied among the acetylated CNCs. For example, the CrI value was lower for DA2 (85.97%) than for pristine chitin (86.48%). This difference is ascribed to the presence of the acetyl groups and the use of mechanical force, which loosened the crystalline structure in a similar manner to the way that

high shear forces loosen the crystalline structure of cellulose (Selkala et al., 2016). Interestingly, the CrI value of DA5 (89.81%) and DA8 (88.30%) decreased with the amount of water added to the reaction system. A plausible explanation is that acetic acid (a product of the hydrolysis of acetic acid) aided the hydrolysis of the amorphous region and resulted in a higher CrI value. Conversely, the esterification and acetylation reactions are still proceeding, and this could weaken the hydrogen bonding interactions of chitin and decrease the CrI value. This phenomenon was evident when acetic acid was used for esterification (DA10).

Based on the FTIR analysis, DA10 possessed the highest ester group content. Therefore, the CrI value of DA10 (84.5%) was also the lowest among all the tested samples. Although ZnCl_2 was used in this system, no traces of zinc were observed in the XRD pattern, as reported previously (Feng et al., 2018). This indicated that Zn^{2+} did not absorb onto the chitin nor did it form any zinc compounds with chitin.

3.5 Thermal stability of chitin nanocrystals

Thermogravimetric analysis (TGA) and the derivative differential (DTG) analysis curves indicate thermal stability, but these curves can also reflect the differences in the crystallinity (Sajomsang & Gonil, 2010; Wang et al., 2013) and molecular weight (Hong, Yang, Yuan, Chen, Song & Lian, 2019; Hong, Yuan, Yang, Zhu & Lian, 2018) of chitin. The thermal stability and degradation profiles of pristine chitin and acetylated CNC are shown in **Figure 7**. All samples have similar TGA curves and unimodal DTG curves. The onset temperature (T_{onset}) and maximum weight-loss rate temperature (T_{max}) of the chitins showed slight differences: 245 °C and 373.9 °C for pristine chitin, 245 °C and 354.5 °C for DA2, 245 °C and 354.5 °C for DA5, 245 °C and 354.5 °C for DA8, and 200 °C and 315.8 °C for DA10, respectively. After the evaporation of water, the chitins began to degrade. During this stage of decomposition, the temperature ranged between 200°C and 420°C due to the degradation of the saccharide backbones (Peesan, Rujiravanit & Supaphol, 2003).

Figure 7. TGA (a) and DTG (b) curves of chitins.

The total weight losses in this range of chitins were 62.71%, 53.94%, 54.04%, 55.28%, and 44.61% for pristine chitin, DA2, DA5, DA8 and DA10, respectively. The residue chars for pristine chitin, DA2, DA5, DA8, and DA10 were 24.73%, 32.32%, 30.58%, 29.06%, and 38.72%, respectively. The reason for this high yield of char is that the acetylated chitin had fewer free hydroxy groups for dehydration during this stage.

After acetylation, the thermal stability of the obtained chitins decreased markedly, in agreement with previous work (Oun & Rhim, 2017; Selkala et al., 2016). The decreased thermal stability of the obtained chitin nanocrystals, when compared with pristine chitin, could be attributed to their smaller particle size and, as a result, their high specific surface area (Fan, Fukuzumi, Saito & Isogai, 2012; Jiang & Hsieh, 2013) and their lower molecular weight (Hong et al., 2019; Hong et al., 2018). Note that the T_{onset} and T_{max} of DA10 were 45 °C and 38.7 °C lower, respectively, than the nanocrystals acetylated with acid anhydride in DES. One explanation for this is that the DA10 had a lower CrI value (Sajomsang et al., 2010; Wang et al., 2013) and a smaller particle size, as shown in the morphology analysis. Similar to a carboxyl group, the ester group is a thermal-sensitive group (Sharma & Varma, 2014). The number of ester groups in chitin can be quantitatively determined according to the FTIR spectrum. The data presented here clearly showed that the absorbance at 1734 cm^{-1} derived from C=O stretching of the ester linkage is the highest for DA10, with a DS of 0.34. Therefore, DA10 had the lowest thermal stability.

4. Conclusions

This study raised a new method for one pot production of acetylated and esterified chitin nanocrystal. ChCl-ZnCl₂ DES was selected as the reaction solvent and catalyst. Several factors were investigated which may affect the acetylation, esterification and nanofibrillation of chitin, including reaction time, acetic anhydride content and water content. The acetylation, esterification and hydrolysis of chitin were simultaneous. In this study, the best reaction condition at 90 °C for production of acetylated chitin nanocrystal is 3 h with mass ratio of chitin to DES is 2:40, containing 4.736 g acetic anhydride with yield of 61.6% with DS of 0.23. For esterification of chitin nanocrystals, same reaction condition was used with 5.572 g acetic acid and the yield is 62.0% with DS of 0.34. The morphology of CNC showed that they are all rod-like shape with nanoscale dimension.

The obtained chemical structures of CNCs were intact according to the results of fourier transform infrared, solid-state NMR spectrum and X-ray diffraction. The result of XRD pattern showed obtained chitin is intact without any zinc compounds. The physical properties of esterified and acetylated chitin showed a little difference, namely, the thermal stability esterified CNC is lower than acetylated CNC because of the more thermal sensitive group were introduced and its lower CrI value. The CNCs had a rod-like shape with lateral dimensions ranging from 20–80 nm in width and 100–700 nm in length. This DES-based approach provides a potentially green and environmentally friendly method for a one-step acetylation, esterification, and hydrolysis of chitin to obtain functionalized CNCs.

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References:

- Abbott, A. P., Bell, T. J., Handa, S., & Stoddart, B. (2005). O-acetylation of cellulose and monosaccharides using a zinc based ionic liquid. *Green Chemistry*, 7(10), 705-707.
- Abbott, A. P., Boothby, D., Capper, G., Davies, D. L., & Rasheed, R. K. (2004). Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids. *Journal of the American Chemical Society*, 126(29), 9142-9147.
- Abbott, A. P., Capper, G., Davies, D. L., & Rasheed, R. (2004). Ionic liquids based upon metal halide/substituted quaternary ammonium salt mixtures. *Inorganic Chemistry*, 43(11), 3447-3452.
- Araki, J., & Kurihara, M. (2015). Preparation of Sterically Stabilized Chitin Nanowhisker Dispersions by Grafting of Poly(ethylene glycol) and Evaluation of Their Dispersion Stability. *Biomacromolecules*, 16(1), 379-388.
- Arias, A., Heuzey, M.-C., Huneault, M. A., Ausias, G., & Bendahou, A. (2015). Enhanced dispersion of cellulose nanocrystals in melt-processed polylactide-based nanocomposites. *Cellulose*, 22(1), 483-498.
- Braun, B., & Dorgan, J. R. (2009). Single-Step Method for the Isolation and Surface Functionalization of Cellulosic Nanowhiskers. *Biomacromolecules*, 10(2), 334-341.
- Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Argüelles-Monal, W., Desbrières, J., & Rinaudo, M. (2001). An infrared investigation in relation with chitin and chitosan characterization. *Polymer*, 42(8), 3569-3580.
- Bulut, E., Sargin, I., Arslan, O., Odabasi, M., Akyuz, B., & Kaya, M. (2017). In situ chitin isolation from body parts of a centipede and lysozyme adsorption studies. *Materials Science and Engineering C*, 70, 552-563.
- Chen, C. C., Deng, S. W., Yang, Y. N., Yang, D., Ye, T., & Li, D. G. (2018). Highly transparent chitin nanofiber/gelatin nanocomposite with enhanced mechanical properties. *Cellulose*, 25(9), 5063-5070.
- Chen, C. C., Li, D. G., Yano, H., & Abe, K. (2019). Bioinspired hydrogels: Quinone crosslinking reaction for chitin nanofibers with enhanced mechanical strength via surface deacetylation. *Carbohydrate Polymers*, 207, 411-417.
- Chen, C. C., Wang, Y. R., Yang, Y. N., Pan, M. Z., Ye, T., & Li, D. G. (2018). High strength gelatin-based nanocomposites reinforced by surface-deacetylated chitin nanofiber networks. *Carbohydrate Polymers*, 195, 387-392.
- Croisier, F., & Jérôme, C. (2013). Chitosan-based biomaterials for tissue engineering. *European Polymer Journal*, 49(4), 780-792.

- Dasgupta, F., Singh, P. P., & Srivastava, H. C. (1980). Acetylation of carbohydrates using ferric chloride in acetic anhydride *Carbohydrate Research*, 80(2), 346-349.
- Del Monte, F., Carriazo, D., Serrano, M. C., Gutierrez, M. C., & Ferrer, M. L. (2014). ChemInform Abstract: Deep Eutectic Solvents in Polymerizations: A Greener Alternative to Conventional Syntheses. *ChemSusChem*, 45(24), 999-1009.
- Du, Q., Sun, J., Li, Y., Yang, X., Wang, X., Wang, Z., & Xia, L. (2014). Highly enhanced adsorption of congo red onto graphene oxide/chitosan fibers by wet-chemical etching off silica nanoparticles. *Chemical Engineering Journal*, 245(6), 99-106.
- Duan, B., Huang, Y., Lu, A., & Zhang, L. N. (2018). Recent advances in chitin based materials constructed via physical methods. *Progress in Polymer Science*, 82, 1-33.
- Fan, Y., Fukuzumi, H., Saito, T., & Isogai, A. (2012). Comparative characterization of aqueous dispersions and cast films of different chitin nanowhiskers/nanofibers. *International Journal of Biological Macromolecules*, 50(1), 69-76.
- Fan, Y., Saito, T., & Isogai, A. (2008). Chitin nanocrystals prepared by TEMPO-mediated oxidation of alpha-chitin. *Biomacromolecules*, 9(1), 192-198.
- Feng, M., Lu, X., Jiang, K., Zhang, J., Xin, J., Shi, C., Wang, K. F., & Zhang, S. (2018). One-Step Preparation of Antibacterial Chitin/Zn Composite from Shrimp Shells by Urea-Zn(OAc)₂•2H₂O Aqueous Solution. *Green Chemistry*, 20(10), 2212–2217.
- Feng, M., Lu, X., Zhang, J., Li, Y., Shi, C., Lu, L., & Zhang, S. (2019). Direct conversion of shrimp shells to O-acylated chitin with antibacterial and anti-tumor effects by natural deep eutectic solvents. *Green Chemistry*, 21(1), 87-98.
- Focher, B., Beltrame, P. L., Naggi, A., & Torri, G. (1990). Alkaline N -deacetylation of chitin enhanced by flash treatments. Reaction kinetics and structure modifications. *Carbohydrate Polymers*, 12(4), 405-418.
- Goodrich, J. D., & Winter, W. T. (2007). Alpha-chitin nanocrystals prepared from shrimp shells and their specific surface area measurement. *Biomacromolecules*, 8(1), 252-257.
- Hatanaka, D., Yamamoto, K., & Kadokawa, J. (2014). Preparation of chitin nanofiber-reinforced carboxymethyl cellulose films. *International Journal of Biological Macromolecules*, 69, 35-38.
- Hirayama, H., Yoshida, J., Yamamoto, K., & Kadokawa, J.-i. (2018). Facile acylation of α -chitin in ionic liquid. *Carbohydrate Polymers*, 200, 567-571.
- Hong, S., Lian, H. L., Sun, X., Pan, D., Carranza, A., Pojman, J. A., & Motamoraes, J. D. (2016). Zinc-based deep eutectic solvent-mediated hydroxylation and demethoxylation of lignin for the production of wood adhesive. *Rsc Advances*, 6(92), 89599-89608.

- Hong, S., Yang, Q., Yuan, Y., Chen, L., Song, Y., & Lian, H. (2019). Sustainable co-solvent induced one step extraction of low molecular weight chitin with high purity from raw lobster shell. *Carbohydrate Polymers*, 205, 236-243.
- Hong, S., Yuan, Y., Yang, Q., Zhu, P., & Lian, H. (2018). Versatile acid base sustainable solvent for fast extraction of various molecular weight chitin from lobster shell. *Carbohydrate Polymers*, 201, 211-217.
- Huang, W.-C., Zhao, D., Guo, N., Xue, C., & Mao, X. (2018). Green and Facile Production of Chitin from Crustacean Shells Using a Natural Deep Eutectic Solvent. *Journal of Agricultural and Food Chemistry*, 66(45), 11897-11901.
- Ifuku, S. (2014). Chitin and Chitosan Nanofibers: Preparation and Chemical Modifications. *Molecules*, 19(11), 18367-18380.
- Ifuku, S., Morooka, S., Morimoto, M., & Saimoto, H. (2010). Acetylation of Chitin Nanofibers and their Transparent Nanocomposite Films. *Biomacromolecules*, 11(5), 1326-1330.
- Jiang, F., & Hsieh, Y.-L. (2013). Chemically and mechanically isolated nanocellulose and their self-assembled structures. *Carbohydrate Polymers*, 95(1), 32-40.
- Kadokawa, J., Takegawa, A., Mine, S., & Prasad, K. (2011). Preparation of chitin nanowhiskers using an ionic liquid and their composite materials with poly(vinyl alcohol). *Carbohydrate Polymers*, 84(4), 1408-1412.
- Kadokawa, J. I. (2013). Ionic Liquid as Useful Media for Dissolution, Derivatization, and Nanomaterial Processing of Chitin. *Green and Sustainable Chemistry*, 3(2), 19-25.
- Kaya, M., Mujtaba, M., Ehrlich, H., Salaberria, A. M., Baran, T., Amemiya, C. T., Galli, R., Akyuz, L., Sargin, I., & Labidi, J. (2017). On chemistry of γ -chitin. *Carbohydrate Polymers*, 176, 177-186.
- Kaya, M., Sofi, K., Sargin, I., & Mujtaba, M. (2016). Changes in physicochemical properties of chitin at developmental stages (larvae, pupa and adult) of *Vespa crabro* (wasp). *Carbohydrate Polymers*, 145, 64-70.
- King, C., Shamshina, J., Gurau, G., Berton, P., Khan, N. F. A. F., & Rogers, R. D. (2017). A platform for more sustainable chitin films from an ionic liquid process. *Green Chemistry*, 19(1), 117-126.
- Klemm, D., Kramer, F., Moritz, S., Lindstrom, T., Ankerfors, M., Gray, D., & Dorris, A. (2011). Nanocelluloses: A New Family of Nature-Based Materials. *Angewandte Chemie International Edition*, 50(24), 5438-5466.

- Kumirska, J., Czerwicka, M., Kaczyński, Z., Bychowska, A., Brzozowski, K., Thöming, J., & Stepnowski, P. (2010). Application of Spectroscopic Methods for Structural Analysis of Chitin and Chitosan. *Marine Drugs*, 8(5), 1567-1636.
- Kurita, K. (2001). Controlled functionalization of the polysaccharide chitin. *Progress in Polymer Science*, 26(9), 1921-1971.
- Lu, Y. S., Weng, L. H., & Zhang, L. N. (2004). Morphology and properties of soy protein isolate thermoplastics reinforced with chitin whiskers. *Biomacromolecules*, 5(3), 1046-1051.
- Mariano, M., El Kissi, N., & Dufresne, A. (2014). Cellulose nanocrystals and related nanocomposites: review of some properties and challenges. *Journal of Polymer Science Part B: Polymer Physics*, 52(12), 791-806.
- Martin, A. M. (1998). *Fisheries waste biomass: biconversion alternatives*. Springer US.
- Morales, J. D. M., Sánchez-Leija, R. J., Carranza, A., Pojman, J. A., Monte, F. D., & Luna-Bárcenas, G. (2017). Free-radical polymerizations of and in deep eutectic solvents: green synthesis of functional materials. *Progress in Polymer Science*, 78, 139-153.
- Morin, A., & Dufresne, A. (2002). Nanocomposites of chitin whiskers from *Riftia* tubes and poly(caprolactone). *Macromolecules*, 35(6), 2190-2199.
- Mukesh, C., Mondal, D., Sharma, M., & Prasad, K. (2014). Choline chloride-thiourea, a deep eutectic solvent for the production of chitin nanofibers. *Carbohydrate Polymers*, 103(4), 466-471.
- Oun, A. A., & Rhim, J.-W. (2017). Effect of oxidized chitin nanocrystals isolated by ammonium persulfate method on the properties of carboxymethyl cellulose-based films. *Carbohydrate Polymers*, 175, 712-720.
- Peesan, M., Rujiravanit, R., & Supaphol, P. (2003). Characterisation of beta-chitin/poly(vinyl alcohol) blend films. *Polymer Testing*, 22(4), 381-387.
- Pereira, A. G. B., Muniz, E. C., & Hsieh, Y. L. (2014). Chitosan-sheath and chitin-core nanowhiskers. *Carbohydrate Polymers*, 107, 158-166.
- Phongying, S., Aiba, S., & Chirachanchai, S. (2007). Direct chitosan nanoscaffold formation via chitin whiskers. *Polymer*, 48(1), 393-400.
- Prasad, K., Murakami, M., Kaneko, Y., Takada, A., Nakamura, Y., & Kadokawa, J. (2009). Weak gel of chitin with ionic liquid, 1-allyl-3-methylimidazolium bromide. *International Journal of Biological Macromolecules*, 45(3), 221-225.
- Ramírezwong, D. G., Ramírezcardona, M., Sánchezleija, R. J., Rugerio, A., Mauriciosánchez, R. A., Hernándezlandaverde, M. A., Carranza, A., Pojman, J. A., Garaytapia, A. M., & Prokhorov, E. (2016). Sustainable-solvent-induced polymorphism in chitin films. *Green Chemistry*, 18, 4303-4311.

- Roberts, G. A. F. (1992). *Chitin chemistry*. Macmillan.
- Sajomsang, W., & Gonil, P. (2010). Preparation and characterization of α -chitin from cicada sloughs. *Materials Science and Engineering C*, 30(3), 357-363.
- Schlubach, H. H., & Repenning, K. (1959). Zur Acetylierung von Kohlenhydraten. *Angewandte Chemie*, 71(5), 193-194.
- Selkala, T., Sirvio, J. A., Lorite, G. S., & Liimatainen, H. (2016). Anionically Stabilized Cellulose Nanofibrils through Succinylation Pretreatment in Urea-Lithium Chloride Deep Eutectic Solvent. *ChemSusChem*, 9(21), 3074-3083.
- Selkala, T., Suopajarvi, T., Sirvio, J. A., Luukkonen, T., Lorite, G. S., Kalliola, S., Sillanpaa, M., & Liimatainen, H. (2018). Rapid uptake of pharmaceutical salbutamol from aqueous solutions with anionic cellulose nanofibrils: The importance of pH and colloidal stability in the interaction with ionizable pollutants. *Chemical Engineering Journal*, 350, 378-385.
- Sharma, M., Mukesh, C., Mondal, D., & Prasad, K. (2013). Dissolution of α -chitin in deep eutectic solvents. *Rsc Advances*, 3(39), 18149-18155.
- Sharma, P. R., & Varma, A. J. (2014). Thermal stability of cellulose and their nanoparticles: Effect of incremental increases in carboxyl and aldehyde groups. *Carbohydrate Polymers*, 114, 339-343.
- Sikorski, P., Hori, R., & Wada, M. (2009). Revisit of alpha-chitin crystal structure using high resolution X-ray diffraction data. *Biomacromolecules*, 10(5), 1100-1105.
- Siró, I., & Plackett, D. (2010). Microfibrillated cellulose and new nanocomposite materials: a review. *Cellulose*, 17(3), 459-494.
- Sirviö, J. A., Visanko, M., & Liimatainen, H. (2016). Acidic deep eutectic solvents as hydrolytic media for cellulose nanocrystal production. *Biomacromolecules*, 17(9), 3025-3032.
- Sirvio, J. A., & Heiskanen, J. P. (2017). Synthesis of Alkaline-Soluble Cellulose Methyl Carbamate Using a Reactive Deep Eutectic Solvent. *ChemSusChem*, 10(2), 455-460.
- Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep Eutectic Solvents (DESs) and Their Applications. *Chemical Reviews*, 114(21), 11060-11082.
- Tang, L. R., Huang, B., Lu, Q. L., Wang, S. Q., Ou, W., Lin, W. Y., & Chen, X. R. (2013). Ultrasonication-assisted manufacture of cellulose nanocrystals esterified with acetic acid. *Bioresource Technology*, 127, 100-105.
- Wang, Q., Yan, X. X., Chang, Y. J., Ren, L. L., & Zhou, J. (2018). Fabrication and characterization of chitin nanofibers through esterification and ultrasound treatment. *Carbohydrate Polymers*, 180, 81-87.

- Wang, Y. C., Chang, Y. G., Yu, L., Zhang, C. Y., Xu, X. Q., Xue, Y., Li, Z. J., & Xue, C. H. (2013). Crystalline structure and thermal property characterization of chitin from Antarctic krill (*Euphausia superba*). *Carbohydrate Polymers*, 92(1), 90-97.
- Wu, H., Nagarajan, S., Shu, J., Zhang, T. P., Zhou, L. J., Duan, Y. X., & Zhang, J. M. (2018). Green and facile surface modification of cellulose nanocrystal as the route to produce poly(lactic acid) nanocomposites with improved properties. *Carbohydrate Polymers*, 197, 204-214.
- Zeng, J. B., He, Y. S., Li, S. L., & Wang, Y. Z. (2012). Chitin Whiskers: An Overview. *Biomacromolecules*, 13(1), 1-11.
- Zhao, H. P., Feng, X. Q., & Gao, H. J. (2007). Ultrasonic technique for extracting nanofibers from nature materials. *Applied Physics Letters*, 90(7).
- Zhu, P., Gu, Z., Hong, S., & Lian, H. (2017). One-pot production of chitin with high purity from lobster shells using choline chloride-malonic acid deep eutectic solvent. *Carbohydrate Polymers*, 177, 217-223.
- Zou, Y., & Khor, E. (2005). Preparation of C-6 Substituted Chitin Derivatives under Homogeneous Conditions. *Biomacromolecules*, 6(1), 80-87.