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Sustainability Spotlight Statement

under

Ionic liquid strategy for chitosan production from chitin and molecular insights

Maximizing biomass waste utilization is a societal priority. Of marine biomass byproducts, chitin is found abundantly and holds importance for chitosan production in diverse industries. Traditional chitosan manufacturing methods rely on harsh alkaline/acidic solutions, detrimental to water ecosystems. We have proposed an energy-efficient, sustainable chitosan production method, replacing even harmful reagents. Shrimp chitin was pretreated in [Emim][OAc] ionic liquid and subjected for microwave-mediated deacetylation using [TBA][OH] or NaOH solution. This synergistic approach improved chitosan production, reaching 85 %DDA in two hours (40 wt.%-NaOH) and 71 %DDA (40 wt.%-[TBA][OH]), surpassing traditional methods. Both [Emim][OAc] and [TBA][OH] could be regenerated, with 97% and 83%, respectively. Our research supports UN-Sustainable Development Goals: industry, innovation, infrastructure (SDG-9), responsible consumption/production (SDG-12), life below water (SDG-14).

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Ionic liquid strategy for chitosan production from chitin and molecular insights

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To produce chitosan is an interesting research. Chitosan is an important polysaccharide in terms of its various applications in industries and is produced from chitin, an abundant biopolymer in crustacean shell biomass wastes. Traditional processes for chitosan manufacture are commonly based on highly concentrated alkaline or acid solutions which are, however, severely eroding and harmful to the environment. In this study, we have described a 'greener' method using 1-ethyl-3-methylimidazolium acetate, [Emim][OAc] ionic liquid (IL), for decrystallization of shrimp crystalline chitin flakes followed by a microwave-mediated NaOH or tetrabutylammonium hydroxide, [TBA][OH], solution-based deacetylation step for chitosan production. The decrease in crystallinity in IL-pretreated chitin was confirmed by XRD and SEM analysis which subsequently benefited chitosan production with up to 85% degree of deacetylation (%DDA) in shorter time periods (1-2 hours) and lower alkaline concentrations (20-40 wt.%). The %DDA in chitin/chitosan was estimated *via* FT-IR and NMR analysis. Notably, we could regenerate the ionic liquids: in case of [Emim][OAc] 97 wt.% and in case of [TBA][OH] 83 wt.% could be reused. Roles of ionic liquids in the process were discussed. Molecular dynamics (MD) simulations showed roles of [TBA]$^+$ cations in the molecular driving forces of [TBA][OH]-induced deacetylation mechanism. The strategy promises a sustainable and milder approach to the existing highly corrosive alkaline- or acid-involved processes for chitosan production.

Introduction

Deacetylation of chitin into chitosan is a key industrial process. Since being less acetylated and crystalline, chitosan has various technical applications compared to chitin.[1,2] Besides, the deacetylation process makes use of chitin – a main component (15-40 wt.%) of crustacean shell biomass wastes, which is released every year in million tons.[1,3,4] While chitin valorization is highly demanded, chitosan, being biocompatible, biodegradable, less toxic and having antibacterial properties, has important industrial roles in diverse fields. Examples include drug delivery in medicals, coating films in pharmaceuticals, carriers in agriculture, membranes for water purification, so forth.[5–8] Additionally, chitosan is also explored as nanosupports for heterogeneous reactions[9] as well as a precursor for synthesis of low molecular weight chemicals such as glucosamine and hydroxymethylfurfural.[10–12]

Chemistry of the deacetylation comprises cleavage of acetyl groups in chitin for production of chitosan with free -NH$_2$ groups (Figure 1), where to be called chitosan needs at least 50% degree of deacetylation (%DDA or molar fraction of non-acetylated D-glucosamine unit).[13] Thermal treatment of chitin with the use of alkaline solution of NaOH is one of the traditional and industrially applied methods for the chitosan production.[14] However, this method is time- and energy consuming (up to days and/or at high temperatures and pressures) when, for instance, crystalline chitin microfibrils are used.[14] Besides, the method needs also large amounts of NaOH (60-70 wt.% solutions) which is a highly corrosive reagent, affecting not only the reaction equipment but also the environment. Recently, some methods have been introduced to improve the chitin deacetylation process, for example, deep eutectic solvents,[15] alkali treatment at high temperature with intermittent washing with water,[16] mechanochemistry with repeated ball milling cycles,[17] ultrasound-assisted alkali treatment,[18] alkali deacetylation at high temperature in presence of glycerol,[19] or use of water-miscible organic solvents together with alkali solution.[20] However, a few challenges still exist in terms of processing time, energy
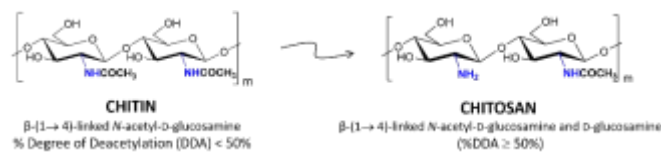
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Figure 1. Transformation of chitin to chitosan proceeds by cleaving off acetyl groups.

efficiency, or highly corrosive chemicals, which we aim to deal with.

Chitin has a highly crystallized and rigid structure due to an extended intra- and intermolecular hydrogen bonding network, which is considered as a bottleneck for its direct dissolution as well as its processing in common solvents or reagents.(21,22) We hypothesize that when being less crystalline, chitin might efficiently interact with various reagents as well as solvents, allowing for desired application-oriented structural modifications. In order to diminish the crystalline nature of the chitin, several aqueous as well as non-aqueous solvent media have been used for chitin pretreatment, including alkali aqueous solvent,(23,24) inorganic salt aqueous solvent, ionic liquid (IL), deep eutectic solvent (DES), etc. (13,25) Out of these studied solvent systems, IL gained a considerable attention for dissolution and derivatization of chitin. Generally being liquids below 100 °C, ILs are special non-aqueous solvent media commonly composed of an organic cation and an organic/inorganic anion.(13,25–27) Owing to their special solvent properties such as high thermal stability, low volatile nature, negligible vapour pressure, high ionic conductivity and large window of liquid phase, ILs have been extensively used as a solvent medium for organic transformations and biopolymer processing (cellulose, lignin, polyhydroxyalkanoate) as well as an electrolyte in batteries, etc.(28–31) In case of chitin processing, some types of ILs including different types of cations and anions in their composition have been previously applied to obtain a semi-crystalline or amorphous material *via* its dissolution under thermal treatments.(32,33) In this report, we used IL 1-ethyl-3-methylimidazolium acetate, [Emim][OAc] (Figure 2), for the pretreatment of shrimp crystalline chitin and subsequently employed the obtained pretreated chitin for further reaction. Amongst common ILs, [Emim][OAc] is popularly known for the pretreatment of biopolymers considering its highly basic nature and short cationic alkyl chains which effectively access the crystalline phase by entering the gap between the polymeric chains.(34)
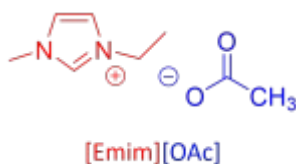

Figure 2. Structure of 1-ethyl-3-methylimidazolium acetate, [Emim][OAc] IL.

The main step, which is the chitin deacetylation to chitosan, requires energy for cleaving off acetyl groups. A method that can provide energy for alkaline deacetylation of chitin is microwave irradiation(19) – a rapidly emerging energy efficient technique applied for various types of organic reactions as well as biomass processing.(35,36) We hypothesize that alkaline NaOH-induced deacetylation system can use energy from microwave irradiation more effectively than from conventional heating. It was reported that aqueous ions are excellent candidates that absorb highly the microwave irradiation, thus can use the most of the energy from microwave irradiation.(37)

In addition to NaOH, aqueous solution of tetrabutylammonium hydroxide, [TBA][OH], was also observed being able to cleave off acetyl groups of chitin.(38) However, potential for [TBA][OH] to be applied for chitosan production under microwave mediation has not been investigated. We therefore wished to study also the ability of [TBA][OH] in chitosan production, and later employ molecular dynamics (MD) simulation to understand the driving forces of [TBA][OH] in the chitin deacetylation process.

With these motivations, we aim in this study to (a) test whether combining IL pretreatment and microwave mediation improves the chitin deacetylation; and (b) investigate the potential to use [TBA][OH] in chitosan production as well as discuss [TBA][OH]-related mechanism using the MD simulation.

Experimental

Chemicals

Practical grade shrimp chitin flakes were received and air-dried at room temperature overnight before further processing. Water was filtered and deionized before use (Milli-Q water). NaOH micropearls were purchased from Sigma-Aldrich Co. LLC (Sweden) and were ground before each single use. Amberlite IRN-78 (hydroxyde form) was purchased from Supelco, Sigma-Aldrich Co. LLC (Sweden). Deuterium oxide (99.9% D), deuterium chloride (99.5% D), dimethyl sulfoxide-d_6 (99.9% D), chloroform-d (99.9% D), [Emim][OAc] ionic liquid, 40 wt.% aqueous [TBA][OH], LiCl, dimethylacetamide, glacial acetic acid and NaCl were purchased from Sigma-Aldrich Co. LLC (Sweden). Acetone and ethanol were purchased from VWR Co. LLC (Sweden). All these purchased chemicals were used without further purification.

Ionic liquid pretreatment of chitin

For the pretreatment, 2.5 wt.% chitin (~500 mg) was added in a round-bottom flask containing 20 mL [Emim][OAc] IL and heated at 120 °C in 1 hour under magnetic stirring. When complete, the mixture was cooled down to ambient temperature and water was added to precipitate the dissolved chitin out of IL. The obtained mixture was stirred at 60 °C in 20 minutes and chitin was separated by filtration. The washing process was repeated twice followed by a final washing step with acetone. The collected chitin was dried at 40 °C under vacuum for 2 hours to obtain a yield of 98 wt.% and saved in a desiccator for further deacetylation experiments (Figure 3a). The recovery of chitin was calculated using equation (1).

$$\text{Recovery yield of chitin \%} = \frac{m_{\text{regenerated chitin}}(g)}{m_{\text{initial chitin}}(g)} \times 100\% \qquad (1)$$

The pretreatment of chitin in IL was also explored at different temperatures and processing time. Specifically, the mixture with 2.5 wt.% chitin (~50 mg) in a glass vial containing 2 mL [Emim][OAc] IL was treated at different temperatures (80 °C, 100 °C, 120 °C, and 140 °C) for 1 to 16 hours. The recovery of the chitin was carried out by the same process as above.

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To recover the [Emim][OAc] IL, the liquid fractions after the pretreatment were collected to remove water by rotary evaporator and dried under vacuum at 40 °C to regenerate the ionic liquid. Regeneration yield of [Emim][OAc] was 97 wt.%. The recovery of IL was calculated using equation (2).

$$\text{Recovery yield IL \%} = \frac{m_{\text{regenerated [Emim][OAc]}} (g)}{m_{\text{initial [Emim][OAc]}} (g)} \times 100\% \quad (2)$$
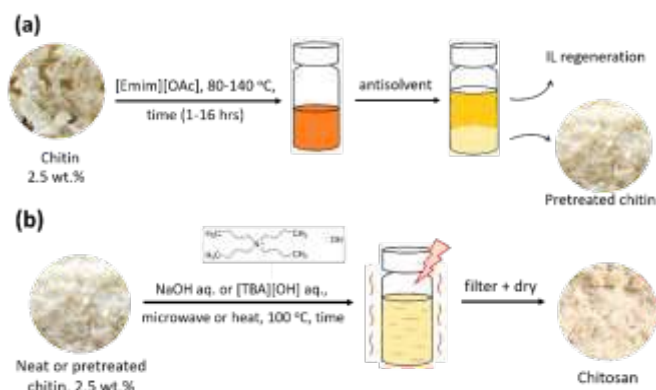

Figure 3. (a) Ionic liquid pretreatment of chitin in [Emim][OAc] at different temperatures and time; and (b) deacetylation of chitin for chitosan at various conditions.

Chitosan production by deacetylation of chitin

To investigate the chitosan production, 75 mg chitin (IL-pretreated or non-pretreated) was added in 3 mL of 20, 40, or 60 wt.% aqueous NaOH solution. The reaction proceeded under stirring at 100 °C either by conventional heating in 24 hours or by microwave mediation in 20 to 120 minutes (Microwave Initiator, Sweden, ~50 W). When complete, the reaction mixture was cooled down to ambient temperature and filtered to separate out the solid. The obtained solid (chitin/chitosan) was washed with water (60 mL x 3 times) until the neutral pH, dried at 40 °C under vacuum for 2 hours (Figure 3b), and saved in a desiccator for further characterization.

Chitin deacetylation with [TBA][OH] solution was also studied using the same microwave irradiation method. Typically, 75 mg of IL-pretreated chitin was mixed with 3 mL of 40 wt.% aqueous [TBA][OH] solution. The reaction proceeded for 20−120 minutes under microwave mediation at 100 °C (~50 W) and stirring. When the reaction completed, the product was obtained following the same procedure as described above.

To regenerate the [TBA][OH], the aqueous fractions after the reaction were collected to perform acetate exchange with hydroxide anions using an ion-exchange resin column. Specifically, 60 g of resin Amberlite IRN-78 (hydroxide form) was dispersed in Milli-Q water and loaded into a glass column (3 cm internal diameter), giving a bed volume of 120 mL after equilibration. The column resin was washed with one column volume (CV) of water to remove any associated impurity prior to use. Next the collected aqueous solution of [TBA][OH] after reaction (20 mL) was then applied onto the column with a flow rate of around 5 mL/min (≈3 drops/second). Water was then

added to elute the sample using a flow rate of 2 mL/min (slowly ≈1-2 drops/second). Fractions were collected, evaporated and dried overnight at 60 °C under vacuum to remove the water. Meanwhile, the resin column was rejuvenated with 1 M aqueous NaOH solution. The regeneration yield of [TBA][OH] was 83% according to equation (3).

$$\text{Regeneration yield IL \%} = \frac{m_{\text{regenerated [TBA][OH]}}(g)}{m_{\text{initial [TBA][OH]}}(g)} \times 100\% \quad (3)$$

Analysis and characterization

Nuclear magnetic resonance (NMR). NMR was used for structural analysis of ionic liquids and chitin/chitosan. For ionic liquids, the samples were diluted with DMSO-d_6 or CDCl$_3$ and the ^{1}H and ^{13}C NMR measurements were conducted at 400 MHz (Bruker Avance 400 MHz NMR instrument), 25 °C.

For chitin and chitosan samples, ^{1}H NMR was used to determine the degree of deacetylation according to equation (4).[39] In this case, the samples were dissolved in 20 wt.% DCl/D$_2$O at 70 °C in 30 minutes, and measured at 400 MHz, 80 °C.

$$\%DDA = \frac{\text{integrals }_{\text{H1D (H1 deacetylated units)}}}{\text{integrals }_{\text{H1D (H1 deacetylated units)}} + \text{integrals }_{\text{H1A (H1 acetylated units)}}} \times 100\% \quad (4)$$

Besides, ^{13}C cross-polarization magic angle spinning NMR (^{13}C CP-MAS-NMR) was employed for solid chitin/chitosan samples. NMR spectra were recorded at 500 MHz using a Bruker Avance III 500 MHz spectrometer with 4 mm zirconia rotors spun at a magic angle of 10 kHz and 25 °C. All the NMR spectra were assigned using Bruker's Topspin (4.0.9) software.

Fourier-transform infrared spectroscopy (FTIR). To acquire structural information of chitin and chitosan samples, FT-IR spectra were recorded in transmittance range from 4000 cm^{-1} to 400 cm^{-1}. The samples were ground with KBr before measurements. %DDA was estimated by comparing the absorption of the amide band at 1560 cm^{-1} and C-O-C band at 1070 cm^{-1} as the reference peak.[40] Details of FT-IR for %DDA calculation are demonstrated in SI 3.

X-ray powder diffraction (XRD). To investigate crystallinity of the samples, XRD measurements were recorded from 7° to 57° of 2θ angle (step size 0.0167°) using a PANalytical X'Pert X-ray diffractometer equipped with a Cu-Kα (λ = 1.54 Å) source and a split of 1/16.

Scanning Electron Microscope (SEM). Surface morphologies of the samples were observed on a Carl Zeiss Merlin field-emission SEM (FE-SEM, Carl Zeiss Merlin GmbH) operating at 40 kV.

Viscosity. Viscosity measurements were used to estimate molecular weight of the chitin/chitosan samples. A typical chitosan sample was prepared by dissolving 10 mg chitosan in 5 mL aqueous solution of 0.1 M acetic acid and 0.2 M NaCl.[41] Three dilutions with the solvent were done for each chitosan sample and the viscometric measurements were performed at 25 °C. Viscosity data was collected to determine reduced viscosity (η_{red}, mL.g^{-1}) by subtracting the solvent viscosity from

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sample values and then intrinsic viscosity ([η], mL.g⁻¹) by extrapolating the reduced viscosity versus concentration in a linear regression. Finally, the viscosity-averaged molecular weight (Mv) of the samples was estimated using Mark–Houwink equation below with K and α are constants which depend on the nature of the polymer, solvent, and temperature. For chitosan samples: a = 0.93, K = 1.81 × 10⁻³ mL.g⁻¹.

$$[\eta] = \mathbf{K} \times \mathbf{M_v^a} \quad (5)$$

Chitin samples were prepared similarly by dissolving 10 mg chitin in 20 mL of 5% (w/w) LiCl/dimethylacetamide.(42) The measurements were performed at 30 °C and Mark–Houwink constant values for chitin samples were taken as a = 0.95, K = 7.60 × 10⁻³ mL.g⁻¹.

Computational simulations

To observe the initial arrangements of [TBA][OH]-chitin system prior to the chitin deacetylation, short-time scaled MD simulations were performed. A chitin model (6-mer × 4-chain) was built for β-chitin based on experimental crystallographic data, showing regularly arranged chito-oligomers.(43,44) The model was introduced in a periodic cubic box of 45 Å side length containing a total of 45 ion pairs [TBA][OH] solvated with water molecules. [TBA]⁺ and [OH]⁻ ions were optimized using the B3LYP method and the 6-311G** basis set with Maestro 11.2 (Schrödinger, LLC).(45) Chitin and ionic liquid systems were generated using Packmol package (v20.3.3).(46) All MD calculations for the system were carried out in MOE platform (v2018.01, Chemical Computing Group ULC) using Nosé-Poincaré-Andersen (NPA) method for the thermostat and the barostat simulator and together with the Amber10:EHT force field.(47)(48) An initial equilibration step of 100 ps was performed and followed by data production with further 200 ps at 373 K. Molecular graphics were constructed with the MOE platform and VMD 1.9.3 software.(49)

Results and discussion

Ionic liquid pretreatment and its regeneration

The [Emim][OAc] IL has an ability to dissolve various types of rigid and crystalline biopolymers.(50,51) Besides, its water-tolerant nature is a benefit considering a certain level of moisture in naturally occurring polymers. Here, chitin was pretreated in [Emim][OAc] IL at different temperatures and time, and the crystallinity of chitin was recorded using XRD measurements (Figure 4). The XRD patterns of neat chitin showed three intense reflections at 2θ 9.7° (020), 19.8° (110), and 26.8° (013) and minor reflections at 12.9° (021) and 23.9° (130), corresponding mostly to α-chitin.(52) After IL-pretreatment at increasing temperatures from 80 to 100, 120 and 140 °C in 1 hour (Figure 4a), the characteristic peaks in chitin were steadily shortened and broadened, implicative of the decreased crystallinity and more amorphous nature of the sample. It is also noticeable that a complete dissolution of chitin was obtained at 120 °C and above (SI 1) while a partly dissolved mixture was occurring at 80 and 100 °C. This depicts a relationship between the processing temperature, chitin

dissolution and the crystallinity of the material. Further, it was recorded that the processing time had also significant influence on the IL-pretreatment of chitin (Figure 4b). Observing the progress of the IL-pretreatment at 100 °C for varying time from 1 hour to 16 hours, the intensity of the characteristic peaks in chitin decreased from 1 to 8 hours and even disappeared upon further pretreatment, indicating the decrystallization of chitin during the process. Considering the obtained results, we therefore chose the IL pretreatment condition as follow: 120 °C and 1 hour, since it provided the treated material with significantly amorphous nature in a short processing time. The
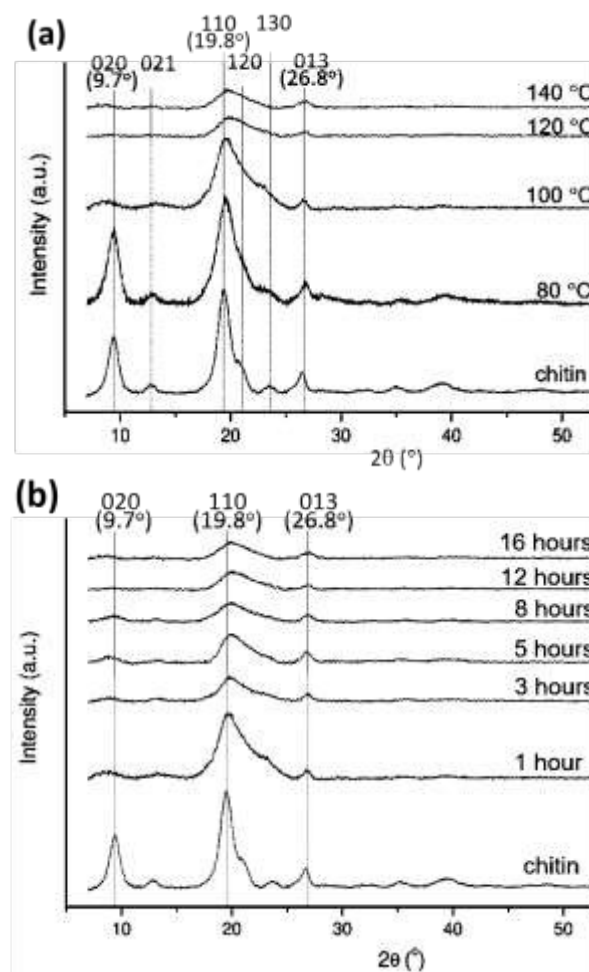

Figure 4. XRD shows a decrease of chitin crystallinity when treated with [Emim][OAc] IL over (a) temperatures (80-140 °C, 1 hour); and (b) time (1-16 hours, 100 °C).

approach of chitin pretreatment using imidazolium-based ILs was also reported previously. Xie and coworkers described the dissolution of pure chitin using 1-butyl-3-methylimidazolium chloride [Bmim][Cl] at 110 °C in 5 hours.(53) 1-allyl-3-methylimidazolium bromide [Amim][Br] was also used by Yamazaki *et al.* to obtain a similar solubility at 100 °C in 24 hours.(54) More recently, Jaworska *et al.* used [Emim][OAc] to achieve the chitin dissolution at 105 °C in 48 hours.(55)

Upon the IL-pretreatment of chitin at the chosen conditions 120 °C and 1 hour, SEM analysis was performed to compare the IL-pretreated chitin and the neat sample. As shown in Figure 5, we
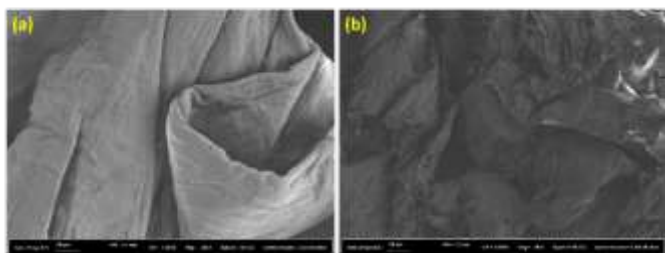
Figure 5. SEM imaging (at 200X magnification) illustrates chitin being more amorphous during IL pretreatment: (a) non-pretreated; and (b) IL-pretreated at 120 °C, 1 hour.

observed a major change in the surface morphology of the samples. While the neat chitin presented a well-organized surface, the IL-pretreated sample exhibited a more irregular and damaged structure. The damaging in the IL-pretreated chitin may represent the amorphous nature and the breakage of the intramolecular or intermolecular hydrogen bond networks in chitin.

Considering the alkaline characteristics of [Emim][OAc] IL, we also studied whether there happened any deacetylation of chitin during the IL-pretreatment process. By using FT-IR, structural changes of chitin after the pretreatment at 100 °C would be recorded for various reaction times. SI 2 shows the common peaks of chitin and chitosan at 3450 and 3300 cm^{-1}, attributed to the O-H and N-H stretching vibrations and the extensive inter- and intra-molecular hydrogen bonding network. The bands in the region of 3000–2800 cm^{-1} correspond to the -CH$_2$ symmetrical and asymmetrical stretching vibrations of polysaccharides. Chitin exhibits the doublet amide I band at 1655 and 1637 cm^{-1} representing the presence of H-bonding in the C=O group with the NH group of the adjacent chain and the O-H group of the inter-chain. The amide II band is located at 1560 cm^{-1}. The peak at 1320 cm^{-1} corresponds to the amide III band (in-plane mode of CONH group). The vibrations at around 1070 cm^{-1} are attributed to the C-O stretching of the polysaccharide. Upon deacetylation, the acetyl groups in chitin are cleaved, leaving chitosan structure with a decreased intensity of amide group signals at 1320 and 1560 cm^{-1}. By calculations we observed that [Emim][OAc] IL were not able to induce the deacetylation of chitin samples, where %DDA were almost unchanged during the process *i.e.* 11-12 %DDA (SI 3).

Roles of the [Emim][OAc] IL in dissolution and decrystallization of chitin were reported.(56,57) In contact with chitin microfibrils, anionic acetate [OAc]$^-$ species, as a small-sized component with ability to form H-bonds with −OH and −NHCO− groups in chitin, penetrate easily into the crystalline structure and disturb the highly ordered intra- and intermolecular hydrogen bonding network of the chitin. The [Emim]$^+$ species, following the acetate anions *via* ionic interactions, can also access inside the chitin lattice. With its large size and highly basic property, [Emim][OAc] limits the re-bonding of intermolecular H-bonds in chitin microfibrils, enabling the chitin to be decrystallized and become porous, less crystalline with

more open structural sites. This open and reactive state of chitin can be beneficial for reagents to enter the lattice, creating more interactions with the functional groups −NHCOCH$_3$ on chitin.

Another interesting point of this pretreatment process is the ability to regenerate the [Emim][OAc] IL. We were able to regenerate up to 97% yield of [Emim][OAc] for reuse. The IL structure remained intact during the process (confirmed by NMR analysis). ^{1}H and ^{13}C NMR results (Figure 6) demonstrated spectroscopic features between fresh and regenerated ionic liquids. Signals of [Emim]$^+$ and [OAc]$^-$ species remained the same while the only difference is water peak at 4.55 ppm (*singlet*) in the ^{1}H NMR spectrum of the regenerated sample.(58–60) Success of this regeneration step implies ability to reuse the chemical and diminish effects on the environment, making the ionic liquid a 'green' solvent.
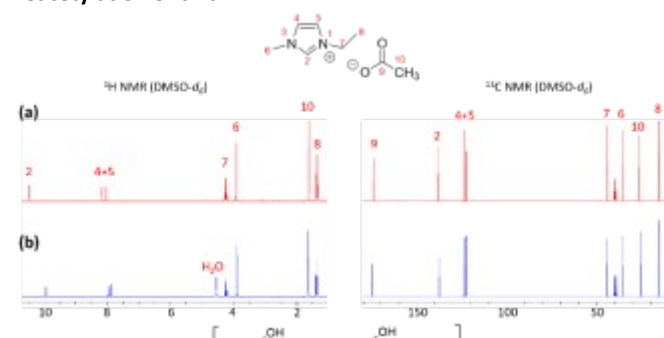
Deacetylation of chitin

Figure 6. Recovery of [Emim][OAc] IL was confirmed by spectroscopic data (1H and 13C NMR) with (a) fresh IL; and (b) recovered IL.
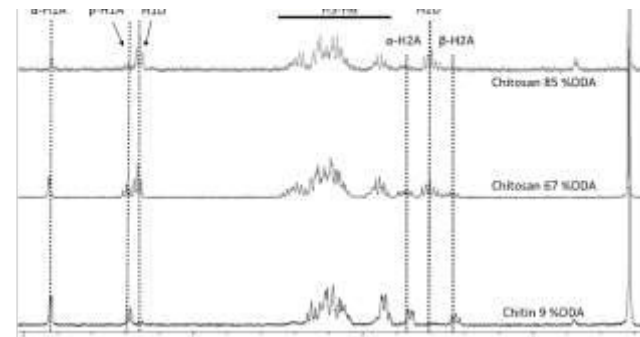

Figure 7. ^{1}H NMR analysis gave structural information and the degree of deacetylation in chitin/chitosan samples.

Chitin deacetylation proceeded while ^{1}H NMR and ^{13}C CP-MAS-NMR analysis were used to study the formation of chitosan (Figure 7 and SI 4) and estimate %DDA according to the equation (4). One benefit of using DCl as solvent in ^{1}H NMR measurements is that the resonance of the solvent (HDO) does not interfere with any carbohydrate protons. Figure 7 shows the characteristic resonances of the acetylated α and β-H1 anomers at 5.84 and 5.38 ppm, respectively. Besides, H2 of the acetylated α and β-anomeric units appear respectively at 3.74 and 3.46 ppm. Meanwhile, H1 of deacetylated units resonates at 5.36 ppm and H2 resonates at 3.61 ppm. Acetyl-protons appear at 2.42 ppm, while the remaining ring protons appear between 3.8 and 4.5 ppm. It can be seen that after

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deacetylation, signals of acetyl groups and acetylated units decreased while signals of deacetylated units aroused.

For the deacetylation, two types of chitin (IL-pretreated or non-pretreated) were processed at different conditions (microwave irradiation or conventional heating) with different concentrations of aqueous NaOH solution from 20 to 60 wt.% (Figure 8). It was observed with increasing NaOH concentration, %DDA increased accordingly. NaOH is the reagent that gave rise to the deacetylation by breaking acetyl groups out of chitin. Comparing IL-pretreated samples with non-pretreated ones, it can be seen that the IL-pretreatment accelerated the deacetylation reaction. This is in agreement with the discussion about IL-pretreatment mentioned above, where the decrease in crystallinity of chitin allowed for more accessible sites towards the reagent. Regarding the heating methods, the microwave-assisted reactions were speeded up compared to the traditional heating. The combination of IL pretreatment and microwave mediation allowed to reduce time to one hour with less amount of NaOH needed (40 wt.% NaOH) while giving a higher %DDA (approximately 70 %DDA), compared to around 60 %DDA for non-treated chitin with 60 wt.% NaOH in 24 hours of conventional heating. Besides, microwave mediated-
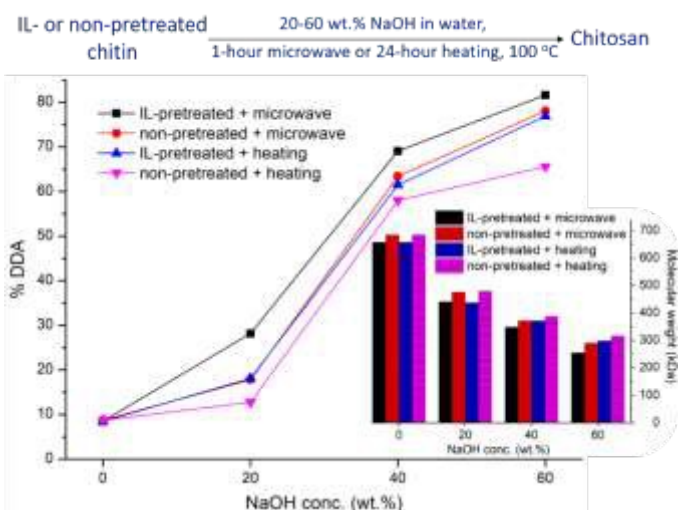

Figure 8. %DDA and molecular weight (kDa) of chitin/chitosan against NaOH concentration (20-60 wt.%) were plotted for different reaction conditions, where deacetylation of IL- or non-pretreated chitin (75 mg) proceeded at 100 °C with either 1 hour of microwave irradiation or 24 hours of conventional heating.

deacetylation of IL-pretreated chitin was studied over time to observe changes in %DDA (SI 5). With two hours of microwave-mediated reaction in 40 wt.% aqueous NaOH solution could give up to 85 %DDA. Microwave irradiation method for chitin deacetylation was also previously reported where 82−85 %DDA was obtained using 40% w/v NaOH solution at 150 °C in a microwave chamber for 3 to 6 hours, conducted by Lertwattanaseri and coworkers.(61) However in our study, IL-pretreatment allowed for the chitin deacetylation to be achieved at even milder conditions (100 °C, ~50 W, 2 hours).

Molecular weights (MW) of chitin/chitosan were also of interest and estimated using viscosity measurements and the Mark–

Houwink equation. Since viscometric analysis requires the polymers to be soluble in a medium, we used acetic acid/NaCl/water for dissolving chitosan and LiCl/dimethylacetamide for chitin. By measuring viscometric values we could determine intrinsic viscosity of the samples before calculating their molecular weights. It can be seen that molecular weight of the obtained materials decreased corresponding to an increase in concentration of aqueous NaOH solution or reaction time, irrespective of microwave or conventional heating (Figure 8 and SI 5). Additionally, we noted that chitosan produced by the microwave irradiation method gave a more extensive decrease in molecular weight than the one from conventional heating. It was described in the previous reports that the removal of acetyl groups (primary reaction) and the cleavage of glycoside bonds (side reaction, depolymerization) due to energy impact (microwave irradiation or heating), and chemicals such as NaOH and [Emim][OAc] induced the decrease in molecular weight of the obtained products.(62–64) The deacetylation process gave a medium to high molecular weight (∼250–500 kDa and above), depending on the reaction condition involved. Hence this method can be useful for the synthesis of medium to high molecular weight chitosan materials with mild reaction conditions.(65)

Besides IL-pretreatment, use of microwave heating was a benefit to the chitin deacetylation. Microwave irradiation, which has its energy at the molecular level, interacts strongly with ionic molecules (conduction mechanism) by collisions and polar molecules (dipolar polarization mechanism) by rotations.(66,67) Hence these molecules will absorb very well the microwave irradiation to convert into heat energy. When using an aqueous solution of NaOH, polar water molecules and ionic features of Na^+ and OH^- were being taken advantage, allowing for the reagent to use most of the energy from microwave irradiation. Hence OH^- anions could rapidly attack the acetamido groups in chitin and then effectively cleave off the acetyl groups upon the deacetylation reaction (Figure 9). Meanwhile, conventional heating requires a regular energy transfer that distributes evenly to the whole system, implicative of an energy waste towards unnecessary sites during the

Figure 9. Mechanism of deacetylation reaction using NaOH or [TBA][OH] as reagents.

reaction, for example, the reactor jacket.

[TBA][OH] in chitin deacetylation and possible molecular roles

With the benefits of IL pretreatment and microwave irradiation over conventional heating, we continued studying the
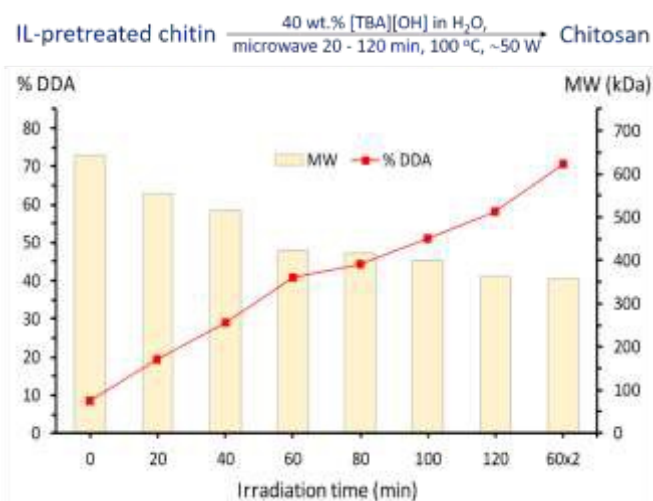

Figure 10. Aqueous 40 wt.% [TBA][OH] solution was used for microwave-mediated deacetylation of IL-pretreated chitin (75 mg) at 100 °C over time, plotted with %DDA and molecular weight (MW, kDa) against irradiation time (minutes). Experiment 60x2 implies two times of 60-minute reaction on the same chitin with fresh [TBA][OH] solutions.

microwave-assisted deacetylation of the IL-pretreated chitin over time using aqueous [TBA][OH] 40 wt.% solution as the reagent (Figure 10). The reaction was investigated up to two hours at 100 °C and %DDA was estimated from ^{1}H NMR analysis. It can be seen that an increased deacetylation was obtained over time to 59 %DDA in 120 minutes and reached up to 71 %DDA when performed twice on the same chitin (60 minutes × 2 times of reaction with fresh 40 wt.% aqueous [TBA][OH] solution). Although the %DDA is still slightly worse than in case of aqueous NaOH (85 %DDA), it is higher than conventional heating (60 %DDA, 24 hours) and similar to commercial chitosan (69 %DDA). A decreasing trend in molecular weight was also observed when using aqueous [TBA][OH] solution in the deacetylation process over time (Figure 10).

It was also noticeable that [TBA][OH] could be regenerated with 83% yield using ion exchange resin, making it promising compared to highly corrosive NaOH solutions at elevated temperature. Spectroscopic results of the recovered [TBA][OH] are shown in Figure 11, confirming success of regeneration. The ^{1}H and ^{13}C NMR spectra demonstrates that [TBA][OH] was recovered with high purity. For the liquid after deacetylation reaction, we could also observe the presence of acetate anion signals at 1.54 ppm (−CH$_3$, *singlet*) on ^{1}H NMR and 25.6 ppm (−CH$_3$) and 173.5 ppm (−CO−) on ^{13}C NMR. These peaks disappeared when [TBA][OH] was regenerated.

To acquire insights into the roles of [TBA]$^+$ and [OH]$^-$ ions, MD simulations were performed to visualize the initial arrangements and motions of the system prior to the chitin deacetylation. As shown in Figure 12, we constructed a model of chitin (6-mer × 4-chain) featuring regularly arranged chito-

oligomers as well as [TBA]$^+$ and [OH]$^-$ ions (Figure 12a). To represent the amorphous state of IL-pretreated chitin, polymer chains were introduced randomly into the MD simulation box. Although the importance of [OH]$^-$ ions in cleaving off acetyl groups in chitin is already known, from experimental and theoretical perspectives,[68–70] the roles of [TBA]$^+$ cations in the system are still pending. Through simulations, we observed a notable preference of [TBA]$^+$ species to selectively bind to the chitin surface. As depicted in Figures 12b and 12c, [TBA]$^+$ cations, being the larger constituents of the ionic liquid, exhibited a tendency to aggregate around the chitin fiber, attracting [OH]$^-$ anions of opposite charge. This electrostatic attraction facilitated the penetration of [OH]$^-$ anions into previously inaccessible regions of the chitin structure for further deacetylation reaction.
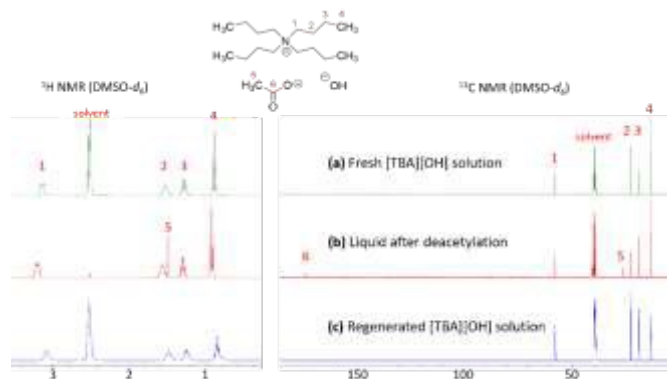

Figure 11. Regeneration of [TBA][OH] was confirmed by 1H and 13C NMR analysis of (a) fresh solution, (b) liquid after the reaction, and (c) recovered solution.

Intermolecular bonding involving [TBA]$^+$, [OH]$^-$, and chitin can be attributed to: (1) electrostatic attraction among quaternary nitrogen of the cation and oxygen of hydroxyl or acetamido groups in chitin; (2) hydrophobic interactions among butyl groups and carbohydrate backbone; and (3) hydrogen bonds between hydroxyl or acetamido groups on chitin and [OH]$^-$ anions.[71,72] These interactions are likely responsible for maintaining proximity between [OH]$^-$ anionic reagents and the chitin surface. This spatial closeness provides numerous opportunities for effective collisions between the [OH]$^-$ anions and acetamido groups during the deacetylation process, as illustrated in Figure 12c. The theoretical insights gained from these observations offer a rationale for understanding how the [TBA][OH] solution can effectively proceed the deacetylation of chitin without requiring additional NaOH reagent.

Conclusions

In this study, we succeeded in improving the deacetylation of crystalline chitin for chitosan production using milder conditions. The developed method was composed of two steps: ionic liquid pretreatment and microwave-mediated deacetylation. The [Emim][OAc] IL pretreatment effectively
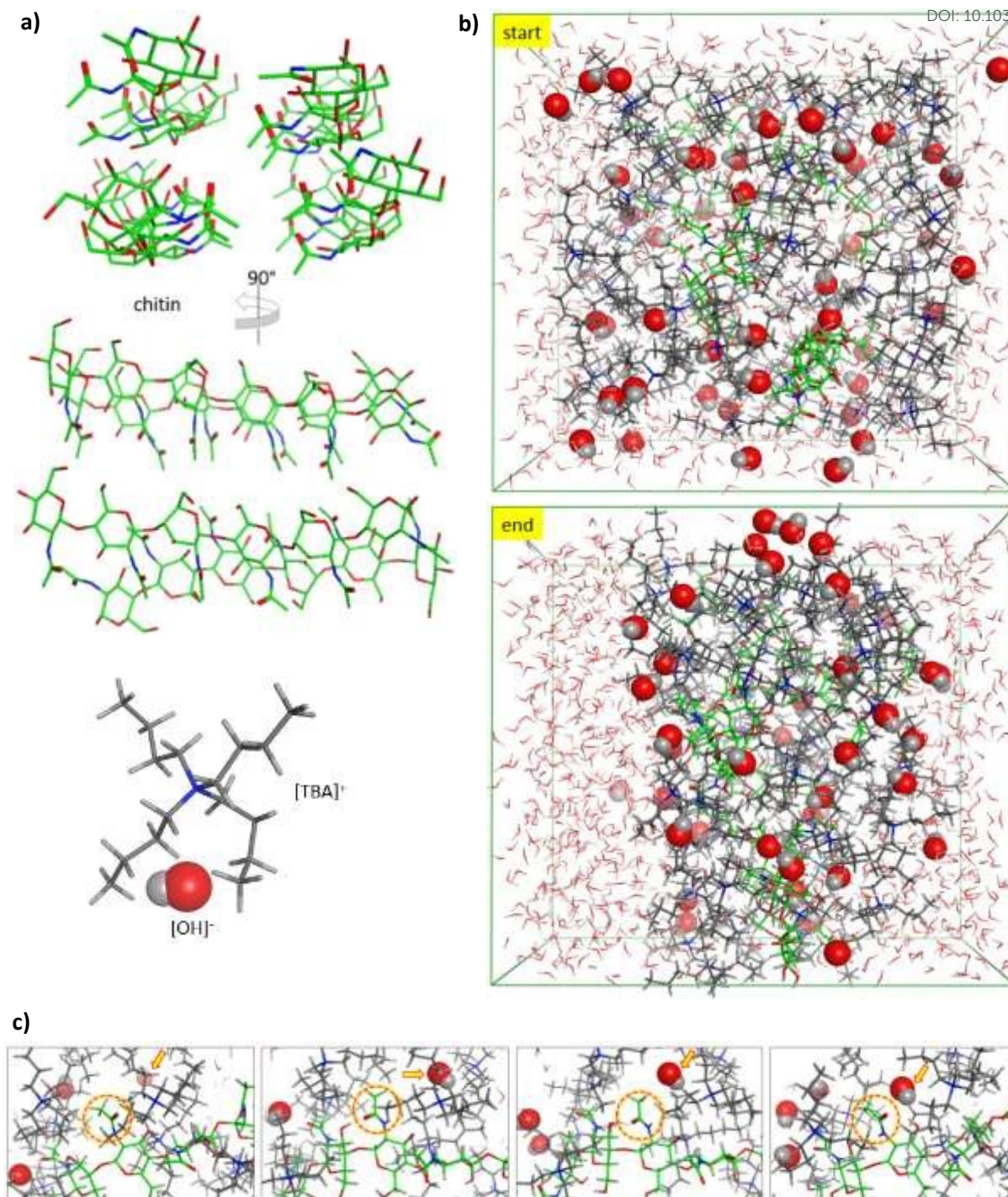
Figure 12. a) Structures of chitin (6-mer x 4 chains) and [TBA][OH]. Color scheme: red (oxygen), green or gray (carbon), blue (nitrogen) and white (hydrogen); b) Illustrations of relative positions between [TBA]+, [OH]- and chitin at the start and end simulations; c) Dynamic attractions (from left to right) of [TBA]+ and [OH]- towards chitin for further collisions between [OH]- and acetamido groups.

decreased the crystallinity in chitin by disturbing the intermolecular hydrogen bonding among its polymeric chains, confirmed by XRD analysis. At 120 °C in 1 hour of pretreatment, amorphous/semi-crystalline chitin was obtained for using in further decetylation reactions. Upon the chitin deacetylation, combination of IL pretreatment and microwave mediation proved to increase significantly the %DDA of chitosan products

up to 85% in two hours of reaction time using 40 wt.% NaOH solution. In comparison, non-pretreated chitin with conventional heating method gave merely 60 %DDA chitosan in 24 hours of reaction time and 60 wt.% NaOH solution. For the deacetylation of IL-pretreated chitin with 40 wt.% [TBA][OH] solution under microwave irradiation, chitosan products were obtained with 59-71 %DDA. Even though [TBA][OH] solution is

slightly less effective compared to NaOH solution, the %DDA of the obtained chitosan was better compared with the conventional heating approach. Noticeably, both the [Emim][OAc] IL and the reagent [TBA][OH] were regenerated with 97 wt.% and 83 wt.%, respectively. MD simulations revealed the molecular driving forces and motions of [TBA][OH] in contact with chitin before the deacetylation process. By intermolecular interactions in the system, OH$^-$ anions remained close to the chitin polymeric chains, enabling the deacetylation of chitin through the cleavage of acetyl groups. With the 'greener', milder, and more sustainable approach in this report, we hope it can further more explorations for scalable production of chitosan considering the recoverability of the solvent/reagent systems and industrially feasible reaction parameters.

Author Contributions

We strongly encourage authors to include author contributions and recommend using CRediT for standardised contribution descriptions. Please refer to our general author guidelines for more information about authorship.

Van Minh Dinh: Investigation, Methodology, Software, Data curation, Writing – original draft.

Santosh Khokarale: Investigation, Writing – review & editing.

Pedro Ojeda May: Software, Methodology, Writing – review & editing.

Tobias Sparrman: Methodology, Software, Data curation.

Knut Irgum: Conceptualization, Methodology.

Jyri-Pekka Mikkola: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Conflicts of interest

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

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Notes and references

‡ Footnotes relating to the main text should appear here. These might include comments relevant not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.
§
§§
etc.

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