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Solubility of Chitin: Solvents, Solution Behaviors and Their Related Mechanisms

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Abstract

Chitin is a natural polysaccharides having a unique molecular arrangement of 2-(acetylamino)-2-deoxy-D-glucose, it possesses multifunctional properties and is suitable for various applications mainly in pharmaceutical, biomedical food, textiles and packaging fields. Therefore, being considered as a superior material for a sustainable future of industrial development, chitin perfectly meets up the demands with diversified functionalities in applications, excellent biocompatibility and biodegradability. Non-toxicity to human and environment (air, water and soil) is a great opportunity for this revolutionary, innovative and sustainable material. Moreover, antibacterial potency and low immunogenicity of chitin have broadened the aspects of research and development on structure-function relationship toward biological tissues and activities. Despite abundance, low cost and availability, many experimental data from potential studies, reproducibility problems of chitin solubility measurement still limit the development of products and access to the market in large volume. Batch-to-batch variability, non-precise characterization and randomly distributed acetyl groups of chitin structure eventually results in a bad reproducibility of chitin solubility. Therefore, the chapter aims to organize the information of chitin structure at molecular level and correlate solubility with chitin structure. Moreover, the dissolution mechanism and solution behaviors in different solvents will be discussed in this chapter.

Keywords: polysaccharide, chitin, chitosan, solubility, dissolution, hydrolysis

1. Introduction

Chitin is a polysaccharide consisting of glycosidic bonds in linear or branched fashion between two adjacent monosaccharides, 2-(acetylamino)-2-deoxy-D-glucose. In general, monosaccharides

undergo a polycondensation reaction to link more than 20 units of oligosaccharides by glycosidic linkages. Most polysaccharides show the degree of average polymerization (number average DP) around 200–3000 while longer polysaccharide (like cellulose) exhibits DP around 7000–15,000. The presence of acetyl, amino and hydroxyl groups in the polysaccharide chain, due to the generation of hydrogen bonds (inter and intramolecular) makes the chitin highly aggregated. Therefore, it is insoluble in all regular solvents such as water, organic solvents, mild acidic or basic solution, etc. Chitin insolubility affects the scaling up of the processes for the production of chitin-based products. The first study on solubility determination of chitin was done by Austin who tested chitin in different solvents [1]. It was a well-organized evaluation of chitin solubility in different types of solvents such as dichloroacetic (DCA) and trichoroacetic (TCA) acids in presence or absence of alcohol, etc. Later on, many studies were conducted with the same intention by many other scientists and chitin solubility was verified in many solvents such as dimethylacetamide (DMA)/LiCl mixture [2], $CaBr_2 \cdot H_2O$ saturated methanol [3], hexafluoroisopropyl alcohol and hexafluoraceton [4], lithium thiocyanate [5], phosphoric acid [6] and N-methyl-2-pyrrolidone [7], etc. Although dissolution of chitin is possible by these solvents many of them are toxic, scarcely degradable, corrosive, or mutagenic. Therefore, the choice of an appropriate solvent for chitin and chitosan solubilization is important and primary issue for lab scale research and scaling up for industrial practices. The acetyl groups in chitin can be removed by deacetylation to convert insoluble chitin into a more soluble compound, namely chitosan (this name is given to chitin with at least 50% degree of deacetylation, DD). Therefore, this chapter will deal with the deacetylation process and the changes in molecular orientations and bonds after the deacetylation reaction. Moreover, the chapter will revise technical details regarding different aspects of solution behavior of chitin and chitosan. The parameters influencing solubility and their action mechanisms, theoretical discussions and recent relevant research findings on chitin and chitosan will be found in this chapter. Finally, modification of polysaccharides and their enhanced solubility will be discussed.

2. Molecular structure of chitin and chitosan

Polysaccharides mimic protein and amino acids structure consisting of special conformation of secondary, tertiary and quaternary architectural structures. Chitin is arranged as crystalline microfibrils clustered with six-stranded helixes of a protein structure. Polymerization of the monosaccharides, β-(1-4)-2-acetamido-2-deoxy-β-ᴅ-glucosamine units exhibit three different polymorphism (α, β and γ sheets) whereas the N-acetyl glycosyl moiety is a common crystallographic unit in all [2]. Electron diffraction studies reveal a highly crystalline nature of chitin in the α and β conformation. The α-conformation is one of the most abundant allomorphs in which the unit polymer chains are arranged in an antiparallel fashion whereas the adjacent chains are always in the opposite direction [3]. In β, less frequent in nature sheet all chains are in the same direction and parallel, γ conformation is a variant of the α arrangement in which two parallel, adjacent and unidirectional chains are arranged with one opposite directional chain (**Figure 1**). Both α and β conformations maintain a strong network dominated by intra-chain hydrogen bonds between the groups of C=O⋯NH and C=O⋯OH within a distance of 0.47 nm. In α-chitin conformation, additional inter-chain hydrogen bonds bind the hydroxymethyl groups while this type of interaction is not observed in the β conformation. Thus,
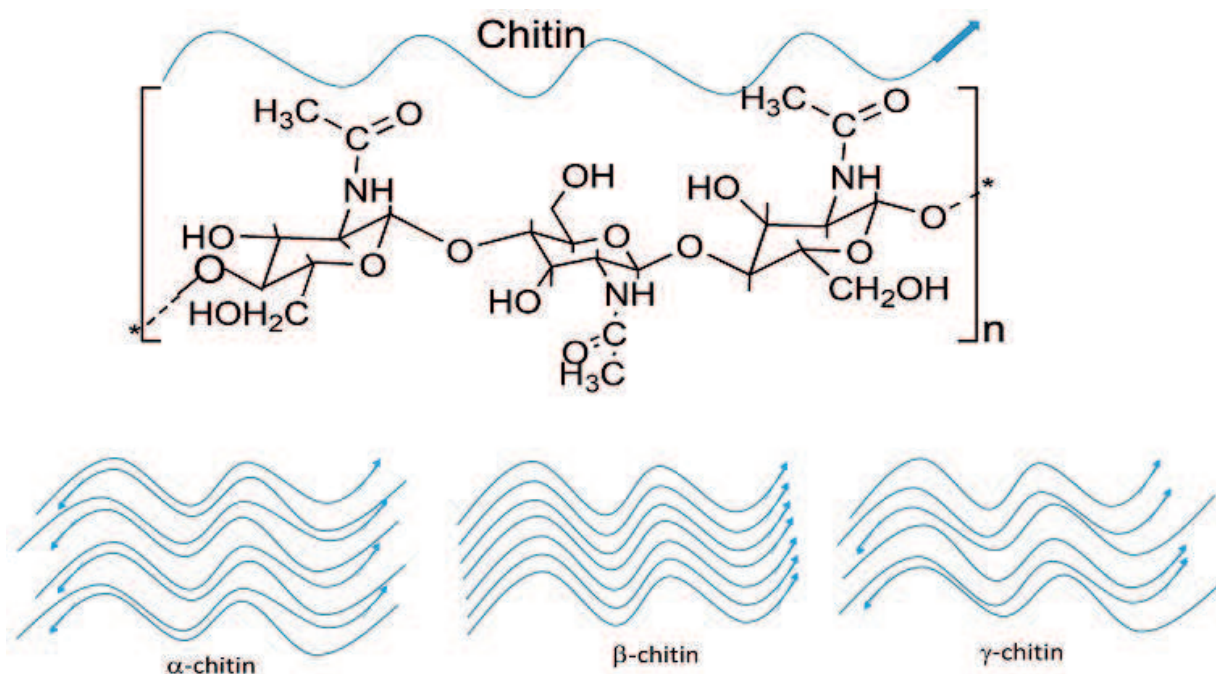
Figure 1. Structure of different chitin conformations (α, β and γ-chitin domain conformation).

β-chitin conformation is more prone to intra-crystalline swelling than α-chitin conformation. Different structural arrangement of α-chitin and β-chitin provide the reason why water, alcohol and amines are able to get access to the β conformation by swelling with and without disrupting the chain arrangement and crystalline structure [4]. For example, the swelling of β sheets in concentrated acidic solution of HNO_3 or HCl solution exhibits a permanent transformation into α-chitin conformation. In this case, a partial dissolution occurs driven by hydrolysis induced by the acid treatment. The recrystallization process of the smaller hydrolyzed chain starts on the un-hydrolyzed chitin sheets, which are called "epitaxy". Therefore, no single crystal growth is observed during the recrystallization and new α-chitin conformation crystals are observed. The transformation $\beta \rightarrow \alpha$ indicates that the α-chitin sheet is thermodynamically stable and stability is achieved only via recrystallization [3]. The α-chitin sheets are not swelled by water and alcohol while aliphatic diamine or highly basic solutions can diffuse into the crystalline structure to promote the formation of chain complexes. The parameter value of inter-chain expansion is same as β-sheet around 0.7 nm. Therefore, a simple processing of β-chitin with 20% NaOH results into the conversion of α to β-conformations.

Chitin polysaccharides contain functional amino groups in its backbone to provide positively charged polysaccharide upon solubilization. The amount of reactive amino groups can be increased by increasing deacetylation which is quantified by the degree of deacetylation (%DD). Chitin is the only positively charged polysaccharide among all other naturally occurred biopolymers which allows a wide range of biological applications. There are two main groups in the chitin structure influences the functionality of chitin: (i) amino groups and (ii) hydroxyl groups (**Figure 1**). The amino sites might react with aldehyde and ketone groups for the Schiff Bases formation and influence solubility. In addition, two hydroxyl groups in chitin structure provide excellent pathways for modification and functionalization in view of

an increase of solubility. Those hydroxyl groups involve in the O-acetylation, O-alkylation, H-bonds formation, etc. [5]. Also, the amino groups are responsible for the short-range primary and secondary electrostatic interaction while the second one involves the formation of hydrogen bonds. Moreover, the available unhydrolysed acetyl groups in chitin molecules form hydrophobic bonds in the solution and get aggregated [6].

3. Factors affecting the solubility of chitin

3.1. Effect of N-acetyl-D-glucosamine units

Chitins may have different acetylation depending on the sources such as fungi, insects, crustaceans or molluscs. Due to its crystalline structure with strong hydrogen bonds and cohesive forces, highly aggregated three-dimensional network is developed which leads to insolubility in conventional solvents. Pure chitin contains around 90% N-acetyl groups in its backbone and some deacetylation reactions take place due to the extraction process of chitin from the natural sources. There are two monomer units present in the chitin structure in different fraction: (i) 2-acetamino-2-deoxy-D-glucopyronase (N-acetyl-D-glucosamine) and (ii) 2-amino-2-deoxy-D-glucopyronase (N-amino-D-glucosamine). The first one, 2-acetamino-2-deoxy-D-glucopyronase, displays insolubility due to the strong hydrogen bonds between the acetyl groups of the same or adjacent chitin chains. Hydrogen bonds network builds a three-dimensional crystalline matrix by sequencing the following bonds —NH···O═C and —OH···O═C. The other unit, N-amino-D-glucosamine shows a distinct property such as hydrophilic nature and positively charged in acidic solution. The domination of the hydrophilic character with a high amount of N-amino-D-glucosamine unit in the chitin backbone can be determined by degrees of deacetylation (DD). The DD is determined from the ratio of N-amino-D-glucosamine to N-acetyl-D-glucosamine while the degree of acetylation (DA) represents the deduction from 100 (i.e. 100—DD). When DD is between 60 and 90% a new chemical entity "Chitosan" is baptized which is soluble in organic acids such as acetic acid. Alternatively, the structure with a DD value less than 60% is regarded as chitin and insoluble in acidic solutions. Chitin is treated with alkali solution (NaOH) for deacetylation to occur. The type of deacetylation process provides a different distribution of acetyl groups in the chitin backbone: micro-block and random. The micro-block domain chitosan is easily susceptible to aggregation along the extended series of acetyl units which leads to a complete insolubility in the majority of the common solvents. In the case of high DA > 60% or DD < 40%, chitosan is very prone to association and aggregation while chitin with low DA = 1.5% or DD = 98.5 did not show any aggregation [7]. Simina and coworkers reported the aggregation behavior for a wide range of DD at pH 4.5 and determined the hydrodynamic radius at DD – 98.5, 63 and 31%. For very high DD (>75%), protonated charge condensation occurs in the chitosan solution due to large charge density which leads to electrostatic repulsion and high solubility. As a result, single size distribution of chitin molecules was observed with an average diameter of 40 nm at DD 98.5% [7]. When the DD was moderated (75–50%), an additional size distribution of 300 nm (an average diameter) with the previous size distribution (40 nm) was displayed which indicated the aggregated form of chitin. The circumstance asserted that the hydrophilic chitin exhibited a transition of hydrophilic

character to the hydrophobic nature due to the rise of hydrogen bonds contributed by the acetyl groups or decrease of DD value. Further decrease of DD (<50%) influenced the hydrophobic nature of the polymer chains and three different size distributions were achieved including two previous size distribution. The aggregation and agglomeration of chitin chains increased the size distribution to micrometer sized (average diameter). It indicated the development of complete hydrophobic bonds influenced by higher amount of acetyl groups than the higher DD (>50%). Chitin structure at low DD (<50%) contains the largest amount of N-acetyl-D-glucosamine units and exhibits distinct domains of crystallinity. Therefore, the fraction of N-acetyl-D-glucosamine units has high influence on the solubility and solution property.

3.2. Effect of solution pH

The aggregation behavior of chitosan is strongly influenced by the pH of the solvent medium. In general, chitosan molecules are more or less ionized up to pH 6.0, and the ionization increases as the pH moves to low values. Therefore, the amino groups of chitin chains (low DA) at a particular pH (<6.0) capture H^+ solution ions and exhibited positive surface charge which can be determined by zeta potential value. The charged amino groups resist the aggregation of chitosan in the solution, but when the DA value increases from zero to higher value the aggregation starts to dominate over the coulombic repulsion forces of the charged groups. The pH at which the net charge of a chitosan solution prevents aggregation is called critical pH. Therefore, the aggregation behavior of chitosan can be divided into two distinct types—closed and open type aggregation based on pH values. The closed type aggregation is observed at very low pH when the radius of gyration becomes constant and insensitive to chitosan concentration. This indicates that chitosan is completely protonated and the solubilized chitosan molecules maintain stable aggregation. This phenomenon can be related to the classical Rayleigh theory in all charged species fragmented into smaller charged species beyond a certain critical point of net charge. On the other hand, open type aggregation takes place when aggregation and association forces overcome the repulsive effect at pH higher than the critical pH. The radius of gyration increases with the incorporation of more chitosan molecules in the solution system. In addition, the high pH value (>6.0) also raises the number of deprotonated species in the solution and aggregation moves to agglomeration due to the generation of hydrogen bonds involving the neutralized NH_2 groups of chitosan chains [8]. Moreover, the hydrolytic cleavage occurs when chitin is treated by strong acid such as highly concentrated acetic acid or HCl by involving the glycosidic bond (**Figure 2**: hydrolytic degradation). The detail mechanism has been discussed in Section 4.1.

3.3. Effect of molecular weight

Apart from the degree of deacetylation and pH, molecular weight influences the conformational changes and solubility of chitosan. Chitosan solubility increases with the decrease of molecular weight [9]. The solubilization process of chitosan, as it happens for functionalized polymers, involves different types of chemical and physical interactions such as hydrogen bonds, hydrophobic interactions, van der Waals forces, etc. The effect of DA on the
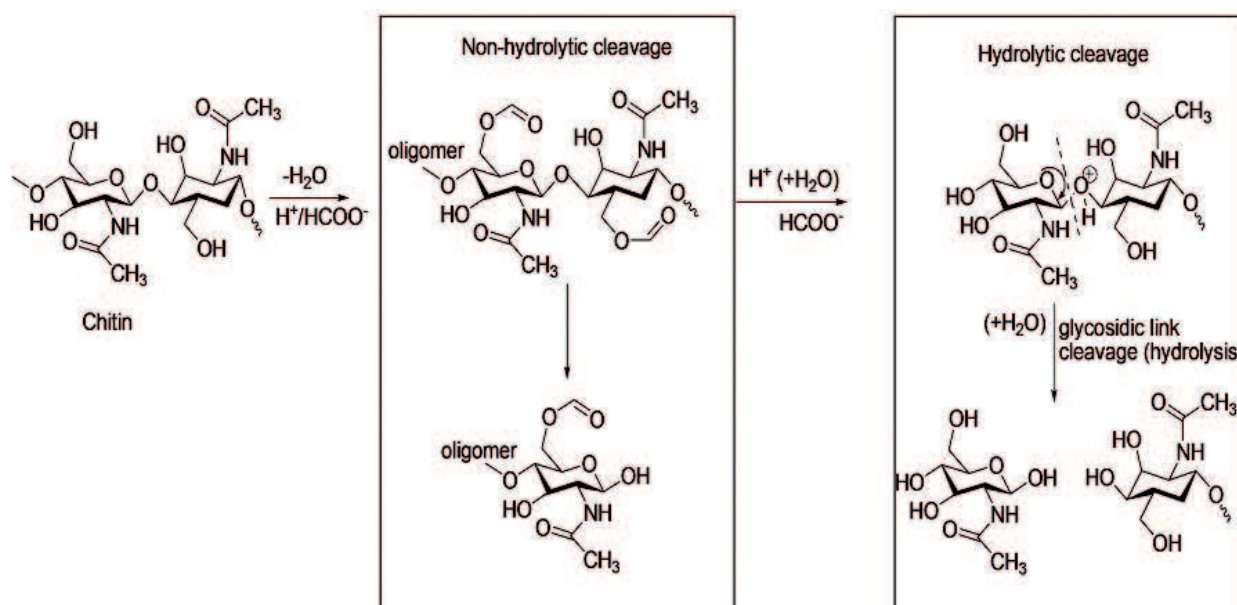
Figure 2. Mechanism of hylytic and non-hydrolytic degradation by organic acid (FA).

solubility of chitosan has already been discussed in previous section in which the hydrogen bonds involving acetyl groups played a dominant role. When DA value is lower than 50%, the protonated amino groups dominate the electrostatic repulsions between chitin chain and the hydrogen bonds collapse. As a result, chitin with low DA (<50%) become soluble at acidic pH. Chitin with low DA is fully ionized at pH 3.0 while deprotonation reaches to the highest value at pH 6.0 and precipitate occurs. Therefore, a transition between dissolved and undissolved chitin is mainly controlled by the medium pH and the degree of deacetylation or number of amino groups present in the chitin structure. The formation of hydrogen bonds and the impact of hydrophobic interaction on the chain aggregation are observed even though chitin molecules are fully deacetylated [6]. Therefore, deprotonation, $\beta \rightarrow \alpha$ phase transition and precipitation represents the scheme for the formulation of α-chitin by aggregation. Chitosan at high molecular weight (MW 300 kDa) exhibit the α-chitin crystalline structure upon aggregation. Aggregation determines a conformational entropy loss due to the arrangement of the molecular chains in a regular crystalline array [10]. The release of water compensates the loss of entropy during chain aggregation resulting in an overall decrease of Gibbs free energy, which is a thermodynamic criterion for a process to be spontaneous. However, the circumstance changes for chitosan oligomers of low MW (2.43 kDa). In this case, the aggregation is not favored due to the formation of shorter chains which reduce the hydrogen bonds formation between macromolecules and lack of amino groups for the formation of intermolecular hydrogen bonds. As a result, the pH for the transition between dissolved and undissolved chitosan in aqueous medium shifted to pH 8.0 from pH 6.0. Therefore, the soluble and insoluble transition of chitosan occurs when the MW (weight average) range exists in the range of 3.82–4.67 kDa. The transition moves to complete solubility when the MW decreases to the monomer scale at below 3.28 kDa because no intermolecular hydrogen bond leads to chitosan aggregation and solubilization is observed at neutral pH [9].

3.4. Salt effect/ionic strength and temperature

Ionic strength is a measure of the total concentration of ions present in a solution. In general, charged particles exhibit a net electrostatic effect up to a distance in the solution which is indicated as Debye screening length (k^{-1}). The electric field affects the electrokinetic phenomena and migration of the charged colloidal particles in the solution. As a result, a double layer or boundary layer is developed in chitosan solution due to the total polysaccharide charged particles and the counterions around the particles. Debye screening length (k^{-1}) can be calculated as a function of ionic strength (I) using Eq. (1),

$$k^{-1} = \sqrt{\frac{1000\varepsilon\, k_b\, T}{8\pi\, e^2\, N_{av} I}} \tag{1}$$

where the parameter ε indicates water permittivity, k_b is to Boltzmann constant, T is the temperature (K), e is the electronic charge (coulombic) and N_{av} is the Avogadro number (6.022×10^{23}). It is observed that k^{-1} and I are inversely related to each other; however, the relationship is not linear but expressed by the square root. Chitosan acts as polyelectrolyte, when it is dissolved in a solvent. The polycationic solution develops electrostatic repulsive interactions while masking other available interactions. The addition of a salt or the increase of ionic strength from 9×10^{-3} to 0.46 M of the chitosan solution results in an inversion from repulsive to attractive interaction and the k^{-1} value decreases from 3.0 to 0.45 nm. The attraction inspired by the screening of amino-charged chitosan chains with anions such as CH_3COO^- and Cl^- increases the tendency of flocculation or precipitation of chitosan. Therefore, the increase of ionic strength (>0.46 M) enhances the aggregation by chitosan-chitosan attraction over the chitosan-solvent interaction and influences chitosan solubility [11]. Chitosan in acidic medium shows an expanded conformation structure since the amino groups exert repulsive force with each other, but the addition of salt or increase of ionic strength shrink the structure by increasing chain flexibility. As a result, the occupied volume of chitosan chains in solution is reduced by increasing the ionic strength and a decrease of intrinsic viscosity of chitosan solution is observed. The intrinsic viscosities of chitosan solution decreased from 2.9 to 0.71 L/g for the increase of ionic strength from 9×10^{-3} to 0.46 M. Temperature dominates the formation and dissolution of hydrogen bonds between acetyl and hydroxyl groups up to a threshold limit of ionic strength. The temperature at which the dissolution occurs is called dissolution temperature and can be defined as a function of ionic strength above the threshold value of ionic strength (i.e. critical ionic strength, I_c). Above I_c, the dissolution temperature is proportional to the ionic strength, therefore the dissolution temperature increases as the ionic strength of the chitosan solution increases [12].

4. Solvability of different solvents

4.1. Organic acids as solvent

Chitosan or modified chitin is readily soluble in dilute acidic medium below its pKa (pH = 6.5) while chitin is insoluble in organic and regular solvents. The amino groups in chitosan backbone

enhance ionization at low pH by forming chit-NH_3^+ and increase the solubility of the polysaccharide while at higher pH value (>pH 6.0), it precipitates. Therefore, the solubility of chitin can be increased by converting to chitosan (by deacetylation reaction) which depends on the pKa value and also on the DD. The ability of acidic media to protonate chitosan mainly controls the ionization and solubility of the polyelectrolytes. Chitosan exhibits solubility in acid media (1%) such as acetic acid, formic acid [13], L-glutamic acid, lactic acid, succinic acid [14], etc. Tsao and coworkers proposed a mechanism for depolymerization of chitosan in acetic acid. The process involves two main pathways by chain scission, that is, (i) the depolymerization of glycosidic linkage by hydrolysis and (ii) the deacetylation of the *N*-acetyl groups. In the initial stage of dissolution, the protonation of glycosidic oxygen atom occurs at C─O bonds and form a conjugated acid. The process of protonation involves the cleavage of the exocyclic part of glucosamine structure from O-5 to C1. In this step, the cleavage of the glycosidic linkage may follow two distinct paths based on two conformations, that is, (i) chair (high energy) and (ii) half-chair (low energy) which are related to the required energy of proton transfer. Since the oxonium ion resulting from the protonation of both distorted conformation is unstable, a small energy pathway is observed to form oxocarbenium ion followed by the half-chair conformation [15]. The water molecules in the form of H_3O^+ due to a nucleophilic attack in protonation, lead to the formation of reducing sugar and these hydrolysis products are readily soluble in water. At the end of the depolymerization reaction, the resulted products are monomers, acetic acids and some other molecules (**Figure 2**: hydrolytic cleavage).

4.1.1. Formic acid (FA)

Chitin liquefaction is one of the simple processes in which chitin is transformed into small soluble molecules. Formic acid (FA) can be used as liquefaction agent for chitin. Moreover, due to high vapor pressure FA can be evaporated without leaving any residue. Three different types of products are formed after the liquefaction of chitin, that is, (i) N-acetyl glucosamine having formate functional groups (NAGF), (ii) dehydrated N-acetyl glucosamine (DH) and (iii) 5-(formyloxymethyl)furfural (FMF). The total yield in this process was achieved around 16.1% in which the highest fraction of yield was found at 10.5% for N-acetyl glucosamine having formate functional groups. In addition, the dehydrated products are achieved around 3.6% while the FMF fractions are significantly lower around 2.0%. The yield of these end products depends on the time and temperature of the liquefaction process. For example, the total yield is increased to 60% (i.e. NAGF 32.7%, DH 11.3% and FMF 16.0%) when the temperature is raised to 100°C and keeping other parameters constant. In contrast, the total yields of 28% (i.e. NAGF 12.7%, DH 11.1% and FMF 4.7%) and 57.8% (i.e. NAGF 13.2%, DH 10.0% and FMF 34.6%) are achieved after only increasing the reaction times (from 12 h) to 24 and 168 h, respectively. [16]. The breakage of glycosidic linkages does not occur in the presence of strong acidic solution, due to insufficient amount of water in the reaction. Therefore, the first step of the reaction pathway follows the generation of monomers and oligomers in the form of soluble chitin by the modification of hydroxyl groups and followed by non-hydrolytic cleavage. As the water concentration increases and reaction proceeds, the depolymerization kinetics increases with the higher supply of water leading to hydrolysis and liquefaction reaction **(Figure 2)**. Anhydrous formic acid can also be as a solvent for chitin [13].

4.2. Inorganic solvents

Many inorganic acids, bases and salts are used for the dissolution of chitin and chitosan. The extensive decomposition and deacetylation of chitin can be obtained by alkali treatment, which increases the solubility in water of the regenerated chitin. The alkali chitin solution is prepared by using 10 times more alkali than chitin. The precipitation of chitin occurs by pouring the solution into acetone followed by neutralization with HCl [17]. The obtained precipitates are insoluble in water, but after 104 h of reaction the alkali treatment allows to reach the aqueous solubility. The enhanced solubility was due to the cleavage of chitin chain and led to the destruction of the crystalline structure of chitin. A prolonged treatment with NaOH also increases the degree of deacetylation up to 90% while 50% and more deacetylated chitin is dissolved in water. Einbu and coworkers analyzed the random degradation of chitin in 2.77 M NaOH and observed random coil conformation of chitin chains regardless of molecular weight [18]. Depolymerization, deacetylation and stability of chitin solution can be enhanced when urea is added to the alkali medium. Hu et al. dissolved chitosan in a mixture continuing 8 wt% NaOH and 4 wt% urea at the temperature of −20°C and stirred for 36 h. Chitin solubilization was not possible below 4 wt% NaOH and solution instability arises upon increasing the alkali solution above 12% NaOH. The addition of 2–8% urea in the 6–10% NaOH increases the solubility and stability of depolymerized chitin fragments in the solution. The explanations behind the achieved solubility and stability is the destruction of inter- and intramolecular hydrogen bonds and the role of urea is to limit aggregation leading to the stability of the solution [19]. The entire process of chitin solubilization is also dependent on the freezing temperature of chitin in a particular mixture of solvent. For example, the chitin solution in 8 wt% NaOH and 4 wt% urea exhibits the freezing point at −19°C. The presence of more than 4 wt% NaOH enables water molecules to get access into the chitin chain matrix; water is expanded and separated from the NaOH molecules at temperature below freezing point. The volume expansion of the chitin matrix upon freezing-induced stretch and collapses of the hydrogen bonds, which brought to depolymerization and solubilization of chitin chains (**Figure 3**). In contrast, the extent of chitin deacetylation in the alkali solution was greater than the same process in presence of urea. The DA value reduced from 94 to 84% after 480 h storage in the mixture of 8 wt% NaOH and 4 wt% urea indicating that urea stabilized the chitin solution and stopped the deacetylation process. Another similar study was carried out by Fang and coworkers who described the insight mechanism of dissolution property of chitin in NaOH-urea mixture [20]. The combined system (NaOH/urea) was quite suitable to prepare a chitin solution at −30°C. The hydrated NaOH captured the chitin chains by hydrogen bonds and formed complexes while urea clusters surrounded outside the complexes as a shell-like structure. The chitin chains were separated by the hydrated NaOH and urea disrupting the inter- and intramolecular hydrogen bonding network and displayed a complete dissolution. The solution was sensitive to temperature and concentration and formed an extended worm-like structure confirmed by TEM, AFM and DLS analysis [21]. Gong and coworkers have already reported a study recently with KOH and KOH-urea as a solvent for chitin dissolution. The chitin solubility was around 80% in the aqueous KOH solution (8.4–25 wt%) and the dissolution power of bases was in the order KOH > NaOH > LiOH at −30°C. Importantly, the degree of acetylation decreased only 12.5% after the treatment with KOH and storage at 4°C
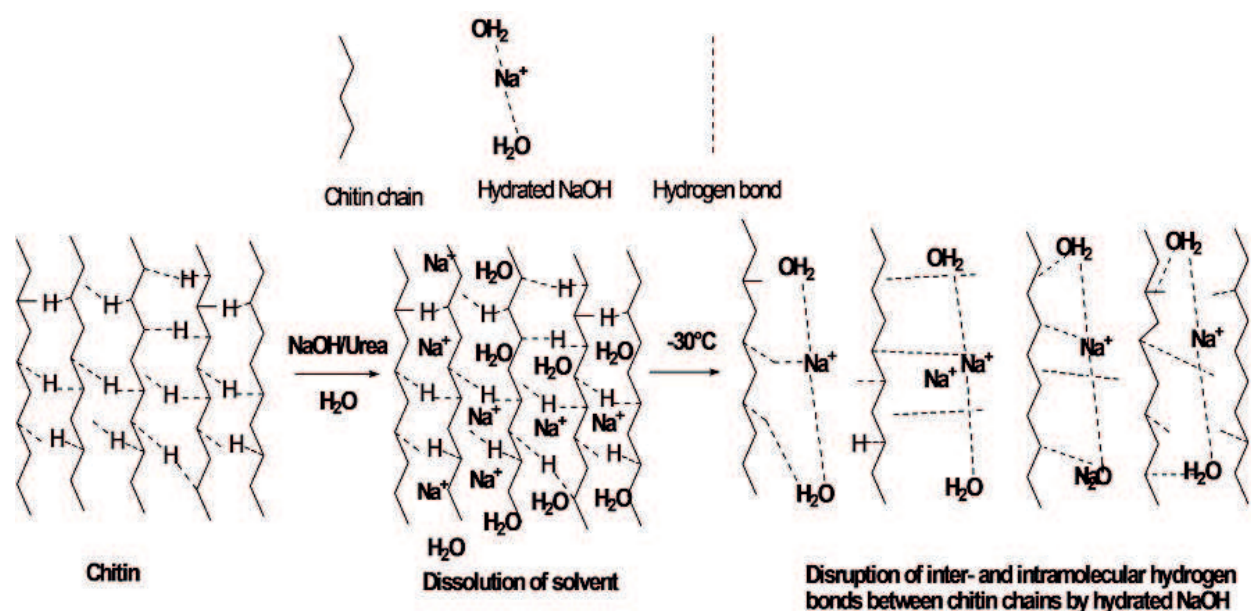
Figure 3. Hydrolysis by NaOH.

for 15 days. Urea did not exhibit any significant effect for enhancing the solvent capability of KOH [21]. Moreover, chitin solubility was also observed in a mixture of 5% LiCl and N,N-dimethylacetamide (DMA). The solution obtained from the mixture solubilized only 2 wt% chitin at 120°C and produced a gel, but 3 wt% chitin was dissolved when lithium thiocyanate was used as a solvent at 100°C [22]. Also, the recovery of the product in a strong acidic environment is quite difficult and expensive. For example, the hydrolysis of chitin/chitosan or depolymerization by 12.07 M inorganic acid (HCl) at 40°C for 28 h does not produce any N-deacetylated moiety [23]. Therefore, use of nitrous acid is a cheaper alternative for chitosan depolymerization by the cleavage of glycosidic bonds but the stoichiometry of the reaction depends on the amount of acidic solution. Water soluble chitosan oligosaccharides and highly deacetylated chitosan oligosaccharides were synthesized using nitrous acid [24]. The depolymerization process was carried out by adding sodium nitrite (NaNO$_2$) to the chitosan solution (2% acetic acid) and keeping the solution for 3 h at pH 7.0, then the excess water in the reaction was evaporated at 50°C. The fractionation and extraction were carried out by methanol and filtrated for separation. The depolymerization started with the deamination of the 2-amino-2-deoxy-β-D-glucopyranose (GlcNH$_2$) units and produced a new reducing sugar of 2,5-anhydro-D-mannose (M-units). The M-units did not exhibit any interconversion between anomers of the reducing sugar. The aldehyde groups of the M-unit were quite reductive to amino groups by avoiding any intramolecular hemiacetal formation **(Figure 4)**. Therefore, two M-units form imino linkage from the reaction between NH$_2$ and –CHO by Schiff base reaction at pH > 5.0 and undergoes several steps of water elimination in acidic medium. As a result, 5-hydroxymethyl-2-furfural (HMF), depolymerized and fully deacetylated chitosan were achieved. The depolymerization process provided water-soluble chitosan of different molecular weights, which can be collected from various methanol fractions. Some solvent systems for chitin have been reviewed by Pillai and coworkers [25]. They reviewed some solvent systems from previous works, highlighting that LiCl-tertiary amide solvent system can

dissolve 5% (w/v) chitin. Moreover, LiCl also forms a coordination complex with chitin which is dissolved in dimethylacetamide (DMA) and N-methyl-2-pyrrolidone (NMP). Calcium chloride-methanol system as a solvent for chitin was also reported in the review. Chitin can form a complex with calcium and dissolve in methanol but the limitation arises due to very high viscosity, which makes the scale up difficult.

4.3. Ionic liquids (ILs) as solvent

Strong organic and inorganic acids, strong alkali solution or other inorganic solvents such as LiCl-tertiary solvent, $CaCl_2$-MetOH system possess some disadvantages like corrosiveness, volatility, toxicity and so on. Moreover, inappropriate segmentation of chains, unstable yields occur during the hydrolysis or depolymerization in those solvents. As a suitable alternative, ionic liquids (ILs) are considered as green solvents due to their non-volatility, excellent solvation power, wide temperature ranges in the liquid phase, strong polarity and stability of end products. 1-butyl-3-methylimidazolium chloride (BminCl) is an ionic liquid (IL) which gives a swelled state of 5 wt% chitin after treatment at 130°C for 5 h [3]. The swelling of chitin in the IL, [BminCl] occurs due to the strong coordination of the Cl^- ions and partially break the hydrogen bonds of chitin chains. The complete solubility of chitin is only possible when a stronger coordinating anion than Cl^- ion will destroy the entire hydrogen bonds network ($-NH\cdots O=C$ and $-OH\cdots O=C$) produced by *N*-acetyl groups. Therefore, 1-butyl-3-methylimidazolium acetate (BminAc) was used as solvent and found better solubility than the (BminCl). The acetate ions in (BminAc) exhibit itself as a strong
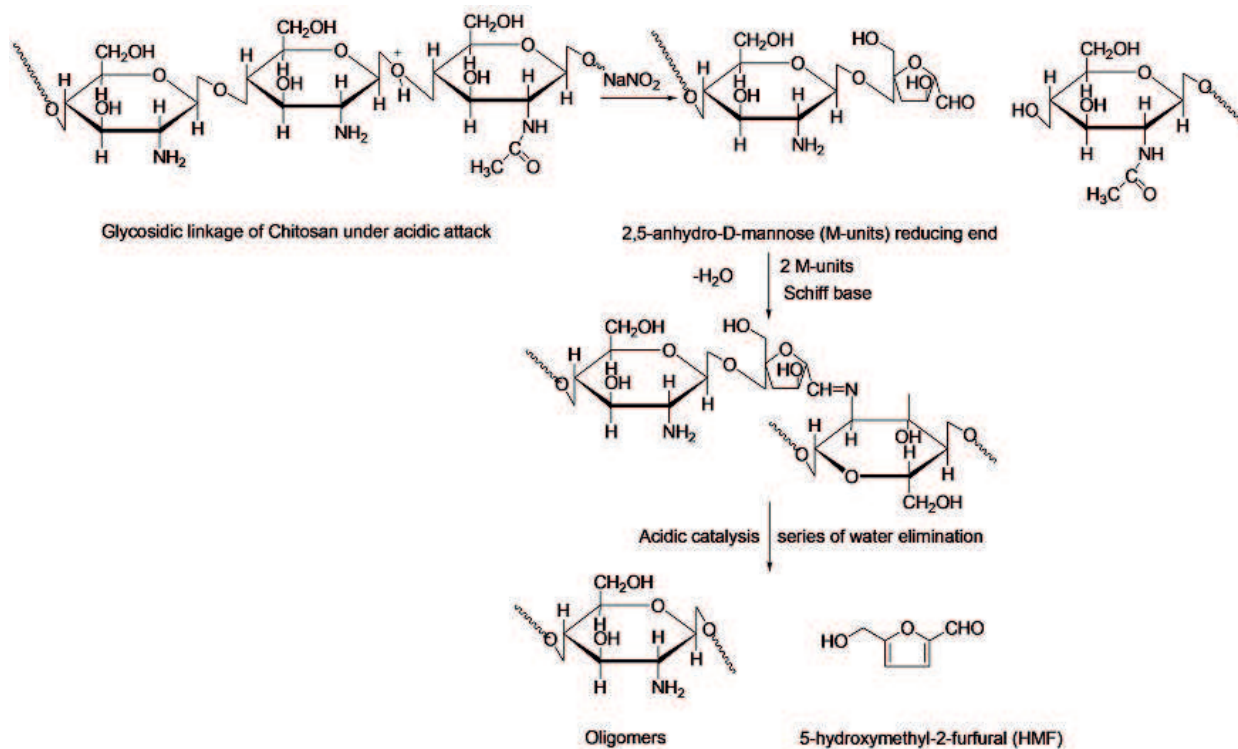

Figure 4. Hydrolysis by $NaNO_2$.

conjugate base of a weak acid, which can interact with the H-bonds of chitin. It destroys the hydrogen bonds and solubilizes the crystal chitin. Xie and coworkers investigated the IL, 1-butyl-3-methyl-imidazolium chloride ([Bmim]Cl) as a solvent but they achieved partial dissolution of chitin and chitosan at 110°C probably due to the moderated polarity of the IL [26]. Prasad and coworkers found that 1-allyl-3-methylimidazolium bromide exhibited good solvent property for the dissolution of 5 wt% chitin when it was heated at 100°C for 48 h [27]. The obtained chitin solution was quite clear and homogeneous as confirmed by scanning electron microscopy analysis. The chitin powder recovered and regenerated by methanol treatment possessed the same crystalline structure as that of the crab shell. No degradation of chain or decrease of molecular weight had been occurred. Upon increasing the chitin amount to 7 wt%, a gel was obtained. Wang and coworkers studied the effect of three ILs: alkyl imidazolium chloride ([AMIM]Cl), alkyl imidazolium dimethyl phosphate ([MMIM] [Me$_2$PO$_4$]) and 1-allyl-3-methyl-imidazolium acetate ([AMIM]Ac) on the solubility of a series of different DA (degree of acetylation) of chitin [28]. The dissolution of 5 wt% chitin occurs at 110°C in the [AMIM]Ac while [AMIM]Cl and [MMIM][Me$_2$PO$_4$] can dissolve 0.5 wt% and 1.5 wt% chitin at 45 and 60°C, respectively. The observation showed that acetate anions are more efficient to break down the network of hydrogen bonds than the chloride and dimethyl phosphate anion. Similar findings were observed when 1-butyl-3-methylimidazolium acetate ([BMIN]Ac) and 1-butyl-3-methylimidazolium chloride ([BMIN]Cl) were used as solvent at room temperature [3]. The study showed that limited chitin solubility (1 wt%) was achieved in [BMIN]Cl while the solubility increased to 5 wt% in [BMIN]Ac at room temperature. Qin and coworkers reported on the dissolution of chitin using IL, 1-ethyl-3-methylimidazolium acetate for which the required temperature was 100°C while the dissolution of cellulose with the same IL was obtained at 40°C [26]. The difference was due to the structural arrangement of chitin with acetamide group on the C2 position while cellulose shows a hydroxyl group in the same position. The major obstacle to dissolution was the strong hydrogen bonds between C = O and NH groups of the adjacent chitin chains distributed in the chitin cluster. Thanks to their strong polarity, ILs overcome the energy barrier and makes it possible to dissolve in the molten state (40°C). Shimo and coworkers used tris(2-hydroxyethyl)methylammonium (THEMA) type ILs in the absence and presence of ethylenediamine (EDA) to dissolve chitin at mild non-aqueous conditions [29]. Four different THEMA-type ILs were used to dissolve chitin: Tris(2-hydroxyethyl)methylammonium acetate ([THEMA][OAc]), Tris(2-hydroxyethyl) methylammonium methyl sulfate ([THEMA][MeOSO3]), Tris(2-hydroxyethyl) methylammoniumtrifluoromethansulfonate ([THEMA][CF3SO3]). Partial and total solubility was observed when THEMA-type ILs used as solvents in the absence and presence of EDA, respectively. For example, ([THEMA][OAc]) exhibited excellent solubility at room temperature in the presence of EDA. The reason behind the complete dissolution was revealed with the help of X-ray diffraction analysis. The analysis exhibited that the EDA penetrated into the crystalline α-chitin and formed a complex. Therefore, when EDA was added to the system of IL and chitin, the EDA easily broke the hydrogen bonds present in the α-chitin and created strong hydrogen bonds of with the IL. This mechanism leads to dissolving the dissolution of chitin at room temperature by loosen their inter-chain hydrogen bonds between chitin chains. Some references about the use of ILs in the modification of chitin and chitosan to enhance the solubility are given in **Table 1**.

Chitin/chitosan	Solvent	References
Acetylated chitin	Dimethyl sulfoxide (DMSO)	[30]
Chitin-graft-polystyrene	Dimethyl sulfoxide (DMSO)	[31]
Monomethyl-modified chitosan	Water	[32]
O-alkylated chitosan	Chloroform, ethanol, water and acetic acid	[33]
Chitosan-graft-polycaprolactone	Dimethylformamide (DMF), DMSO, ethanol and toluene	[34]

Table 1. Modified chitin and chitosan with different ionic liquids and solubility in solvents.

4.4. Enzymatic hydrolysis

Enzymatic hydrolysis is a green process to achieve chitosan excellent solubility in water by producing chitooligosaccharides (COS). The process does not require extreme conditions (very low pH or high concentration of acids) and the tuning of molecular weight, and DD of final product can be achieved by avoiding any unwanted yield. Chitinase, chitosanase are specific enzymes and many other nonspecific enzymes such as glycanases, proteases, lipase are isolated from many biological sources. Unlike acid hydrolysis, enzymatic hydrolysis affects both the depolymerization and deacetylation of chitin or chitosan through the catalytic activities, which mainly depends on the molecular structure of chitinase (enzyme). Chitinase contains four catalytic domains in its structure, that is, (i) signal sequence, (ii) catalytic domain, (iii) serine/threonine region which can accept O-glucosylation and (iv) C-terminal chitin-binding domain [41]. The depolymerization occurs in the similar style of classical acid-base catalytic reaction (**Figure 3**: hydrolytic cleavage) followed by retention (two steps) or inversion (one step) reaction. In the retention mechanism, firstly, the acidic residues release protons, cleavage of glycosidic bonds and subsequently positively charged oxocarbonium ions intermediates are produced. Then, secondly, the intermediates are stabilized with the help of intermediate covalent bonds (glycosal-enzyme) but the subsequent reaction with the water molecules leads to the retention of anomeric configuration again. However, the inversion mechanism involves negative-charged residue, carbonium intermediate and water molecules at a time for the degradation of chain and inversion of the anomeric configuration. Moreover, the deacetylation of chitin molecules takes place by deacetylase treatment. The chitin deacetylase enzyme isolates from *Mucor rouxii* and forms enzyme-polymer complexes with chitin. The hydrolysis of acetyl groups occurs by the enzymatic attack to three acetyl groups (maximum) before the reaction proceeds to next complex formation. The enzymatic deacetylation yields randomly distributed GlcNAc and GlcN residues in the form of block copolymers. Pronase was reported to increase the solubility and decrease the degree of acetylation (DA) [42]. The chitosan solution in 1% acetic acid was treated with pronase at 100:1 ratio at 37°C for 1–5 h with subsequent and re-precipitation by using NaOH (2 M). The treated chitosan exhibited the molecular weight of 8.5 kDa while the native chitosan was 71 kDa. Moreover, the DA value decreased from 25 to 14% after the isolation of new chitosan. Consequently, the chitosan after pronase digestion can be solubilized around 66–74 wt% in 0.01% acetic acid which was 13 wt% for the raw chitosan. The other enzymes used for chitosan enzymatic treatments and the operation conditions have been summarized in **Table 2**.

Enzyme	Water soluble modified chitosan	References
Lysozyme	Chitooligosaccharides (COS)	[35, 36]
Papain		
Cellulase		
O-glycoside hydrolase (EC 3.2.1)	Low MW chitosan (3–6 kDa)	[37]
Chitosanase and β-D-glucosaminidase	D-glucosamine	[38]
Chitin deacetylases	Chitin and chitosan oligomers	[39]
Carbohydrases from Myceliophthora sp	Low MW chitosan (4–28 kDa with 85% DD)	[40]

Table 2. List of enzymes for the hydrolysis of polysaccharide (chitin, chitosan).

5. Modified chitosan

Modification of molecular structure can enhance the solubility of chitosan in water. Phosphorylated chitosan, quaternized chitosan derivatives and carboxymethyl chitosan can be solubilized

Modified chitosan	Solubility in	References
Phosphorylated chitosan		
N-methylene phosphonic chitosan	Water, HCl, acetic acid	[43]
Chitosan diethyl phosphate	Diluted organic or mineral acid	[44]
α-Galactosyl-chitosan conjugates	Water	[45]
Chitosan-dendrimer hybrid	Water	[46]
Quaternized chitosan derivatives		
N-phenmethyl-N,N-dimethyl chitosan (PDCS) N-(1-pyridylmethyl-2-ylmethyl)-N,N-dimethyl chitosan N-(1-pyridylmethyl-3-ylmethyl)-N,N-dimethyl chitosan N-(1-pyridylmethyl-4-ylmethyl)-N,N-dimethyl chitosan	0.1–1.6 mg/ml in water	[47]
N-[(2-hydroxy-3trimethylammonium)propyl]chitosan chloride (HTACC)	Water	[48]
Carboxymethyl chitosan		
O-carboxymethyl chitosan sodium	N-methylmorpholine-N-oxide (NMMO)	[49]
N,N-dicarboxymethyl chitosan	Water	[50]
N,O-carboxymethyl chitosan	Water	
Photosensitive chitosan with benzene group	Benzene and toluene	[5, 51]
Dibutyryl chitin	DMF, DMSO, dimethylacetamide (DMAc) and ethanol	[52]

Table 3. Modified chitosan and solubility in different solvents.
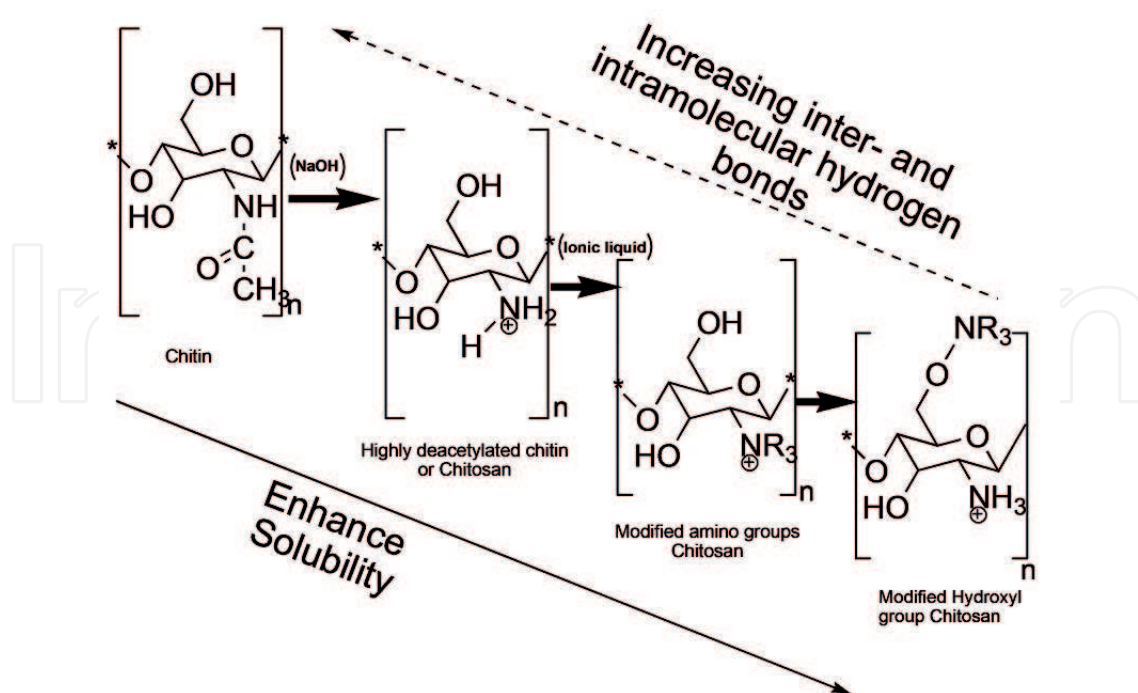
Figure 5. Trend of solubility and intermolecular hydrogen bond between acetyl groups.

in different solvents at ambient conditions (**Table 3**). The solubility trend of chitin based on the modification of the molecular structure has been clearly displayed in **Figure 5**.

6. Conclusion and future perspectives

Chitin and chitosan have shown a big potential in pharmaceuticals, biomedical, agricultural sectors as well as food and textiles industry. Despite the myriad of opportunities, chitin and chitosan poor solubility in the most common solvents is a greatly limitation for scaling up the process from lab to industrial level. High viscosity of chitin and chitosan solution is another drawback with great impact of processing operations and equipment requirements. Even though chitin is sparingly soluble in strong acidic solution, corrosive and hazardous solvents should not be practiced to meet up regulatory compliances concerning chemical safety management. In conclusion, much works is still required to exploit the opportunities of this futuristic material which can contribute to economically feasible industrial growth.

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References

[1] Austin PR. Solvents for and Purification of Chitin. United States Patent1973. p. 2-4

[2] Agboh OC, Qin Y. Chitin and chitosan fibers. Polymers for Advanced Technologies. 1997;**8**:355-365

[3] Wu Y, Sasaki T, Irie S, Sakurai K. A novel biomass-ionic liquid platform for the utilization of native chitin. Polymer. 2008;**49**(9):2321-2327

[4] Rinaudo M. Chitin and chitosan: Properties and applications. Progress in Polymer Science (Oxford). 2006;**31**(7):603-632

[5] Guiping M, Yang L, Kennedy JF, Nie J. Synthesize and properties of photosensitive organic solvent soluble acylated chitosan derivatives (2). Carbohydrate Polymers. 2011;**84**:681-685

[6] Philippova OE, Korchagina EV, Volkov EV, Smirnov VA, Khokhlov AR, Rinaudo M. Aggregation of some water-soluble derivatives of chitin in aqueous solutions: Role of the degree of acetylation and effect of hydrogen bond breaker. Carbohydrate Polymers. 2012;**87**(1):687-694

[7] Popa-Nita S, Alcouffe P, Rochas C, David L, Domard A. Continuum of structural organization from chitosan solutions to derived physical forms. Biomacromolecules. 2010;**11**(1):6-12

[8] Philippova OE, Korchagina EV. Chitosan and its hydrophobic derivatives: Preparation and aggregation in dilute aqueous solutions 1. Polymer Science Series A. 2012;**54**(7):552-572

[9] Tian M, Tan H, Li H, You C. Molecular weight dependence of structure and properties of chitosan oligomers. RSC Advances. 2015;**5**(85):69445-69452

[10] Recillas M, Silva LL, Peniche C, Goycoolea FM, Rinaudo M, Argüelles-Monal WM. Thermoresponsive behavior of chitosan-g-N-isopropylacrylamide copolymer solutions. Biomacromolecules. 2009;**10**(6):1633-1641

[11] Cho J, Heuzey M-C, Bégin A, Carreau PJ. Viscoelastic properties of chitosan solutions: Effect of concentration and ionic strength. Journal of Food Engineering. 2006;**74**(4):500-515

[12] Tanaka K, Nishida K, Gabrys BJ, Lawrence MJ, Kanaya T. Critical dissolution ionic strength of aqueous solution of chitosan hydrochloride salt. Journal of the Society of Fiber Science and Technology. 2014;**70**(9):225-231

[13] Hunt S, Huckerby TN. A comparative study of molluscan and crustacean chitin proteoglycans by carbon-13 NMR spectroscopy. Identification of carbohydrate and amino acid contributions and the determination of amino acid chemical shifts in anhydrous formic acid. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 1987;**88**(4):1107-1116

[14] Romanazzi G, Gabler FM, Margosan D, Mackey BE, Smilanick JL. Effect of chitosan dissolved in different acids on its ability to control postharvest gray mold of table grape. Phytopathology. 2009;**99**(9):1028-1036

[15] Smith BJ. A conformational study of 2-oxanol: Insight into the role of ring distortion on enzyme-catalyzed glycosidic bond cleavage. Journal of the American Chemical Society. 1997;**119**(11):2699-2706

[16] Zhang J, Yan N. Formic acid-mediated liquefaction of chitin. Green Chemistry. 2016;**18**(18):5050-5058

[17] Sannan T, Kurita K, Iwakura Y. Studies on chitin, 1-effect of deacetylation on solubility. Die Makromolekulare Chemie. 1976;**177**(12):3589-3600

[18] Einbu A, Naess SN, Elgsaeter A, Varum KM. Solution properties of chitin in alkali. Biomacromolecules. 2004;**5**(5):2048-2054

[19] Hua X, Dua Y, Tanga Y, Wanga Q, Fenga T, Jianhong Yanga JFK. Solubility and property of chitin in NaOH/urea aqueous solution. Carbohydrate Polymers. 2007;**70**:451-458

[20] Fang Y, Duan B, Lu A, Liu M, Liu H, Xu X, Zhang L. Intermolecular interaction and the extended wormlike chain conformation of chitin in NaOH/urea aqueous solution. Biomacromolecules. 2015;**16**(4):1410-1417

[21] Gong P, Wang J, Liu B, Ru G, Feng J. Dissolution of chitin in aqueous KOH. Cellulose. 2016;**23**(3):1705-1711

[22] Gagnaire D, Saint-Germain J, Vincendon MNMR. Studies of chitin and chitin derivatives. Die Makromolekulare Chemie. 1982;**183**(3):593-601

[23] Einbu A, Grasdalen H, Varum KM. Kinetics of hydrolysis of chitin/chitosan oligomers in concentrated hydrochloric acid. Carbohydrate Research. 2007;**342**(8):1055-1062

[24] Lin CW, Lin JC. Characterization and blood coagulation evaluation of the water-soluble chitooligosaccharides prepared by a facile fractionation method. Biomacromolecules. 2003;**4**(6):1691-1697

[25] Pillai CKS, Paul W, Sharma CP. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Progress in Polymer Science (Oxford). 2009;**34**(7):641-678

[26] Qin Y, Lu X, Sun N, Rogers RD. Dissolution or extraction of crustacean shells using ionic liquids to obtain high molecular weight purified chitin and direct production of chitin films and fibers. Green Chemistry. 2010;**12**(6):968

[27] Kamalesh P, Masa-aki M, Yoshiro K, Akihiko T, Yoshifumi N, Jun-ichi K. Weak gel of chitin with ionic liquid, 1-allyl-3-methylimidazolium bromide. International Journal of Biological Macromolecules. 2009;**45**:221-225

[28] Wang WT, Zhu J, Wang XL, Huang Y, Wang YZ. Dissolution behavior of chitin in ionic liquids. Journal of Macromolecular Science, Part B. 2010;**49**(3):528-541

[29] Shimo M, Abe M, Ohno H. Functional comparison of polar ionic liquids and onium hydroxides for chitin dissolution and deacetylation to chitosan. ACS Sustainable Chemistry and Engineering. 2016;**4**(7):3722-3727

[30] Mine S, Izawa H, Kaneko Y, Kadokawa J. Acetylation of α-chitin in ionic liquids. Carbohydrate Research. 2009;**344**(16):2263-2265

[31] Yamamoto K, Yoshida S, Mine S, Kadokawa J. Synthesis of chitin-graft-polystyrene via atom transfer radical polymerization initiated from a chitin macroinitiator. Polymer Chemistry. 2013;**4**(11):3384

[32] Zhaodong W, Liuchun Z, Chuncheng L, Dong Z, Yaonan X, Guohu G, Wenxiang Z. Modification of chitosan with monomethyl fumaric acid in an ionic liquid solution. Carbohydrate Polymers. 2015;**117**:973-979

[33] Chen H, Cui S, Zhao Y, Wang B, Zhang S, Chen H, Peng X. O-alkylation of chitosan for gene delivery by using ionic liquid in an in-situ reactor. Engineering. 2012;**4**(10):114-117

[34] Yang L, Zhang J, He J, Zhang J, Gan Z. Homogeneous synthesis of amino-reserved chitosan-graft-polycaprolactone in an ionic liquid and the application in cell cultivation. Polymer International. 2015;**64**(8):1045-1052

[35] Laokuldilok T, Potivas T, Kanha N, Surawang S, Seesuriyachan P, Wangtueai S, Phimolsiripol Y, Regenstein JM. Physicochemical, antioxidant, and antimicrobial properties of chitooligosaccharides produced using three different enzyme treatments. Food Bioscience. 2017;**18**(April):28-33

[36] Zimoch-Korzycka A, Gardrat C, Castellan A, Coma V, Jarmoluk A. The use of lysozyme to prepare biologically active chitooligomers. Polímeros. 2015;**25**(1):35-41

[37] Il AV, Varlamov VP, Melent AI, Aktuganov GE. Depolymerization of chitosan with a chitinolytic complex from bacteria of the genus Bacillus sp. 739. Applied Biochemistry and Microbiology. 2001;**37**(2):142-144

[38] Sun Y, Zhang J, Wu S, Wang S. Preparation of d-glucosamine by hydrolysis of chitosan with chitosanase and β-d-glucosaminidase. International Journal of Biological Macromolecules. 2013;**61**:160-163

[39] Hamer SN, Cord-Landwehr S, Biarnés X, Planas A, Waegeman H, Moerschbacher BM, Kolkenbrock S. Enzymatic production of defined chitosan oligomers with a specific

pattern of acetylation using a combination of chitin oligosaccharide deacetylases. Scientific Reports. 2015;**5**(1):8716

[40] Khasanova LM, Il'ina AV, Varlamov VP, Sinitsyna OA, Sinitsyn AP. Hydrolysis of Chitozan with an enzyme complex from Myceliophthora sp. Applied Biochemistry and Microbiology. 2014;**50**(4):422-428

[41] Struszczyk MH. Chitin and chitosan: Part III. Some aspects of biodegradation and bioactivity. Polimery/Polymers. 2002;**47**(9):619-629

[42] Vishu Kumar AB, Varadaraj MC, Gowda LR, Tharanathan RN. Low molecular weight chitosans-preparation with the aid of pronase, characterization and their bactericidal activity towards *Bacillus cereus* and *Escherichia coli*. Biochimica et Biophysica Acta—General Subjects. 2007;**1770**(4):495-505

[43] Heras A, Rodri'guez NM, Ramos VM, Agullo E. N-methylene phosphonic chitosan: A novel soluble derivative. Carbohydrate Polymers. 2001;**44**:1-8

[44] Cardenas G, Cabrera G, Taboada E, Rinaudo M. Synthesis and characterization of critosan alkyl phosphate. Journal of the Chilean Chemical Society. 2006;**51**(1):815-820

[45] Sashiwa H, Thompson JM, Das SK, Shigemasa Y, Tripathy S, Roy R. Chemical modification of chitosan: Preparation and lectin binding properties of alpha-galactosyl-chitosan conjugates. Potential inhibitors in acute rejection following xenotransplantation. Biomacromolecules. 2000;**1**(3):303-305

[46] Sashiwa H, Yajima H, Aiba S. Synthesis of a chitosan – Dendrimer hybrid and its. Biomacromolecules. 2003;**4**:1244-1249

[47] Wei L, Li Q, Tan W, Dong F, Luan F, Guo Z. Synthesis, characterization, and the antioxidant activity of double quaternized chitosan derivatives. Molecules. 2017;**22**(3):501

[48] Noppakundilograt S, Buranagul P, Graisuwan W, Koopipat C, Kiatkamjornwong S. Modified chitosan pretreatment of polyester fabric for printing by ink jet ink. Carbohydrate Polymers. 2010;**82**:1124-1135

[49] Liu X, Wang Q, Zhuang X, Wu B, Yang F, Zeng A. Study on antibacterial activity of O-carboxymethyl chitosan sodium salt and spinnability of O-carboxymethyl chitosan sodium salt/cellulose polyblends in N-methylmorpholine-N-oxide system. Carbohydrate Polymers. 2012;**89**(1):104-110

[50] An NT, Thien DT, Dong NT, Le DP. Water-soluble N-carboxymethylchitosan derivatives: Preparation, characteristics and its application. Carbohydrate Polymers. 2009;**75**(3): 489-497

[51] Ma G, Qian B, Yang J, Hu C, Nie J. Synthesis and properties of photosensitive chitosan derivatives(1). International Journal of Biological Macromolecules. 2010;**46**(5):558-561

[52] Guo C, Zhou L, Lv J. Effects of expandable graphite and modified ammonium polyphosphate on the flame-retardant and mechanical properties of wood flour-polypropylene composites. Polymers and Polymer Composites. 2013;**21**(7):449-456