

## Review

## Preparation of nanochitin using deep eutectic solvents

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## SUMMARY

**Chitin is an abundant and renewable non-wood biopolymer. Nanochitin is formed by the assembly of chitin molecules, which has the advantages of large tensile strength, high specific surface area, and biodegradability, so it has been widely used. However, the traditional methods of preparing nanochitin have many drawbacks. As the new generation of green solvents, deep eutectic solvents (DESs) have been successfully applied in the fields of chitin dissolution, extraction, and nanochitin preparation. In this review, the relevant knowledge of chitin, nanochitin, and DESs was first introduced. Then, the application status of DESs in the fields of chitin was summarized, with a focus on the preparation of nanochitin using DESs. In conclusion, this review provided a comprehensive analysis of the published literature and proposed insights and development trends in the field of preparation of nanochitin using DESs, aiming to provide guidance and assistance for future researchers.**

## INTRODUCTION

With the continuous development of the social economy and the increasing improvement of human living standards, a large amount of energy consumption has brought serious environmental pollution and ecological damage. In order to protect the earth's environment on which humans rely, green chemistry and sustainable development have become the focus of attention of all countries.<sup>1,2</sup> In order to achieve this goal, we need to avoid the use of fossil energy and seek renewable and sustainable resources to meet the needs of human production and living. With the continuous efforts of researchers, functional materials have been successfully prepared from biomass materials widely existing in nature to effectively solve energy and environmental problems.<sup>3,4</sup> Among these biomass materials, chitin meets the current requirements of sustainability, renewability, functionality, biodegradability, and biocompatibility, which has attracted the great attention of researchers.<sup>5-8</sup>

Chitin is a linear natural polymer composed of N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) units linked by  $\beta$ -(1  $\rightarrow$  4)-glycoside bonds.<sup>9</sup> Chitin is the most abundant non-wood biopolymer, which exists in crustaceans, insects, beaks of mollusks, and cell walls of fungi, making it an inexhaustible renewable resource.<sup>9,10</sup> However, crustaceans are more abundant on earth than other organisms. According to statistics, in 2018, about 9.4 million tons of crustaceans were caught through aquaculture. In addition, wild capture added another 6.3 million tons.<sup>11</sup> As a result, 6 to 8 million tons of crustacean shell waste is discarded every year.<sup>12</sup> Therefore, considering the environmental pollution and economic benefits, researchers have concentrated on treating crustaceans to extract chitin. Because of its wide availability, chitin has attracted great attention in a wide range of fields, such as food, medicine, textiles, bioengineering, and functional materials.<sup>5,13,14</sup>

The extraction and dissolution of chitin are two key factors for the successful application of chitin. Generally speaking, chitin, protein, and  $\text{CaCO}_3$  form the shell of crustaceans. Therefore, in order to extract chitin efficiently, the acid-base method is traditionally used to extract chitin.<sup>15</sup> That is to say, acidic reagents (such as HCl) are used to dissolve  $\text{CaCO}_3$ , alkaline reagents (such as NaOH) are used to remove protein, and the last remaining material is chitin. Obviously, this traditional extraction method can get pure chitin, but the chemical reagents are harmful to human health and pollute the environment. In addition, chitin has poor solubility in most solvents due to its high crystallinity, rich hydrogen bond network, and high molecular weight, which limit its wide application. Traditionally, the systems for dissolving chitin are very limited, mainly including strong acid, alkaline aqueous solution, LiCl/DMAC, saturated  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ /methanol, and ionic liquids.<sup>16-19</sup> However, the aforementioned dissolution methods have the disadvantages of high toxicity, high cost, and complex preparation, so it is difficult to realize the industrial application of chitin.

Deep eutectic solvents (DESs) are homogeneous and stable liquids formed by hydrogen bond interactions between hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs).<sup>20</sup> Earlier, ionic liquids (ILs) were considered as green solvents. However, after intensive research, ILs do not fulfill the concept of green development due to the presence of toxicity.<sup>21-23</sup> In addition, ILs have the disadvantages of high viscosity, high corrosiveness, poor degradability, and high production cost.<sup>23</sup> DESs have been recognized as suitable alternatives to ILs because they have similar characteristics and properties to ILs, and also have unique features such as sustainability, recyclability, easy

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preparation, low toxicity, and cost-effectiveness.<sup>24</sup> DESs have been extensively researched over the past two decades, with a significant increase in related articles and more than 2,000 articles published in 2022.<sup>23</sup> The melting point of DESs is lower than that of the constituents, and electrostatic interactions, hydrogen bonding, van der Waals interactions, and lattice energies play crucial roles in the formation of DESs.<sup>25–27</sup> The physicochemical properties of DESs can be adjusted by changing the types of HBAs and HBDs, varying the molar ratios of HBDs to HBAs, and adding co-solvents.<sup>28</sup> DESs are recognized as the new generation of green solvents and have a wide range of applications in biomass extraction and processing, polymer synthesis, film science, analytical chemistry, and other fields.<sup>29–33</sup> Of course, through the long-term research of researchers, DESs have been successfully applied in the fields of extracting and dissolving chitin, which can solve the problems caused by traditional methods.<sup>34,35</sup>

Nanochitin, an important form of chitin, refers to the aggregation of chitin molecules with nanometer size in micro dimension. Compared with chitin, nanochitin has more excellent properties due to its nanoscale. The linear structure of nanochitin with a high aspect ratio makes it have excellent mechanical properties, such as high tensile strength, high toughness, and high elastic modulus. In addition, due to the nanostructure, nanochitin has a high specific surface area and its surface has characteristic functional groups (acetylamino, hydroxyl, etc.), which make it have higher reaction activity. Therefore, nanochitin is easier to be modified by reaction reagents to obtain functionalized nanochitin. Moreover, nanochitin also has the advantages of rich hydrogen bonding functional groups, low density, anisotropy, biocompatibility, biodegradability, and renewability.<sup>7,9</sup> For example, the hydrophilic property of nanochitin makes it compatible with hydrophilic polymers, so the nanochitin can be uniformly dispersed in the polymer matrix to maximize filler-substrate adhesion. Based on the aforementioned theory, nanochitin composites of hydrophilic polymers such as poly (vinyl alcohol),<sup>36,37</sup> aqueous polyurethanes,<sup>38</sup> and soy protein isolate<sup>39</sup> have been fabricated. At present, nanochitin has been successfully prepared into a wide range of functional materials, such as Pickering stabilizers, gels, films, and high-performance composites, which can be used in different fields.<sup>7,9,40</sup>

There are two traditional methods to prepare nanochitin: chemical methods (acid hydrolysis and oxidation) and mechanical decomposition.<sup>9</sup> Chemical methods can remove the disordered and defective region in chitin, retain the crystalline region, and finally form nanochitin with a low aspect ratio.<sup>41,42</sup> Mechanical decomposition only destroys the hydrogen bonds between chitin molecules through strong mechanical action, without removing the amorphous region in chitin, and finally forms nanochitin with a high aspect ratio.<sup>7,40</sup> It is obvious that chemical methods require the use of highly corrosive and highly reactive chemicals, which are harmful to human health and destroy the ecological environment. Mechanical decomposition requires special instruments, which are costly and energy-consuming, so it does not meet the requirements of sustainable production. In summary, traditional methods of preparing nanochitin have many drawbacks, so it is particularly important to develop an economical, efficient, and environmentally friendly method to prepare nanochitin. As green solvents, DESs have achieved success in extracting and dissolving chitin, which brings hope to the preparation of nanochitin using DESs. After a long period of research, researchers have successfully utilized DESs to prepare nanochitin, which has been prepared into different functional materials.<sup>34,35</sup> At present, there are fewer studies on the preparation of nanochitin using DESs, so the field needs more effort to be gradually perfected.

In this review, the relevant knowledge of chitin and nanochitin was first introduced, including the source, structure, and properties of chitin, as well as the classification, properties, traditional preparation methods, and applications of nanochitin. After that, DESs were briefly introduced, including classification, basic properties, and advantages. Then, the application status of DESs in the fields of chitin was summarized, and the preparation of nanochitin using DESs was emphasized. Finally, this review analyzed the published relevant literature on preparing nanochitin by DESs, and put forward the future challenges and development trends, hoping to provide guiding help for future researchers.

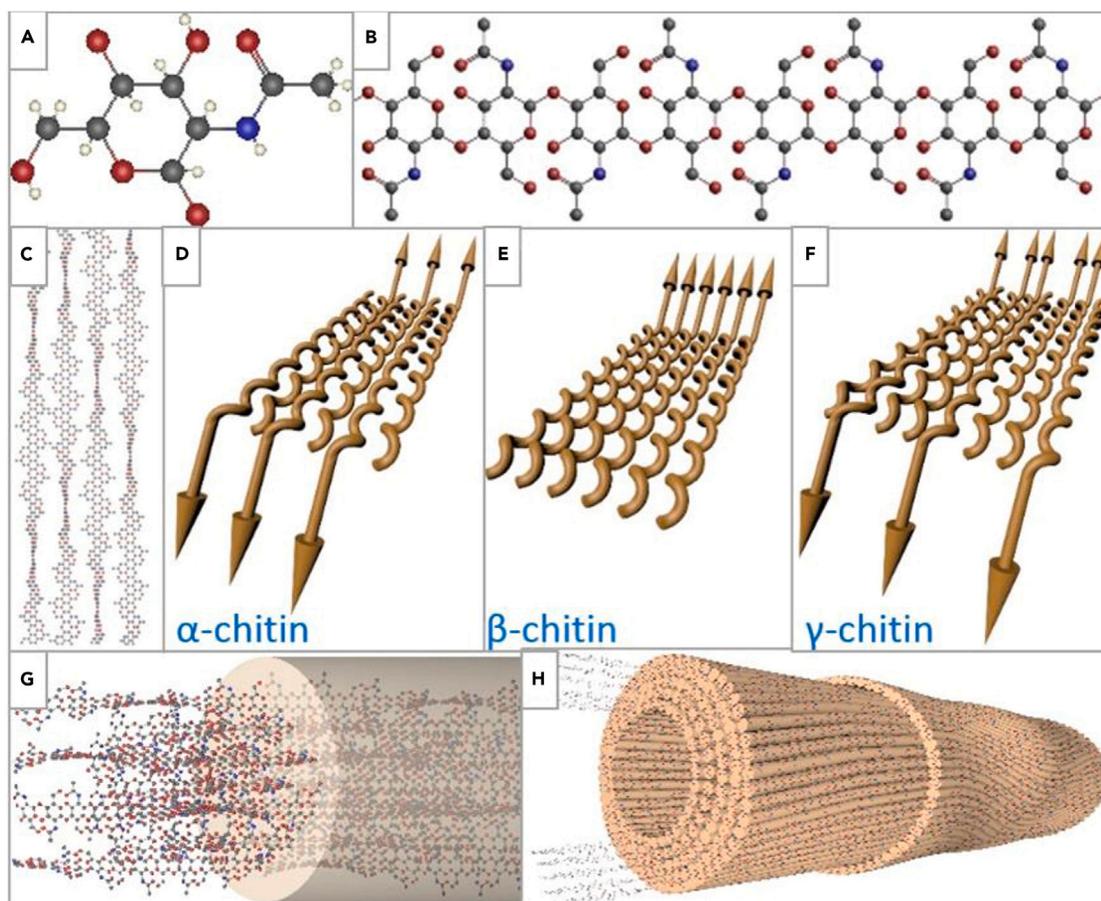
## CHITIN

### The source of chitin

Chitin is one of the most abundant renewable biological resources in nature and the second largest natural polysaccharide biopolymer after cellulose.<sup>35</sup> Chitin is the major component of the exoskeleton of arthropods, including crustaceans, insects, beaks of mollusks, and cell walls of fungi.<sup>9</sup> Chitin is composed of linear molecular chains, which are combined with different substances in different organisms to exhibit different crystal forms.

In nature, chitin mainly has three crystalline allomorphs with different arrangements and the relative directions of adjacent chains (Figure 1).<sup>43</sup>  $\alpha$ -Chitin mainly exists in crustaceans, insect cuticles, and fungi, and its molecular chains are antiparallel, showing the orthorhombic crystal of both inter-sheet and intra-sheet bonds.  $\beta$ -Chitin mainly exists in squid pens, sea tubeworms, and diatoms, and its molecular chains are arranged in parallel, showing the monoclinic crystal without the inter-sheet bonds.  $\gamma$ -Chitin rarely exists in nature and is only found in some species of squids and beetles. Its molecular structure is composed of two parallel chains and an adjacent antiparallel chain.<sup>7</sup>

Compared with other organisms, crustaceans are more abundant on earth, and  $\alpha$ -chitin forms strong intermolecular hydrogen bonds due to the antiparallel arrangement of molecular chains. Therefore,  $\alpha$ -chitin is the most abundant, stable, and important form at present.<sup>9</sup> The shell of crustaceans is mainly composed of three substances:  $\text{CaCO}_3$ , protein, and chitin, which are assembled in a certain order at the molecular level to form the shell of crustaceans. Generally speaking, chitin molecules exist in the inner layer of the exoskeleton, while protein covers chitin. These two substances tightly bind together through strong hydrogen bonds, forming chitin-protein fibrils.  $\text{CaCO}_3$  serves as the matrix, which can provide strength and hardness, enveloping chitin-protein fibrils to form the twisted plywood structure. These three substances form the compact exoskeleton through such assembly (Figure 2).<sup>44</sup>



**Figure 1. Structure and polymorphic configuration of chitin**

(A) and ball-and-stick model of dGlcNAc monomer (B). The schematic view of the alignment of the molecular chains in chitin (C). Schematic diagrams of the molecular chain orientations in the polymorphic configuration of different types of chitins (D–F). Nanofiber packaging diagram and molecular chain packaging diagram in chitin (G and H). Copyright 2021 Elsevier Ltd.

### The structure and properties of chitin

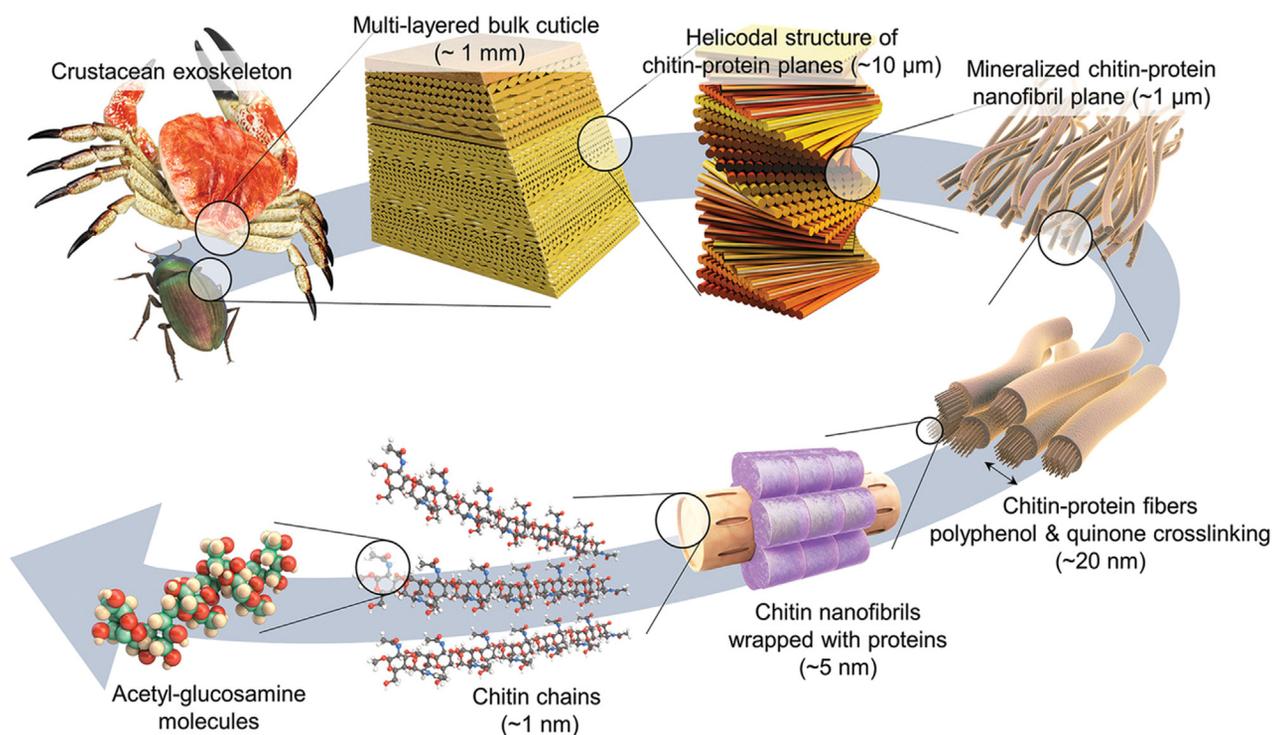
Similar to cellulose structure, chitin is a linear natural polymer composed of GlcNAc and GlcN units linked by  $\beta$ -(1  $\rightarrow$  4)-glycoside bonds. Chitin has three important structural parameters, including the degree of acetylation ( $D_A$ : the mole fraction of GlcNAc residues), the pattern of acetylation (the sequence of GlcN and GlcNAc units along the polymer chain), and molecular weight.<sup>9</sup> In addition, chitin is highly crystalline due to the strong hydrogen bonding interactions between the molecular chains of chitin.<sup>45–47</sup> Because of these factors, chitin is insoluble in most solvents, which greatly limits its industrial application.

In order to solve the problems of low solubility and limited application fields of chitin, researchers try to give coping strategies from different angles. First, chitin can be modified to obtain more valuable derivatives (such as chitosan). When  $D_A$  is greater than 0.5, the polymer turns into chitosan.<sup>48</sup> Compared with chitin, the molecular weight of chitosan is reduced, and its solubility and reactivity are greatly improved due to more amino groups in its structure. Chitosan is considered to be the most valuable derivative of chitin, which has a wide range of applications, including medicine, agriculture, food, and so on.<sup>49–52</sup> In addition, suitable solvents can be found to directly dissolve chitin, thereby further producing functional materials. At present, the systems for dissolving chitin are very limited, mainly including strong acid, alkaline aqueous solution, LiCl/DMAC, saturated  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ /methanol, and ionic liquids.<sup>16–19</sup> However, these systems have the disadvantages of high pollution, high cost, and low solubility, so researchers have been trying to find a green solvent to replace the traditional solvents.

## NANOCHITIN

### The classification of nanochitin

Nanochitin refers to the aggregation of chitin molecules whose microdimension is nanosize, and its surface is covered with functional groups such as acetamino and hydroxyl groups. Nanochitin mainly includes chitin nanofibers (ChNFs), chitin nanocrystallines (ChNCs), and chitin nanoparticles.<sup>7,34</sup> Because chitin molecules are linear, ChNFs and ChNCs are more common nanostructures of chitin. The molecular structure of chitin



**Figure 2.**  $\alpha$ -chitin

The multiscale structure of  $\alpha$ -chitin in nature. Copyright 2022 Wiley.

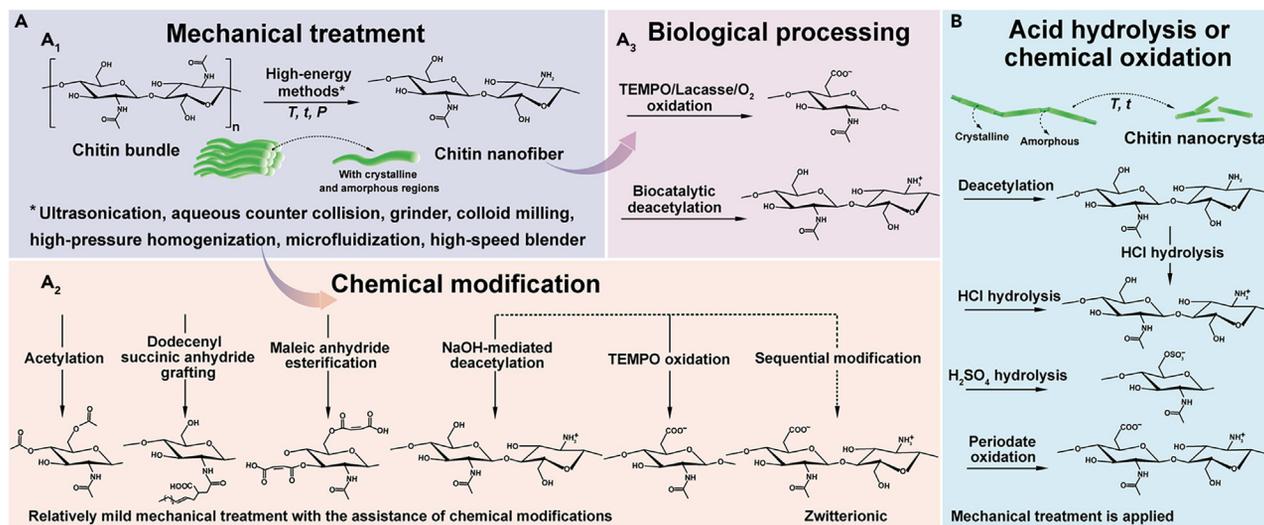
is composed of amorphous and crystalline regions, and the crystalline regions are composed of neatly arranged chains of chitin molecules. Mechanical fibrillation of the chitin molecular bundles through strong mechanical interaction leads to increased nanoscale lateral dimensions and inter-fibre repulsion, while preserving the amorphous and crystalline regions of chitin, resulting in fibrillar ChNFs with high aspect ratios.<sup>7</sup> The use of highly corrosive and highly reactive chemicals enables the stripping of chemicals from the surface of chitin nanofibrils and the removal of the amorphous structure of chitin, resulting in rod-like ChNCs with low aspect ratios.<sup>7</sup> In this review, we mainly introduce ChNFs and ChNCs.

### The properties of nanochitin

The mechanical properties of nanochitin are mainly determined by its size. The tensile strength ( $\sigma$ ) and diameter (width) of nanochitin have the relationship of  $\sigma \sim 1/\sqrt{D}$ .<sup>53</sup> Therefore, the tensile strength of nanochitin increases exponentially with the diameter (width). For example, the tensile strength of the film made of 6 nm wide nanofibers is  $\approx 200$ – $250$  MPa, while the tensile strength of the film made of 19 nm wide nanofibers is  $\approx 100$  MPa.<sup>54</sup> In addition to the width, the length of nanochitin also significantly affects its mechanical properties. The longer the nanofibers, the more serious the entanglement between the fibers. That is to say, the fibers are arranged more closely without defects, so the mechanical strength is greatly improved. For example, the tensile strength of the film prepared by ChNFs is more than 100 MPa, while the tensile strength of the film prepared by ChNCs is less than 50 MPa.<sup>54–56</sup> In general, compared with low aspect ratio ChNCs, the nanocomposites prepared from high-aspect-ratio ChNFs have higher mechanical properties, which are mainly due to the interaction between long fibers and the enhancement of entanglement. Moreover, the long fibers are closely packed without pores, which increases the cohesion strength.

In addition, nanochitin suspension also has a colloidal behavior. According to aspect ratio multiplied by concentration (Onsager's theory), the suspension of high-aspect-ratio ChNFs has lower critical gel concentration than that of low aspect ratio ChNCs in a monodisperse system, which is mainly because the entanglement and interaction between long fibers are stronger and it is easier to form the network structure.<sup>9,57</sup> Generally speaking, nanochitin is stable in an acidic environment, but unstable in an alkaline environment. According to surface potential (Derjaguin – Landau – Verwey – Overbeek theory), the amino groups on the surface of nanochitin are protonated in an acidic environment and stabilized by electrostatic repulsion. In an alkaline environment, the amino groups on the surface of nanochitin are deprotonated, resulting in aggregation and instability between fibers. However, surface modification of nanochitin can be performed to make them stable in an alkaline environment.<sup>5,58</sup> Functional groups (such as through sulfation, guanylation, and oxidation) that can ionize in an alkaline environment are introduced into the nanochitin molecular chain, which can stabilize the nanochitin suspension.<sup>9</sup>

The transparency related to nanochitin is also a property that attracts a lot of attention. The suspension of nanochitin is transparent, which makes it possible to prepare transparent materials with high performance. The transparency of the nanochitin suspension is related to the size of the fiber. Generally speaking, the shorter the fiber is, the higher the transparency of the suspension is.<sup>59,60</sup> In addition to the size of the fiber,



**Figure 3. Overview of the methods for preparing ChNFs**

(A) and ChNCs (B). ChNFs containing crystalline and disordered structures are produced by mechanical treatment (a<sub>1</sub>) or mechanical treatment assisted by chemical modification (a<sub>2</sub>) and biological processing (a<sub>3</sub>). Copyright 2022 American Chemical Society.

the transparency of the nanochitin suspension is also affected by the concentration, pH value, and ionic strength of the suspension.<sup>9</sup> Except for the aforementioned properties, nanochitin also has other superior properties, such as high and active surface area, excellent mechanical toughness, biocompatibility, biodegradability, and renewable, which has aroused great interest in many fields.<sup>40,61</sup>

### The traditional methods for preparing nanochitin

In order to prepare nanochitin, there are two approaches: top-down and bottom-up.<sup>9</sup> For the top-down approach, macro chitin particles are gradually decomposed into nanoscale molecular aggregate. For the bottom-up approach, macro chitin particles are first dissolved to form the single-molecule solution, and then the molecules are self-assembled to form nanochitin.<sup>42,48,62</sup> Therefore, the bottom-up approach needs the ideal solvent to dissolve chitin, and the obtained nanochitin cannot maintain the same morphology and crystal form as natural chitin. In the top-down approach, the chitin particles are decomposed step by step from large to small, and the self-assembly of molecules is not involved. Therefore, the structure of the final nanochitin has not changed. Considering the aforementioned factors, the preparation of nanochitin is mainly through the top-down approach at present.<sup>42,48,62–64</sup>

Traditionally, the preparation of nanochitin by the top-down approach can be divided into chemical methods (acid hydrolysis and oxidation) and mechanical decomposition.<sup>9</sup> Chemical methods can remove the disordered and defective region in chitin, retain the crystalline region, and finally form low aspect ratio ChNCs.<sup>41,42</sup> In addition, chemical reagents can modify the surface of chitin while treating chitin, such as deacetylation and esterification.<sup>9</sup> Compared with chemical methods, mechanical decomposition only destroys the hydrogen bonds between chitin molecules through strong mechanical action, without removing the amorphous region in chitin, and finally forms a high-aspect-ratio ChNFs.<sup>9,40</sup> Mechanical treatment consumes a lot of energy, so mechanical decomposition is usually combined with chemical methods (chemical and biological treatment) to promote fiber peeling and reduce the energy used in mechanical treatment.<sup>65</sup> The preparation methods of ChNCs and ChNFs are shown in Figure 3.<sup>7</sup>

The relevant studies on the preparation of nanochitin by traditional methods (chemical methods and mechanical decomposition) are listed in Table 1. Mechanical decomposition is simple and adaptable, which mainly includes ultrasound, grinding, high-pressure homogenization, microfluidization, and magnetic stirring. They all promote the peeling of chitin fibers through strong mechanical action. In addition, there is no chemical reaction, and most of the structural characteristics of natural chitin are retained.<sup>7,9,40</sup> Therefore, ChNFs with a high aspect ratio can be obtained. However, mechanical decomposition often requires special instruments, which are expensive and energy-consuming, so it is difficult to achieve large-scale production. Chemical methods include inorganic acid hydrolysis, organic acid hydrolysis, and oxidation. Chemical reagents can remove the disordered structure in chitin, so as to obtain high-crystallinity ChNCs.<sup>7,9,40</sup> Obviously, the chemical methods require the use of highly corrosive and highly active chemicals, which harm human health and destroy the ecological environment. To sum up, the traditional methods of preparing nanochitin have many defects, so it is particularly important to develop a cost-effective and environment-friendly method to prepare nanochitin.

### The applications of nanochitin

Nanochitin has been widely used in Pickering stabilizers due to its self-assembly in different environments. The size of the emulsion droplet is a key index.<sup>81</sup> Generally speaking, the smaller the emulsion droplet, the higher its stability. Compared with other types of nano-polysaccharides

**Table 1. A summary of studies on the preparation of nanochitin using traditional methods**

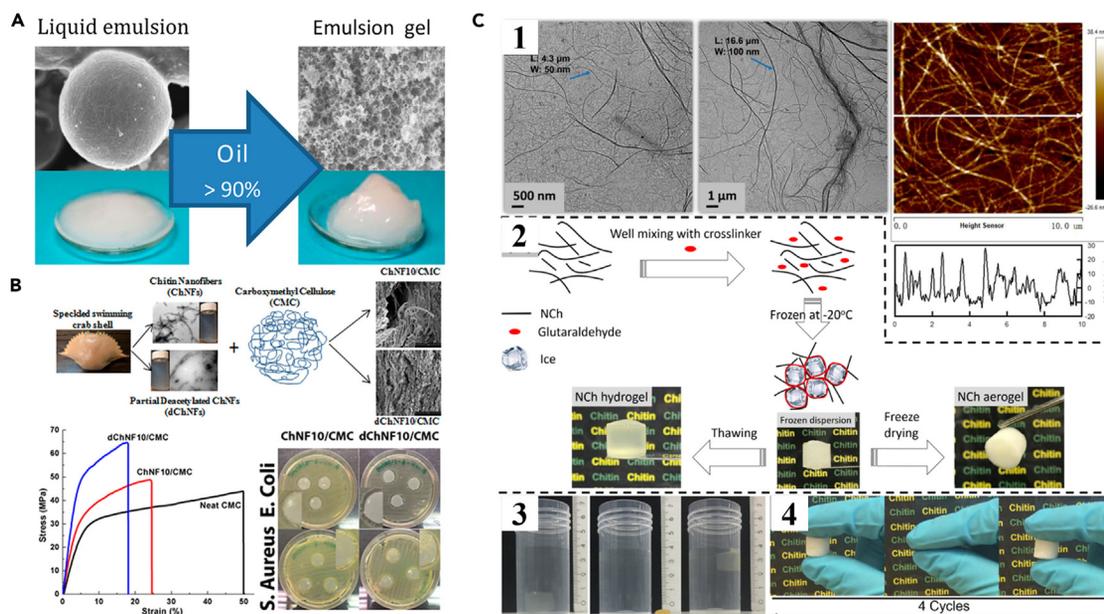
Production route	Reagents/methods	Chitin crystal allomorph and source	Average size (nm)		Crystallinity index (%)	Reference
			Width	Length		
Mechanical disintegration	Ultrasonication	$\beta$ -Chitin (squid pen)	3–4	>500	37	Fan et al. <sup>66</sup>
	Grinding	$\alpha$ -Chitin (crab shell)	10–20	NR <sup>a</sup>	NR	Ifuku et al. <sup>67</sup>
	High-pressure homogenization	$\alpha$ -Chitin (lobster waste)	80–100	>5000	85	Salaberria et al. <sup>68</sup>
	Microfluidization	$\beta$ -Chitin (squid pen)	2–7	>1000	89	Wu et al. <sup>69</sup>
		$\alpha$ -Chitin (lobster exoskeleton)	3–4	1000–1500	NR	Mushi et al. <sup>70</sup>
	Magnetic stirring	Partly deacetylated $\alpha$ -chitin	6.2	250	57	Fan et al. <sup>71</sup>
Inorganic acid hydrolysis	HCl	$\alpha$ -Chitin (shrimp shell)	15–30	150–250	87	Tran et al. <sup>60</sup>
	H <sub>2</sub> SO <sub>4</sub>	$\alpha$ -Chitin (crab shell)	8.0	>1000	86–93	Dutta et al. <sup>72</sup>
	H <sub>3</sub> PO <sub>4</sub>	$\alpha$ -chitin (crab shell)	7.0	>1000	89–93	Dutta et al. <sup>72</sup>
Organic acid hydrolysis	Maleic acid	$\alpha$ -Chitin (shrimp shell)	Nano	Nano	72–82	Liu et al. <sup>73</sup>
	Oxalic acid	$\alpha$ -Chitin (shrimp shell)	12.5–13.0	600–1100	>80	Ma et al. <sup>74</sup>
Oxidation	(2,2,6,6-Tetramethylpiperidin-1-yl) oxyl radical (TEMPO)/NaClO/NaBr	$\alpha$ -Chitin (crab shell)	7–20	50–500	93	Fan et al. <sup>75</sup>
	TEMPO/NaClO <sub>2</sub> /NaClO	$\alpha$ -Chitin	5–15	200–600	92–95	Pang et al. <sup>76</sup> ; Jiang et al. <sup>77</sup>
	Ammonium persulfate	$\alpha$ -Chitin (crab shell)	15	400–500	79–94	Oun et al. <sup>78</sup> ; Oun et al. <sup>79</sup>
	Potassium periodate	$\alpha$ -Chitin (shrimp shell, crab shell)	12	220–250	97	Liu et al. <sup>80</sup>

<sup>a</sup>NR: Not reported.

(such as nanocellulose), Pickering emulsion droplets stabilized by nanochitin are usually smaller and show higher storage stability.<sup>82</sup> For example, Pang et al. used a TEMPO/NaClO<sub>2</sub>/NaClO system to prepare zwitterion ChNFs through the combination of partial deacetylation and oxidation.<sup>76</sup> At the same time, the double pH-responsive Pickering emulsion stabilized by zwitterion ChNFs was successfully prepared to replace the traditional pH-responsive Pickering emulsion. Another application of nanochitin as the Pickering stabilizer is to prepare an adjustable internal phase Pickering emulsion (HIPPE). As shown in Figure 4A, Perrin et al. successfully prepared oil/water (O/W) HIPPE gel with 96% internal phase (hexadecane) by using the stability of ChNCs on the oil/water interface.<sup>83</sup> The softness of the gel can be adjusted by concentration, pH value, and ionic strength. In another study, Zhu et al. used ChNFs to prepare O/W HIPPE gel, whose internal phase was edible oil with a volume fraction of 88%.<sup>84</sup> Moreover, this gel can be used as gel ink for 3D printing to prepare materials of various shapes.

Because of the inherent morphology and chemical characteristics of nanochitin, such as its large aspect ratio, low density, anisotropy, flexibility, and rich surface functional groups, nanochitin can be used as functional additives or nanofillers to prepare high-performance functional materials.<sup>7</sup> The strengthening ability of nanochitin is usually attributed to the formation of the hydrogen bonding permeation network in the matrix. Wang et al. used TEMPO/NaClO<sub>2</sub>/NaClO system to prepare ChNFs with cation (NH<sub>3</sub><sup>+</sup>) and anion (COO<sup>-</sup>), which can be used to improve the rheological property of smart ink of anthocyanin.<sup>85</sup> The results showed that the yield stress and shear viscosity of the ink were improved, resulting in excellent printability. Moreover, the friction resistance and tape fastness were 97.8% and 98.9%, respectively. In another study, Li et al. extracted deacetylated ChNFs from crab shells, which were used as enhancers and antibacterial agents for carboxymethyl cellulose (CMC) films (Figure 4B).<sup>10</sup> The results showed that the mechanical properties and antibacterial properties of the ChNFs/CMC film were greatly improved, which had a great application prospect in the field of antibacterial food packaging.

Nanochitin has the advantages of high longitudinal elastic modulus (more than 150 GPa in theory), high aspect ratio, and high specific surface area, and it is easy to obtain self-entangled or aggregated nanostructure by simply concentrating or drying nanochitin suspension.<sup>86,87</sup> Therefore, nanochitin can be prepared into high-performance films by suspension casting or drying. Fan et al. used four different types of nanochitin (TEMPO-oxidized  $\alpha$ -ChNCs, partially deacetylated  $\alpha$ -ChNCs/ $\alpha$ -ChNFs mixture, HCl hydrolysis  $\alpha$ -ChNCs and  $\beta$ -ChNFs) to prepare four films by suspension casting, which all had good transmittance and mechanical strength.<sup>55</sup> Zhang et al. prepared the partially deacetylated ChNFs film, TEMPO-oxidized ChNFs film, and the composite film by drying the nanosuspension at 40°C.<sup>88</sup> It was found that the three kinds of films had similar mechanical strength, antibacterial properties, and biodegradability.



**Figure 4. ChNCs at the interface**

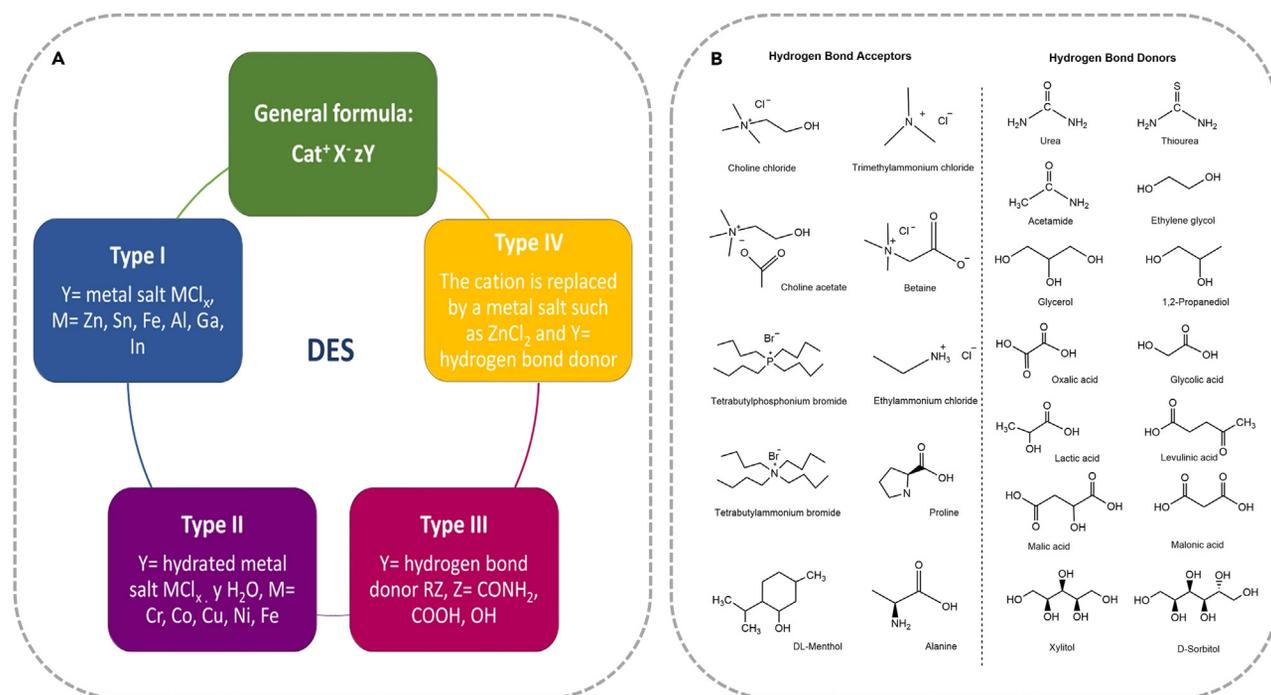
The photo of O/W HIPPE gel containing 96% internal phase (hexadecane) by using the stability of ChNCs at the oil/water interface (A). Copyright 2014 American Chemical Society. The ChNFs/CMC films prepared by using extracted ChNFs as the enhancer exhibit excellent mechanical and antibacterial properties (B). Copyright 2016 American Chemical Society. Transmission electron microscopy and atomic force microscopy images of ChNFs ( $c_1$ ). Schematic illustration of the formation of nanochitin hydrogels and cryogels via ice templating and cross-linking ( $c_2$ ). The photographs of cylindrical nanochitin hydrogels ( $c_3$ ). The photographs of cylindrical nanochitin cryogels ( $c_4$ ). Copyright 2019 American Chemical Society.

Due to the flexible long-chain structure and abundant hydrogen bonding functional groups, nanochitin can be assembled into the gel through simple physical crosslinking, such as chain entanglement, hydrophobic interaction, and hydrogen bonding interaction.<sup>82,89</sup> At present, a large number of studies have been reported to prepare the gel by using the powerful three-dimensional network structure formed by nanochitin.<sup>90–92</sup> Mushi et al. prepared ChNFs hydrogels with different concentrations.<sup>93</sup> It was found that the gelation of nanochitin occurred at 0.4 wt %, and the hydrogels with the concentration of 2.0 and 3.0 wt % had the maximum compression modulus and storage modulus, respectively. In addition to the preparation of hydrogel by nanochitin alone, nanochitin can be mixed with other substances to form a mixed hydrogel, which can improve the performance of a hydrogel. For example, Yao et al. successfully prepared composite hydrogel by uniformly dispersing ChNCs in sodium alginate aqueous solution.<sup>94</sup> Due to the strong electrostatic interaction between ChNCs and sodium alginate, the mechanical properties and swelling stability of the hydrogel were greatly improved. Moreover, the composite hydrogel had good biocompatibility and low cytotoxicity, which was successfully applied in the field of bone scaffold. In addition to physical crosslinking, the introduction of chemical crosslinking in nanochitin gel can form a covalent entangled network structure, which further improves the performance and expands the application. As shown in Figure 4C, Liu et al. first prepared ChNFs with a high aspect ratio by microfluidization method and then prepared hydrogel by chemical crosslinking of ChNFs and glutaraldehyde through ice template method.<sup>95</sup> It was found that when the concentration of ChNFs was as low as 0.4 wt %, it could form hydrogel with high strength, high viscoelasticity, and shape recovery. In addition to the hydrogel, the functional aerogel can also be prepared by using nanochitin. Lindy et al. treated ChNCs with ultrasound in water, then exchanged solvents with ethanol, and finally dried samples under supercritical carbon dioxide to form aerogel.<sup>96</sup> The prepared aerogel had high porosity (97%), low density (0.043 g/cm<sup>3</sup>), large specific surface area (261 m<sup>2</sup>/g), very low shrinkage (4%), and excellent mechanical properties (modulus of 9.3 MPa), which had broad application prospects in the fields of thermal insulation and biocatalysis.

Because of the excellent properties of nanochitin, researchers have conducted a lot of studies on the application of nanochitin. Due to the limited space, this review only lists a small part of them, and readers can refer to other wonderful reviews on other applications of nanochitin.<sup>7,9,40</sup> It should be emphasized that although nanochitin is widely used, there are many defects in the traditional methods of preparing nanochitin. Therefore, it is urgent to develop a green method for the preparation of nanochitin to replace the traditional methods, which makes the applications of nanochitin more economical, efficient, and environmentally friendly.

## DESs

In 2003, Abbott et al. first found that choline chloride and urea could form a solvent with a low melting point by mixing, which was named as DES.<sup>97</sup> DESs are usually homogeneous and stable liquids formed by simple physical mixing of HBAs and HBDs in a certain proportion at a certain temperature.<sup>20</sup> DESs can usually be represented by the general formula Cat<sup>+</sup>X<sup>-</sup>+Y, where Cat<sup>+</sup>X<sup>-</sup> represents salts and Y represents



**Figure 5. General formula and classification of DESs**

(A) Common HBAs and HBDs are used for the preparation of DESs (B). Copyright 2021 Springer.

HBDs (such as Lewis acid and Brønsted acid). As shown in Figure 5A,<sup>98</sup> DESs can be divided into four types according to the types of HBAs and HBDs. Among the four types of DESs, DESs of type III are easy to prepare, low cost, stable chemical properties, easy to obtain, and biodegradable, so they have the most extensive applications. Common HBAs and HBDs used to prepare DESs are shown in Figure 5B.<sup>98</sup>

Performance determines the applications, so understanding the physicochemical properties of DESs is crucial for their applications. The freezing point of DESs is lower than that of any of its components, and the freezing point of most DESs is in the range of  $-85^{\circ}\text{C}$  to  $95^{\circ}\text{C}$ .<sup>99</sup> The freezing point of DESs can reflect the interaction between HBAs and HBDs, which is mainly affected by ion symmetry, hydrogen bonding formation ability, and other factors.<sup>100</sup> For example, with the weakening of ionic symmetry, the freezing point decreases. The density of most DESs is greater than that of water, ranging from  $1.0$  to  $1.35\text{ g/cm}^3$ . The density of DESs varies with temperature, the properties, and the molar ratio of each component.<sup>98,100</sup> For example, with the increase in temperature, the fluidity of molecules increases, which leads to an increase in the molar volume of DESs and a decrease in the density of DESs. The viscosity of most DESs is in the range of  $100$ – $1000\text{ mPa s}$ , which is generally higher than that of organic solvents. The viscosity of DESs is mainly determined by intermolecular interactions, which may be affected by many factors, including the chemical properties of DESs components, the types and molar ratio of HBAs and HBDs, temperature, and water content.<sup>26,99</sup> DESs with low viscosity are the ideal industrial solvents, while DESs with high viscosity hinder the migration and diffusion of molecules, resulting in more energy consumption in the production process and increased production costs.<sup>98,101,102</sup> In addition, DESs synthesized by different HBAs and HBDs have different pH values, which are commonly represented by Kamlet Taft parameters, including polarity ( $\pi$ ), hydrogen bonding acidity ( $\alpha$ ), and hydrogen bonding alkalinity ( $\beta$ ).<sup>75</sup> The Kamlet Taft parameter is often used to evaluate the influence on molecular solubility, reaction rate, and reaction mechanism.

DESs not only overcome the shortcomings of traditional solvents such as high toxicity, high cost, and complex preparation but also have unique characteristics, such as low melting point, incombustibility, high chemical and thermal stability, and excellent biodegradability. DESs have been widely used in the dissolution, extraction, and separation of biomass materials; smelting of precious metals; preparation of functional materials; synthesis of organic reactions; and so on.<sup>29–33</sup> Due to the limited space, this review focuses on the applications of DESs in the field of chitin, especially the preparation of nanochitin. For the development and applications of DESs in other fields, readers can refer to other wonderful reviews.<sup>26,99,100</sup>

## THE APPLICATION STATUS OF DESs IN THE CHITIN FIELD

### Dissolving chitin with DESs

Chitin is difficult to dissolve in most solvents because of its high molecular weight, high crystallinity, and rich hydrogen bond network.<sup>14,103,104</sup> Therefore, a solvent system that can dissolve chitin must have high ionic strength and the ability to break hydrogen bonds. There are a lot of hydrogen bonds and ionic bonds between DESs components, which provide the theoretical basis for the dissolution of chitin. As shown in Table 2, researchers used different DESs to dissolve chitin.

**Table 2. A summary of studies on dissolution of chitin using DESs**

Chitin sources	HBA	HBD	HBA/HBD molar ratio	Reaction conditions	Maximum solubility of chitin (%)	Reference
Crab shell	Choline chloride	Urea	1:2	Heating at 100°C, 10 h	6.0	Sharma et al. <sup>105</sup>
				Ultrasonication and heating at 80°C, 1 h	5.5	
				Microwave irradiation at 80°C, 2 h	7.0	
	Choline bromide	Urea	1:2	Heating at 100°C, 10 h	6.5	
				Ultrasonication and heating at 80°C, 1 h	6.0	
				Microwave irradiation at 80°C, 2 h	7.0	
	Chlorocholine chloride	Urea	1:2	Heating at 100°C, 10 h	8.0	
				Ultrasonication and heating at 80°C, 1 h	6.0	
				Microwave irradiation at 80°C, 2 h	7.5	
	Betaine	Urea	1:4	Heating at 100°C, 10 h	5.0	
				Ultrasonication and heating at 80°C, 1 h	5.0	
	Choline chloride	Ethylene glycol	1:2	Heating at 100°C, 10 h	NS <sup>a</sup>	
Ultrasonication and heating at 80°C, 1 h				NS		
Microwave irradiation at 80°C, 2 h				NS		
Choline chloride	Glycerol	1:2	Heating at 100°C, 10 h	NS		
			Ultrasonication and heating at 80°C, 1 h	NS		
			Microwave irradiation at 80°C, 2 h	NS		
Choline chloride	Thiourea	1:2	Heating at 100°C, 10 h	9.0		
			Ultrasonication and heating at 80°C, 1 h	7.0		
			Microwave irradiation at 80°C, 2 h	8.0		
Crab shell	AMIMCl	Thiourea	1:0.5	Heating at 100°C, 24 h	5.0	Idenoue et al. <sup>106</sup>
	BMIMCl	Thiourea	1:0.5		NS	
	EMIMCl	Thiourea	1:0.3		2.0	
	BMIMBr	Thiourea	1:0.5		5.0	
	EMIMBr	Thiourea	1:0.5		2.0	
Crab shell	Choline chloride	Formic acid	1:2	Heating at 115°C, 14 h	11.0	Bisht et al. <sup>107</sup>
	Choline chloride	Lactic acid			10.0	
	Betaine	Formic acid			10.0	
	Betaine	Lactic acid			9.0	

<sup>a</sup>NS: Not soluble.

The use of DESs to dissolve chitin can be traced back to 2013. Sharma et al. successfully dissolved chitin by using choline chloride (ChCl), choline bromide, chlorocholine chloride, betaine (Bet) as HBAs, and urea and thiourea as HBDs.<sup>105</sup> It was found that ultrasonic heating and microwave radiation heating can replace the traditional water bath or oil bath heating and achieve similar solubility in a shorter time. When urea and thiourea were HBDs, chitin was successfully dissolved, and the maximum solubility of ChCl/thiourea DES was 9.0 wt %. However, when the HBDs were ethylene glycol and glycerol, the dissolution of chitin could not be achieved, which may be attributed to their poor ability to destroy the intermolecular hydrogen bonds of chitin. From this research results, it can be seen that the selection of HBDs was crucial for achieving the dissolution of chitin. This study was a successful attempt to dissolve chitin by DESs, and researchers have continued to develop new DESs systems to achieve chitin dissolution since then.

Satoshi et al. mixed ionic liquid with thiourea to form a new DES, which can dissolve chitin under the condition of heating at 100°C for 24 h.<sup>106</sup> In a recent study, Meena Bisht et al. used ChCl, Bet, and urea as HBAs, and formic acid (FA), acetic acid, propionic acid, and lactic acid (LA) as HBDs, and achieved greater solubility of chitin by heating at 115°C for 14 h in a 1:2 M ratio.<sup>107</sup> Among them, the maximum solubility of ChCl/FA, ChCl/LA, Bet/FA, and Bet/LA DESs can reach 11.0, 10.0, 10.0, and 9.0 wt %, respectively.

The aforementioned researches show that DESs have great potential in dissolving chitin. In order to realize the dissolution of chitin, HBDs and HBAs must cooperate with each other to destroy the original hydrogen bond network structure of chitin, which can establish the new hydrogen bond network structure between chitin and DESs. At present, there are few studies on the dissolution of chitin by DESs, and

the maximum solubility is only about 11 wt %. The dissolution of chitin is an important method to convert it into high-value-added products, so it is necessary to focus on optimizing the chemical structure and composition of DESs to further expand the solubility of chitin in the future.

### Extracting chitin with DESs

Compared with the dissolution of chitin by DESs, the extraction of chitin from crustacean shells by DESs is more widely studied. Traditionally, the extraction of chitin from crustacean shells usually includes the following three key steps: demineralization, deproteinization, and decolorization.<sup>15</sup> Demineralization refers to dissolving  $\text{CaCO}_3$  with acid reagents (such as HCl), leaving chitin-protein fibrils. Deproteinization refers to the use of alkaline reagents (such as NaOH), which can remove protein from chitin-protein fibrils. Decolorization refers to the further treatment of chitin samples with bleaching agents (such as  $\text{H}_2\text{O}_2$ ) to remove the residual pigment. This traditional extraction method can get pure chitin, but the chemical reagents are harmful to human health and cause pollution to the environment. Because DESs are environmentally friendly solvents, the extraction of chitin from crustacean shells by DESs has become a research hotspot.<sup>34,35</sup>

The extraction of chitin from crustacean shells by DESs includes two key steps: demineralization and deproteinization.<sup>108,109</sup> Demineralization is achieved by releasing hydrogen ions from DESs, which can react with  $\text{CaCO}_3$  to produce soluble calcium salts, water, and  $\text{CO}_2$ . Subsequently, due to the abundant active functional groups (such as hydroxyl, carboxyl, and amino groups) in protein, the hydrogen bonds in the chitin-protein fibrils are broken after DESs treatment, and new hydrogen bonds are formed between protein and DESs. As a result, the protein is separated and dissolved in DESs at high temperatures.

Saravana et al. successfully extracted chitin from shrimp shells using 14 kinds of DESs.<sup>110</sup> Among them, ChCl/malonic acid DES had the best effect, and high-purity chitin can be obtained with a yield of  $19.41\% \pm 1.35\%$ . As shown in Figure 6A, Zhu et al. used ChCl/thiourea, ChCl/urea, ChCl/glycerol, and ChCl/malonic acid DESs to extract chitin from lobster shell, and ChCl/malonic acid had the strongest ability to extract pure chitin from lobster shell.<sup>111</sup> In another study, Huang et al. used ChCl/malic acid DES to extract high-purity chitin from shrimp shells (Figure 6B).<sup>108</sup> In a recent study, Meena et al. used ChCl/LA and Bet/LA DESs to obtain high-purity chitin from crayfish waste, and the yield of chitin reached 85% (Figure 6C).<sup>107</sup>

Because DESs are green solvents, DESs have great potential in the field of chitin extraction. At present, there are more and more reports about the extraction of chitin by DESs, and there are also more and more systems available for selection. Published studies show that acidic DESs are more conducive to the extraction of chitin and can obtain higher purity chitin.<sup>112,113</sup> However, the current relevant reports are based on small-scale research in the laboratory, and cannot be applied to industrial production.<sup>34,35</sup> Therefore, the extraction mechanism needs to be further improved in the future, including cost, the mass ratio of chitin raw material to DESs, the composition of DESs, extraction conditions, and so on, in order to gradually replace the traditional chitin extraction industry.

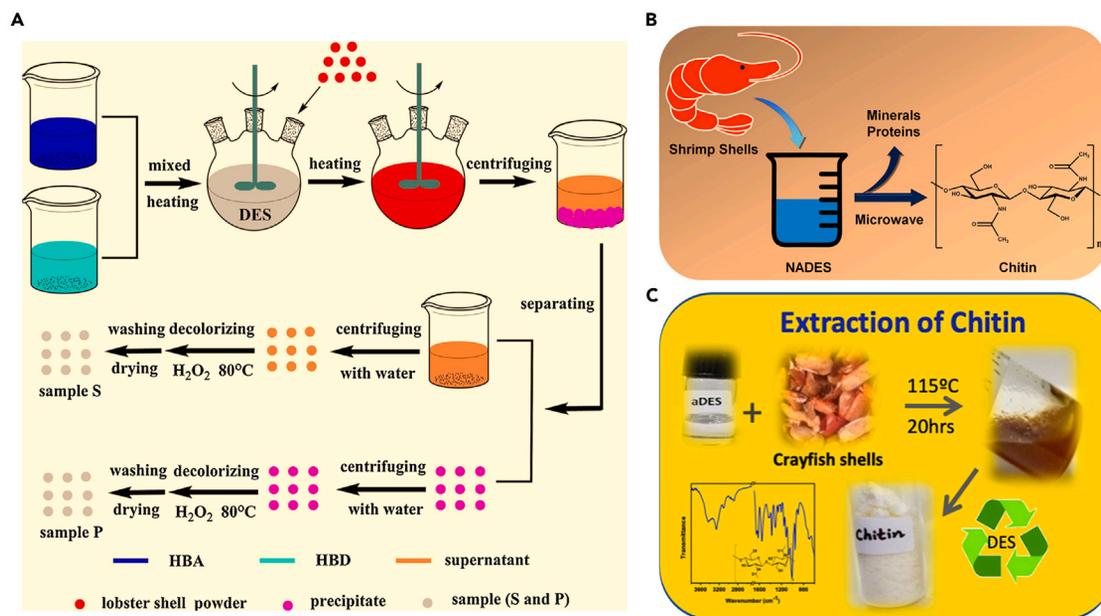
### PREPARING NANOCHITIN WITH DESs

As mentioned previously, the traditional methods of preparing nanochitin have many defects, so researchers are constantly trying to develop a new system to prepare nanochitin. As the new generation of green solvents, DESs have been successfully applied in the fields of chitin dissolution and extraction, so researchers have gradually applied DESs to the preparation of nanochitin. It is found that ChNFs and ChNCs can be prepared separately using DESs. In general, chitin is treated by acidic DESs, which can effectively remove amorphous regions in chitin, resulting in rod-shaped or needle-like ChNCs. When non-acidic DESs are used, due to the inability to effectively remove an amorphous region, they can only disrupt the hydrogen bonds between chitin molecules, ultimately forming fibrous ChNFs.<sup>34</sup> Therefore, by rationally designing the composition and structure of DESs, nanochitin with different morphology and structure can be obtained. As shown in Table 3, the researchers used different DESs to prepare nanochitin.

### Preparing ChNFs with non-acidic DESs

The use of DESs to prepare nanochitin can be traced back to 2014. Mukesh et al. first suggested that DESs could be used to prepare ChNFs.<sup>114</sup> As shown in Figure 7A, Mukesh et al. pretreated chitin with ChCl/thiourea DES, and then obtained ChNFs by ultrasound treatment for 40 min. Moreover, the DES solution can be recovered by evaporation to remove water, so the DES can be reused to prepare ChNFs. Finally, the yield of ChNFs reached as high as 84%. The transmission electron microscopy clearly showed that the prepared ChNFs had a diameter of  $20 \pm 6$  nm and a length of  $1000 \pm 500$  nm. Interestingly, when the HBD was changed from thiourea to urea, ChNFs could not be obtained, but the aggregated state was obtained. This phenomenon suggested that the composition and chemical structure of DESs were crucial for the formation of ChNFs.

In order to apply the prepared ChNFs, the authors mixed ChNFs with sodium alginate and  $\text{CaCl}_2$  to prepare Ca-Alg/ChNFs bio-nanocomposite gel beads. The rheological test showed that the modulus of the Ca-Alg/ChNFs bio-nanocomposite gel beads was three times higher than that of Ca-Alg gel beads without ChNFs, which suggested that the bio-nanocomposite gel beads had higher elasticity in the presence of ChNFs. In addition, the authors investigated the ability of Ca-Alg/ChNFs bio-nanocomposite gel beads to control drug release. For the anti-cancer drug (5-FU), Ca-Alg/ChNFs bio-nanocomposite gel beads can release about 70% of the drug after 24 h, while the control group of Ca-Alg gel beads can only rapidly release 39% of the drug after 3 h. The high release rate and long sustained release time of bio-nanocomposite gel beads indicated that ChNFs played a crucial role in the sustained release of drugs. In conclusion, nanochitin has the potential to control the release of drugs, so it has a broad application prospect in the field of medicine.<sup>13,120,121</sup>



**Figure 6. Extraction of chitin**

Schematic depiction for the extraction of chitin from lobster shell by DESs (A). Copyright 2017 Elsevier Ltd. Schematic depiction for the extraction of chitin from shrimp shell by DESs (B). Copyright 2018 American Chemical Society. Schematic diagram of extracting chitin from crayfish shell waste by DESs (C). Copyright 2021 American Chemical Society.

### Preparing ChNCs with acidic DESs

Cao et al. first used acidic DES to prepare ChNCs.<sup>115</sup> As shown in Figure 7B, chitin was mixed with *p*-toluenesulfonic acid/ChCl DES with continuous stirring at 95°C for 30 min, and then ethanol was added to quench the reaction, ultimately obtaining ChNCs. It can be clearly seen that the production of ChNCs did not require additional steps, which greatly reduced energy consumption. The scanning electron microscopy clearly showed that the ChNCs presented a rod-shaped structure and were monodisperse in length and width, with average length and width of about 293.4 and 26.6 nm, respectively. For comparison, the authors treated chitin with urea/ChCl and thiourea/ChCl DESs at 95°C for 30 min, which showed that ChNCs cannot be obtained. However, when the treatment temperature exceeds 100°C and the treatment time exceeds 2 h by using thiourea/ChCl DES, nanochitin can be obtained. Compared with thiourea/ChCl DES, *p*-toluenesulfonic acid/ChCl DES had lower treatment temperatures and shorter treatment times, which were mainly because the acidity of DES promoted the formation of ChNCs. In addition, in order to apply the prepared nanochitin, porcine pancreatic lipase (PPL) was successfully immobilized on ChNCs to form immobilized PPL (PPL@ChNC). The enzyme loading and activity recovery rate of PPL@ChNC were 35.6 mg/g and 82.5%, respectively. However, when nanochitin was replaced with chitin, the enzyme loading and activity recovery rate were only 30.6 mg/g and 75.8%, respectively, which indicated that the size of the carrier greatly affected the enzyme loading and activity. Moreover, the authors investigated the thermal stability, pH, and storage stability of PPL@ChNC. The PPL@ChNC showed higher activity at higher pH and higher temperature compared to free PPL. When the PPL@ChNC was stored at 4°C for 20 days, PPL@ChNC still maintained more than 94.5% of its initial activity, which demonstrated the high storage stability of this prepared biocatalyst. This study suggests that nanochitin has great potential in biocatalysis and biomedicine.<sup>13,122</sup>

Hong et al. successfully achieved efficient one-step production of acetylated and esterified ChNCs by using ChCl/ZnCl<sub>2</sub> DES (Figure 8A).<sup>116</sup> For comparison, the authors selected four DESs: ChCl/ZnCl<sub>2</sub>, ChCl/urea, ChCl/thiourea, and ZnCl<sub>2</sub>/urea, which reacted with acetic anhydride under the same conditions. After ultrasonic treatment (600 W, 45 min), only the chitin by ChCl/ZnCl<sub>2</sub> treatment showed uniform suspension, while others showed a flocculent structure, which suggested that the structure and composition of DESs determined the formation of nanochitin. Moreover, the addition of reactive reagents (acetic anhydride and acetic acid) to DESs enabled the one-step preparation of acetylated and esterified nanochitin. The prepared ChNCs exhibited a rod-shaped structure with a width of 20–80 nm and a length of 100–700 nm. This study indicated that using ChCl/ZnCl<sub>2</sub> DES alone was not sufficient to hydrolyze chitin, but it became an efficient solvent for catalyzing the hydrolysis reaction of chitin in the presence of acetic anhydride or acetic acid and promoted the surface functionalization of the prepared ChNCs. Because DESs are green solvents, this study provides a new approach to producing functional ChNCs.

As shown in Figure 8B, Shu et al. used FeCl<sub>3</sub>·6H<sub>2</sub>O/betaine hydrochloride DES to prepare ChNCs.<sup>117</sup> The synergistic action of Lewis acid and released Brønsted acid from betaine hydrochloride enabled efficient hydrolysis of chitin, which was combined with low-energy ultrasonication (320 W, 5 min) to prepare ChNCs. The authors investigated the effects of reaction time and reaction temperature on the yields and morphological size of ChNCs. When the reaction time was extended from 1 to 3 h and the reaction temperature was increased

**Table 3. A summary of studies on the preparation of nanochitin using DESs**

Chitin sources	HBA	HBD	HBA/HBD molar ratio	Reaction conditions	Post-treatment	Yield (wt %)	Dimension of ChNMs		Applications	Reference	
							Width (nm)	Length (nm)			
Crab shell	ChCl <sup>a</sup>	Thiourea	1:2	100°C, 2 h	Ultrasonication 40 min	84.0	20 ± 6	1000 ± 500	Controlled drug release	Mukesh et al. <sup>114</sup>	
Commercial chitin	ChCl	PTA <sup>b</sup>	1:1	95°C, 30 min	Nothing	NR <sup>k</sup>	26.6	293.4	Enzyme carriers	Cao et al. <sup>115</sup>	
Shrimp shell	ChCl	ZnCl <sub>2</sub>	1:2	90°C, 3 h 2.368 g Ac <sub>2</sub> O <sup>c</sup> 4.736 g Ac <sub>2</sub> O	Ultrasonication 600 W, 45 min	77.2	NR	NR	NR	NR	Hong et al. <sup>116</sup>
						61.6	30–80	200–700			
						55.2	NR	NR			
						73.8	NR	NR			
						59.0	30–60	100–650			
						54.0	NR	NR			
						55.4	NR	NR			
						58.8	20–60	150–500			
						53.8	NR	NR			
						62.0	20–50	100–400			
Shrimp shell	Bet <sup>e</sup>	FeCl <sub>3</sub> ·6H <sub>2</sub> O	1:1	70°C, 3 h	Ultrasonication 320 W, 5 min	88.5	4–18	120–530	Pickering emulsion stabilizer	Hong et al. <sup>117</sup>	
						86.7	NR	NR			
						85.2	NR	NR			
						86.5	4–18	120–310			
						88.5	4–22	160–440			
						87.1	NR	NR			
						83.2	NR	NR			
Crab shell	ChCl	Oa <sup>f</sup>	1:2	100°C, 1 h	Ultrasonication 1000 W, 30 min	79.5	NR	NR	NR	Yuan et al. <sup>118</sup>	
				100°C, 3 h		78.0	49 ± 8	530 ± 89			
		La <sup>g</sup>		100°C, 1 h		87.5	44 ± 11	367 ± 48			

(Continued on next page)

Table 3. Continued

Chitin sources	HBA	HBD	HBA/HBD molar ratio	Reaction conditions	Post-treatment	Yield (wt %)	Dimension of ChNMs		Applications	Reference
							Width (nm)	Length (nm)		
				100°C, 3 h		84.0	NR	NR		
		Ma <sup>h</sup>		100°C, 1 h		84.1	47 ± 10	257 ± 59		
				100°C, 3 h		82.3	NR	NR		
		Ca <sup>i</sup>		100°C, 1 h		82.3	42 ± 10	306 ± 58		
				100°C, 3 h		77.9	NR	NR		
		Ma <sup>j</sup>		100°C, 1 h		79.6	NR	NR		
				100°C, 3 h		79.5	49 ± 10	670 ± 92		
Shrimp shell	ChCl	Ma	1.5:1	80°C overnight	Rotor-stator colloidal mill 30 min	NR	15–30	100–500	Film reinforcing filler	Nguyen et al. <sup>119</sup>

<sup>a</sup>ChCl: Choline chloride.

<sup>b</sup>PTA: P-toluenesulfonic acid.

<sup>c</sup>Ac<sub>2</sub>O: Acetic anhydride.

<sup>d</sup>AcOH: Acetic acid.

<sup>e</sup>Bet: Betaine hydrochloride.

<sup>f</sup>Oa: Oxalic acid dihydrate.

<sup>g</sup>La: Lactic acid.

<sup>h</sup>Ma: Malonic acid.

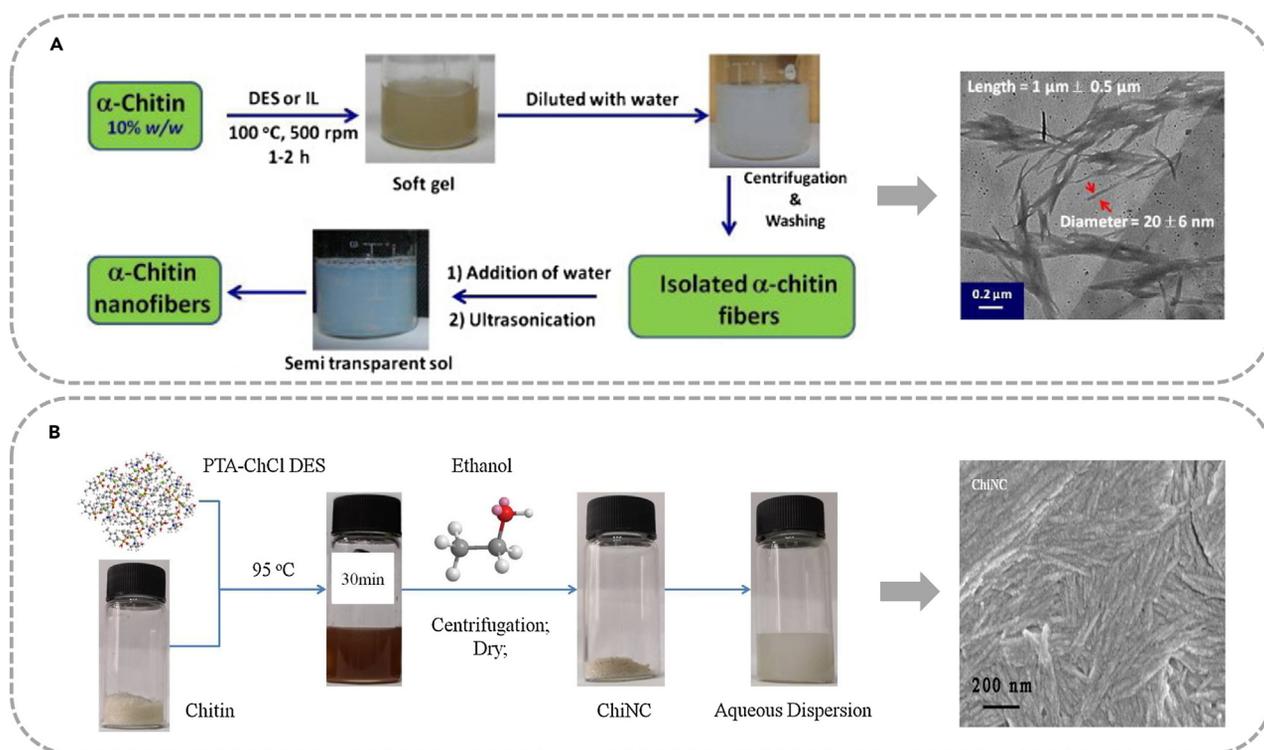
<sup>i</sup>Ca: Citric acid monohydrate.

<sup>j</sup>Ma: Malic acid.

<sup>k</sup>NR: Not reported.

from 70°C to 100°C, the yields of ChNCs showed a decreasing trend, and the appearance of ChNCs became more and more transparent, which might be due to the excessive hydrolysis of chitin molecular chains at higher temperature and longer reaction time. Moreover, the yields of ChNCs were above 85% under all treatment conditions. The transmission electron microscopy images showed that the average diameter of all ChNCs was around 10 nm, while the length of ChNCs depended on the treatment conditions. For example, the length distribution of ChNCs prepared by DES70-3 (70°C, 3 h), DES100-1 (100°C, 1 h), and DES100-3 (100°C, 3 h) were 120–530, 160–440, and 120–310 nm, respectively. Therefore, the increase in treatment time and reaction temperature promoted the hydrolysis of chitin, and the length distribution of ChNCs became more uniform. Due to the excellent stability of the prepared ChNCs suspension, ChNCs were successfully used as the stabilizer in the preparation of soybean O/W Pickering emulsion. The authors investigated the droplet size distribution as well as the storage stability of emulsion with different O/W mass ratios (20/80, 30/70, 40/60, and 50/50) and different ChNCs concentrations (0.1, 0.3, and 0.5 wt %). Under all conditions, the emulsion formed a smooth and creamy structure and remained highly stable without phase separation for 24 h. The authors determined the diameter distribution of emulsion droplets by using the laser diffraction particle size analyzer. The results showed that when the concentration of ChNCs was fixed, the average diameters of emulsion droplets were very similar regardless of the oil-water mass ratio. This indicated that ChNCs were effectively adsorbed at the oil/water interface and ChNCs acted as the intermediate medium to prevent oil droplet aggregation and stabilize the emulsion. When the oil-water mass ratio was fixed, the droplet diameter gradually increased with the decrease of ChNCs concentration, which was attributed to the low loading of ChNCs and the decrease of ChNCs coverage at the oil/water interface. Therefore, ChNCs can be used as green and non-toxic additives in food, cosmetics, and other functional products.<sup>123–125</sup>

In another study, Yang et al. prepared ChNCs using five different DESs, which consisted of ChCl and organic acids (oxalic acid dihydrate, lactic acid, malonic acid, citric acid monohydrate, and malic acid) (Figure 9A).<sup>118</sup> Chitin was treated by five DESs followed by ultrasonic treatment (1000 W, 30 min), all of which produced high transmittance and stable suspension of ChNCs. Moreover, the yields of all ChNCs were in the range of 78%–87.5%, which were similar to or better than conventional inorganic acid hydrolysis. In the preparation process of ChNCs, acidic DESs had dual effects, which promoted the hydrolysis of chitin and served as the acylation reagent, thereby blocking the hydrogen bonding connection between chitin molecular chains and improving the colloidal stability of ChNCs suspension. Further research showed that the selection of HBDs affected the morphology and size of prepared ChNCs. The length of ChNCs prepared from malonic acid was the shortest, indicating that malonic acid can easily penetrate into the amorphous region of chitin and promote hydrolysis reactions. ChNCs prepared from lactic acid showed more needle-like structures and exhibited less aggregated structures in aqueous solution, which was mainly attributed to the fact that lactic acid had the highest pKa value (3.86) among all the organic acids and effectively removed amorphous region in chitin. The atomic force microscopy showed that ChNCs had an average diameter of 42–49 nm and an average length



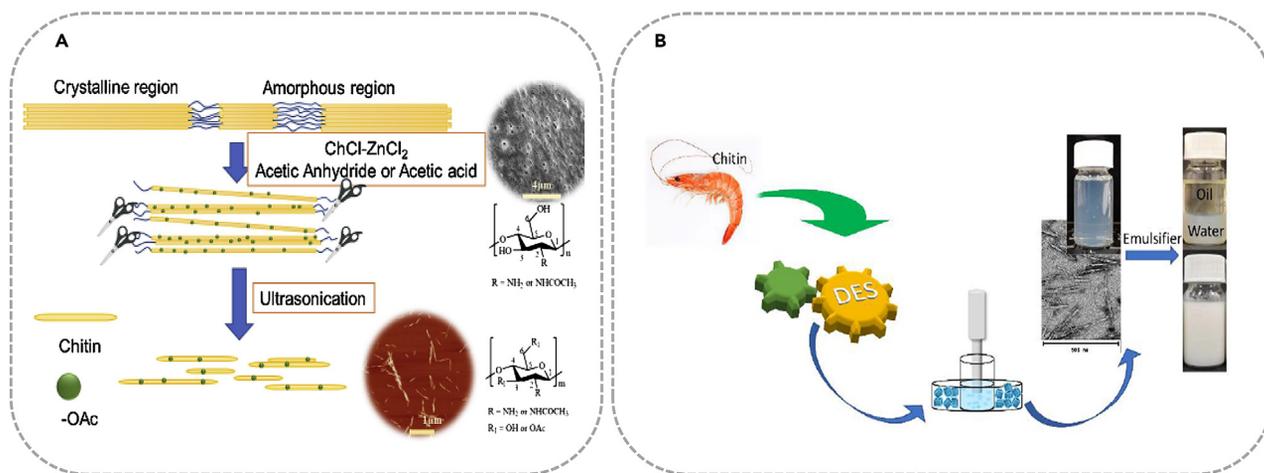
**Figure 7. ChNFs prepared by ChCl/thiourea DES**

Schematic illustration and TEM photo of ChNFs prepared by choline chloride/thiourea DES (A). Copyright 2014 Elsevier. Schematic illustration and SEM photo of ChNCs prepared by choline chloride/*p*-toluenesulfonic acid DES (B). Copyright 2019 Elsevier.

of 257–670 nm. ChNCs with high thermal stability can be used as potential reinforcing fillers for thermoplastics, while stable nanochitin suspension can be used as an emulsifier.<sup>126–128</sup>

Recently, Nguyen et al. used ChCl/malic acid DES to prepare ChNCs with mild mechanical treatment (rotor-stator colloid milling) (Figure 9B).<sup>119</sup> Compared with acid hydrolysis (Ac-ChNCs), DES-ChNCs and Ac-ChNCs were similar in length (100–500 nm), but the width of DES-ChNCs (15–30 nm) was smaller than that of Ac-ChNCs (20–65 nm). In this study, the authors proposed the concept that the amorphous structure in chitin was not completely removed after DESs treatment and the residual amorphous chitin attached to the surface of the nanochitin. This meant that the DESs treatment mainly swelled the amorphous region or removed part of the amorphous region in chitin, causing a certain degree of damage to the hydrogen bonds in the crystalline region of chitin. However, during the acid hydrolysis process, the amorphous region in chitin was basically completely removed due to the strong acidity. To validate this idea, the authors investigated the colloidal stability of two ChNCs suspensions. Through the measurement of fluid dynamics diameter and zeta potential, the results showed that the DES-ChNCs suspension exhibited higher stability in a larger pH range and higher ion strength, which may be related to the stereostability effect caused by the presence of amorphous chitin. In addition, the authors investigated the effect of the concentration of ChNCs on stability. The results showed that the DES-ChNCs suspension can remain stable above 2.0 wt % at pH = 6 without phase separation, forming a self-supporting elastic network. In contrast, Ac-ChNCs suspension required a concentration of 10 wt % or more to remain stable. This phenomenon suggested that due to the short-range steric repulsion, DES-ChNCs can form looser and softer aggregate, which formed the self-supporting network at lower concentration and prevented deposition. Moreover, composite films were prepared by mixing ChNCs and PVOH. Compared with Ac-ChNCs composite films, DES-ChNCs composite films were smoother and more uniform, with better thermal stability, slower weight loss rate, and higher weight loss temperature. Due to the better compatibility between DES-ChNCs and PVOH matrix, DES-ChNCs composite films had stronger tensile strength, elongation at break, and Young's modulus. Therefore, ChNCs prepared by using DESs have great application prospects in the field of functional materials.<sup>129–131</sup>

In summary, the preparation of nanochitin by DESs is a feasible pathway. However, there are few studies on the preparation of nanochitin using DESs and the studies that can be found are the ones mentioned previously, so this field needs to be further studied through a lot of work in the future. At present, HBAs used for the preparation of nanochitin are mainly composed of choline chloride and betaine, whereas HBDs are mainly composed of thiourea, inorganic salts, and organic acids, which suggest that the composition of DESs is very important for the formation of nanochitin. DESs are classified as acidic and non-acidic, and can be used to prepare ChNCs and ChNFs, respectively. When non-acidic DESs are used to treat chitin, the amorphous regions can only swell, so the amorphous regions cannot be removed to obtain ChNFs with a high aspect ratio. In acidic DESs, small-molecule acids (e.g., lactic acid, malonic acid) can penetrate into the amorphous regions



**Figure 8. ChNFs prepared by ChCl/ZnCl<sub>2</sub> DES**

Schematic illustration and AFM photo of ChNCs prepared by choline chloride/ZnCl<sub>2</sub> DES (A). Copyright 2019 Elsevier. Schematic illustration of ChNCs prepared by betaine hydrochloride/ferric chloride hexahydrate DES (B). Copyright 2020 MDPI.

of chitin, thus effectively removing the amorphous regions and facilitating the hydrolysis reaction, which results in the formation of ChNCs with low aspect ratios. Moreover, acidic DESs can also act as acylating reagents, thereby blocking the hydrogen bonding connections between the chains of chitin molecules and promoting the formation of ChNCs. Recently, Danbi et al. used acidic DESs to prepare chitin nanofibers while the nanofibers could be esterified.<sup>132</sup> DESs with strong dicarboxylic acids promoted nanofibrillation by uniformly dispersing the substrate, swelling tightly packed fibers, and facilitating acidity-dependent esterification leading to spatial and electrostatic repulsions.

In addition, the combination of DESs treatment and auxiliary fibrillation techniques (ultrasound or colloid milling) is necessary to successfully prepare nanochitin. The main function of DESs is to promote the hydrolysis of chitin, while the auxiliary fibrillation techniques are to peel off the chitin fibers through strong physical interaction, which can ultimately form nanochitin. Interestingly, when HBD is *p*-toluenesulfonic acid, nanochitin can be obtained without the need for additional fibrillation techniques. *p*-toluenesulfonic acid is an organic strong acid, so the DES has strong acidity and strong hydrogen bonding acidity, which can fully hydrolyze chitin and peel off chitin fibers to form nanochitin under a high-temperature environment. Therefore, the pH and KT parameters of DESs can be adjusted by controlling the types and proportions of HBAs and HBDs, which can efficiently prepare nanochitin in one step.

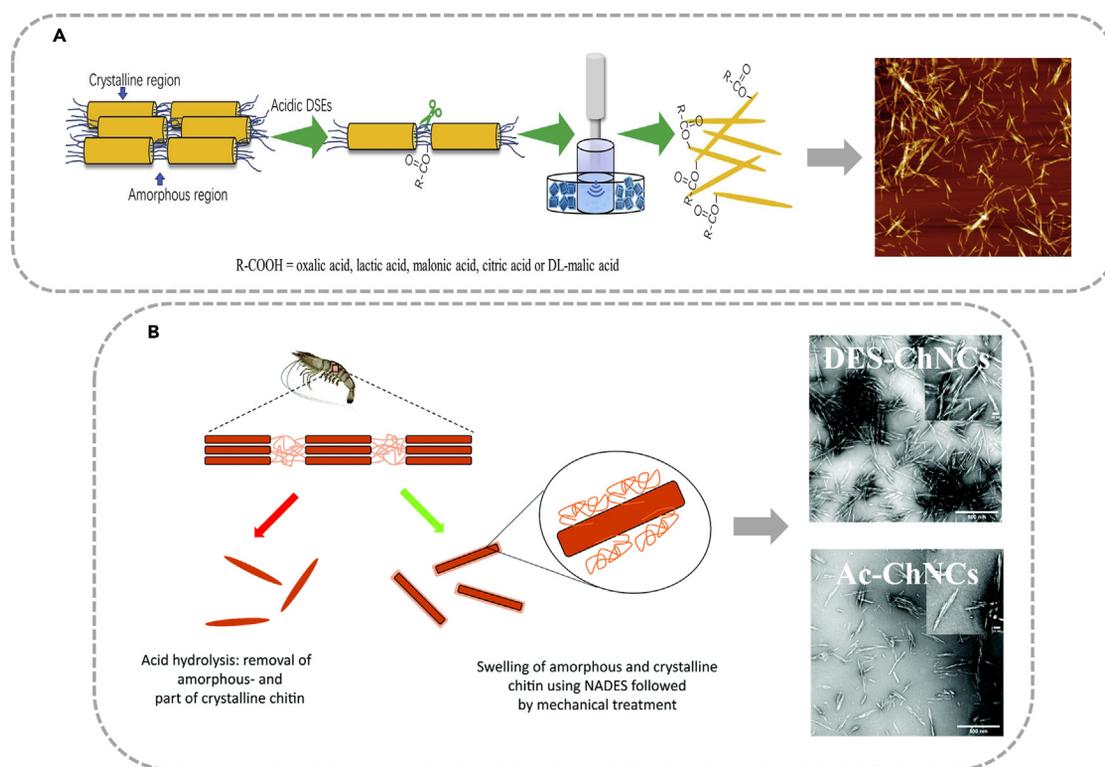
Due to its many superior properties, nanochitin prepared by traditional methods has been widely used. In this review, nanochitin prepared by DESs has also been applied in multiple fields, such as controlling drug release, enzyme carriers, Pickering stabilizers, and enhancers for functional materials. As the new generation of green solvents, DESs have a lot of advantages, such as low toxicity, recyclability, and environmental friendliness. Therefore, compared to traditional methods, nanochitin prepared by DESs will have more extensive applications in the future.

## CONCLUSION AND OUTLOOK

Nanochitin has a lot of advantages such as high and active surface area, excellent mechanical toughness, biocompatibility, biodegradability, and renewability, which make it an ideal raw material with high application potential. At present, nanochitin has been successfully prepared into different functional materials, such as Pickering stabilizer, film, and gel. However, considering the many shortcomings of traditional methods for preparing nanochitin, such as high energy consumption, high cost, and high pollution, DESs are considered the ideal method to replace traditional methods for preparing nanochitin. DESs have been applied in the fields of dissolution and extraction of chitin, so researchers are attempting to use DESs to prepare nanochitin.

At present, there are few studies on the preparation of nanochitin using DESs, so this field is in its early stages. Analyzing the published research, it can be seen that there are a few types of DESs to prepare nanochitin. HBAs mainly include ChCl and Bet, while HBDs mainly include thiourea, inorganic salts, and organic acids. In addition, in order to successfully prepare nanochitin, DESs treatment generally needs to be combined with auxiliary fibrillation techniques (ultrasound or colloid milling). The main function of DESs is to promote the hydrolysis of chitin, while auxiliary fibrillation techniques use strong physical interaction to peel off chitin fibers and form nanochitin. Moreover, nanochitin prepared by DESs has also been successfully applied, such as controlling drug release, enzyme carriers, Pickering stabilizers, and enhancers for functional materials. DESs have great attractive advantages, so it can be foreseen that nanochitin prepared by DESs will be more widely used in the future.

Based on the aforementioned analysis, the possible future development directions in this field are proposed. Firstly, there are few types of DESs to prepare nanochitin, and the impacts of the structure and composition of DESs on the yield, morphology, and surface chemical structure of nanochitin are unknown. In fact, the types and properties of HBAs and HBDs significantly affect the properties of DESs. Therefore, it is necessary to continuously try different HBAs and HBDs through a large number of comparative experiments to obtain the optimal DESs system. From the comparison process, the impacts of the structure and composition of DESs on the prepared nanochitin can be summarized,



**Figure 9. ChNFs prepared by ChCl/OA DES**

Schematic illustration and AFM photo of ChNCs prepared by choline chloride/organic acids DESs (A). Copyright 2020 Elsevier. Schematic illustration and TEM photo of ChNCs prepared by choline chloride/malic acid DES (B). Copyright 2022 Royal Society of Chemistry.

which can provide the theoretical basis for the industrial production of nanochitin prepared by DESs. Secondly, at present, DESs require a combination of auxiliary fibrillation techniques (ultrasound or colloid milling) to prepare nanochitin, which still has a larger size. Therefore, other more efficient fibrillation techniques (such as microfluidization and high-pressure homogenization) can be considered in order to prepare nanochitin of ideal size. In addition, when HBD is *p*-toluenesulfonic acid (an organic strong acid), nanochitin can be obtained without the need for additional fibrillation techniques. This can give us important insights that by controlling the types and ratios of HBAs and HBDs to adjust the pH and KT parameters of DESs, it is possible to achieve the preparation of nanochitin in only one step. Thirdly, at present, DESs treat chitin through traditional heating methods (water or oil bath). Microwave heating is an efficient heating method that has the characteristics of fast heating speed and low heat loss, and can significantly shorten treating time, improve productivity, and reduce costs. Therefore, in future research processes, microwave heating can be used to prepare nanochitin. Fourthly, during the preparation of nanochitin, reagents that can react with functional groups in chitin are added to the system. This can prepare functionalized nanochitin, thereby avoiding subsequent modification of nanochitin, which will be one of the hot research directions in the future. Fifthly, recyclability is one of the important advantages of DESs, but current research studies have not yet mentioned it. Generally speaking, the recycling and utilization of DESs involve multiple steps such as centrifugation, filtration, and rotary evaporation, which consume a lot of energy and are not environmentally friendly. Therefore, it is very important to find an efficient recovery method in the future, which is conducive to the large-scale production of nanochitin prepared by DESs.

### Limitations of the study

At present, the preparation of nanochitin by DESs is at an early stage. This review summarizes and outlooks the field of preparation of nanochitin by DESs based on the published literature.

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### AUTHOR CONTRIBUTIONS

L.Z. reviewed the literature and wrote the manuscript. B.Z. reviewed the literature. L.Y. revised the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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