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REVIEW

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Preparation, characterization and application in environmental protection of low-molecular-weight chitosan: a review

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Abstract

Chitosan is a biopolymer derived from chitin, which is the second most abundant and renewable polymer in nature after cellulose. Low-molecular-weight chitosan (LMWC) is the degradation product of chitosan through depolymerization. Compared with conventional chitosan, LMWC is considered as one of the most promising functional materials due to its characteristics of lower polymerization degree, lower viscosity, good water solubility, reactivity and degradability. This review focused on the preparation and characterization methods as well as the application in environmental remediation of LMWC. The three main methods of LMWC preparation including chemical, physical and enzymatic methods were summarized and compared in this paper. The mechanism, advantages and disadvantages of various preparation methods were also discussed. In addition, the applications of LMWC in environmental fields such as water treatment, soil remediation and air purification were briefly reviewed. With the continuous progress of science and technology and the improvement of environmental awareness, it is believed that more efficient, economical and environmentally friendly chitosan degradation methods will be developed, providing strong support for the wide application of LMWC in the field of environmental protection.

Keywords Chitosan, Degradation, Low molecular weight, Environmental remediation

1 Introduction

Chitin is the second most abundant polysaccharide polymer in nature after cellulose, which mainly is derived from the exoskeletons of crustaceans and insects, bacteria, fungi and mushrooms, and is an important natural renewable biomass resource [1, 2]. Chitosan is a partially or wholly deacetylated derivative of chitin, and N-acetylation degree (DA) is used to distinguish chitin from chitosan [3]. Chitosan is a weak base and the pK_a value of the amino group on the molecular chain is 6.3 ~ 7.2 [4]. Therefore, chitosan is soluble in acetic acid, formic acid, citric acid solution and hydrochloric acid, perchloric acid and phosphoric acid solution, but insoluble in water, organic solvents and alkaline solutions [5]. Due to the excellent physical and chemical properties such as low toxicity, biocompatibility and biodegradability, chitosan has been widely used in agriculture, aquaculture, food and nutrition, biomedicine, water treatment, soil remediation, beauty, hygiene and personal care, textile and paper industry, packaging, biotechnology, chemistry and catalysis, chromatography, photography and other fields [6–9].

It is well known that chitosan has poor water solubility, which is mainly due to the strong interaction between amino and hydroxyl groups on its molecular chain, which leads to the easy formation of hydrogen bonds between

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molecules, and thus reduces its solubility in water [10]. Particularly, the solubility of chitosan is also related to the degree of deacetylation, the degree of polymerization, the molecular weight, the degree of neutralization of amine group, the ionic strength of solvent, the pH of chitosan solution, the concentration of polymer, the distribution of N-acetylglucosamine and glucosamine residues along the main chain of macromolecular chains [11–13]. In order to improve the water solubility and broaden the application range of chitosan, reducing the molecular weight of chitosan and generating chitosan derivatives are two commonly used modification methods [14, 15]. The molecular weight of chitosan can be reduced by acid hydrolysis, enzymatic hydrolysis, mechanical method or chemical oxidation method to reduce the molecular size of chitosan and improve its solubility in water [16]. Chitosan can be divided into low-molecular-weight (LMWC, < 100 kDa), medium-molecular-weight (MMWC, 100–1000 kDa) and high-molecular-weight (HMWC, > 1000 kDa) chitosan [17]. Compared with HMWC, LMWC has more excellent physical and chemical properties, such as low viscosity, good water solubility, good biocompatibility, biodegradability, moisture absorption, chelation and good anti-tumor, antibacterial, anti-inflammatory and other biological activities [16]. Therefore, it is urgent to effectively reduce the molecular weight of conventional chitosan to prepare and obtain LMWC so as to improve the solubility of chitosan in aqueous solution and broaden the application range of chitosan. At present, the preparation methods of LMWC are various, but each method is accompanied by its own limitations and shortcomings. More importantly, the specific route and the degradation mechanism of LMWC have not been fully clarified, which hinders the further optimization and innovation of the preparation technology to a certain extent.

In view of the above background, this paper aims to review the latest research progress of preparation methods of LMWC, in-depth analysis of the advantages and disadvantages of various methods, explore the similarities and differences in their degradation mechanisms, and look forward to future research directions. It is hoped that this review can provide valuable reference information for researchers, promote the in-depth development of preparation technology for LMWC and its application in more fields, so as to contribute to the realization of sustainable development goals. We also call on more scholars and scientific research institutions to devote themselves to research in this field, jointly overcome technical difficulties, and promote innovation and breakthroughs in the preparation technology of LMWC.

2 Preparation of LMWC

Chitin is composed of 2-acetamido-2-deoxy-D-glucose (GlcNAc) as the basic unit by β-(1–4) glucoside bonds [18, 19], as shown in Fig. 1. The production process of chitosan from chitin (Fig. 1) consists of four steps [20]: (1) deproteinization, heating at 60–100 °C for 1–72 h

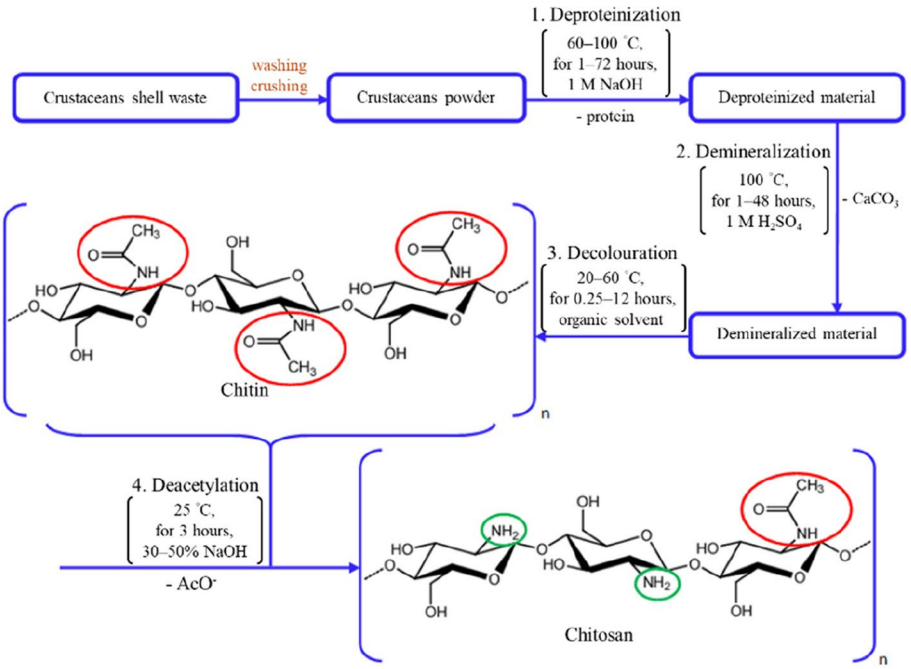

Fig. 1 Diagram of the preparation process from chitin to chitosan [20]

in the presence of 0.125–2.5 M NaOH, Na_2CO_3, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_3; (2) Demineralization, treatment in HCl, HNO_3, H_2SO_4, CH_3COOH and HCOOH at 100 °C for 1–48 h; (3) decolorization, dissolved in organic solvents, bleached with KMnO4, heated at 20–60 °C for 0.25–12 h; and (4) Deacetylation, 30–50% NaOH solution. Chitosan is a natural linear polysaccharide formed by the β-(1–4) glucosamine bond, which is relatively unstable and sensitive [21]. Therefore, HMWC can be degraded through the break of the glucoside bond, resulting in the shortening of the molecular chain and obtaining of LMWC [22]. At present, the main degradation methods of chitosan are chemical, physical and enzymatic methods [22, 23].

2.1 Chemical degradation method

Chemical degradation method is one of the most commonly used methods for polysaccharide degradation in industrial production, and is also the first choice for degradation of chitosan [15, 24, 25]. Chemical degradation methods mainly include acid hydrolysis method and oxidation degradation method [26, 27]. Chemical degradation of chitosan is a complex and multi-factorial process involving a variety of chemical and physical factors, which can significantly affect the degradation rate, efficiency and product properties. In order to gain a deeper understanding of this process, several key factors were selected for detailed discussion. In practical application, it is necessary to consider the interaction between various factors to optimize the degradation conditions and obtain the ideal degradation products.

2.1.1 Acid hydrolysis method

Acid hydrolysis is a mature and simple method for preparing LMWC. The acid hydrolysis of chitosan consists of two processes, namely the hydrolysis of the acetyl group and the hydrolysis of the glycoside bond on the main chain, as shown in Fig. 2 [28]. The hydrolysis rate of acetyl group is basically the same as that of glycoside bond on the main chain under dilute acid conditions, however, the hydrolysis rate of glycoside bond (k_2) is 10 times that of acetyl group (k_1) in concentrated acid solutions [29]. Common acidic reagents for chitosan hydrolysis include hydrochloric acid, sulfuric acid, nitric acid, nitrous acid, phosphoric acid, hydrofluoric acid, acetic acid [30] and organic acids such as acetic acid, lactic acid, citric acid, succinic acid and tartaric acid [31].

(1) Acid concentration is an important parameter affecting the hydrolysis rate of chitosan. In general, the acid concentration is proportional to the hydrolysis rate. For example, chitosan with moderate molecular weight (199 kDa) was obtained by hydrolyzing chitosan with 5% acetic acid for 30 min. The molecular weight of chitosan hydrolyzed at 1% and 3% concentrations for 30 min was 593 and 282 kDa, respectively [17]. Results showed that the degradation rate of chitosan increased with the increase of organic acid concentration. Generally, the polymerization degree and molecular weight of chitosan products decreased with the increase of acid concentration, reaction time and reaction temperature, while the yield of chitosan products showed a decreasing trend.

(2) The reaction temperature is another parameter affecting the rate of acid hydrolysis. Il'ina and Varlamov [32] studied the hydrolysis properties of high molecular weight (726 kDa) and low molecular weight (28 kDa) chitosan in lactic acid solution. Results showed that temperature had a significant effect on the acid hydrolysis of high molecular weight chitosan, and the hydrolysis rate increased by 25–50% with the increase of temperature. Chitosan was hydrolyzed with 5% acetic acid at 30, 40, 50 and 60 °C for 90 min, and the molecular weights of the degradation products were 383, 323, 224 and 166 kDa, respectively [17]. Therefore, increasing the temperature is conducive to the acid hydrolysis of chitosan.

(3) The deacetylation degree (*DD*) is also a key factor that influences acid hydrolysis of chitosan. The solubility, biodegradability, aggregation, and pK_a value of chitosan depend on the ratio between N-acetylated glucosamine and glucosamine units. The main technical methods for determining the values of DD include colloidal/conductometric/potentiometric titration, UV-Vis spectrophotometry, infrared spectroscopy, elemental analysis, thermal analysis with differential scanning calorimetry and nuclear magnetic resonance [33, 34]. Chitosan is more prone to hydrolysis in the presence of acetyl amino group, because the salting of amino group in the presence of free amino group at the C2 position reduces the charge density of oxygen on the glycoside bond, resulting in a difficult hydrolysis reaction. Therefore, the higher the degree of acetylation of chitosan, the faster the rate of acid hydrolysis. In other words, the DD is inversely proportional to the rate of acid hydrolysis. The lower the DD of chitosan, the higher the rate of acid hydrolysis. The rate constant of hydrolysis of glycosidic acid in chitosan depends on the relationship between two adjacent sugar residues [29]. The degradation rate constant between two N-acetylglucosamine residues or between N-acetylglucosamine and glucosamine is much higher than that between glucosamine and N-acetylglucosamine or two glucosamine residues. This may be due to the following two effects: (i) protonation of the positively charged amino group adjacent to the glycoside bond inhibits hydrolysis; and (ii) the acetyl amino group adjacent to the glycoside bond can promote hydrolysis.

In summary, acid hydrolysis method is a main method for large-scale production of LMWC, which is simple,

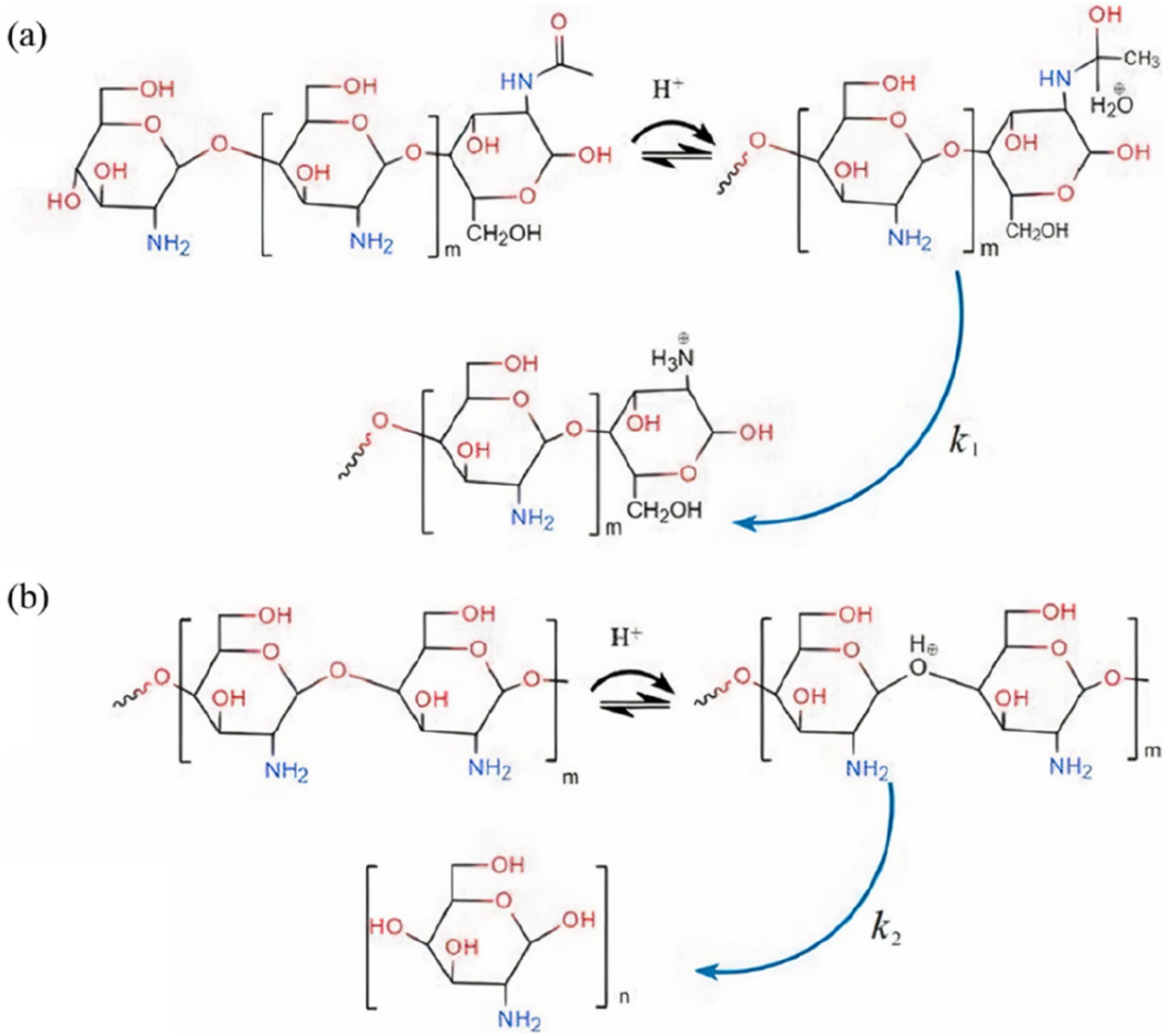

Fig. 2 Mechanism of acid hydrolysis of chitosan [28, 29]: the hydrolysis of the acetyl group (a) and the hydrolysis of the glycoside bond on the main chain (b)

mature and low cost. However, this method also has certain negative effects and disadvantages [35]: (i) acid degradation of chitosan usually requires a drastic reaction, which is due to the inhibition of acid penetration in the crystalline zone of chitosan particles, resulting in the hydrolysis process requiring a higher concentration of acid reagents and a greater acid load; (ii) the reaction process is not easy to control, so that the molecular weight distribution of the product is wide, and the generated byproducts are difficult to separate; and (iii) strong acids may cause degradation of glucosamine, resulting in changes in the structure and properties of the end product. At present, an effective solution to reduce the negative effects of acid hydrolysis is to combine acids with other reagents (such as ionic liquids) to enhance the hydrolysis efficiency of chitosan. Zhang et al. [36] investigated the co-hydrolyzation of chitosan with imidazolyl ionic liquid and hydrochloric acid. Results showed that the total reducing sugar yield of chitosan depolymerized by ionic liquid combined with hydrochloric acid solution was higher than that of hydrochloric acid solution alone. Moreover, the high solubility of chitosan in ionic liquid is also conducive to promoting the hydrolysis of chitosan, and it has been proved that imidazolyl ionic liquid is a good catalyst for chitosan hydrolysis [37]. Table 1 compared different LMWC products obtained by acid

Table 1 Comparison of LMWC products obtained by acid hydrolysis method

Acid	T (°C)	Time	M_w/M_v (kDa)	Ref.
5% HAc	60	90 min	M_v,166	[17]
1% lactic acid	8	10 d	M_w, 3.0	[32]
1% lactic acid	22	20 d	M_w, 2.3	[32]
1% lactic acid	37	30 d	M_w, 1.8	[32]
85% H_3PO_4	80	35 d	M_w, 20	[38]
1% HAc-10 M HCl	105	6 h		[39]
(4.5:1.5 M) HCl-H_3PO_4	110	24 h		[17]

(M_v the viscosity-average molecular weight, M_w weight-average molecular weight, Dalton (Da) is the standard unit that is used for indicating molecular weight)

hydrolysis method. As can be seen from the Table 1, the acid degradation of chitosan required a longer time and a higher temperature, which were also important reasons limiting the wide application of this method.

2.1.2 Oxidative degradation method

Oxidative degradation method is a commonly used method to degrade polysaccharide polymers by chemical oxidants. The commonly used oxidants mainly include hydrogen peroxide (H_2O_2), ozone (O_3), sodium hypochlorite (NaClO), potassium persulfate ($K_2S_2O_8$), sodium nitrite ($NaNO_2$) and chlorine gas (Cl_2) [20]. In particular, oxidative degradation with hydrogen peroxide is a commonly used method for the industrial preparation of LMWC, which has the characteristics of simple process, high efficiency and low production cost. Hydrogen peroxide can depolymerize chitosan by breaking the 1,4-β-D-glucoside bond on the sugar chain, and then reduce the molecular weight of chitosan. Tian et al. [40] used hydrogen peroxide to degrade chitosan at different temperatures and pH values, and proposed the degradation mechanism.

(1) Temperature is the first important factor affecting the oxidative degradation of chitosan. The low temperature will result in the incomplete reaction, and the higher temperature (> 50 °C) is conducive to improving the degradation reaction rate [27]. Additionally, increasing the temperature is conducive to the decomposition of hydrogen peroxide to produce active free radicals, which is conducive to the reduction of molecular weight of chitosan. However, the temperature is too high (> 65 °C), Maillard side reactions will occur, resulting in darkening of product color or changes in product properties [40, 41].

(2) Oxidant concentration is the second factor affecting the degradation efficiency, which is directly related to the degradation rate of chitosan and the yield of LMWC.

Table 2 Comparison of LMWC products obtained by oxidative degradation method

Oxidizing agent	T (°C)	Time	M_W/M_V (kDa)	Ref.
2% H_2O_2	Room temperature	16 ~ 24 d	M_W, 2	[43]
30% HNO_2	90	1 h		[44]
H_2O_2	Room temperature	180 min	M_v, 889	[45]
0.3% H_2O_2	Room temperature	12 h	M_v, 65	[46]
2.0 M H_2O_2	60	4 h	M_V, 11	[40]
H_2O_2/AA (5.7:1 mM)	50	8 h	M_W, 6.6	[47]
12% O_3	Room temperature	20 min	M_v, 104	[48]
0.3% H_2O_2	Room temperature	12 h	M_W, 140	[42]

It is generally believed that the concentration of hydrogen peroxide is proportional to the degradation rate and yield. However, too high concentration of hydrogen peroxide will destroy hydroxyl radicals, but reduce the reaction efficiency and yield. Similar to acid hydrolysis, reaction time is a third factor that affects the degradation efficiency and product properties. Minh et al. [42] used hydrogen peroxide to decapsulate chitosan in solid state, and found that the molecular weight and viscosity of chitosan decreased with the extension of reaction time within the first 15 h, and the molecular weight and viscosity of chitosan remained unchanged after 3 h. Du et al. [25] used hydrogen peroxide to prepare water-soluble chitosan, and the results showed that with the increase of reaction time, the yield of the product initially increased and then decreased. This is mainly due to the fact that oxidants must penetrate into the reticular structure of chitosan during the degradation process, so the maximum value of degradation occurs in the early stage of the reaction. As the reaction time continues to increase, ring-opening of sugar units may occur, and the color of the product will be deepened. The preparation conditions of LMWC obtained by oxidative degradation method are shown in Table 2.

At present, the hydrogen peroxide degradation method has been widely used and studied because it is easy to implement, available in the market of chemical agents and relatively low cost. However, if hydrogen peroxide is used alone to degrade chitosan, the degradation efficiency is relatively low. Moreover, hydrogen peroxide degradation method also has other problems worthy of further attention and research: (i) for example, the deamination and ring-opening of sugar units may occur, changing the chemical structure and basic properties of products; (ii) the use

of a large number of chemicals may cause environmental pollution; (iii) the degradation process produces harmful by-products; and (iv) the complex degradation composition makes it difficult to separate and purify the product.

2.2 Physical degradation method

Physical degradation method refers to the degradation technology that uses radiation, ultrasound or moving mechanical parts to cut polymer chains, mainly including ultrasonic method, gamma ray method, X-ray method, ultraviolet method and microwave radiation method [22, 49]. Ultrasonic method is the most commonly used physical method to change the molecular weight and deacetylation degree of chitosan. When any heterogeneous material is exposed to ultrasonic waves with the intensity of up to 100 Hz, the structure of the material can change due to the adiabatic implosion of the resulting microbubbles. The cavitation effect when the microbubble burst makes the local temperature increase instantaneously (>5000 K), accompanied by shear expansion between bubbles. When the frequency exceeds 100 kHz, the system generates hydroxyl radicals, which in turn causes the polymer to decompose. Furthermore, ultrasonic degradation of chitosan is mainly driven by mechanical force, and the degradation rate is usually proportional to the molecular weight. At the same time, the degradation rate is significantly correlated with factors such as ultrasonic intensity, solution temperature, chitosan deacetylation degree, chitosan concentration and ionic strength [50]. Kim et al. [51] used gamma ray radiation to degrade chitosan and believed that the degradation mechanism was as follows:

$$R - H \xrightarrow{\gamma} H \cdot (C4 - C6) + H\cdot$$

$$R - H + H\cdot \rightarrow R \cdot (C4 - C6) + H_2$$

$$R \cdot C1,\ C6) \rightarrow F_1 \cdot + F_2 \text{ (Scission)}$$

$$R - NH_2 + H\cdot \rightarrow R \cdot (C2) + NH_3$$

where, R-NH_2 represented the chitosan macromolecule, R·(Cn) represented the chitosan molecule linked to the carbon atom at the Cn position, and $F_1\cdot$ and F_2 represented the molecular fragments after the break of the main chain.

The physical degradation method has a high degradation rate and uses no or very few additional chemicals, so it is relatively efficient and environmentally friendly. However, the physical degradation method also has some disadvantages: (1) the reaction rate is fast and difficult to control; (2) the distribution range of molecular weight of degradation products is wide; and (3) the degradation process requires special equipment with higher prices. Based on this, scholars proposed to combine physical methods with chemical methods or biological methods to synergistically degrade chitosan, so as to learn from each other and minimize negative effects and costs as much as possible. Li et al. [52] firstly used microwave-induced plasma desorption/ionization (MIPDI) technology to degrade chitosan and prepare LMWC. Results showed that the [·OH] radical content of MIPDI was the most abundant at the air-liquid interface, and the chitosan with molecular weight of 540 kDa could be degraded into soluble chitosan (≤10 kDa), and the yield of LMWC could reach 61% within 60 min. Savitri et al. [31] studied the ultrasonic treatment of chitosan at different concentrations of acetic acid (0.2 −1% v/v) for 30 min and 120 min at 40 and 60 °C, respectively. The results showed that the molecular weight of chitosan decreased with the increase of acetic acid concentration during ultrasonic treatment. The results also show that the method can produce a large amount of LMWC even at a very low acetic acid concentration. Table 3 illustrated the results and preparation conditions of LMWC products obtained by some physical methods.

Table 3 Comparison of LMWC products obtained by physical degradation method

Type	Conditions	Solvent/gas	T (°C)	Time (min)	M_w (kDa)	Ref.
plasma	100 W	nitrogen	Room temperature	5	41.0	[53]
ultrasound		0.8% HAc	60	30	137.9	[31]
ultrasound		0.5% HAc	60	30	221.8	[31]
ultrasound		0.5% HAc	40	30	251.9	[31]
γ ray	10 kGy	0.1 M HAc		20–180	0.97–67	[54]
γ ray	6 kGy	water			79.2	[55]
microwave	2.46 GHz	0.1 M HAc		20	30	[56]
microwave		0.1 M HCl		19	12.6	[57]

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis refers to the hydrolysis of high molecular weight chitosan with various enzymes to obtain LMWC. The enzymes that hydrolyze the glycoside linkages in carbohydrates are called glycosyl hydrolases, including non-specific enzymes (cellulase, lipase, pectinase, papain, protease, etc.) or specific enzymes (chitinase, chitanase and lysozyme). The process of polysaccharide hydrolysis by glycosylase mainly follows two mechanisms: retention mechanism and reversal mechanism [58, 59], as shown in Fig. 3. In the retention mechanism, one residue acts as a nucleophile while the other provides protons to the leaving group, resulting in the hydrolyzation of glycosylase by water molecules. In the reversal mechanism, the protonation of glycoside oxygen and the substitution of aglycones are accompanied by the attack of water molecules, which are activated by nucleophilic amino acids, thus directly displacing the ectopic substituents. At present, the most effective active enzymes for chitosan degradation are cellulase and chitosan enzyme. In particular, chitosanase is a specific enzyme that degrades chitosan and catalyzes the hydrolysis of β−1,4-glucoside bonds in chitosan. In addition, the binding mechanism between chitanase and chitosan is related to its spatial structure, electrification and hydrophobicity of amino acids. Most chitanases recognize ligands through electrostatic interactions between the negative charge of the acidic group of the enzyme protein and the positive charge of the amino group of chitosan. Compared with chemical and physical methods, enzyme degradation method has many advantages: (1) specific degradation without changing the basic structure of chitosan, ensuring the basic function of chitosan; (2) the method is environmentally friendly and does not need to consume a large amount of acidic reagents; and (3) the reaction conditions are mild and easy to control. The molecular weight distribution of LMWC degraded by different enzymes may be significantly different even though they have similar average molecular weight. In addition, the properties of LMWC obtained by enzymatic degradation also have an important relationship with the DD of the initial chitosan. Table 4 illustrated the results and preparation conditions of LMWC products obtained by enzymatic hydrolysis method.

In Table 5, the advantages and disadvantages of physical, chemical and biological methods are compared and summarized in detail, and the possible application fields are proposed. In fact, the mechanisms of LMWC products prepared by various methods are not very clear. To gain a deeper understanding and optimize the preparation process, a cutting-edge approach combining experimental techniques such as quantum chemistry with theoretical calculations is urgently needed. This interdisciplinary research will not only reveal the degradation mechanism of LMWC but also provide theoretical and experimental guidance for the development of novel,

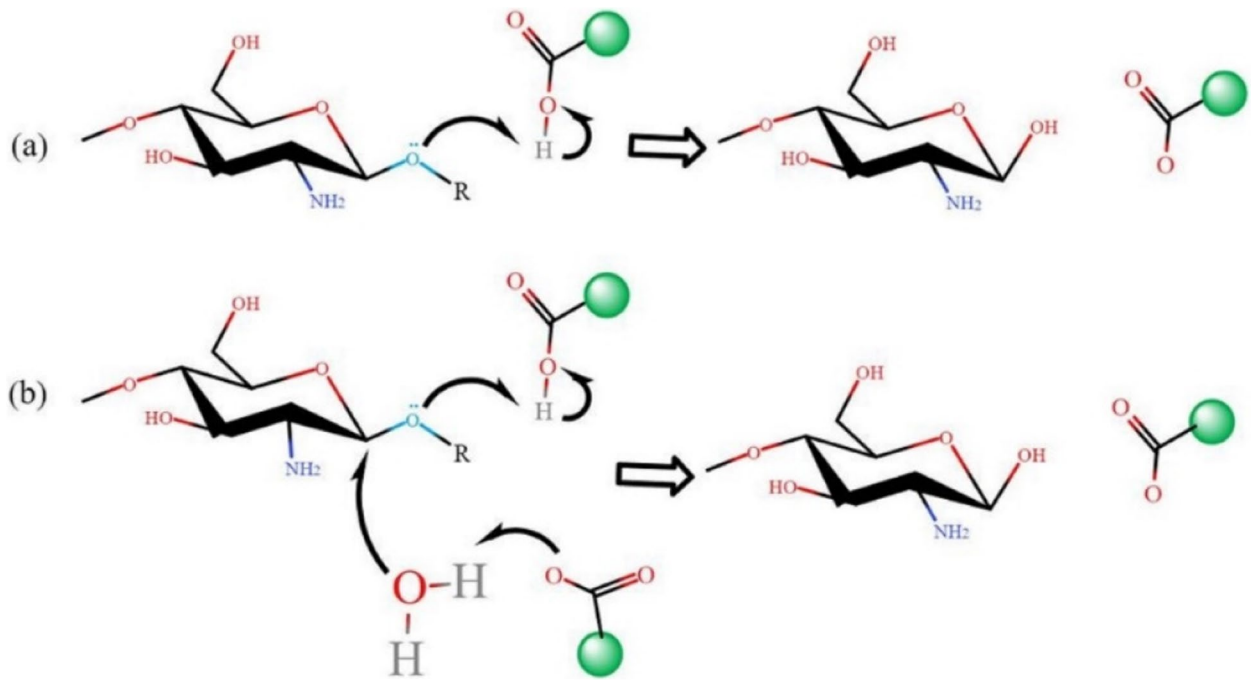

Fig. 3 Mechanism of enzyme-catalyzed hydrolysis of glycoside bond: retention mechanism (a) and reversal mechanism (b)

Table 4 Comparison of LMWC products obtained by enzymatic hydrolysis method

Enzyme	pH	T (°C)	Time (h)	Yield (%)	M_W (kDa)	Ref.
pepsin	5.0	38	4		3.0	[60]
pepsin + papain	4.8	50	24	13	0.6	[61]
chitinase + cellulase	5.3	45	6	80	1.3	[62]
muramidase	4.0	42	72		22.2	[63]
amylase	5.5	55	4	96		[64]
pectinase	3.0	38	6	86		[65]
cellulase	5.2	55	24		2.2	[66]
neutral protease	5.4	50	4	94	25.7	[67]
cellulase	5.2	55	18		3.3	[68]
chitosanase	5.6	55	1.5		1–100	[69]

efficient, and environmentally friendly chitosan degradation technologies, further promoting the widespread application and development of chitosan in biomedicine, agriculture, environmental protection, and other fields.

3 Characterization analysis

The basic properties of chitosan have great influence on its physical, chemical and biological properties and applications. The main parameters that affect the properties of LMWC are solubility, *DD*, average molecular weight, crystallinity and so on.

3.1 Solubility

The solubility of LMWC was determined according to mass difference method [70] and spectrophotometry [71]. 0.05 g water-soluble chitosan was mixed with 5 mL distilled water, stirred for 5 h, and filtered with 0.45 μm filter paper. The solubility is estimated by the change in the weight of the filter paper. Spectrophotometry is quick and easy to use a spectrophotometer to measure the transmittance of each suspension or solution at 600 nm.

3.2 DD

Chitosan is a copolymer of *N*-acetylglucosamine (GlcNAc) and *D*-glucosamine (GlcN) units. The molar fraction of the *N*-acetylglucosamine units in the chain is defined as the *DD*. In some literatures, the degree of acetylation is used, *DA* = 100-*DD*. *DD* is related to the properties of chitosan in different applications, such as the ability to chelate metal ions, acid-base properties, adsorption properties, self-aggregation, solubility, and biodegradability. The most widely used methods to determine the degree of chitosan deacetylation include potentiometric titration, first derivative ultraviolet (FDUV) spectroscopy [72], Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy [13] and elemental analysis, as well as gel permeation chromatography (GPC) and differential scanning calorimetry and thermogravimetry.

3.2.1 Potentiometric titration [73, 74]

In brief, chitosan (0.2 g) was dissolved in 30 mL 0.1 M HCl aqueous solution with 5–6 drops of methyl orange at room temperature. The red chitosan solution was titrated 0.1 M NaOH solution until the color turned orange. In addition, the end point can be determined by plotting the first derivative curve in relation to the NaOH volume.

$$DD = \frac{(V_1 - V_2) \times 16}{V_1 \times 9.94 \times m} \times 100 \quad (1)$$

where, m was the chitosan weight; V_1 (mL) was volume of chitosan solution; V_2 (mL) was NaOH volume; 9.94 was the theoretical value of -NH_2 content of chitosan; and 16 was the gram equivalent weight of the amino group.

3.2.2 FDUV spectroscopy

The calibration curve was carried out by plotting the first derivative UV values at 203 nm as a function of GlcNAc and glucosamine hydrochloride. The standard solutions of GlcNAc and GlcN were prepared with 0.85% phosphoric acid. About 100 mg samples were mixed in 20 mL 85% phosphoric acid. UV/Vis spectral analysis was performed in the range 190–400 nm. DA was calculated as [72]:

$$DA = \frac{\frac{m_1}{208.21} \times 100}{\frac{m_1}{208.21} + \frac{m_2}{161.17}} \quad (2)$$

where, m_1 was the GlcNAc mass calculated from the calibration curve, m_2 was the GlcN mass in the solution, $m_2 = M$ - m_1. M is the chitosan mass in the solution.

Table 5 Comparison of different degradation methods and their advantages and disadvantages

Methods	Conditions		Advantages	Disadvantages	Application field
Chemical	Acid hydrolysis method (hydrochloric, nitric, phosphoric, hydrofluoric, acetic, etc.)		Simple operation, low cost, easy to obtain reagents, wide range of application	Difficult to control the degree of degradation, low degree of polymerization of degradation products, environmental pollution and high equipment requirements	Suitable for the occasions that are sensitive to cost and have a certain tolerance for environmental pollution, such as industrial production and wastewater treatment.
	Oxidative degradation	Hydrogen peroxide (H_2O_2)	Fast reaction rate, high efficiency, low cost, high yield, environmentally friendly	Poor reaction stability and repeatability, wide molecular weight distribution, difficult separation and purification	
		Sodium hypochlorite (NaClO)	High efficiency, low cost	Environmental pollution, difficult purification, safety issues	
		Chlorine gas (Cl_2)	High efficiency	High toxicity, complicated operation, environmental pollution	
		Ozone (O_3)	High efficiency, no secondary pollution, mild conditions	High equipment requirements and high energy consumption	
Physical	Ultrasonic, microwave, gamma rays, ultraviolet rays		Easy controlling of the reaction process, no chemicals required, less environmental impact, better uniformity and quality of the products	Low yield, high equipment requirements, limited reaction conditions, difficult to control the degree of degradation	Suitable for the occasions with high product quality requirements and strict environmental pollution control, such as medicine, food and other fields.
Enzymatic	Non-specific enzymes (cellulase, lipase, pectinase, protease, etc.)		Rich source of enzymes, relatively lower potential costs	Low efficiency, poor product uniformity and stability, harsh reaction conditions	Suitable for applications requiring high biocompatibility and bioactivity, such as biomedicine, tissue engineering and other fields.
	Specific enzymes (chitinase, chitanase)		High efficiency, high product quality, mild conditions, environmental friendliness	High cost and limited application range	

$$M = \frac{M_1 \times M_3}{M_1 + M_2} \tag{3}$$

where M_1 was the mass of solid chitosan sample, M_2 was the mass of 20 mL 85% phosphoric acid (34 g), M_3 was the mass of 1 mL chitosan solution in concentrated phosphoric acid.

3.2.3 UV-Vis spectroscopy [72]

Different proportions of GlcN and GlcNAc solution were used to simulate the chitosan samples with different *DD*. UV spectra of these solutions were recorded. The ratio of absorbance to total concentration was correlated with the degree of acetylation to generate a calibration curve. Absorbance was measured at λ = 201 nm. The *DA* of the solution was defined as the concentration of N-acetylglucosamine divided by the total concentration of *N*-acetylglucosamine and *D*-glucosamine hydrochloride.

$$DD = (1 - \frac{161.1AV - Cm}{km \times 42.1 \times AV}) \times 100 \tag{4}$$

where, 161.1 (g mol^{-1}) was the molecular weight of GlcN residue, 42.1 (g mol^{-1}) was the difference between the molecular weight of GlcNAc and GlcN, *A* was the absorbance at λ = 201 nm, *V* (L) was the solution volume, *m* (mg) was the sample mass. *C* and *k* were the intercept with the X-axis and the slope of the standard curve.

3.2.4 FTIR spectroscopy

About 20–30 mg of dried chitosan sample and 60 mg of KBr were mixed evenly. 30 mg of the mixture was used to prepare small discs at a pressure of 80 MPa for 60 s. FTIR spectra was recorded by Fourier infrared spectrometer at the range of 4000–400 cm^{-1}. *DD* can be calculated by Eq. (5) [75]:

$$DD = 100 - \frac{A_{1655}/A_{3450}}{1.33} \times 100 \tag{5}$$

where, A_{1655} and A_{3450} represent the absorbance of the sample at 1655 cm^{-1} (N-acetyl group) and 3450 cm^{-1} (hydroxyl bond), respectively. The factor 1.33 is the ratio of A_{1655}/A_{3450} for fully N-acetylated chitosan.

3.2.5 Elemental analysis

The compositions of C and N elements were determined by microelement analyzer. DD was calculated by the following formula [76]:

$$DD = \left(1\frac{\mathrm{C/N} - 5.145}{6.861 - 5.145}\right) \times 100 \tag{6}$$

where, C/N was carbon to nitrogen ratio.

3.2.6 ^{1}H-NMR

Chitosan samples were dissolved in D_2O-DCl solution and freeze-dried twice with D_2O to exchange the unstable protons with deuterium atoms. *DD* was calculated as follows [77]:

$$DD = 100 \times \frac{\text{H-1D}}{\text{H-1D} + \text{HAc}/3} \tag{7}$$

where, H-1D and HAc were represented as the integrals of peak of the H-1 anomeric proton of deacetylated monomer (H-1D) and the three protons of N-acetyl group (H-Ac), respectively.

3.3 Intrinsic viscosity

The viscosity depends on the molecular weight and the DD of chitosan, which decreases with the decrease of the molecular weight of chitosan. In addition, viscosity can be used to determine the stability of the polymer in solution. The intrinsic viscosity of chitosan was determined by Ubbelohde viscometer with capillary diameter of 0.63 mm in a temperature controlled bath apparatus [78]. The chitosan oligomers were accurately weighed and dissolved in 100 mL 0.1 M CH_3COOH-0.2 M NaCl solution. The intrinsic viscosity [η] was defined as the Eq. (8):

$$[\eta] = \lim_{c \to 0}[\eta_{sp}/C] \tag{8}$$

where, η_{sp} is the specific viscosity, which is defined by Eq. (9):

$$\eta_{sp} = (\eta - \eta_0)/\eta_0 \tag{9}$$

where, η is the viscosity of chitosan and η_0 is the viscosity of pure solvent.

3.4 Average molecular weight

The intrinsic viscosity was related to the average molecular weight (M_v) according to the Mark-Houwink-Sakurada equation [78]:

$$[\eta] = K(M_v)^{\alpha} \tag{10}$$

where, [η] (mL g^{-1}) was the intrinsic viscosity, *K* (cm^3 g^{-1}) and *a* were related to the viscosity constants, which varied in function of the nature of the solvent, temperature and chemical structure of the polymer as well as molar mass. $K = 1.64 \times 10^{-30} \times DD^{14}$, $\alpha = -1.02 \times 10^{-2} \times DD + 1.82$ [79].

The number average molecular weight (M_n) and weight average molecular weight (M_w) values were measured by GPC method [80]. A sample of chitosan (1.0 mg L^{-1}) was dissolved in a sodium acetoacetate buffer (0.33 M acetic

acid, 0.1 M NaOH, pH = 3.9). The standard curve was established with ethylene glycol or dextran as the standard material.

The weight average degree (DP_w) and viscosity average degree (DP_v) of polymerization of chitosan were obtained by calculating the relative quantities of GlcNAc (203 g mol^{-1}) and GlcN (161 g mol^{-1}) as the following Equation [81]:

$$DP = \frac{M \times 100}{(203 \times DA) + [161 \times (100 - DA)]} \quad (11)$$

where, DP and M were the average polymerization degree and average molecular weight, respectively.

3.5 Crystallinity

DD and crystallinity of chitosan greatly affect its solubility in acid or water. X-ray powder diffraction (XRD) pattern was used to analyze the crystallinity of chitosan [82]. The crystallinity index (CrI, %) was calculated as the following Equation [81]:

$$CrI_{020} = \frac{I_{020} - I_{am}}{I_{020}} \times 100 \quad (12)$$

$$CrI_{110} = \frac{I_{110} - I_{am}}{I_{110}} \times 100 \quad (13)$$

where, I_{020} and I_{110} were the maximum intensities of the (020) and (110) plane in the XRD profile at $2\theta \approx 10^{\circ}$ and 20°, respectively. I_{am} was the intensity of the amorphous diffraction region at $2\theta \approx 16^{\circ}$.

3.6 Ash content

Ash was decomposed by pyrolysis and weighing. 1.0 g of chitosan was put into a quantitative crucible and heated in a Muffle furnace at 600 °C for 2 h. The ash content was determined using the following equation [83]:

$$\text{Ash content (\%)} = \frac{\text{Mass of residue (g)}}{\text{Sample mass (g)}} \times 100 \quad (14)$$

4 Application of LMWC in environmental protection

As an eco-friendly biopolymer, chitosan has been widely used in the field of environmental remediation, such as water purification, wastewater treatment, sludge dewatering, membrane filtration and soil remediation. However, most of commercial chitosan is insoluble in water, which greatly limits their application in wastewater treatment, soil remediation and air pollution control. LMWC has better water solubility and reactivity, and can have reactions such as acylation, acylation, etherification, alkylation, graft copolymerization and crosslinking [58], so that it can be modified under different conditions for further expanding the application scale and scope of chitosan.

4.1 Water treatment

Chitosan is mainly used as coagulant and chelating agent in water treatment, which can efficiently remove heavy metals and organic substances in water [84]. As a coagulant, the amino group in chitosan molecules will be protonated in acidic solution, so that the molecular chain of chitosan exhibits positive charge, which becomes a typical cationic flocculant. It can effectively neutralize the negatively charged colloidal particles in water, so that condensation occurs between particles, thus accelerating the precipitation process. Chitosan coagulant has the advantages of non-toxic, harmless, easy biodegradation and will not cause secondary pollution to the environment. As a chelating agent, active groups such as amino and hydroxyl in chitosan molecules can chelate with metal ions to form stable chelates to remove heavy metal ions (Cu, Pb, Cd, Hg, etc.) from water. Besides, chitosan chelating agent has high selectivity and adsorption capacity in the treatment of heavy metal wastewater, and can separate heavy metal ions from water, so as to achieve the purpose of purifying water quality. In addition, chitosan chelating agent can also be used to recover precious metals and rare metals, which has high economic value. Similarly, LMWC also has the same function in the field of water treatment. LMWC can be combined with other functional materials to prepare composite coagulants or chelators with multiple functions to meet the needs of different water quality treatment. Denisova et al. [85] studied the effect of chitosan nanoparticles with different molecular weights on the activity of *Escherichia coli* in drinking water. Geetha Devi et al. [86] investigated the dairy wastewater treatment using LMWC, and results showed that LMWC was an effective coagulant and could reduce chemical oxygen demand, total suspended solids and turbidity in dairy wastewater. LMWC is rich in amino and hydroxyl groups, which can form coordination bonds with metal ions and remove heavy metals from aqueous solutions through complexation [59]. Shukla et al. [87] used LMWC coated iron oxide nanoparticles (CSO-INPs) to remove chromium from wastewater, and investigated the effects of factors such as pH, temperature, dose and time on the removal efficiency of Cr by CSO-INPs. LMWC grafted onto maleic anhydride/polyvinyl alcohol/fibroin protein had a good removal effect on lead ions in aqueous solution [88]. Functional groups that bind to heavy metal ions usually exist in the form of amines ($-NH_2$) and hydroxyl groups (-OH). In addition, the interaction mechanism between metal ions

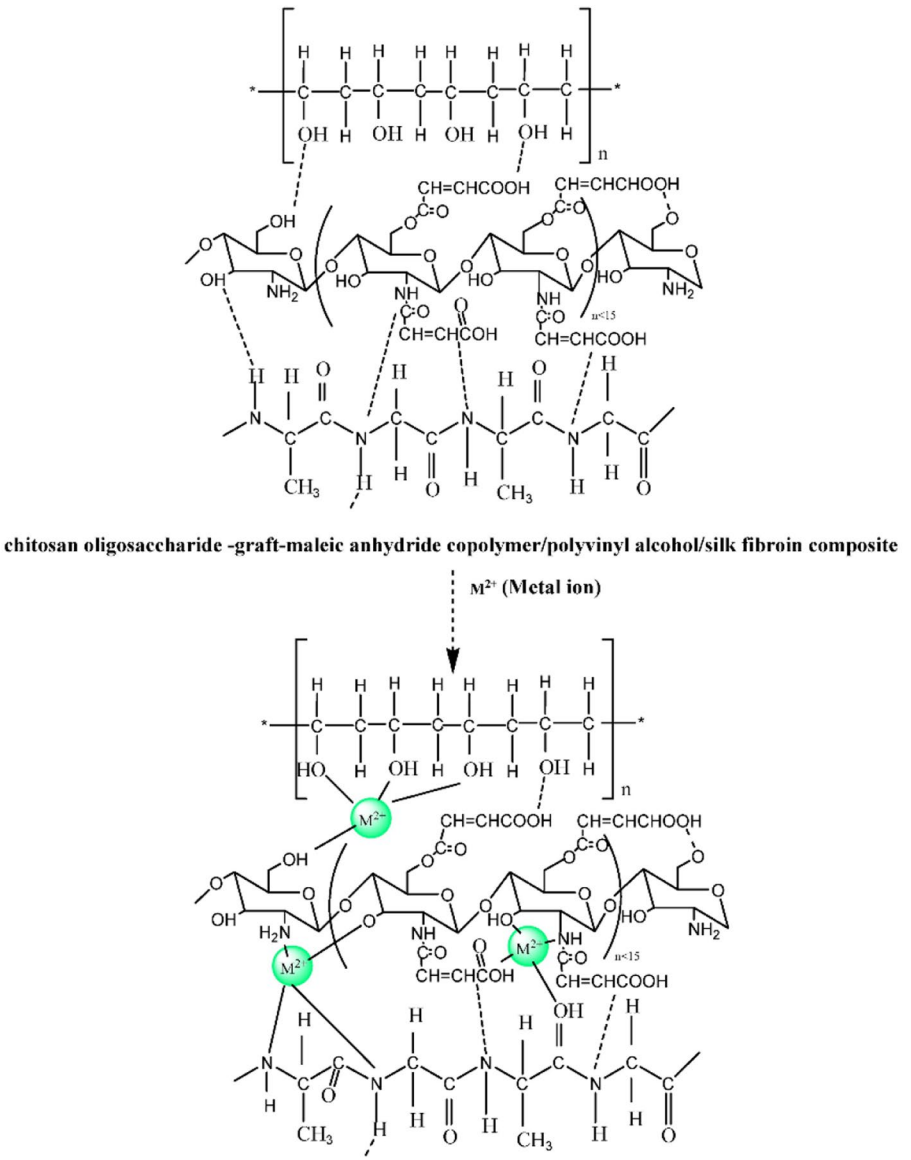

Fig. 4 Interaction mechanism of metal ion (II) onto chitosan oligosaccharide-graft-maleic anhydride copolymer/polyvinyl alcohol/silk fibroin composite [88]

and chitosan-grafted maleic anhydride/polyvinyl alcohol/fibroin composites is shown in Fig. 4.

4.2 Soil remediation

Chitosan, as a natural polysaccharide, has a variety of functional groups and rich cations, which can form complexes with organic pollutants or heavy metals in soil to reduce the activity and migration of these harmful substances. This complexation makes harmful substances inactive and difficult to migrate, which is conducive to soil remediation. Chitosan contains a large number of active groups, such as amino and hydroxyl groups, which can interact with heavy metal ions and organic pollutants in soil through hydrogen bonding, ion exchange, chelation, etc., so as to remove or stabilize pollutants from the soil [7]. chitosan can also promote the structural properties and water retention of soil by improving the physical and chemical properties of soil, which is beneficial to improve soil fertility and self-purification and can provide a better environment for plant growth. Besides,

chitosan is a powerful chelating agent and can easily form complexes with transition metals and heavy metals [89]. Even if K^+, Cl^- and NO- 3 exist in the soil, chitosan and its derivatives can still bind with heavy metals in the soil to form complexes through coordination bonds [90]. Particularly, biopolymers with low molecular weight, low viscosity and high DD can improve the water stability of soil more than those with high molecular weight, high viscosity and DD [91]. Consequently, it is feasible to use LMWC and its derivatives as soil amendments. In addition, LMWC can also expand the pore structure of soil and improve the water retention and aeration of soil, which is conducive to plant growth and root development [92]. LMWC can also be used to prepare soil remediation materials, such as soil curing agents and plant root protectors, which can effectively repair contaminated soil. Adamczuk and Jozefaciuk [91] found that LMWC could dissolve better and faster than high molecular weight chitosan in organic acids of soil. It should be noted that the application effectiveness of LMWC in soil remediation is affected by various factors, such as soil type, types and concentrations of contaminants, the amount of chitosan as well as the application method.

4.3 Air pollution control

Chitosan itself has strong adsorption capacity and surface activity, which can absorb harmful gases in the air, such as benzene, formaldehyde and ammonia in the air atmosphere [93, 94]. Chitosan can reduce the content of suspended particulate matter in the air by adsorbing particulate matter, thus improving air quality. Moreover, chitosan can also be used as a carrier of photocatalyst, composite with other photocatalytic materials, and use photocatalytic oxidation to convert harmful gases into harmless substances. For example, chitosan as a precursor of in situ spinning was used to capture $PM_{2.5}$ [95]. Due to the strong polarity, electrostatic spinning nanofibers with chitosan have strong synergistic effect of electrostatic adsorption and surface adhesion [96]. After effective degradation, the molecular weight of chitosan decreased, the molecular chain became shorter and the specific surface area would increase, leading to the fact that LMWC had more adsorption sites and higher adsorption efficiency and capacity. Therefore, LMWC can be used as an adsorbent or catalyst carrier to remove harmful gases or pollutants from the air and as a catalyst carrier to catalyze the conversion of harmful gases in the air into harmless substances. With the increasingly serious problem of air pollution and the continuous development of air treatment technology, the application potential of LMWC in air treatment will gradually become prominent. Lee et al. [97] investigated the anti-oxidant activity and dust-proof effect of chitosan with different molecular weights, indicating that chitosan hydrolysate had obvious free radical scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid. Furthermore, LMWC could effectively remove fine dust in the air that may contain particulate matter and unknown microbial species [97].

Although the research and application of LMWC in the field of air purification is still in the initial stage, it has shown great potential in the field of air purification due to its unique physical and chemical properties and biocompatibility. In the future, the efficiency and selectivity of LMWC in air purification is expected to be significantly improved through further functional modification and composite material research, and its application in the field of air purification will be more widely promoted and applied.

5 Conclusions

In this paper, the preparation strategies of LMWC and its detailed physicochemical properties were reviewed, and its broad application prospects in environmental protection were discussed. This review not only reveals the innovative potential of LMWC as biomaterials in environmental protection, but also provides a scientific basis and forward-looking perspective for its further optimization and application, which is of great significance for promoting the development of green and sustainable environmental protection technologies.

(1) The current preparation techniques of LMWC, including physical method, acid hydrolysis method and oxidative degradation method, are systematically reviewed. We realize that each method has its own characteristics, but it faces certain limitations. This paper discussed the basic principle, operation process, advantages and disadvantages of these methods in detail, and emphasized that the selection and optimization of these methods should be carried out according to the characteristics of target products and application requirements. This paper not only deepens the understanding of the existing technology, but also lays a theoretical foundation for exploring new, efficient and environmentally friendly LMWC preparation methods. Therefore, the exploration of more efficient, environmentally friendly and controllable new degradation methods is of vital significance to promote the industrialization process of LMWC and meet the needs of diverse applications.

(2) The performance characterization and analysis methods of chitosan and its degradation products were comprehensively summarized, including the detection methods of key indicators such as solubility, deacetylation degree, molecular weight and ash content. These standardized analysis methods laid a solid foundation for

the performance evaluation and application expansion of LMWC.

(3) Although the research and application of LMWC in the field of environmental remediation such as water pollution, soil and air treatment are still in the infancy, the unique physical and chemical properties and biological activities of LMWC indicate huge application potential and broad market prospects. With the in-depth exploration of adsorption, complexation, catalysis and other mechanisms, the performance of LMWC will be further optimized, and the application conditions will be clearer, which is expected to play an irreplaceable role in many environmental protection fields such as water purification, soil improvement and air purification, and contribute an important force to promote environmental protection and sustainable development.

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Authors' contributions
Conceptualization, N.-Y. L. and H. W.; writing-original draft, N.-Y.L. and H.W.; funding acquisition, N.-Y. L. and H. W.; writing- review & editing, H.W. All authors read and approved the final manuscript.

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Not applicable.

Declarations

Competing interests
The authors declare they have no competing interests.

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