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Minireview

Recent research progress on preparation and application of *N*, *N*, *N*-trimethyl chitosan

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ABSTRACT

N,*N*,*N*-trimethyl chitosan (TMC) is a quaternized chitosan derivative with excellent solubility in aqueous solutions. It has been extensively studied as an absorption enhancer, antibacterial agent and gene vector due to its ability to form complexes with anionic gels or macromoleculars. However, the research which describes the process of TMC preparation and its new applications has not been fully reviewed. In this paper, recent progress regarding different TMC preparation methods and its characterization and application in different fields is presented. Key findings are compared and summarized and some topics for further study are suggested.

1. Introduction

Chitosan, β-(1–4)-linked D-glucosamine, is a fully or partially deacetylated chitin which is one of the most abundant natural polymers. Due to its biocompatibility, biodegradation, antibacterial activity and good film-forming properties, chitosan is used in papermaking, food packaging, agriculture, medicine, cosmetics, etc. [1,2] Generally, chitosan must be modified because of its lack of solubility, high molecular chain rigidity and high brittleness of its film. Presently used methods for the modification of chitosan mainly include acylation, alkylation, phosphorylation, carboxylation, sulphonation [3–9] and a crosslinking reaction with aldehyde or another crosslinking agent [10,11]. Quaternary ammonium chitosan salt, phosphorylated chitosan and carboxymethyl chitosan are used when good solubility is required, whereas crosslinked chitosan is used when less solubility is required [12,13]. In addition, new types of functional chitosan are being developed to expand its range of applications.

Trimethyl chitosan iodide was first described in 1977, but the full preparation process for *N*, *N*, *N*-trimethyl chitosan chloride (TMC) was reported by Muzzarelli and Tanfani [14] in1985. Nowadays, TMC is well-known as a kind of quaternary ammonium derivative of chitosan, which can be used as a permeation enhancer and a gene vector due to its good solubility in wide range of pH values, antibacterial activity and absorption enhancing properties [15–17]. An increasing number of researchers are studying its properties and exploring different applications of TMC and a large body of research has already been published.

2. The structure and properties of TMC

2.1. The structure of TMC

TMC is one of the *N*-alkyl chitosan derivatives but some of the reviewed studies have indicated that *O*-methylation also occurs during the alkylation reaction. Therefore, the products after alkylation may include TMC, 6-O methylated and 3-O methylated TMC, and *N*-monomethylated and *N*, *N*-dimethylated chitosan. The ^{1}H and ^{13}C NMR spectra of TMC are shown in Fig. 1 [18]. Initially, for the ^{1}H NMR spectrum, the peak at 3.0 ppm was assigned to $N(CH_3)_3^+$ and the peak for $N(CH_3)_2$ was thought to occur at 3.2 ppm according to Dung et al. [19] However, these results were subsequently proved to be incorrect by Sieval et al. [18] Actually, the peak for $N(CH_3)_3^+$ occurs at around 3.3 ppm and the peak for $N(CH_3)_2$ occurs at 2.8 ppm (D_2O) to 3.1 ppm (D_2O/DCl) depending on the pH of the solvent. In addition, the peaks for OCH_3 (in the case of *O*-

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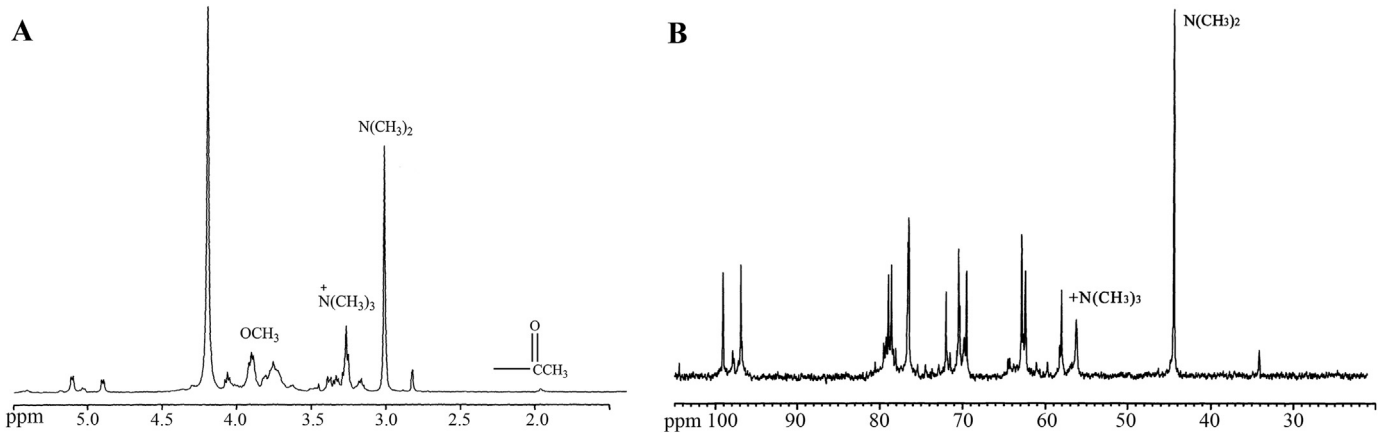

Fig. 1. ^{1}H (a) and ^{13}C (b) NMR spectra of TMC dissolved in D_2O [18].

methylation) and the acetyl group occur at around 3.5 and 2.0 ppm respectively in the ^{1}H NMR spectrum. For the ^{13}C NMR spectrum, the signals at 55 ppm and 44 ppm are attributed to $N(CH_3)_3^+$ and $N(CH_3)_2$ respectively [18,20–22]. Furthermore, the DQ (degree of quaternization) and degree of *O*-methylation can be calculated according the NMR spectra.

2.2. The solubility of TMC

The solubility of TMC is influenced by deacetylation degree (DD) and the molecular weight of the original chitosan, the DQ and the percentage of *O*-methylation. TMC, is more soluble than chitosan because of the greater number of hydrophilic amino groups on the main chain after quaternization. Generally, the lower the deacetylation degree, the less chance of forming quaternary ammonium salt during methylation, which directly results in lower solubility. This was proven by Muzzarelli and Tanfani [14], and it was reported that the solubility of TMC with a DQ of 60%, obtained from modifying the chitosan with a DD of 40%, was still very poor. However, for the TMC prepared from chitosan with a DD of 95%, even a DQ of 25% enabled the TMC to be dissolved over the full pH range [23]. Moreover, DQ is the most important evaluate index of water solubility. Some studies have been performed via synthesizing the TMC with a higher DQ by using different preparation methods, or repeating methylation process steps according to the application. However, the amount of *O*-methylated TMC is increased with the increasing number of process steps [24]. It was proven that when the DQ was lower than 24%, the solubility of TMC was almost the same as that of *O*-methylated TMC with a pH of less than 7, but when the DQ was higher than 33%, only the TMC without *O*-methylation had better water solubility [25]. In addition, although the product with DQ>60% could still be dissolved in water when the concentration of tertiary amine was lower than 5% [18], the percentage of dimethylation was increased with the prolonged treatment, which had a negative effect on solubility because insoluble *N*,*N*-dimethyl chitosan was created [26]. Therefore, reducing the proportion of monomethylation and demethylation is necessary in the process of preparing TMC.

2.3. The antibacterial properties of TMC

The mechanism of the antibacterial action of chitosan is still not very well understood even though chitosan has been widely reported as a natural antibacterial agent [27–30]. It is believed that there are interactions between the positive charge on the amino group of chitosan and the negative charge on the surface of the bacterial cell [31–34]. Therefore, the antibacterial activity of TMC is higher than that of the parent chitosan because of the increased positive charge after quaternization. Moreover, compared with HTCC (*N*-([2-hydroxy-3-trimethylammonium])propyl chitosan chloride), which was used as an antibacterial agent under alkaline conditions [35], TMC had higher antibacterial activity against *Salmonella cholerae-suis*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* according to MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) tests, and the antibacterial activity of TMC was significantly decreased by the presence of divalent cations (Ba^{2+} or Ca^{2+}) [36–39]. In addition, TMC exhibited better antibacterial properties against Gram-positive *Staphylococcus aureus* than against Gram-negative *Staphylococcus aureus* as measured by the inhibition zone method and the turbidity tests [40,41]. This may be because of the enhanced autolysis activity of Gram-positive bacteria in the presence of TMC. The effectiveness of autolysis can be enhanced as the positive charge is increased [3]. However, the antibacterial activity of carboxymethylated TMC against *Staphylococcus aureus* and *Escherichia coli* was weaker than that of TMC and was significantly influenced by pH value [41].

It was proven that the antibacterial activity was improved with the increasing chain length of alkyl substituent [42,43], and it was higher under acidic conditions than under neutral conditions [44]. Recent study has shown that alkyl chain length of *N*-alkyl or *N*,*N*-dialkyl derivatives can have positive or negative effect on antibacterial activity depending on the bacteria species, but the increased length of the alkyl chain will lead to increased hemolysis [45]. Therefore, it is important to prepare TMC with higher purity to replace the unmodified chitosan as a more effective antibacterial agent.

2.4. The thermal stability of TMC

It has been reported that the thermal stability of chitosan was reduced by introducing substituents onto the amino group [46–48]. The thermal stability of TMC showed the same trend as the original chitosan by TG analysis, and the thermal stability and activation energy decreased with increasing DQ [49]. This is probably because TMC has stronger hydrophilic properties than the parent chitosan, and the interchain interaction of TMC is weakened with the increase of the water content, leading to lower thermal stability. According to the experiments of TMC film, no glass transition temperature was observed, but a discrete transition at 25 °C