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RECENT ADVANCES IN THE SYNTHESIS AND CHARACTERISATION OF METHYLATED CHITOSAN DERIVATIVES

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Abstract: The synthesis, characterization, and physicochemical characteristics of water-soluble methylated chitosan derivatives are the main topics of this research. There are several uses for trimethylchitosan and N-[(2-hydroxyl-3-trimethyl ammonium) propyl] chitosan (HTCC) in biomedicine, and the degree of quaternization has a significant impact on the physicochemical characteristics of HTCC. N,O-substituted quaternized chitosan is frequently obtained by directly replacing the hydrogen atoms from the associated amino and hydroxyl functional groups from chitosan at the C6 position with glycidyl trimethylammonium chloride (GTMAC).

Keywords: trimethylchitosan, 6-O-methylated chitosan, N, N-dimethylated chitosan, glycidyl trimethylammonium chloride, antimicrobial activity, quaternized chitosan, N,O-substituted quaternized chitosan.

Introduction. At present, the synthesis of materials with antimicrobial, antioxidant, and antifungal properties is a vital problem. Treatment of diseases caused by microorganisms such as gram-positive and gram-negative bacteria, viruses, and fungi is one of the main problems in clinical practice. Chitosan and its water-soluble derivatives play an important role among the many materials used to solve these problems. Chitosan is a natural aminopolysaccharide consisting of repeating units of N-acetylglucosamine and glucosamine. It has a unique chemical structure as a linear polycation with a high charge density and reactive amino and hydroxyl groups. Chitosan belongs to the category of non-toxic, biodegradable, and biocompatible polymers, which is a significant advantage over other polymeric materials. Modification of chitosan using reactive groups with ionic substituents helps to solve the problem of solubility in a wide range of pH values. Chitosan and its derivatives have gained significant attention in biomedical applications due to their biodegradability, biocompatibility, non-toxicity, and antimicrobial properties. Chemical modifications, such as carboxymethylation, quaternization, acetylation, and sulfation, improve their solubility and functionality, making them suitable for various medical applications. Particular attention in recent years has been given to the study of polysaccharides of quaternary ammonium groups. Quaternization of chitosan involves the introduction of a hydrophilic group by any of three methods: direct quaternary ammonium substitution, open-loop epoxy, and N-alkylation. Highly substituted quaternized chitosan (QCh) provides better water solubility and enhanced antimicrobial activity and reduces cytotoxicity due to innate mucoadhesiveness and efficient penetration with high potential. Moreover, the elaboration of a new methodology for the determination of molar mass characteristics of QCh derivatives is crucial. Here are some key biomedical applications of QCh: it is widely explored in the development of drug delivery systems due to its enhanced solubility and biocompatibility. It can be used to encapsulate various drugs, including hydrophilic ones, which are difficult to

deliver with traditional chitosan. QCh nanoparticles, micelles, and hydrogels can provide controlled and sustained release of therapeutic agents, improving the bioavailability and reducing side effects of drugs. QCh possesses inherent antimicrobial properties due to the positively charged quaternary ammonium groups, which can interact with the negatively charged surfaces of bacterial cell membranes. These properties make QCh an effective material for applications such as wound dressings, surgical masks, and antimicrobial coatings for medical devices. It can help in preventing infections and accelerating wound healing. It has shown promise in gene therapy applications. Its positive charge allows it to bind to negatively charged DNA or RNA molecules, forming complexes (such as nanoparticles) that can protect nucleic acids from degradation. These complexes can then be used to deliver genetic material to cells for therapeutic purposes, such as in the treatment of genetic disorders or cancer. In tissue engineering, QCh can be used as a scaffold material for cell growth and tissue regeneration. Its biocompatibility, biodegradability, and ability to support cell attachment and proliferation make it a suitable candidate for creating 3D structures for tissue regeneration. Furthermore, QCh scaffolds can be functionalized to improve their performance, such as enhancing cell-matrix interactions. QCh-based hydrogels, films, and dressings are utilized in wound healing due to their ability to create a moist healing environment, promote cell migration, and possess antimicrobial properties. They can also promote faster tissue regeneration and reduce the risk of infection. Additionally, quaternization can enhance the material's adhesion to tissue surfaces, making it more effective in wound management. QCh has been investigated for its potential in cancer therapy, both as a drug carrier and as a direct therapeutic agent. The ability to deliver chemotherapeutic agents directly to tumor cells while minimizing systemic side effects is a significant advantage. Additionally, QCS can be combined with other agents like photosensitizers for photodynamic therapy or with antibodies for targeted drug delivery to cancer cells. The versatility of QCh in forming films and nanoparticles has also been explored in biosensor development. The material can be functionalized to detect specific biomolecules or pathogens, making it a valuable tool in diagnostic applications, such as detecting infection markers or analyzing blood samples. Some studies suggest that QCh exhibits anticancer activity, inhibiting the proliferation of cancer cells and inducing apoptosis. The antioxidant properties of QCh may also provide protective effects against oxidative stress, which plays a significant role in the development of various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders [1]. Infections caused by microorganisms such as gram-positive and gram-negative bacteria, viruses, and fungi are one of the main problems in clinical practice, pharmaceutical and food industries [2]. Chitosan and its derivatives play an important role among the many materials used to solve these problems. Chitosan is a natural amino polysaccharide consisting of repeating units of N-acetylglucosamine and glucosamine. It has a unique chemical structure in the form of a linear polycation with high charge density, and reactive amino and hydroxyl groups. Chitosan is classified as a non-toxic, biodegradable and biocompatible polymer, which is a significant advantage compared to other polymeric materials [3]. However, chitosan is insoluble in most organic solvents and in aqueous solutions at neutral pH, and this places limitations on its use. In addition, at $\text{pH} > 6.5$, chitosan almost loses cationic properties, which are responsible for its solubility and interaction with negatively charged surfaces of bacteria. Decreasing the molecular weight and increasing the degree of deacetylation is accompanied by an increase in solubility and a decrease in the viscosity of the solutions [4]. The modification of chitosan using the reactive groups with ionic substituents helps to solve the

solubility problem over a wide range of pH values. The synthesis of various water-soluble derivatives of chitosan has been described in several works [5]. Especially, the investigation of polymeric quaternary ammonium compounds has received much attention in the past years [6]. Chitosan's molecular weight, degree of acetylation, charge density, and degree of quaternization all influence its biological activities. Chitosan's biological and physicochemical characteristics are mostly influenced by the level of deacetylation, which has a direct bearing on its molecular weight, pKa, hydrophilicity, crystallinity, degradation, and biological activities [7]. While transitional values of the degree of deacetylation show rapid rates of chitosan degradation, a degree of deacetylation value near 0% or 100% prolongs biodegradation and cell adhesion. Because the QCh derivative and the peptidoglycan of the bacterial cells formed polyelectrolyte complexes, which in turn prevented the growth of the bacteria, the produced QCh demonstrated antibacterial activity. At a 50% degree of quaternization, the antibacterial effectiveness of TMC and DMCHT is compared. Because TMC's smaller alkyl groups made it easier for it to engage with the bacteria's cell wall, the results demonstrated that it had superior antibacterial efficacy against *S. aureus*. Heavy N-ethyl functional groups make up the structure of voluminous DMCHT [8]. Figure 1 shows the synthesis routes for the multifunctional quaternized chitosan derivatives. Because of their unfavorable characteristics, other byproducts such as 6-O-methylated, 3-O-methylated, and N,N-dimethylated chitosan are rarely used. Terayama and associates synthesized the first trimethyl chitosan, known as macamin.

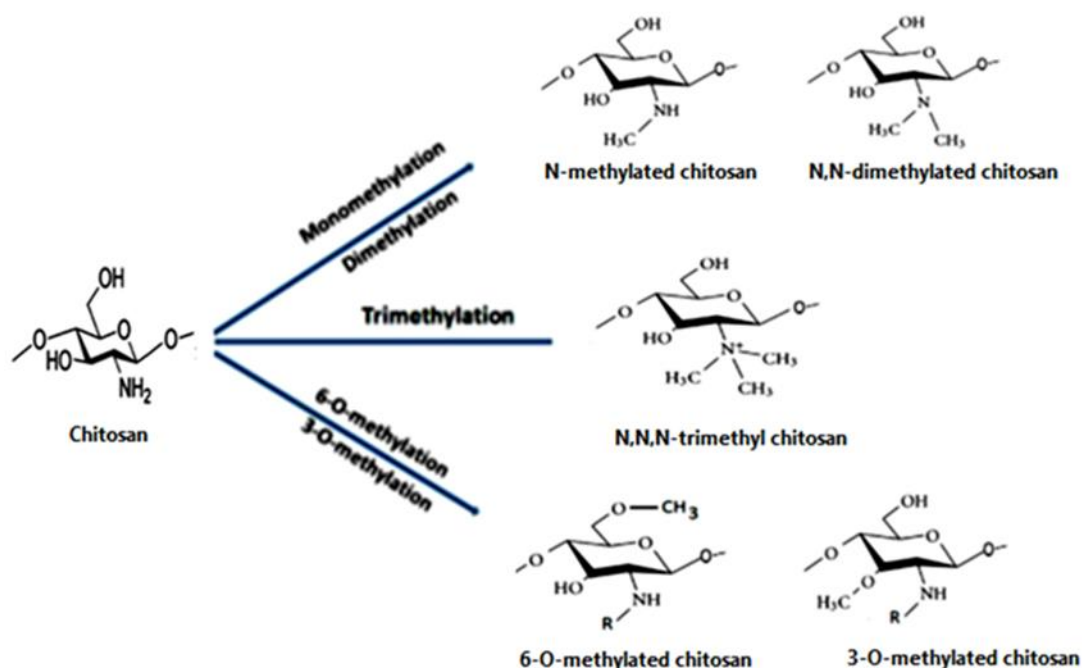


Figure 1. Different pathways for synthesis of methylated chitosan from native chitosan

The second most popular quaternized chitosan is N-[(2-hydroxy-3-trimethyl ammonium) pyropyl] chitosan, also known as HTCC. It is made by an alkylation process that adds a quaternary ammonium group outside the chitosan structure. The reagent GTMAC is commonly used to directly exchange hydrogen from linked amino and hydroxyl functional groups from chitosan at the P6 position to generate HTCC, which improves water solubility and antibacterial activity (Figure 2). The degree of positive charge is reflected in the quaternization levels of the gene-modified HTCC polymers. It has improved water solubility, modified antibacterial activities, and

non-toxicity in addition to biocompatibility and bioavailability characteristics. According to Xiao et al. [10], HTCC is less structured than crystalline chitosan. The process of quaternization produces a disordered water polymer that enhances absorption and allows for solubilization through the diffusion of free water molecules from the polymer chains by weakening both intramolecular and intermolecular hydrogen bonds. Furthermore, the high positive charge density on HTCC contributes to its enhanced antimicrobial properties, which is attributed to the negative surface charge of bacterial cell membranes. This results in a surface that is consistently positively charged. The extent of quaternization plays a significant role in determining the physicochemical characteristics of HTCC. Wang et al. [11] examined the influence of quaternization on the physicochemical and biological traits of HTCC. Their research indicated that as the degree of quaternization decreased during storage, properties such as apparent viscosity, solubility, and elasticity increased. A lower degree of quaternization leads to hydrophobic HTCC polymers, which are effectively utilized as vaccine adjuvants for the adsorption of antigens through hydrogen bonding facilitated by unoccupied amino groups. Additionally, Shagdarova et al. [12] observed that a higher degree of quaternization resulted in enhanced antibacterial efficacy against *S. epidermidis* and *E. coli*.

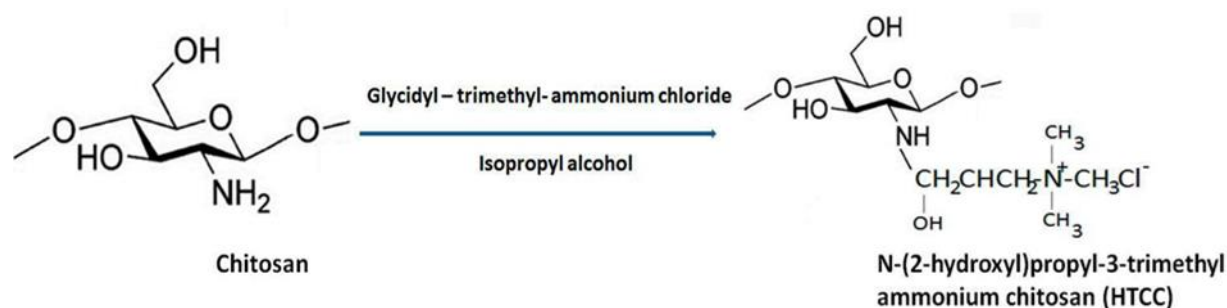


Figure 2. General synthesis of HTCC from chitosan

HTCC will be synthesized using the subsequent process: After 30 milliliters of distilled water at 85 degrees Celsius have been used to dissolve 3 grams of low molar mass chitosan, 10.7 milliliters (12 grams) of GTMAC, the alkylating reagent, will be added in three equal parts spaced one hour apart. At 85°C, the reaction mixture will be swirled for five hours. One hundred milliliters of cooled acetone will be added gradually to the reaction mixture. Decantation will separate the precipitate, which will then dissolve in 50 milliliters of methyl alcohol and precipitate in a 4:1 v/v acetone: ethanol combination. Along with decantation, the resultant precipitate will also be dialyzed against distilled water, cleaned with hot ethanol, and dried lyophilically. The resulting chitosan derivative should have a degree of quaternization (DQ) of about 98%. Similar results will be obtained for the synthesis of chitosan derivatives with DQs of 10, 40, and 53%, with low molar mass chitosan: GTMAC weight ratios ranging from 1:0.6 to 1:4. 60–70% chitosan derivatives should be produced. The results showed that a decrease in the degree of quaternization during storage increased the apparent viscosity, solubility, and elasticity. A hydrophobic HTCC polymer, which is produced by a lower degree of quaternization, is used as an effective vaccination adjuvant to adsorb antigens by hydrogen bonding created by vacant amino groups [11]. The superiority of the new scientific development in the synthesis and

application of biomedicine is that chitin and chitosan will be used from local sources—substandard cysts of crustacean species *Artemia Parthenogenetica* inhabits in the Aral Sea.

Conclusions Methylated aqueous derivatives of chitosan represent promising materials with a wide range of biomedical applications due to their unique combination of properties, such as biocompatibility, biodegradability, antimicrobial activity, and the ability to be easily modified for specific therapeutic purposes. Its potential use in drug delivery, gene therapy, tissue engineering, and wound healing highlights its versatility and importance in modern biomedical research and clinical applications.

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