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BIOLOGICAL ACTIVITY OF QUATERNARY AMMONIUM SALTS DERIVED FROM AROMATIC TERTIARY AMINES

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Abstract: Reactions between pyridine and certain esters of monochloroacetic acid were carried out to obtain quaternary ammonium salts. As a result of these reactions, a quaternary ammonium salt of pyridine was synthesized. The optimal reaction conditions were studied, and the yields of the resulting salts were calculated. Although chitosan is known for its high biological activity, its limited solubility in water restricts its range of applications. Therefore, the synthesis of quaternary ammonium salts based on chitosan is considered an important reaction. A novel quaternary ammonium salt of chitosan was synthesized using the quaternary ammonium salt of pyridine. The resulting cationic chitosan derivative exhibited antibacterial properties. The synthesized compounds were analyzed using physicochemical methods, including IR and NMR spectroscopy. The results confirmed the formation of the quaternary ammonium salt of chitosan.

Keywords: Pyridine, chitosan, esters of monochloroacetic acid, DMF, ethanol, acetone, temperature, IR, NMR.

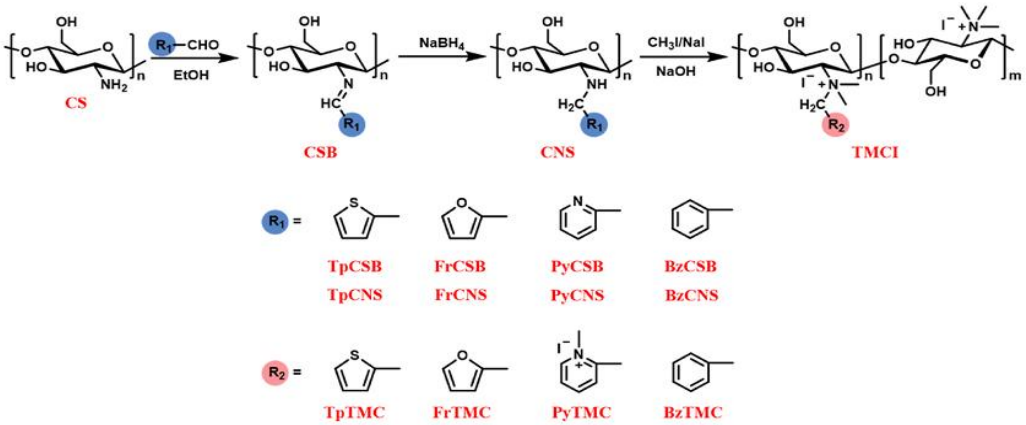
Introduction

Chitosan is a versatile biopolymer with broad-spectrum antibacterial activity, biocompatibility, and biodegradability, which can be enhanced by modifying its functional groups. These properties make chitosan and its derivatives suitable for applications in medicine, agriculture, cosmetics, textiles, and food packaging. [1–7] However, chitosan's limited solubility in water and relatively low antibacterial activity restrict its widespread application. Incorporation of nitrogen-containing heterocycles, long-chain alkyl groups, and quaternary ammonium salts into the chitosan structure can significantly enhance its antibacterial performance. [8–9]

Conversion of chitosan into quaternary ammonium salts increases its cationic charge density, thereby improving its antimicrobial activity. One of the most common methods for synthesizing quaternary ammonium chitosan derivatives is by modifying the amino groups in the chitosan molecule with small molecules that contain quaternary ammonium structures. There are two main synthetic approaches: direct alkylation using alkyl halides or the reaction via Schiff base

intermediates followed by quaternization. [10]

Fungal pathogens pose significant threats to global agriculture, reducing both crop yield and quality. Although traditional fungicides are effective, they raise environmental and health concerns, including toxicity, resistance development, and harmful ecological effects. This highlights the growing demand for alternative, environmentally friendly antifungal agents. Owing to its multifunctional and non-toxic nature, chitosan has attracted attention in various fields. [11–12] Based on these insights, we have synthesized novel quaternary ammonium chitosan derivatives to explore their biological activities.



The synthetic pathway of chitosan derivatives involves the following key steps.

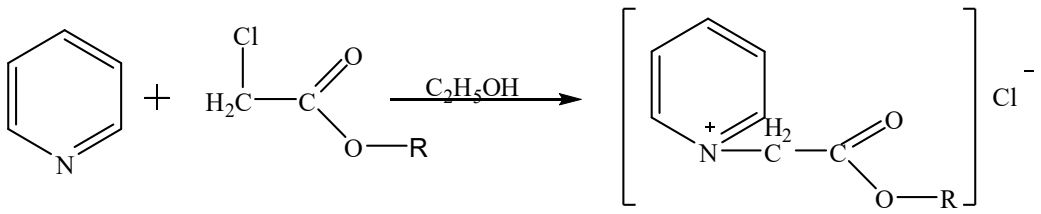
Object and Methodology of the Research.

To determine the specific physicochemical properties of the synthesized compounds, modern instrumental analytical techniques were employed. Infrared (IR) spectra of the compounds were recorded using a Perkin-Elmer Spectrum 2000 FT-IR spectrometer in KBr pellets. Proton (^1H) and carbon-13 (^13C) nuclear magnetic resonance (NMR) spectra were obtained using Unity-400+ (400 MHz) and Jeol-600 (600 MHz) spectrometers. The internal standard was tetramethylsilane (TMS), and spectra were recorded in deuterated solvents such as CD_3COOD and DMSO-d_6.

The melting points of the synthesized compounds were determined using a MEL-TEMP apparatus (USA). All synthetic procedures were conducted using thermally and mechanically stable transparent quartz glassware (Borosilicate 3.3 grade), including flasks and beakers.

Results and Discussion

The reactions of pyridine with the pentyl, iso-pentyl, and hexyl esters of monochloroacetic acid were investigated, and optimal reaction conditions for each were established. The reaction between pyridine and the esters of monochloroacetic acid proceeded according to the following general scheme:



R –C_5H_{11}, i-C_5H_{11}, C_6H_{13},.

These studies confirmed the formation of pyridinium-based quaternary ammonium salts, which were subsequently used for further modifications, including the synthesis of chitosan-based derivatives. The influence of different ester substituents on the reaction yield and efficiency was also analyzed and discussed.

Experimental Optimization of the Reaction Conditions

For the synthesis, pyridine and esters of monochloroacetic acid were dissolved in ethanol and mixed in a 1:2 molar ratio. Initial trials showed that cooling the reaction vessel resulted in a low product yield. Therefore, the reactions were carried out at different temperature intervals: 30–40 °C, 50–60 °C, and 70–80 °C. Among these, the highest product yields were consistently obtained in the 50–60 °C range.

Based on these observations, all subsequent experiments were performed by heating the reaction mixture at 50–60 °C for 2–4 hours. After completion, the resulting quaternary ammonium salts were dried under vacuum for 24 hours.

From the results of the reactions between pyridine and the esters of monochloroacetic acid, the optimal reaction conditions were systematically studied. The influence of solvent type, reaction temperature, reaction time, and reagent molar ratios on the yield of the final product was investigated. The results are summarized in the following table:

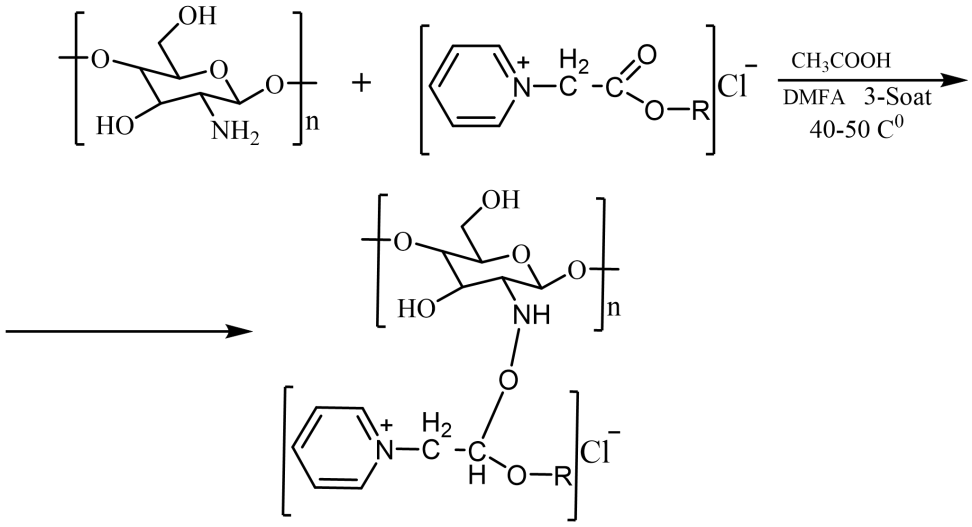
Table 1.1

Optimization of Reaction Conditions for the Synthesis of Pyridinium Salts with Esters of Monochloroacetic Acid

№	Tertiary Amine	Monochloroacetic Acid Ester	Solvent	Reaction Time (h)	Temperature (°C)	Molar Ratio (Amine:Ester)	Yield (%)
2	Pyridine	Pentyl	Ethanol	3	60	1:2	84
3	Pyridine	Iso-pentyl	Ethanol	3	60	1:2	84
4	Pyridine	Hexyl	Ethanol	3	60	1:2	80

To purify the obtained salts, they were dissolved in ethanol, treated with activated charcoal, and heated. The hot solution was then filtered and recrystallized from ethanol. As a result, white crystalline salts were obtained. [13–17]

The synthesized quaternary ammonium salts were then reacted with chitosan. Through the quaternization of chitosan, a water-soluble derivative was obtained, which not only enhanced the biological activity of chitosan but also expanded its potential application areas.



R –C_5H_{11}, изо-C_5H_{11}, C_6H_{13},.

Optimization of Reaction Conditions

To determine optimal reaction conditions, various solvents were tested, with dimethylformamide (DMF) yielding the best results. Reactions were performed at different time intervals, and the highest product yield was observed after 3 hours of reaction time. Additionally, the influence of reaction temperature on the yield of the synthesized quaternary ammonium salt was studied. The highest yield was obtained when the reaction was carried out at 40–50 °C.

Biological Activity (Antifungal Assay)

Fungal Growth Inhibition Test

This test is used to evaluate the ability of active compounds to inhibit fungal growth and assess their antifungal efficacy. The procedure was as follows:

A 5 mg/mL aqueous solution of the synthesized quaternary ammonium chitosan salt was prepared using deionized water. Serial dilutions were made to prepare test solutions at concentrations of 0.1, 0.5, and 1.0 mg/mL by mixing the chitosan derivative with potato dextrose medium (PDA). The mixtures were sterilized at 120 °C. Once cooled and solidified in Petri dishes (6.5 cm diameter), fungal mycelial discs (5 mm diameter) were placed at the center of each plate. The plates were incubated at 27 °C.

When the fungal growth on the control plate (without sample) reached the edge of the plate, the diameters of fungal growth zones were measured both for the test plate (Da) and the control plate (Db). The inhibition index (I%) was calculated using the following formula:

$$(\%) = \left(1 - \frac{D_a - 5}{D_b - 5}\right) \times 100$$

In this formula, Da represents the diameter of the fungal growth zone on the test plate**,** while Db denotes the diameter on the control plate**.** The equation reflects the difference in fungal growth between the control and treated samples. The inhibition index expresses the percentage reduction in fungal growth and serves as a quantitative measure of antifungal efficacy.

Results of the Study: IR Spectral Analysis

The IR spectra of the synthesized compounds were recorded using SPECORD-75IR and Avatar 360 spectrophotometers with KBr pellets.

Figure 1 shows the IR spectra of chitosan and its quaternary ammonium salt derivative obtained via reaction with the pyridinium-based quaternary ammonium salt. The broad absorption band in the range of 3200–3500 cm^{-1} corresponds to the stretching vibrations of N–H and O–H groups in the chitosan structure.

Characteristic absorption bands were observed at: 2878 cm^{-1} – attributed to C–H stretching vibrations, 1600 cm^{-1} – corresponding to amide bond vibrations, 1156 cm^{-1} and 1078 cm^{-1} – related to C–O–C and secondary hydroxyl (C–O) stretching vibrations, respectively. Out-of-plane deformation vibrations of aromatic C–H bonds were identified in the 650–900 cm^{-1} region, characteristic of substituted aromatic rings.

The appearance of new absorption bands at 1467 cm^{-1}, 1464 cm^{-1}, and 1471 cm^{-1} in the spectrum of the chitosan derivative confirms the presence of $–N^+$ stretching vibrations, indicating the successful formation of the quaternary ammonium salt of chitosan. These spectral changes validate the structural modification of chitosan and support the successful synthesis of its quaternized derivative.

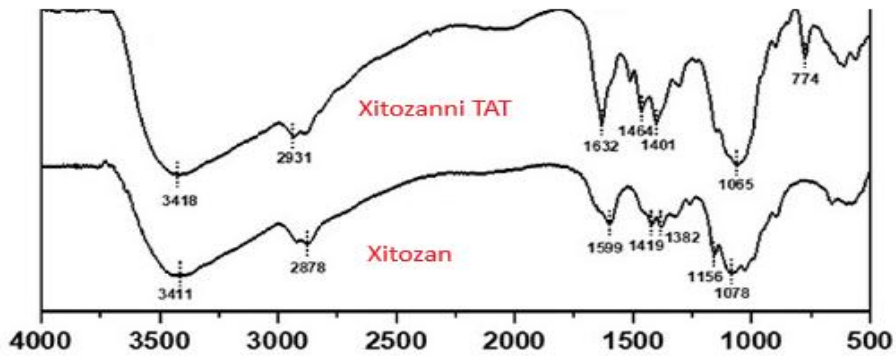


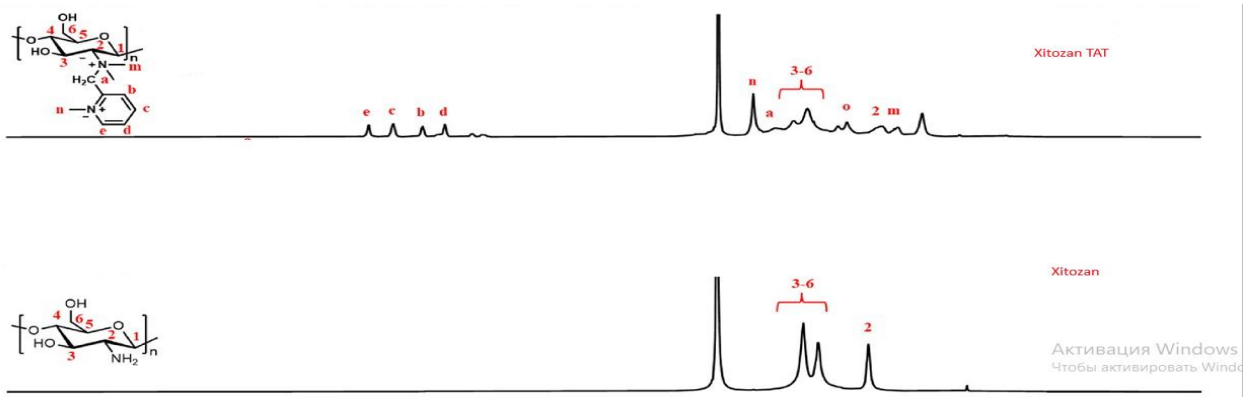
Figure 1. IR Spectra of Chitosan and Its Quaternary Ammonium Salt Derivative.

^{1}H NMR Spectral Analysis

The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Unity-400+ (400 MHz) and Jeol-600 (600 MHz) spectrometers using deuterated solvents (CD_3COOD and DMSO-d_6). Tetramethylsilane (TMS) was used as the internal standard, and chemical shifts are reported in parts per million (δ, ppm) relative to the TMS standard. The corresponding spectra are shown in **Figure 2**.

Chitosan: ^{1}H NMR (500 MHz, D_2O): δ 3.75–3.58 (m, 4H, H_3–H_6), δ 3.02 (s, 1H, H_2)

Quaternized Chitosan Derivative: ^{1}H NMR (500 MHz, D_2O): δ 8.68 (s, 1H, H_e), δ 8.40 (m, 2H, H), δ 8.06 (s, 1H, H_β), δ 7.81 (s, 1H, H), δ 4.31 (s, 1H, H_n), δ 4.06 (s, 1H, H_a), δ 3.85–3.69 (m, 4H, H_3–H_6), δ 3.24 (s, 9H, H_o), δ 2.85 (s, 1H, H_2), δ 2.66 (s, 6H, H_m).



Conclusion

A water-soluble quaternary ammonium salt was successfully synthesized based on pyridine. Considering the known biological activity of chitosan, its modification with the synthesized quaternary ammonium salt was carried out to enhance its functional properties. As a result, a water-soluble derivative of chitosan was obtained. The introduction of positively charged quaternary ammonium groups into the chitosan structure significantly enhanced its antibacterial activity. This structural modification led to a marked increase in the biological efficacy of the chitosan derivative.

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